Volume 45 September 1991 (2) Number 3



# PROGRAM AND ABSTRACTS OF THE 40TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

Grant No. DAMD17-92-J-2008

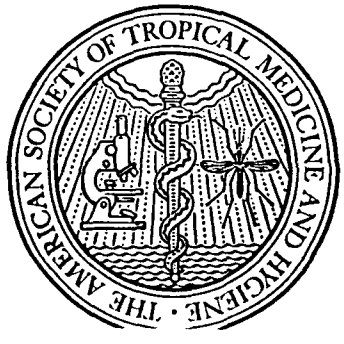


## The Boston Sheraton
## Boston, Massachusetts
## December 1-5, 1991

Final Proceedings

**Supplement to**
**THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE**





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# REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | 1991 September | Final Proceedings |

**4. TITLE AND SUBTITLE** Annual Meeting of the American Society of Tropical Medicine -- Program and Abstracts of the 40th Annual Meeting of the American Society of Tropical Medicine and Hygiene

**5. FUNDING NUMBERS**
Grant No
DAMD17-92-J-2008

**6. AUTHOR(S)**

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**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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School of Medicine
10701 East Boulevard
Cleveland, OH 44106

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

Conference held December 1-5, 1991 at the Sheraton, Boston, Massachusetts

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for public release; distribution unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200 words)*

**14. SUBJECT TERMS**

Conference, Biotechnology, R&I, ID

**15. NUMBER OF PAGES**

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | N/A | N/A |

# AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

## ANNUAL MEETING

## 1-5 DECEMBER, 1991



## SHERATON BOSTON HOTEL

## BOSTON, MASSACHUSETTS

# AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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*AMERICAN SOCIETY OF TROPICAL VETERINARY MEDICINE*

Jim C. Williams, President

## REGISTRATION INFORMATION

Republic Foyer, Sheraton Boston

| | | |
|---|---|---|
| Sunday | December 1 | 12:00 NN- 5:00 PM |
| Monday | December 2 | 7:30 AM- 5:00 PM |
| Tuesday | December 3 | 8:00 AM- 5:00 PM |
| Wednesday | December 4 | 8:00 AM- 5:00 PM |
| Thursday | December 5 | 8:00 AM-12:00 NN |

## SPONSORS

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## EXHIBITORS

Academia Book Exhibits
Airco New England
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Becton Instruments, Inc.
Becton Dickenson Tropical Disease Diagnostics
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Summit Chemical Company
Zoecon

## TIME AND PLACE OF EXHIBITS

Republic Ballroom, Sheraton Boston

| | | |
|---|---|---|
| Monday | December 2 | 9:00 AM- 4:30 PM |
| Tuesday | December 3 | 8:00 AM- 4:30 PM |
| Wednesday | December 4 | 8:00 AM- 4:30 PM |

## ARCHIVE EXHIBIT

## AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE and AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

The Archives exhibit will be located at the **Francis A. Countway Library of Medicine**, Harvard Medical School, 25 Shattuck Street, Boston. The exhibit, co-sponsored by the Library's Rare Books Department, will feature items from the ASTMH and ACAV collections, as well as items from the Library's own archives.

Society members are invited to visit the ASTMH Archives Room, 5th Floor, Countway Library. Wear your ASTMH registration badge to assure entry to the Library.

## LATE BREAKERS IN BIOLOGY AND MOLECULAR BIOLOGY

Thursday, December 5
8:00-10:30 AM
Commonwealth Room

This Session is specifically designed for brief presentation of important, new data obtained after the closing date for abstract submission. Presentations are restricted to 5 minutes plus 5 minutes discussion time. Submit abstracts of 200 words or less to:

Dr. Michael Hollingdale
Biomedical Research Institute
1211 Parklawn Drive,
Rockville, MD 20852.
Phone (301) 881-3300, Fax 881-7640 *or*

Dr. Stephen Meshnick
Department of Microbiology,
Sophie Davis School of Biomedical Education,
City College of New York, 128th St. and Convent Ave.,
New York, NY 10031.
Phone (212) 650-6628, Fax 650-7797

prior to the Meeting or at the Meeting, but no later than 5:00 PM, Tuesday, December 3. The list of presenters will be posted Wednesday morning. Check the bulletin board at the Meeting for further information, including hotel rooms of Drs. Hollingdale and Meshnick.

## AUDIOVISUAL FACILITIES

Slide preview and submission facilities are provided in the Jefferson Room beginning 3:00 PM on Sunday, December 1. Speakers scheduled for AM sessions should preview slides and place them in carousels on the afternoon before their scientific presentation. Speakers scheduled for afternoon sessions should prepare slides on the morning of their presentation.

## MESSAGES AND EMERGENCY CALLS

A message board will be available near the Registration Desk, Republic Foyer, 2nd Floor, Sheraton Boston Hotel. Emergency calls should be directed to (617)-236-2000, the main switchboard of the hotel.

## EMPLOYMENT OPPORTUNITIES

Bulletin boards for posting employment opportunities will be available in the Registration area, Republic Foyer.

## POSTER PRESENTATIONS

Poster Sessions on Tuesday, December 3 and Wednesday, December 4 will be located in the Liberty Complex, 2nd Floor. A Continental Breakfast will be served. Posters may be set up beginning at 7:00 PM on the night before the session or in the morning before the session begins. Numbers on poster boards correspond to abstract numbers in the Program Booklet. Authors should be in attendance from 7:30- 9:30 AM at each poster session. Posters should be taken down during the lunch hour or afternoon following the session.

## CONTINUING MEDICAL EDUCATION

The American Society of Tropical Medicine & Hygiene is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians.

The American Society of Tropical Medicine & Hygiene designates this continuing medical education activity for up to 30 credit hours in Category 1 of the Physician's Recognition Award of the American Medical Association.

Attendees desiring CME credit are advised to preregister for this activity (see materials mailed in the Registration Package) or to register at the ASTMH Registration Desk, Republic Foyer, whereupon they will receive instructions and required forms. Documentation fees will be collected. Certificates based on recorded attendance at medical education activities will be mailed within one month of the meeting.

## ASTMH TRAVEL GRANT AWARDEES

Sponsored by

The Rockefeller Foundation
U.S. Army Medical Research & Development Command
National Institute of Allergy and Infectious Diseases

| | |
|---|---|
| Dr. Roberto Badero | Salvador, Bahia, Brazil |
| Dr. Eleni Dotsika | Athens, Greece |
| Dr. Phyllis Freeman-Junior | Nashville, Tennessee |
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## NOTICES

Badges must be worn to attend all functions.

Smoking will be permitted only where specifically authorized. This rule is in compliance with the Resolution on Smoking adopted at the ASTMH Annual Business Meeting on November 5, 1976. The cooperation and thoughtfulness of smokers is requested to minimize embarrassment and discomfort for all persons.

The time and/or location of all activities are subject to change. Change notices will be posted in the Registration area.

Suggestions for changes in the Annual Meetings may be directed to members of the Scientific Program Committee.

## 1992 ANNUAL MEETING

The 1992 Annual Meeting will be held 15-19 November at the Seattle Sheraton Hotel, Seattle, Washington.

The Deadline for Abstract submission will be 1 June, 1992. A revised Abstract Submission computer software package will be available in early 1992. Watch for announcements and order forms, which will be mailed with the Journal.

# SUMMARY OF

# SCIENTIFIC PROGRAM

## SUNDAY, DECEMBER 1

| | | |
|---|---|---|
| 8:00 AM - 5:00 PM | ASTMH Council | Gardner B |
| 8:30 AM- 4:45 PM | Pre-Meeting Workshop: Mosquito Histology, Ultrastructure, and Methods for the *in situ* Localization of Viruses | Hampton A/B |
| 12:00 AM- 5:00 PM | Registration | Republic Foyer |
| 1:00 - 5:00 PM | SIRACA Subcommittee of ACAV | Board |
| 4:00 - 5:30 PM | ACAV Executive Council | Conference |
| 4:30- 5:30 PM | ACME Council | Berkeley A |
| 6:00 PM | **Opening Reception** | Grand Ballroom |

## MONDAY, DECEMBER 2

| | | |
|---|---|---|
| 7:00 - 8:00 AM | Program Committee Breakfast | Dalton |
| 7:30 AM- 5:00 PM | Registration | Republic Foyer |
| 8:00 AM- 12:00 NN | **Plenary Symposium**: Cholera | Grand Ballroom |
| 9:00 AM- 4:30 PM | **Exhibits** | Republic Ballroom |
| 9:50- 10:20 AM | Coffee Break | Republic Ballroom/ Corridor |
| 1:30 - 5:20 PM | **Symposium**: Malaria Vaccines | Grand Ballroom |
| 1:30 - 6:15 PM | 7th Annual Meeting: **American Committee on Medical Entomology.** Symposium on Vector Control | Independence East |
| 1:30 - 5:15 PM | **Symposium:** Role of Immunological Mechanisms in the Induction and Regulation of Granulomatous Hypersensitivity to Egg Antigens of *Schistosoma mansoni* | Independence W/C |
| 1:30 - 5:15 PM | **Scientific Session A**: Clinical Tropical Medicine | Gardner A/B |
| 1:30 -4:00 PM | **Scientific Session B**: Arbovirus Molecular Biology | Hampton A/B |
| 1:30 - 5:00 PM | **Scientific Session D:** Kinetoplastida Biology and Molecular Biology | Fairfax A |
| 1:30 - 3:00 PM | **Scientific Session E:** Bacteriology and Rickettsiology | Fairfax B |
| 2:55 - 3:40 PM | Coffee Break | Republic Ballroom/ Corridor |
| 3:30 - 5:00 PM | **Scientific Session F**: Medical Malacology | Fairfax B |
| 4:00 - 5:45 PM | **Scientific Session C**: Hepatitis and Retrovirus Epidemiology | Hampton A/B |
| 4:00 - 6:00 PM | **Workshop:** Diseases of the Tropics: Telecommunications and Technology Transfer | Commonwealth |
| 5:30 - 7:00 PM | NIAID Meeting: International Grants on Tropical Diseases | Fairfax B |

## TUESDAY, DECEMBER 3

| | | |
|---|---|---|
| 7:00 - 8:00 AM | Past Presidents' Breakfast | Board |
| 7:00 - 9:00 AM | AJTMH Editorial Board Breakfast | Clarendon |
| 7:30 - 10:00 AM | **Poster Session I**<br>Continental breakfast. | Liberty |
| 8:00 AM - 4:30 PM | Registration | Republic Foyer |
| 8:00 AM- 5:00 PM | **Exhibits** | Republic Ballroom |
| 9:30 AM- 12:00 NN | **Symposium:** Malaria Prevention and Control: From Recommendation to Reality | Grand Ballroom |
| 9:30 AM- 12:00 NN | **Symposium:** Models of Vector-Borne Diseases | Independence East |
| 9:30 AM- 12:00 NN | **Scientific Session G:**<br>Malaria Biology and Molecular Biology | Commonwealth |
| 10:00 AM- 12:00 NN | **Symposium:** Cell-Surface Cysteine-Rich Proteins and the Pathogenesis of Tropical Diseases | Independence W/C |
| 10:00 AM- 12:00 NN | **Scientific Session H:**<br>Helminth Diagnosis and Epidemiology | Hampton A/B |
| 10:00 - 11:45 AM | **Scientific Session I:**<br>Filaria Biology and Molecular Biology | Gardner A/B |
| 10:00 AM- 12:00 NN | **Scientific Session J:**<br>Arbovirus Immunology | Fairfax A |
| 10:00 - 11:45 AM | **Scientific Session K:**<br>Sand Flies and *Leishmania* | Fairfax B |

## TUESDAY, DECEMBER 3, Cont.

| | | |
|---|---|---|
| 1:00 - 1:45 PM | **Film**: Wood Ticks and Lyme Disease | Fairfax A |
| 1:00 - 1:30 PM | **Update**: Global Immunization. The Children's Vaccine Initiative | Independence W/C |
| 1:00 - 1:45 PM | **Commemorative Fund Lecture** | Grand Ballroom |
| 2:00 - 3:00 PM | **ASTMH Presidential Address** | Grand Ballroom |
| 3:00 - 3:15 PM | **Congressional Outlook** | Grand Ballroom |
| 3:15 - 3:45 PM | Coffee Break | Republic Ballroom / Corridor |
| 3:45 - 4:15 PM | **ASTMH Awards Ceremony** | Grand Ballroom |
| 4:15 - 5:15 PM | **ASTMH Business Meeting** | Grand Ballroom |
| 5:15 - 6:15 PM | **Public Policy Workshop**: How to Participate in the Public Policy Process | Grand Ballroom |

## WEDNESDAY, DECEMBER 4

| | | |
|---|---|---|
| 7:30 - 10:00 AM | **Poster Session II**<br>Continental breakfast. | Liberty |
| 8:00 AM- 5:00 PM | Registration | Republic Foyer |
| 8:00 AM- 4:30 PM | **Exhibits** | Republic Ballroom |
| 9:45 AM- 12:00 NN | **Symposium:** Recent Advances in the Exoerythrocytic Stages of the Malaria Parasite | Independence W/C |
| 10:00 AM- 12:00 NN | **Scientific Session L:**<br>Lyme Disease **(Annual Meeting: American Society of Tropical Veterinary Medicine)** | Commonwealth |
| 10:00 AM- 12:00 NN | **Scientific Session M:**<br>Kinetoplastida Chemotherapy and Epidemiology | Independence East |
| 10:00 AM- 12:00 NN | **Scientific Session N:** Intestinal Protozoa | Hampton A/B |
| 10:00 AM- 12:00 NN | **Scientific Session O:**<br>Filariasis Pathology and Diagnosis | Gardner A/B |
| 10:00 - 11:45 AM | **Scientific Session P:** Viral vaccines | Fairfax A |

## WEDNESDAY, DECEMBER 4, cont.

| | | |
|---|---|---|
| 1:00 - 1:45 PM | **Film**: Wood Ticks and Lyme Disease | Grand Ballroom |
| 1:30 - 5:45 PM | **American Committee on Clinical Tropical Medicine and Travelers' Health**<br>V. Marcolongo Lecture | Independence W/C |
| 1:30 - 5:00 PM | **Scientific Session Q:** Malaria Immunology I | Fairfax A/B |
| 1:30 - 5:00 PM | **Scientific Session R:** Helminth Immunology | Commonwealth |
| 1:30 - 5:00 PM | **Scientific Session S:** Opportunistic Infections | Hampton A/B |
| 1:30- 5:45 PM | **American Committee on Arthropod-Borne Viruses** | Gardner A/B |
| 1:30 - 5:30 PM | **Scientific Session T:** Filarial Immunoregulation and Protective Immunity | Independence East |
| 3:00 - 3:35 PM | Coffee Break | Republic Ballroom/ Corridor |
| 6:30 - 8:00 PM | Pre-Banquet Reception | Constitution Ballroom/Foyer |
| 8:00 - 10:30PM | **ASTMH Banquet** | Grand Ballroom |

## THURSDAY, DECEMBER 5

| | | |
|---|---|---|
| 7:00- 9:00 AM | ASTMH Council Breakfast | Exeter |
| 8:00 AM- 12:00 NN | Registration | Republic Foyer |
| 8:00 - 10:30 AM | **Late Breakers in Biology and Molecular Biology** | Commonwealth |
| 8:15 - 10:30 AM | **Scientific Session U:** Malaria Chemotherapy I | Independence Center |
| 8:15 - 10:30 AM | **Scientific Session V:** Schistosomiasis: Molecular Biology and Biochemistry | Independence East |
| 8:00 - 10:45 AM | **Scientific Session W:** Malaria Epidemiology and Field Studies | Fairfax A |
| 8:00 - 10:30 AM | **Scientific Session X:** Amebiasis | Hampton A/B |
| 8:00 - 10:30 AM | **Scientific Session Y:** Arbovirus Epidemiology | Independence West |
| 8:30 - 10:30 AM | **Scientific Session Z:** Filariasis Chemotherapy | Gardner A/B |
| 8:00 - 10:30 AM | **Scientific Session AA:** Entomology | Fairfax B |
| 10:30 - 11:00 AM | Coffee Break | Republic Ballroom/ Corridor |
| 11:00 AM- 12:00 NN | **Fred L. Soper Memorial Lecture** | Grand Ballroom |

## THURSDAY, DECEMBER 5

| | | |
|---|---|---|
| 1:30- 5:00 PM | **Symposium:**<br>Disease Control in Operation Desert Shield/ Storm | Fairfax A/B |
| 1:30 - 5:15 PM | **Scientific Session BB:**<br>Malaria Immunology II | Constitution |
| 1:30 - 5:00 PM | **Scientific Session CC:**<br>Malaria Chemotherapy II | Gardner A/B |
| 1:30 - 4:15 PM | **Scientific Session DD:**<br>Arbovirus Vector Studies | Hampton A/B |
| 1:30 - 4:30 PM | **Scientific Session EE:**<br>Kinetoplastida Immunology | Commonwealth |
| 2:50- 3:30 PM | Coffee Break | Republic Ballroom/ Corridor |

# DETAILED

# SCIENTIFIC PROGRAM

"*" Designates the speaker presenting multiple-author papers.

# PRE-MEETING WORKSHOP

## WORKSHOP ON MOSQUITO HISTOLOGY, ULTRASTRUCTURE, AND METHODS OF THE *IN SITU* LOCALIZATION OF VIRUSES ‡

Sunday, December 1 — Hampton A/B
Organizer: W.S. Romoser
8:30 AM- 4:45 PM

‡ Sponsored by the American Committee of Medical Entomology and the Ohio University Tropical and Geographical Disease Institute. Supported in part by the US Army Medical Research and Development Command.

# PLENARY SYMPOSIUM

## CHOLERA

Monday, December 2
8:00 AM - 12:00 NN — Grand Ballroom
Chairperson: M.M. Levine

8:00 S1 THE EPIDEMIOLOGY OF CHOLERA: FROM SNOW TO CEVICHE. Glass R. Viral Gastroenteritis Unit, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA.

8:35 S2 CHOLERA IN PERU, 1991: THE EXTENT OF THE EPIDEMIC, MODES OF TRANSMISSION AND THE IMPACT OF THE OUTBREAK ON THE COUNTRY AS A WHOLE. Salazar Lindo E. Ministry of Health, Lima, Peru.

9:15 S3 NON-CULTIVABLE *VIBRIO CHOLERAE* O1 IN ENVIRONMENTAL WATERS, ZOOPLANKTON AND EDIBLE CRUSTACEA: IMPLICATIONS FOR UNDERSTANDING THE EPIDEMIOLOGIC BEHAVIOR OF CHOLERA. Colwell R. Maryland Biotechnology Institute, University of Maryland, College Park, MD.

9:50 Coffee Break

10:20 S4 THE MOLECULAR PATHOGENESIS OF CHOLERA: YET FURTHER INSIGHTS. Mekalanos J. Department of Microbiology and Molecular Biology, Harvard Medical School, Boston, MA.

10:55 S5 A PRIMER ON THE THERAPY OF CHOLERA UNDER EPIDEMIC CONDITIONS. Bennish M. Tufts University School of Medicine, Boston, MA.

11:25 S6 OLD AND NEW CHOLERA VACCINES: A 100 YEAR PERSPECTIVE. Levine MM. Center for Vaccine Development, University of Maryland, Baltimore, MD.

# SYMPOSIUM: MALARIA VACCINES

Monday December 2
1:30 - 5:20 PM Grand Ballroom
Chairpersons: W.R. Ballou and E. Nardin

## I. TARGET ANTIGENS

### SPOROZOITE (CS) PROTEIN

1:30 S7 THE DESIGN OF MULTIPLE ANTIGEN PEPTIDE (MAPS) VACCINES BASED ON RESPONSES OF SPOROZOITE-IMMUNIZED VOLUNTEERS. Nardin E*. New York University School of Medicine, New York, NY.

1:50 S8 INDUCTION OF CYTOLYTIC T CELL RESPONSES BY THE REPEATLESS *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN MOLECULE INCORPORATED INTO LIPOSOMES. White K, Gordon D, Gross M, Richards RL, Alving CR, Ballou WR and Krzych U*. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

### BLOOD STAGE ANTIGENS

2:10 S9 IMMUNOGENICITY OF NEW FORMULATIONS OF SYNTHETIC PEPTIDE SPF66 VACCINE. Ballou WR*, Gordon DG, Alving CR, and Sadoff JC. Department of Immunology, Walter Reed Army Institute of Research, Washington DC.

2:30 S10 EXPRESSION IN E. *COLI* AND IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* MSA-2. Anders RF, Dyer S, Kemp DO, Pye D, Wu MJ, Smythe JA, Marshall VM, Kemp DJ, Goss N, Woodrow GC, and Coppel RL. The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia.

### GAMETOCYTE ANTIGENS

2:50 S11 PFS25 TRANSMISSION BLOCKING VACCINES. Kaslow DC*, Bathurst I, Isaccs S, Keister DB, Moss B, and Barr PJ. Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

3:10 Coffee Break

## II. NEW DELIVERY SYSTEMS AND ADJUVANTS

3:40 S12 ROLE OF ADJUVANT FORMULATIONS BASED ON COPOLYMERS IN THE DESIGN OF A MALARIA PEPTIDE VACCINE. Millet P*, Kalish ML, Olsen M, Grady KK, Collins WE, and Hunter RL. Malaria Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA

4:00 S13 THE USE OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBsAg) AS A CARRIER FOR *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN B AND T CELL EPITOPES. Gordon DM*, Cohen J, De Wilde M, Meuwissen JHE, Hollingdale MR, and Ballou WR. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

4:20 S14 MALARIA SPOROZOITE VACCINE PROVIDES MINIMAL PROTECTION DESPITE INDUCTION OF HIGH LEVELS OF ANTIBODIES. Hoffman SL*, Edelman R, Bryan J, Schneider I, Davis J, Sedegah M, Gordon DM, Hollingdale MR, Gross M, Paparello S, and Jones T. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

**III. VACCINE TRIALS UNDER CONDITIONS OF NATURAL EXPOSURE**

4:40 S15 FIELD TRIAL OF A RECOMBINANT *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE (CS) PROTEIN CONJUGATE VACCINE IN THAI SOLDIERS. Brown AE, Singharaj P, Webster HK, Pipithkul J, Gordon DG, Boslego JW, Krinchai K, Su-archawaratana P, Wongsrichanalai C, Hollingdale MR, Ballou WR, Wittis J S, Cryz SJ, and Sadoff JC. U.S. Army Medical Component, AFRIMS, Bangkok, Thailand.

5:00 S16 IMMUNOGENICITY AND EFFICACY OF A *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE VACCINE IN A MALARIA ENDEMIC AREA OF KENYA. Sherwood JA*, Copeland RS, Taylor KA, Abok K, Ruebush TK, Ondolo HAO, Were JBO, Oloo AJ, Githure JI, Mason CJ, Wirtz RA, Schneider IP, Gordon DM, Ballou WR, Hollingdale MR, Gross M, Wittis J, Sadoff JC, and Roberts CR. U.S Army Medical Research Unit-Kenya; and Walter Reed Army Institute of Research, Washington, DC.

## 7th ANNUAL MEETING
## AMERICAN COMMITTEE ON MEDICAL ENTOMOLOGY ‡

## SYMPOSIUM ON VECTOR CONTROL

Monday December 2
1:30 - 6:15 PM Independence East
Chairperson: G. G. Clark

1:30 INTRODUCTION, Clark GG, San Juan Laboratories, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, San Juan, PR.

1:35 S17 OVERVIEW OF VECTOR MANAGEMENT; THE PROBLEMS. Arata AA. VBC Project/Agency for International Development, Arlington, VA.

1:55 S18 THE FUTURE IS HERE: THE EMBATTLED INSECTICIDE ARSENAL. Jany W. American Cyanamid, Lahaska, PA.

2:15 S19 REPELLENTS: AN IMPASSE FOR DISEASE VECTORS OR HUMAN HOSTS? Bowen MF. SRI International, Menlo Park, CA.

2:35 S20 SUCCESSFUL CONTROL OF TSETSE FLIES USING ATTRACTANTS. Brady J. Imperial College, London, UK.

2:55 Coffee Break

3:25 S21 THE ROLE OF BIOLOGICAL CONTROL IN THE INTEGRATED CONTROL OF MOSQUITOES. Lacey LA. USDA, ARS, APO, NY.

3:45 S22 VACCINES AGAINST ARTHROPODS. Kay BH. Queensland Institute of Medical Research (QIMR), Brisbane, Australia.

4:05 S23 GENETIC APPROACHES TO MALARIA CONTROL: HOW LONG IS THE ROAD? Gwadz RW. NIAID, National Institutes of Health, Bethesda, MD.

4:25 S24 APPLICATION OF REMOTE SENSING TECHNOLOGY TO VECTOR CONTROL. Washino K. University of California, Davis, CA.

4:45 S25 IMPREGNATED BEDNETS FOR MALARIA CONTROL. BIOLOGICAL SUCCESS AND SOCIAL RESPONSIBILITY. Sexton JD. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

5:05 S26 COMMUNITY INVOLVEMENT IN THE PREVENTION OF CHAGAS' DISEASE IN BOLIVIA. Bryan RT, Centers for Disease Control, Atlanta, GA.

5:25 S27 COMMUNITY-BASED PREVENTION AND CONTROL OF DENGUE/DENGUE HEMORRHAGIC FEVER. Gubler DJ. Centers for Disease Control, Fort Collins, CO.

5:45 ACME Business Meeting

‡ Supported by Becton Dickinson Tropical Disease Diagnostics

## SYMPOSIUM:
## THE ROLE OF IMMUNOLOGICAL MECHANISMS IN THE INDUCTION AND REGULATION OF GRANULOMATOUS HYPERSENSITIVITY TO EGG ANTIGENS OF *SCHISTOSOMA MANSONI*

Monday, December 2
1:30 - 5:15 PM Independence West/Center
Chairpersons: M. J. Stadecker and D.G. Colley

1:30 INTRODUCTORY REMARKS. Stadecker M. Department of Pathology, Tufts University School of Medicine, Boston, MA.

1:45 S28 CHARACTERIZATION OF SCHISTOSOMAL EGG ANTIGENS RECOGNIZED BY HUMAN CD4 POSITIVE T CELL CLONES. Harn D. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

2:05 S29 THE ROLE OF CD8 T CELLS IN THE REGULATION OF GRANULOMATOUS HYPERSENSITIVITY. Doughty B. Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

2:25 S30 FUNCTION AND REGULATION OF EGG-INDUCED TH2 RESPONSES IN *SCHISTOSOMA MANSONI* INFECTION. Sher A. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

SYMPOSIUM: *SCHISTOSOMA* GRANULOMAS

2:45 S31 THE ROLE OF ANTIGENS AND CYTOKINES IN THE GENERATION AND MAINTENANCE OF THE SCHISTOSOMAL EGG GRANULOMA. Boros D. Department of Immunology/Microbiology, Wayne State University School of Medicine, Detroit, MI.

3:05 Coffee Break

3:35 S32 MOLECULAR BASIS OF GRANULOMA FORMATION: INTERACTION OF T CELLS, THEIR ANTIIDIOTYPIC REGULATORY AND SUPPRESSOR FACTOR ACTIVITY. Phillips SM. Allergy and Immunology Section, University of Pennsylvania, Philadelphia, PA.

3:55 S33 IMMUNE MECHANISMS AND SCHISTOSOME GRANULOMAS: CELLS, IDIOTYPES, CYTOKINES, AND GENES. Colley D. Veterans Administration Medical Center, Vanderbilt University, Nashville, TN.

4:15 S34 THE SCHISTOSOMAL EGG GRANULOMA: NEW APPROACHES TO ITS STUDY AND INTERPRETATIONS OF ITS BIOLOGY. Stadecker M. Department of Pathology, Tufts University School of Medicine, Boston, MA.

4:35 S35 THE INFLAMMATION/FIBROSIS INTERFACE IN THE PATHOGENESIS OF SCHISTOSOMAL LIVER DISEASE. Wyler D. Division of Geographic Medicine and Infectious Diseases, Tufts University School of Medicine, New England Medical Center Hospitals, Boston, MA.

4:55 General Discussion

## SCIENTIFIC SESSION A: CLINICAL TROPICAL MEDICINE

Monday, December 2
1:30 - 5:15 PM Gardner A/B
Chairpersons: P. Kozarsky and P. Weller

1:30 1 ANTHRAX OUTBREAK IN CENTRAL JAVA, INDONESIA. Sianturi L, Koesharyono C, Soerjosembodo S, Suharyono W, Ezzell J, Ksiazek T, and Jennings G*. Ministry of Health, Jakarta, Indonesia; U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD; and U.S. Medical Research Unit No. 2, Jakarta, Indonesia.

1:45 2 THE USE OF TUBERCULIN SCREENING IN SUSPECTED TUBERCULOUS MENINGITIS PATIENTS. Girgis NI*, Kilpatrick ME, Farid Z, Erian M, and Corwin A. US Navy Medical Research Unit No. 3, Cairo, Egypt.

2:00 3 OCCURRENCE AND SELF-TREATMENT OF TRAVELER'S DIARRHEA IN A LARGE GROUP OF AMERICAN TRAVELERS. Hill DR*. International Travelers' Medical Service, University of Connecticut School of Medicine, Farmington, CT.

2:15 4 RABIES VACCINE IN TRAVELERS: A DECISION ANALYSIS. Wilson ME* and Fineberg HV. Mt. Auburn Hospital, Cambridge, MA; and Harvard Medical School, Harvard School of Public Health, Boston, MA.

2:30 5 *BLASTOCYSTIS HOMINIS*: ERADICATIVE THERAPY FOR A PROBABLE PATHOGEN. El-Masry NA*, Bassily SB, Farid Z, Mansour NS, Sabry AG, and Kilpatrick ME. US Naval Medical Research Unit No. 3, Cairo, Egypt.

2:45 6 UPDATE ON DISTRIBUTION, CHARACTERISTICS, AND MANAGEMENT OF THE POLYCYSTIC HYDATID DISEASE IN MAN. D'Alessandro A*. Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA.

3:00 Coffee Break

3:30 7 THE NATURAL HISTORY OF CUTANEOUS LEISHMANIASIS, GUATEMALA. Herwaldt BL*, Arana BA, and Navin TR. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Medical Entomology Research and Training Unit, Guatemala City, Guatemala.

3:45 8 BUBONIC LEISHMANIASIS: A NEW FORM OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION IN CEARA STATE, BRAZIL. Sousa AQ*, Pompeu MM, Gomes TN, Coelho Filho JM, Oliveira EG, Lima FF, Vasconcelos IA, Parise ME, Maguire JH, Vasconcelos AW, and David JR. Hospital Sao Jose, Ceara State, Brazil; Nucleo de Medicina Tropical, Federal University of Ceara, Brazil; Departamento de Patologia e Medicina Legal, Federal University of Ceara, Brazil; Departamento de Medicina Clinica, Federal University of Ceara, Brazil; Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA; Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

4:00 9 RECOMBINANT INTERFERON-γ IN COMBINATION WITH PENTAVALENT ANTIMONY IN THE THERAPY OF LEISHMANIASIS. Badaro R*, Barral-Netto M, Carvalho EM, Teixeira R, Rocha H, Johnson, W., Jr. Department of Medicine, Federal University of Bahia, Brazil; and Division of International Medicine, Cornell University College, New York, NY.

4:15 10 RELATIONSHIP BETWEEN INTENSITY OF *OPISTHORCHIS VIVERRINI* INFECTION AND HEPATOBILIARY DISEASE DETECTED BY ULTRASONOGRAPHY. Elkins DB*, Mairiang E, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Posri S, Sithithaworn P, Haswell-Elkins M. Tropical Health Program, Queensland Institute of Medical Research, Herston, Queensland, Australia; and Departments of Radiology, Parasitology and Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

4:30 11 IVERMECTIN IN ONCHOCERCIASIS AND IN CONCOMITANT *ONCOCERCA VOLVULUS, LOA LOA,* AND *MANSONELLA STREPTOCERCUS*. Grigsby ME*, Olopoenia L, and Sanusi S. Infectious Diseases Division, Department of Medicine, Howard University Hospital, Washington, DC.

4:45 12 CLINICAL DIFFERENCES BETWEEN BLACK AND CHACHI AMERICAN ONCHOCERCIASIS PATIENTS LIVING TOGETHER IN ECUADOR. Proano R*, Mackenzie CD, Guderian RH, and O'Day J. Department of Pathology, Michigan State University, East Lansing, MI.

5:00 13 ANEMIA AND BLOOD TRANSFUSION PRACTICES IN KENYAN WOMEN. Zucker JR*, Lackritz EM, Ruebush TK, Adungosi J, Were JB, and Campbell CC. Malaria Branch, Centers for Disease Control, Atlanta, GA; Siaya District Hospital, Siaya, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

## SCIENTIFIC SESSION B: ARBOVIRUS MOLECULAR BIOLOGY

Monday, December 2
1:30 - 4:00 PM Hampton A/B
Chairperson: J. Patterson

1:30 14 CORRELATION OF AN ANTIGENIC SUBTYPE OF EASTERN EQUINE ENCEPHALITIS VIRUS WITH ALTERATIONS IN THE E2 GLYCOPROTEIN. Repik PM*, Strizki JM, and Calisher CH. The Medical College of Pennsylvania, Philadelphia, PA; and Division of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, CO.

1:45 15 DETECTION OF RIFT VALLEY FEVER VIRUS NUCLEIC ACID IN MOSQUITOES BY *IN SITU* HYBRIDIZATION WITH A DIGOXIGENIN CDNA PROBE Patrican LA*, Hoover TA, Dohm DJ, Lerdthusnee K, and Romoser WS. Department of Entomology, Cornell University, Ithaca, NY; Bacteriology Division, U. S. Army Medical Research of Infectious Diseases, Ft. Detrick, MD; Virology Division, U. S. Army Medical Research of Infectious Diseases, Ft. Detrick, MD; and Geographical Disease Institute, Ohio University, Athens, OH

2:00 16 MOLECULAR ANALYSIS OF YELLOW FEVER VACCINE VIRUSES. Galler R*, Post PR, Santos CN, and Carvalho R. Departmento de Bioquimica e Biologia Molecular, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

2:15 17 DETECTION OF WEST NILE VIRUS BY THE POLYMERASE CHAIN REACTION AND ANALYSIS OF STRAIN VARIATION. Porter KR*, Oprandy JJ, Dubois DR, Summers P, Nelson WM, Henschal EA, and Hayes CG. Infectious Diseases Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD; and Virology Division, Walter Reed Army Institute for Research, Washington, DC.

2:30 18 USE OF POLYMERASE CHAIN REACTION (PCR) FOR THE SENSITIVE DETECTION OF ST. LOUIS ENCEPHALITIS VIRAL RNA Vodkin MH*, Howe DK, Novak RJ, Shope RE, and McLaughlin GL. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; Illinois Natural History Survey, Center for Economic Entomology, Champaign, IL; and Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

2:45 19 CLONING AND SEQUENCE ANALYSIS OF THE GENES ENCODING THE STRUCTURAL AND NONSTRUCTURAL PROTEINS OF LANGAT VIRUS TP-21. Iacono-Connors L*. Virology Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

3:30 20 IDENTIFICATION OF A PUTATIVE RECEPTOR PROTEIN FOR VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS FROM *AEDES ALBOPICTUS* CELLS. Ludwig GV* and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

3:45 21 GENOMIC STRUCTURE, RNA POLYMERASE AND VIRULENCE OF LEISHMANIA VIRUS. Patterson JL*, Widmer G, Cadd T, Titus R, Keenan M, and Armstrong T. Department of Microbiology and Molecular Genetics, Harvard Medical School; Division of Infectious Diseases, Children's Hospital; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

# SCIENTIFIC SESSION C:
# HEPATITIS AND RETROVIRUS EPIDEMIOLOGY

Monday, December 2
4:00 - 5:45 PM Hampton A/B
Chairpersons: J. Ticehurst

4:00 22 PHYLOGENY OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I: INSIGHTS FROM SEQUENCE ANALYSES OF VIRUS STRAINS ISOLATED FROM REMOTE MELANESIAN POPULATIONS. Yanagihara R*, Gessain A, Sherman MP, Franchini G, and Poiesz BJ. National Institutes of Health, Bethesda, MD and Department of Medicine, SUNY Health Science Center at Syracuse, Syracuse, NY.

4:15 23 PREVALENCE OF HIV INFECTION AND AIDS IN EGYPT OVER FOUR YEARS OF SURVEILLANCE (1986-1990). Watts DM*, Constantine NT, Sheba MF, Kamal M, Callahan JD, and Kilpatrick ME. US Naval Medical Research Unit No. 3, Cairo, Egypt (A WHO Collaborating Center for AIDS); University of Maryland School of Medicine, Baltimore, MD; and Ministry of Health, Cairo, Egypt

4:30 24 PREVALENCE OF SERUM ANTIBODIES TO HTLV-1 IN AN ISOLATED COMMUNITY IN THE HIGHLANDS OF IRIAN JAYA, INDONESIA. Jennings GB*, Bangs MJ, Sie A, and Anthony RL. U.S. Naval Medical Research Unit No.2, Jakarta, Indonesia and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

4:45 25 COMPARISON OF TRANSMISSION RATES OF HIV-1 AND HIV-2 IN A COHORT OF PROSTITUTES IN SENEGAL. Donnelly CA*, Leisenring WM, Sandberg S, Kanki PJ, and Awerbuch T. Department of Biostatistics, Harvard School of Public Health, Boston, MA; Department of Mathematics, Framingham State College, Framingham, MA; and Department of Cancer Biology, Harvard School of Public Health, Boston, MA.

5:00 26 EXPERIMENTAL HEPATITIS E FOLLOWS THE HEPATIC REPLICATION OF HEV AND COINCIDES WITH THE APPEARANCE OF SERUM ANTI-HEV. Longer CF*, Denny S, Asher LV, Myint KS, LeDuc JW, Binn LN, Krawczynski K, and Ticehurst JR. Department of Viral Diseases, Walter Reed Army Institute of Research, Washington, DC; Animal Resources Division, US Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; Division of Pathology, Walter Reed Army Institute of Research, Washington, DC; Department of Virology, USA Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Disease Assessment Division, US Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Hepatitis Branch, Centers for Disease Control, Atlanta, GA.

5:15 27 SPORADIC ACUTE HEPATITIS E INFECTIONS IN EGYPTIAN CHILDREN DIAGNOSED BY IgM AND IgG SEROLOGIC TESTS Goldsmith R*, Yarbough PO, Reyes GR, Gabor KA, Kamel M, and Gaffar Y. Department of Epidemiology and Biostatistics, University of Calif, San Francisco, CA; Molecular Virology Department, Genelabs, Inc.; Department of Clinical Pathology, University of Cairo; and Department of Medicine, Ains Shams University Cairo, Egypt.

5:30 28 DETECTION OF ANTIBODIES TO HEPATITIS E VIRUS IN EPIDEMIC AND IMPORTED CASES OF ENTERICALLY-TRANSMITTED NANB HEPATITIS. Yarbough PO*, Gabor KA, Reyes GR, Flower AJ, and Skidmore SJ. Genelabs Inc. Redwood City, CA; Public Health Laboratory, Leicester, England; and Regional Virus Laboratory, East Birmingham Hospital, Birmingham, England.

## SCIENTIFIC SESSION D: KINETOPLASTIDA: BIOLOGY AND MOLECULAR BIOLOGY

Monday, December 2
1:30 PM - 5:00 PM Fairfax A
Chairpersons: D. Wirth and M. Pereira

1:30 29 ARE THERE NATURALLY OCCURRING LEISHMANIAL HYBRIDS? Kreutzer RD*, Tesh RB, Grogl M, Neva FA, Yemma JJ, and Iacoliangelo T. Biology Department, Youngstown State University, Youngstown, OH; Yale Arbovirus Research Unit,Yale Medical School, New Haven, CT; Leishmaniasis Section,Walter Reed Army Institute of Research, Washington, DC; and Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD.

1:45 30 IDENTIFICATION AND CHARACTERIZATION OF EPIDERMAL GROWTH FACTOR RECEPTORS IN *TRYPANOSOMA CRUZI*. Freeman-Junior P* and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

2:00 31 TYROSINE KINASE ACTIVITY IN *TRYPANOSOMA BRUCEI*. Wheeler-Alm E and Shapiro SZ*. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL.

2:15 32 DIFFERENTIAL EXPRESSION OF THE MITOCHONDRIAL OLIGOMYCIN-SENSITIVE ATPASE IN BLOODSTREAM FORMS OF *TRYPANOSOMA BRUCEI*. Bienen EJ* and Shaw MK. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya.

2:30 33 CDNA CLONING OF A NOVEL TRYPANOSOME PROCYCLIC STAGE SURFACE ANTIGEN BY EXPRESSION IN MAMMALIAN (COS7) CELLS. Jackson DG*, Smith DK, Luo G, and Elliott JF. Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, U.K.; Department of Immunology, University of Alberta, Edmonton, Alberta, Canada; and Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada.

2:45 34 PENETRIN, A *T. CRUZI* HEPARIN BINDING PROTEIN THAT PROMOTES TRYPOMASTIGOTE PENETRATION INTO MAMMALIAN CELLS AND CONFERS INVASIVENESS TO *E. COLI*. Ortega-Barria E* and Pereira M. Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospitals, Boston, MA.

3:00 Coffee Break

3:15 35 *LEISHMANIA* DNA SEQUENCE RESPONSIBLE FOR TUNICAMYCIN-RESISTANCE. Liu X* and Chang KP. Department of Microbiology/Immunology, University of Health Sciences, Chicago Medical School, North Chicago, IL.

3:45 36 GENE TARGETING IN *LEISHMANIA ENRIETTII*. Tobin JF* and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

4:00 37 GENE EXPRESSION IN *LEISHMANIA* : IDENTIFICATION OF ESSENTIAL DNA SIGNALS. Lafaille MA*, Laban A, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

4:15 38 TRANSFECTION OF *LEISHMANIA* WITH GP63 METALLOPROTEINASE GENE AND ITS EXPRESSION IN THE TRANSFECTANTS Chang KP*, Liu X, and Du YB. Department of Microbiology/Immunology, University of Health Sciences, Chicago Medical School, North Chicago, IL.

4:30 39 *LEISHMANIA MAJOR* SECRETES CHITINASE THAT FUNCTIONS IN THE SANDFLY VECTOR. Schlein Y, Jacobson RL*, and Shlomai J. Department of Parasitology, Hebrew University - Hadassah Medical School, Jerusalem, Israel.

4:45 40 REVERSAL OF ADRIAMYCIN RESISTANCE IN *LEISHMANIA AMAZONENSIS* BY THE $Ca^{2+}$ ANTAGONIST VERAPAMIL. Lopes UG*, Gueiros-Filho F, Viola JB, Gomes FA, and Campos CB. Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

## SCIENTIFIC SESSION E: BACTERIOLOGY AND RICKETTSIOLOGY

Monday, December 2
1:30 - 3:00 PM Fairfax B
Chairpersons: J. Chulay and J. Williams

1:30 41 A FOODBORNE OUTBREAK OF TYPE E BOTULISM IN CAIRO, EGYPT, APRIL 1991. Hibbs RG*, Mishu B, Darwish A, Weber JT, Hatheway CL, El-Sharkawy S, and Corwin A. US Naval Medical Research Unit No. 3, Cairo, Egypt; Centers for Disease Control, Atlanta, GA; and Infectious Disease Department, Ministry of Health, Cairo, Egypt

2:45 42 POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF *FRANCISELLA TULARENSIS*. Long GW*, Narayanan RB, Fortier AH, Nacy CA, and Oprandy JJ. Infectious Disease Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD and Cellular Immunology Department, Walter Reed Army Institute of Research, Washington, DC.

2:00 43 IgG CLASS ANTIBODIES AGAINST GROUP B *NEISSERIA MENINGITIDIS* OUTER MEMBRANE ANTIGENS OF DIFFERENT SEROTYPES PREVALENT IN BRAZIL De Gaspari EN*, Tavares AV, Ribeiro CL, Farhat CK. Immunology Section, Adolfo Lutz Institute; and Pediatrics Division, Emilio Ribas Hospital, Sao Paulo, Brazil.

2:15 44 THE EFFECTS OF ANTIMALARIAL CHEMOPROPHYLACTIC AGENTS ON THE VIABILITY OF THE TY21A TYPHOID VACCINE STRAIN. Brachman JP*, Metchock B, and Kozarsky P. Emory University School of Medicine, Atlanta, GA.

2:30 45 DIVERGENCE OF SPOTTED FEVER AND TYPHUS GROUP RICKETTSIAE BY SEQUENCING THE P34-17KD COMMON PROTEIN ANTIGEN GENE REGION. Dasch GA* and Swinson KL. Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

2:45 46 OCCURRENCE OF A TYPHUS-LIKE RICKETTSIA ASSOCIATED WITH OPOSSUMS AND THEIR FLEAS IN LOS ANGELES COUNTY. Williams SG*, Sacci Jr. JB, Fujioka K, Sorvillo FJ, Barr RA, and Azad AF. Department of Microbiology, University of Maryland School of Medicine, Baltimore, MD.; Los Angeles County Department of Health, Los Angeles, CA; and University of California, Los Angeles, CA.

## SCIENTIFIC SESSION F: MEDICAL MALACOLOGY

### Annual Meeting: American Committee on Medical Malacology

Monday, December 2
3:30 - 5:00 PM Fairfax B
Chairpersons: E.A. Malek and J. Malone

3:30 47 A MAJOR REPETITIVE ELEMENT IN THE *SCHISTOSOMA MANSONI* SNAIL HOST *BIOMPHALARIA GLABRATA* IS RELATED TO LINE-1 TRANSPOSONS. Knight M*, Miller A, Richards C, and Lewis F. Biomedical Research Institute, Rockville, MD.

3:45 48 OBSERVED LINKAGE BETWEEN SUSCEPTIBILITY TO *SCHISTOSOMA MANSONI* INFECTION AND ABNORMAL EGG PRODUCTION IN *BIOMPHALARIA GLABRATA* SNAILS. Cooper L*, Richards CS, Lewis F, Cousin C, Minchella DJ. University of Maryland, Department of Entmology, College Park, MD; Biomedical Research Institute, Rockville, MD; University of the District of Columbia, Washington, DC; and Purdue University, West Lafayette, IN.

4:00 49 IS *THIARA (TAREBIA) GRANIFERA* (LAMARCK) AN INTERMEDIATE HOST OF *PARAGONIMUS WESTERMANI* (KERBERT)? Sodeman WA*. Division of Gastroenterology, Department of Medicine, Medical College of Ohio, Toledo, OH.

4:15 50 A GEOGRAPHIC INFORMATION SYSTEM FOR HABITAT OF THE LYMNAEID SNAIL INTERMEDIATE HOST OF *FASCIOLA HEPATICA* ON LOUISIANA COASTAL MARSH RANGELAND. Zukowski SH*, Wilkerson GW, Jones FW, and Malone JB. Department of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA and Computer-Aided Design and Geographic Information Systems Laboratory, Louisiana State University, Baton Rouge, LA.

4:30 Business Meeting

# WORKSHOP:
# DISEASE OF THE TROPICS:
# TELECOMMUNICATIONS AND TECHNOLOGY TRANSFER

Monday, December 2
4:00 - 6:00 PM Commonwealth
Chairpersons: L.J. Legters and L.H. Brink

Workshop will demonstrate uses of telecommunications in medical technology transfer between U.S. academic centers and "Third World" institutions. Segments of high definition television footage—both live and transmitted via satellite and previously recorded— will be discussed by panelists. Subject areas include:

- Case Presentations and Clinical Diagnosis
- Field Epidemiologic Investigation
- Vector Biology and Control
- Disaster Relief Operations
- Uplinks from Tropics to the U.S. Classroom

## POSTER SESSION I

*Continental Breakfast*

Tuesday, December 3
7:30 PM - 10:00 PM Liberty

### CLINICAL TROPICAL MEDICINE

51 MORTALITY IN A PROSPECTIVE COHORT OF NEWBORNS IN MALAWI, 1987-1990. Bloland PB*, Steketee RW, Wirima JJ, Breman JG. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.; and Ministry of Health, Lilongwe, Malawi.

52 HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA DONOVANI* S.L. IN KENYA Mebrahtu YB*, Van Eys GJ, Lawyer PG, Pamba H, Koech DK, Roberts CR, Perkins PV, Were JB, and Hendricks LD. United States Army Research Unit and Kenya Medical Research Institute, Nairobi, Kenya; Royal Dutch Tropical Research Institute, Amsterdam, Holland; Walter Reed Army Research Institute, Washington DC; Medical School, University of Nairobi, Nairobi, Kenya; Kenya Medical Research Institute, Nairobi, Kenya; and Trotting Horse Lane, Missoula, MT.

53 GLUTAMINE SUPPLEMENTED ORS IS SUPERIOR TO STANDARD CITRATE GLUCOSE ORS FOR THE MAINTENANCE THERAPY OF ADULT CHOLERA PATIENTS IN JAKARTA. Punjabi NH*, Kumala S, Rasidi C, Witham ND, Pulungsih SP, Rivai AR, Sukri N, Burr DH, Lesmana M, Hisham MA, and Simanjuntak CH. U.S. Naval Medical Research Unit No.2, Jakarta, Indonesia; Infectious Diseases Hospital of Jakarta, Indonesia; Subdirectorate Diarrhea, CDC, Jakarta, Indonesia; and National Institute of Health, Research and Development, Jakarta, Indonesia.

54 THE INTERRELATIONSHIP BETWEEN DIARRHEA AND VITAMIN A DEFICIENCY IN CHILDREN UNDER FIVE YEARS OF AGE IN THE SUDAN. El Bushra HE*, Ash LR, Coulson AH, and Neumann CG. Department of Community Medicine, Faculty of Medicine, University of Khartoum, Khartoum. Sudan; Department of Epidemiology, UCLA School of Public Health, Los Angeles, California.; and Department of Health Sciences, UCLA School of Public Health, Los Angeles.

55 INTESTINAL PARASITOSES AMONG PATIENTS AND STAFF OF AN INSTITUTION FOR THE MENTALLY RETARDED. Ferrer I* and Kozek WJ. Medical Sciences Campus, University of Puerto Rico, Rio Piedras, PR.

56 SYPHILIS AND MALARIA DURING PREGNANCY IN MALAWI. Steketee RW, McDermott JM*, and Wirima JJ. Malaria Branch, Centers for Disease Control, Atlanta GA; Malaria Branch, Centers for Disease Control, Atlanta GA; and Ministry of Health, Malawi.

### MEDICAL ENTOMOLOGY

57 VITELOGENESIS IN THE IXODID TICK, *HYALOMMA DROMEDARI: IN VIVO* AND *IN VITRO* ANALYSIS. Schriefer ME* and Sonenshine DE. School of Medicine, Departments Immunology and Microbiology, University of Maryland at Baltimore, Baltimore, MD; and Department of Biology, Old Dominion University, Norfolk, VA.

58 LABORATORY EVALUATION OF PREDATORY CAPACITY OF CYCLOPOID COPEPODS ON *AEDES AEGYPTI* LARVAE IN PUERTO RICO. Suarez MF*, Clark GG, and Marten G. International Health, Johns Hopkins University, San Juan, PR; San Juan Laboratories, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, San Juan, PR; and Tulane University, New Orleans, LA.

59 DISPERSAL OF THE SAND FLY *LUTZOMYIA LONGIPALPIS* IN AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN COLOMBIA. Ferro C, Morrison AC*, Morales A, Tesh RB, and Wilson ML. Instituto Nacional de Salud, Bogota. Colombia; Yale University School of Medicine, Deparment of Epidemiology and Public Health, New Haven, CT.

**KINETOPLASTIDA**

60 MAXADILAN, A POTENT VASODILATOR FROM SAND FLY SALIVARY GLANDS. Lerner EA*, Vibbard DE, and Shoemaker CB. Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, MA; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

61 TRANSFORMATION FACTORS INDUCING *LEISHMANIA* AMASTIGOTE FORMATION IN A CELL-FREE MEDIUM. Mohareb EW*, Mikhail EM, and Mansour NS. US Naval Medical Research Unit No. 3, Cairo, Egypt.

62 STUDIES ON THE INTRACELLULAR CALCIUM HOMEOSTASIS IN *TRYPANOSOMA BRUCEI BRUCEI*. Vercesi AE*, Docampo RE, and Moreno SN. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; and Departamento de Bioquimica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil

63 IMMUNOTHERAPY AND CHEMOTHERAPY OF MUCOCUTANEOUS AND DISSEMINATED CUTANEOUS LEISHMANIASIS IN A HORSE IN BRAZIL Barbosa-Santos EG*, Marzochi MC, Urtado W, Queiros F, and Chicarino J. Biological Sciences Department, National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; Equine Therapeutic Clinic, Evandro Chagas Hospital, Rio de Janeiro, Brazil.

64 PARTIAL PURIFICATION AND CHARACTERIZATION OF S-ADENOSYLMETHIONINE SYNTHETASE OF *LEISHMANIA*. Nolan LL*, Tang S and Sufrin JR. School of Public Health, University of Massachusetts, Amherst, MA; School of Public Health, University of Massachusetts, Amherst, MA; and Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, NY.

65 ANTI-LEISHMANIAL ACTIVITY OF ETHER ANALOGUES OF LYSOPHOSPHOLIPIDS. Fiavey NP and Ngwenya BZ*. Hahnemann University School of Medicine, Philadelphia, PA.

66 AN ENZYME IMMUNOASSAY AND CONFIRMATORY TEST FOR THE SPECIFIC AND SENSITIVE DETECTION OF ANTIBODY TO *TRYPANOSOMA CRUZI*. Pan AA*, Brashear RJ, Winkler MA, and Lee H. Transfusion Diagnostics, Abbott Laboratories, North Chicago, IL.

67 EVALUATION OF A RAPID DOT ELISA FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS. Thai L, Geronimo SE, Filho JH, Ponce E, Ponce CA, Evans TG*, and Wright JD. Division of Infectious Diseases, University of Utah, Salt Lake City, UT; Univ. Fed. de Natal, Natal, RGN, Brazil; Univ. Fed. do Ceara, Fortaleza, CE, Brazil; Lab. Central, Min. de Salud, Tegucigalpa, Honduras; and Gull Foundation for Medical Research, Salt Lake City, UT.

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68 CANINE LEISHMANIASIS IN A U. S. MILITARY WORKING DOG. Nuzum EO*, Wempe JM, Wilber J, Grogl M. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D. C.; U. S. Army Regional Veterinary Laboratory, Ft Sam Houston, TX; Kirtland Air Force Base Veterinary Services, and William Beaumont Army Medical Center, El Paso, TX.

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69 "TRICKLE" INFECTIONS IN JIRDS: CELLULAR AND HUMORAL RESPONSES TO *BRUGIA PAHANGI*. Chisholm ES* and Lammie PJ. Parasitic Disease Branch, Centers for Disease Control, Atlanta, GA.

70 *ONCHOCERCA VOLVULUS* PARAMYOSIN: CHARACTERISATION OF A FULL-LENGTH cDNA, MOLECULAR CLONING AND EXPRESSION. Dahmen A*, Gallin M, Schumacher M, and Erttmann KD. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Federal Republic of Germany.

71 DAYTIME IDENTIFICATION OF PATIENTS WITH PATENT BANCROFTIAN FILARIASIS WITH A RECOMBINANT FILARIAL ANTIGEN. Dissanayake S*, Xu M, Zheng HJ, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Guizhou Provincial Institute of Parasitic Diseases, Guiyang, People's Republic of China.

72 A HIGHLY ENDEMIC FOCUS OF BANCROFTIAN FILARIASIS IN THE NILE DELTA: ENVIRONMENTAL AND ENTOMOLOGICAL STUDIES. Gad AM*, Sabry Z, Reiad IB, Ramzy RM, Weil GJ, Buck AA, and Faris R. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

73 A MODEL FOR THE SEASONAL TRANSMISSION OF DRACUNCULIASIS IN A RURAL COMMUNITY. Heuschkel C* and Awerbuch T. MPH Program, Harvard School of Public Health, Boston, MA; and Dept of Biostatistics, Harvard School of Public Health, Boston, MA.

74 *ONCHOCERCA* MICROFILARIAE STIMULATE SUPEROXIDE PRODUCTION BY EOSINOPHILS AND SECRETE SUPEROXIDE DISMUTASE *IN VITRO*. James ER*, Tuxworth WJ, Callahan HL, and Crouch RK. Department of Ophthalmology, Medical University of South Carolina, Charleston, SC; and Department of Biological Chemistry, Harvard University Medical School, Boston, MA.

75 ANTIGENICITY AND B CELL EPITOPES OF A PROTECTIVE FILARIAL ANTIGEN IN HUMAN BANCROFTIAN FILARIASIS. Kazura J*, Hazlett F, Nilsen T, Alpers M, and Day K. Case Western Reserve University, Cleveland, OH; Papua New Guinea Institute of Medical Research, Goroka, New Guinea; and Imperial College, London, UK.

76 A RAPID DNA ASSAY FOR THE NON-RADIOACTIVE DETECTION OF *BRUGIA* MICROFILARIAE IN BLOOD SAMPLES Lizotte MR*, Poole CB, and Williams SA. Department of Biological Sciences, Smith College, Northampton, MA; and New England Bioloabs, Beverly, MA.

77 POTENT MACROFILARICIDAL ACTIVITY OF THE BENZIMIDAZOLE CARBAMATE, UMF-078, AGAINST *BRUGIA PAHANGI* AND *ACANTHOCHEILONEMA VITEAE* IN JIRDS. McCall JW*, Dzimianski MT, Elslager EF, Townsend LB, Wise DS, Jun JJ, and Supakorndej P. Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA ; Elslager & Associates, Ann Arbor, MI; and College of Pharmacy, University of Michigan, Ann Arbor, MI.

78 SIGNIFICANCE OF TRANSGLUTAMINASE-CATALYZED REACTIONS IN *BRUGIA MALAYI* FILARIAL PARASITES Mehta K*, Rao UR, Vickery AC, Fesus L. University of Texas MD Anderson Cancer Center, Houston, TX; College of Public Health, University of South Florida, Tampa, FL; and University Medical School of Debrecen, Debrecen, Hungary.

79 INTRACELLULAR DEVELOPMENT OF FILARIAE INFLUENCED BY MOSQUITO HOST TISSUE Nayar JK*, Bradley TJ, LeFevre LC, and Knight JW. Institute of Food and Agricultural Sciences - University of Florida, Medical Entomology Lab, Vero Beach, FL; and Department of Ecology and Evolutionary Biology, University of California, Irvine, CA.

80 STRAIN DEPENDENT DIFFERENCES IN LYMPHATIC DILATATION DUE TO BRUGIAN INFECTION IN THE MOUSE. Nelson FK*, Shultz LD, Greiner DL, and Rajan TV. Department of Pathology, University of Connecticut Health Center, Farmington CT; Department of Medicine, University of Massachussets Medical Center, Worcester MA; and The Jackson Laboratory, Bar Harbor, ME.

81 ONCHOCERCIASIS IN COLOMBIA? AN UPDATE ON THE LOPEZ DE MICAY FOCUS. Palma GI*, Travi BL, Satizabal JE, Martinez F, and Smith DS. Department of Microbiology, School of Health Sciences, Universidad del Valle, Cali, Colombia; Fundacion CIDEIM, Cali, Colombia; Department of Opthalmology, School of Health Sciences, Universidad del Valle, Cali, Colombia; and Fulbright Scholar, School of Medicine, University of Colorado, Boulder, CO.

82 A PRELIMINARY STUDY OF THE FEMALE REPRODUCTIVE SYSTEM OF *SCHISTOSOMA JAPONICUM* AT ULTRASTRUCTURAL LEVEL. Zhou SL*, Yang MX, Kong CH, Li Y, Liang HL, Fang P, and Lei SL. Department of Parasitology, Hubei Medical College, Wuhan, People's Republic of China; and Laboratory of Electron Microscopy, Hubei Medical College, Wuhan, People's Republic of China.

## HELMINTH BIOLOGY

83 USE OF SOIL TYPE AND LANDSAT MSS SATELLITE DATA IN A GEOGRAPHIC INFORMATION SYSTEM TO ASSESS RISK OF FASCIOLIASIS IN CATTLE IN LOUISIANA Malone JB*, Fehler DP, and Loyacano AF. Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA; Landscape Architecture, Louisiana State University, Baton Rouge, LA; and Dean Lee Research Station, Louisiana State University, Alexandria, LA.

84 INCIDENCE AND EFFECT ON COGNITIVE FUNCTION OF SUBCLINICAL *TOXOCARA* INFECTION IN INNER-CITY CHILDREN, CINCINNATI. Schantz PM, Addiss DG*, Succop PA, Fried JA, Wilson M, Roda S, and Bornschein RL. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Institute of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.

85 PREVALENCE OF INTESTINAL PARASITES IN GUATEMALA Gonzalez-Camargo CL*. Central Laboratory Department. Division for Surveillance and Diseases Control, Ministry of Public Health, Guatemala.

86 *STRONGYLOIDES STERCORALIS*: THREE DIMENSIONAL ULTRASTRUCTURAL INVESTIGATION OF NEUROSENSORY AND ASSOCIATED CEPHALIC STRUCTURES OF FILARIFORM LARVAE. Ashton FT, Bhopale VM, Volk SW, and Schad GA*. University of Pennsylvania, Philadelphia, PA.

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87 SERUM ANTIBODY RESPONSES IN HUMAN OPISTHORCHIASIS. Akai PS*, Pungpak S, Ho M, Bunnag D, and Befus AD. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada; Department of Clinical Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada; and Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand.

88 HUMORAL RESPONSE IN RABBITS INOCULATED WITH ADULT *ASCARIS* EXTRACT OR INFECTED WITH *ASCARIS* LARVAE. Barbosa JM* and Kozek WJ. Medical Sciences Campus, University of Puerto Rico, Rio Piedras, PR.

89 IMMUNOLOGIC CHARACTERIZATION OF *ANCYLOSTOMA CANINUM* ESOPHAGEAL EXTRACTS Pedro JF* and Kozek WJ. Medical Sciences Campus, University of Puerto Rico, Rio Piedras, PR.

90 ELEVATED NITRATE EXCRETION IN HUMANS AND EXPERIMENTAL HAMSTERS ASSOCIATED WITH LIVER FLUKE INFECTION. Satarug S*, Haswell-Elkins M, Bygott J, Sithithaworn P, Mairiang E, Mairiang P, Yongvanit P, Elkins D. Departments of Biochemistry, Parasitology, Radiology and Medicine, Faculty of Medicine, Khon Kaen Univ. Thailand; Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Australia; and Department of Medicine, University of Queensland, Brisbane, Australia.

91 CLINICAL, RADIOLOGIC AND EPIDEMIOLOGIC CORRELATIONS OF ELISA AND IMMUNOBLOT ASSAYS FOR *TAENIA SOLIUM* CYSTICERCOSIS IN TWO POPULATIONS IN MEXICO. Schantz PM*, Sarti-G. E, Plancarte A, Wilson M, Roberts J, and Flisser A. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; Direccion General De Epidemiologia, Secretaria de Salud, Mexico D.F., Mexico; Departamento de Immunologia, Instituto de Investigaciones Biomedicas, Mexico DF, Mexico.

92 FURTHER EVIDENCE OF 100% SPECIFICITY IN A RECENTLY DEVELOPED *TAENIA SOLIUM* (CYSTICERCOSIS) IMMUNOBLOT ASSAY. Pilcher JB*, Tsang VC, Gilman RH, Rhodes ML, and Pawlowski ZS. Immunolology and Molecular Biolology Activity, Parasitic Diseases Branch, CID, Centers for Disease Control, Atlanta, GA; The Johns Hopkins University, Baltimore, MD; USDA, ARS, Beltsville, MD; and Institute of Microbiology and Infectious Diseases, Academy of Medicine, Poznan, Poland.

93 FIVE-YEAR IMPACT OF REPEATED, AGE-TARGETED DRUG THERAPY ON THE URINARY TRACT MORBIDITY ASSOCIATED WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION. King CH*, Muchiri EM, and Ouma JH. Division of Geographic Medicine, Department of Medicine, Case Western Reserve University, Cleveland, OH; and Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya.

## OPPORTUNISTIC INFECTIONS

94 *NAEGLERIA FOWLERI* BY SCANNING ELECTRON MICROSCOPY. John DT* and John RA. Oklahoma State University College of Osteopathic Medicine, Tulsa, OK; and Symex Corp., Tulsa, OK.

95 A SPECTRUM OF TOXOPLASMOSIS IN THE IMMUNOSUPPRESSED PATIENT Bertoli F, Espino M, Arosemena VJ, Fishback JL, and Frenkel JK*. Department of Pathology, Metropolitan Social Security Hospital Complex, Panama City, Republic of Panama; and Department of Pathology, University of Kansas Medical Center, Kansas City, KA.

96 SENSITIVITY OF PROTOZOAN PARASITES TO BENZIMIDAZOLES: CORRELATION WITH β-TUBULIN SEQUENCE. Edlind TD*, Li J, and Katiyar SK. Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, PA.

97 OPTIMAL DOSAGE OF PYRIMETHAMINE IN HUMAN TOXOPLASMOSIS. Furmaniuk J, Pawlowski ZS*, Ewertowska D, and Senczuk W. Clinic of Parasitic and Tropical Diseases, Department of Toxicology, University School of Medicine, Poznan, Poland.

98 NEW MANIFESTATIONS & SIMPLIFIED DIAGNOSIS OF HUMAN MICROSPORIDIOSIS. Bryan RT*, Weber R, Stewart JM, Angritt P, and Visvesvara GS. Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA; and AIDS Pathology Division, Armed Forces Institute of Pathology, Washington, DC.

99 EXPERIMENTAL MICROSPORIDIOSIS IN RHESUS (*MACACA MULATTA*) MONKEY Aldras A* and Didier ES. Microbiology Department, Tulane Delta Regional Primate Research Center, Covington, LA.

100 RISK OF DISSEMINATED *MYCOBACTERIUM AVIUM-INTRACELLULARE* INFECTION (DMAI) IN HIV-POSITIVE POPULATION IN A U.S. METROPOLITAN AREA. Fisher EJ*, Hayashi H, and Carleton B. Department of Internal Medicine, Henry Ford Hospital, Detroit MI; and Department of Pathology, Henry Ford Hospital, Detroit MI.

## BACTERIA AND RICKETTSIA

101 POLYMERASE CHAIN REACTION BASED DIAGNOSIS OF *MYCOBACTERIUM TUBERCULOSIS* AND *M. AVIUM* INFECTION IN PATIENTS. Sritharan V*, and Barker, Jr. RH. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

102 CHRONIC CHILDHOOD MALNUTRITION REDUCES ANTIBODY RESPONSE TO *H. INFLUENZAE* TYPE B (HIB)-PROTEIN CONJUGATE VACCINE. Oyango F, Steinhoff MC*, Mbori-Ngacha D, and Siber G. University of Nairobi, Nairobi, Kenya; Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD; and Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

103 DIAGNOSIS AND TREATMENT OF CRYPTOGENIC TUBERCULOSIS, CAIRO, EGYPT - 1990-91. Farid Z*, Kilpatrick ME, and Kamal M. U.S. Naval Medical Research Unit No.3, Cairo, Egypt; and Abbassia Fever Hospital, Ministry of Health, Cairo, Egypt.

104 DETECTION OF STRAIN VARIATION IN *RICKETTSIA TSUTSUGAMUSHI* BY MOLECULAR ANALYSIS OF THE IMMUNODOMINANT 54-56 KD PROTEIN ANTIGEN GENE. Kelly DJ*, Swinson KL, and Dasch GA. Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

105 CHEMILUMINESCENT SUBSTRATE BASED WESTERN BLOTTING FOR DETECTION OF URINARY IgA IN INFANTS WITH *CAMPYLOBACTER* ENTERITIS. Wu SL*, Pazzaglia G, Haberberger RL, Oprandy JJ, Sieckmann DG, and Hayes CG. Infectious Disease Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD; Enteric Immunoprophylaxis Program, Naval Medical Research Institute, Bethesda, MD; and U.S. NAMRU-2 DET, Jakarta, Indonesia.

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## MALARIA

106 POLYMORPHISM OF THE GENE ENCODING *PLASMODIUM FALCIPARUM* LSA-1, A LIVER STAGE-SPECIFIC VACCINE CANDIDATE. Zhu J* and Hollingdale MR. Biomedical Research Institute, Rockville, MD.

107 CYTOKINE PROFILES IN EXPERIMENTAL HUMAN MALARIA. Harpaz R*, Edelman R, and Sztein MB. Center for Vaccine Development, University of Maryland, Baltimore, MD.

108 SYNTHETIC MONOPHOSPHORYL LIPID A AND ITS SUBUNIT ANALOGS AS ADJUVANTS FOR THE *PLASMODIUM FALCIPARUM* GP195 PROTEIN. Hui GS*, Chang SP, Matsuura M, and Hasegawa A. Department of Tropical Medicine, School of Medicine, University of Hawaii, Honolulu, HI.; Department of Microbiology, Jichi Medical School, Tochigi-ken, Japan.; and Department of Agricultural Chemistry, Gifu University, Gifu, Japan.

109 SEQUENCE ANALYSIS OF *PLASMODIUM VIVAX* (VK247 VARIANT) FROM GLOBALLY DIVERSE REGIONS. Lanar DE*, Kain KC, and Wirtz RA. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; and Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.

110 RENAL PATHOLOGY IN *SAIMIRI* MONKEYS DURING A VACCINE TRIAL USING THE RECOMBINANT CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX*. Tegoshi T*, Broderson JR, Iseki M, Oo MM, Nagatake T, Collins WE, and Aikawa M. Case Western Reserve University, Institute of Pathology, Cleveland, OH; Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, GA.

111 CSP-3: A *PLASMODIUM FALCIPARUM* SPOROZOITE-SPECIFIC MALARIAL VACCINE CANDIDATE. Anders J*, Zhu J, Aikawa M, Chen G, Sina B, Offutt S, and Hollingdale MR. Walter Reed Army Institute of Research, Washington, DC; Biomedical Research Institute, Rockville, MD; and Case Western Reserve University, Cleveland, OH.

112 NATURAL EXPOSURE AND IMMUNIZATION WITH A SUBUNIT VACCINE DO NOT INDUCE SIGNIFICANT ANTIBODY LEVELS TO A *PLASMODIUM VIVAX* PROTECTIVE EPITOPE. Jones TR*, Yuan LF, Marwoto H, Gordon DM, Wirtz RA, and Hoffman SL. Naval Medical Research Institute, Bethesda, MD; National Institute for Health Research and Development, Jakarta, Indonesia; and Walter Reed Army Institute of Research, Washington, DC.

113 ESTIMATING MALARIA CHALLENGE ON INDIVIDUAL VOLUNTEERS DURING A *PLASMODIUM FALCIPARUM* VACCINE TRIAL IN WESTERN KENYA. Copeland RS*, Taylor KA, Sherwood JA, Kamanza J, Asiago C, and Roberts CR. U.S. Army Medical Research Unit-Kenya, Kenya Medical Research Institute, Nairobi, Kenya; and Centers for Disease Control, Nairobi, Kenya.

114 FIELD TRIAL OF A MALARIA SPOROZOITE VACCINE. Brown AE*, Singharaj P, Webster HK, Pipithkul J, Gordon DM, Boslego JW, Krinchai K, Su-archawaratana P, Wongarichanalai C, Cryz SJ, and Sadoff JC. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Walter Reed Army Institute of Research, Washington, DC; and Swiss Serum and Vaccine Institute, Berne, Switzerland.

115 COMPARISON OF DISSECTION AND ELISA ANALYSIS OF *ANOPHELES DARLINGI* EXPERIMENTALLY INFECTED WITH *PLASMODIUM VIVAX*. Miller RE, Klein TA, Milstrey EG*, Bento JL, Pereira TR, McGreevy PB, and Wirtz RA. US Army Medical Research Unit, Rio de Janerio, Brazil; Division of Communicable Disease and Immunology, Entomology Branch, Walter Reed Army Institute of Research, Washington, DC; and Division of Experimental Therapeutics, Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, DC.

116 EFFECT OF ADJUVANTS ON THE SPECIFICITY OF ANTIBODY TO WHOLE BLOOD STAGE *PLASMODIUM YOELII*. ten Hagen T, Sulzer A, Lal AA, and Hunter RL*. Department of Pathology, Emory Unversity, Atlanta, GA; and Malaria Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

117 PERSISTENCE OF IRRADIATED *PLASMODIUM BERGHEI* EXOERYTHROCYTIC MALARIA PARASITES. Scheller LF*, Wirtz RA, and Azad AF. University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore, MD.; and Walter Reed Army Institute of Research, Washington, DC.

118 IMMUNE RESPONSE TO PLASMODIAL ANTIGENS AND SPLENOMEGALY IN A HIGHLY ENDEMIC AMERINDIAN COMMUNITY OF VENEZUELA. Torres JR*, Zisman A, DiJohn D, and Sulzer AJ. Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas; Department of Parasitology, University of New York, New York, NY; Centers for Disease Control, Department of Parasitology, Malaria Branch, Atlanta, GA.

119 CHARACTERIZATION OF ANTI-*PLASMODIUM FALCIPARUM* ANTIBODIES IN HUMAN BREAST MILK. Leke R*, Ndansi R, Southerland NJ, Quakyi IA, and Taylor DW*. University Centre for Health Sciences, University of Yaounde, Yaounde, Cameroun; and Department of Biology, Georgetown University, Washington, DC.

120 ANOPHELES MIDGUT ANTIGEN BASED MALARIA TRANSMISSION BLOCKING IMMUNITY. Lal AA*, Schriefer M, Qari SH, Goldman IF, Azad AF, and Collins WE. Division of Parasitic Disease, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Department of Microbiology and Immunology, University of Maryland, Baltimore, MD.

121 AN EVALUATION OF IMMUNE RESPONSES OF *AOTUS* MONKEYS Hickey MA*, Johnson AH, Araujo HA, Mercolino T, Lyon J, and Taylor DW. Department of Biology, Georgetown University, Washington, DC; Department of Pediatrics, Georgetown University Hospital, Washington, DC; Walter Reed Army Institute of Research, Washington, DC; and Becton-Dickinson Immunocytometry Systems, San Jose, CA.

122 ANTIBODY RESPONSE TO THE NONREPEAT REGIONS OF *PLASMODIUM FALCIPARUM* CS PROTEIN IN PERSONS LIVING IN MALARIA ENDEMIC REGIONS. Goldman IF*, Shi YP, Alpers M, Marinette P, Gross M, and Lal AA. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.; Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea; Instituto Evandro Chagas, Belem, Brazil; and Smith Kline Beecham, King of Prussia, PA.

123 IMMUNOENZYMATIC LABELLING OF MULTIPLE PLASMODIAL SALIVARY GLAND SPOROZOITES IN A SINGLE TEST. Golenda CF*, Hall T, Wirtz RA, and Schneider I. Department of Entomology, Walter Reed Army Institute of Research, Washington, DC and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

124 PREVALENCE OF ANTIBODY TO THE VARIANT REPEAT OF THE CS PROTEIN OF *PLASMODIUM VIVAX* IN . .U. Franke ED*, Lucas CM, Cachay M, Covenas H, and Wirtz RA. U.S. Naval Medical Research Institute Detachment - Lima, Peru; and Walter Reed Army Institute of Research, Washington, DC.

125 IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* BLOOD-STAGE ANTIGENS AMONG VENEZUELAN YANOMAMI INDIANS. Di John D*, Torres JR, Murillo J, Murphy JR, and Levine MM. Department of Medical and Molecular Parasitology, New York University Medical Ctr, New York, NY; Inst de Medicina Tropical, Univ Central, Caracas, Venezuela; Centro Medico, Caracas, Venezuela; and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD.

126 STUDY OF THE HUMORAL RESPONSE AGAINST THREE DEFINED *PLASMODIUM FALCIPARUM* ANTIGENS IN DIFFERENT POPULATIONS USING SYNTHETIC PEPTIDES IN AN IMMUNOENZYMATIC ASSAY. Deslandes D, Ferreira-da-Cruz MF, Oliveira-Ferreira J, Druilhe P, and Daniel-Ribeiro C*. Department of Immunology, IOC - FIOCRUZ, Rio de Janeiro, Brazil; and Institut Pasteur, Paris, France.

127 CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PFMDR2 GENE. Zalis MG*, Wilson CM, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

128 GLUCOSAMINE-6-PHOSPHATE DEAMINASE AND N-ACETYL-GLUCOSAMINE-6-PHOSPHATE DEACETYLASE FROM NORMAL AND *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES. Weidanz JA*, Campbell P, Roden L, and Vezza AC. Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; and Laboratory of Metabolic Diseases, University of Alabama at Birmingham, Birmingham, AL.

129 THE *PLASMODIUM FALCIPARUM* G6PD GENE: CLONING, SEQUENCE, AND EXPRESSION Shahabuddin M* and Kaslow DC. Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

130 WIDE GEOGRAPHIC DISTRIBUTION OF THE VARIANT FORM OF THE HUMAN MALARIA PARASITE PLASMODIUM VIVAX. Qari SH*, Goldman IF, Mellit P, Alpers M, Marinette P, Collins WE, and Lal AA. Division of Parasitic Disease, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.; Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea; and Instituto Evandro Chagas, Belem, Brazil.

131 ABSENCE OF PYRIDOXAL KINASE ACTIVITY IN *PLASMODIUM FALCIPARUM* AND *P. BERGHEI*. Inyama JS, Mulaya N, Ofulla OA, Roberts CR, and Martin SK*. Department of General Biology, Kenyatta University, Nairobi, Kenya; Vector Biology Research Center, Kisian, Kenya; and U.S. Army Medical Research Unit, Nairobi, Kenya.

132 *PLASMODIUM FALCIPARUM* SPOROZOITE RELEASE OF CIRCUMSPOROZOITE PROTEIN IN THE MOSQUITO HOST. Beier JC*, Madani A, Vaughan JA, and Noden BH. Department of Immunology and Infectious Diseases, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

133 THE QUANTITATIVE BUFFY COAT SYSTEM (QBC) FOR THE RAPID DIAGNOSIS OF *PLASMODIUM FALCIPARUM, P. VIVAX* AND *P. MALARIAE* IN A HYPERENDEMIC COMMUNITY. Anthony RL*, Purnomo, and Bangs MJ . US Naval Medical Research Unit, No. 2, Jakarta, Indonesia; and The Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

## ARBOVIRUSES AND HEPATITIS

134 HEPATITIS IN NORTHERN PAKISTAN. Bryan JP*, Rauf A, Ahmed A, Perine PL, Malik IA, and Legters LJ. Department of Preventive Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; and Pakistan-U.S. Laboratory for Sero-Epidemiology (PULSE), Rawalpindi, Pakistan.

135 RIFT VALLEY FEVER EPIZOOTIC IN THE CENTRAL HIGHLANDS OF MADAGASCAR . Morvan J, Rollin PE*, Laventure S, Rakotoarivony I, Coudrier D, and Roux J. Institut Pasteur de Madagascar, Antananarivo, Madagascar; and Institut Pasteur, Paris, France.

136 TITERS OF VESICULAR STOMATITIS VIRUS, NEW JERSEY SEROTYPE, IN MALE AND FEMALE *LUTZOMYIA SHANNONI* (DIPTERA: PSYCHODIDAE) COLLECTED IN GEORGIA. Comer JA*, Stallknecht DE, Corn JL, and Nettles VF. Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, GA.

137 NATURAL GENETIC VARIATION AMONG JAPANESE ENCEPHALITIS VIRUS STRAINS; IDENTIFICATION OF A NEW GENOTYPIC GROUP. Chen WR*, Rico-Hesse R, and Tesh RB. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

138 EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE VECTOR COMPETENCE OF *AEDES TAENIORHYNCHUS* FOR VEE AND RVF VIRUSES Turell MJ*. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

139 THE EFFECTS OF TEMPERATURE ON FECUNDITY AND VIRUS REPLICATION IN *AMBLYOMMA CAJENNENSE* INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS. Dohm DJ* and Linthicum KJ. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

140 TRANSMISSION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY HEMATOPHAGOUS MITES. Durden LA*, Linthicum KJ, and Turell MJ. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

141 HUMAN SEROSURVEY FOR LACROSSE (LAC), WESTERN EQUINE (WEE), AND ST. LOUIS (SLE) ENCEPHALITIS VIRUSES IN CENTRAL AND NORTHERN MISSOURI. Frazier CL*. Department of Biology, Southeast Missouri State University, Cape Girardeau, MO.

142 HEMORRHAGIC FEVER WITH RENAL SYNDROME IN FRANCE: UPDATE Rollin PE*, Courdrier D, Saluzzo JF, and Sureau P. Laboratoire des Fievres Hemorragiques Virales; Institut Pasteur, Paris, France.

143 REPLICATION AND PERSISTENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN TWO SPECIES OF WEST AFRICAN TICKS. Gonzalez JP*, Camicas JL, Zeller HG, Cornet JP, Some J, and Wilson ML. Institut Francais de Recherche scientifique pour le Developpement en Cooperation, Dakar, Senegal; Institut Pasteur, Dakar, Senegal; Ministere de l'Agriculture et l'Elevage, Bobo-Dioulasso, Burkina Faso; and Yale University School of Medicine, New Haven, CT.

TUESDAY AM
POSTER I

144 RIFT VALLEY FEVER VIRUS ANTIBODY IN HUMAN SERA COLLECTED AFTER AN OUTBREAK IN DOMESTIC ANIMALS IN KENYA. Logan TM*, Davies FG, Linthicum KJ, and Ksiazek TG. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Veterinary Research Laboratory, Kabete, Kenya; and Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

145 OUTBREAK OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN YUGOSLAVIA ASSOCIATED WITH DOMESTIC RATS AND MICE. Diglisic G*, Gligic A, Stojanovic R, Obradovic M, Velimirovic D, Lukac V, Xiao SY, Rossi CA, and LeDuc JW. Institute of Immunology and Virology, Belgrade, Yugoslavia; Military Medical Academy, Belgrade, Yugoslavia; Medical University of Belgrade, Belgrade, Yugoslavia; US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

146 HANTAAN AND BORRELIA POSTIVITY IN ALPINE MOUNTAIN TROOPS. Nuti M*, Arreghini S, Peragallo M. Institute of Tropical and Infectious Diseases, First University of Rome, Italy; "Cadore Alpine Brigate" Belluno, Italy ; and Preventive Medicine Service, Italian Army Medical Corps, Rome, Italy.

147 A POSSIBLE MOUSE MODEL FOR DENGUE HEMORRHAGIC FEVER. Feighny R*, Dubois D, Putnak R, Burrous J, Summers P, Strupczewski K, LaRussa V, Krishnamurti C, and Hase T. Departments of Virus Diseases, Biologics Research, Hematology, and Ultrastructural Pathology, Walter Reed Army Institute of Research, Washington, DC.

148 MOLECULAR CHARACTERIZATION OF VACCINIA VIRUS SURFACE ANTIGENS. Harrison SA* and Schmaljohn AL. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD.

149 ISOLATION OF A HANTAVIRUS FROM A FATAL HFRS CASE IN SLOVENIA. Avsic-Zupanc T*, Likar M, Furlan P, Kaps R, Xiao SY, Rossi CA, and LeDuc JW. Institute of Microbiology, Medical Faculty of Ljubljana and General Hospital, Novo Mesto, Slovenia; and US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

## SYMPOSIUM: MALARIA PREVENTION AND CONTROL: FROM RECOMMENDATION TO REALITY

Tuesday, December 3
9:30 AM - 12:00 NN Grand Ballroom
Chairpersons: S.L. Hoffman and L.H. Brink

- THE INSTITUTE OF MEDICINE REPORT: REVIEW OF THE WORLDWIDE MALARIA SITUATION. RECOMMENDATIONS FOR CONTROL.
- REALITY IN THE FIELD: A CASE STUDY: IRIAN JAYA, INDONESIA
- PANEL DISCUSSION (Facilitators: S.L. Hoffman and K. Campbell. Panel includes representatives from the Indonesian Malaria Control Program, WHO; USAID; Hoffmann-LaRoche; SmithKline, Beecham; and The Medical Research Council, The Gambia)
- OPEN DISCUSSION

## SYMPOSIUM: MODELS OF VECTOR-BORNE DISEASES

Tuesday, December 3
9:30 AM - 12:00 NN Independence East
Chairpersons: T. Awerbuch and S. Sandberg

9:30 S36 MODELS FOR STUDYING EFFECTS OF DRUGS ON MALARIA. Singer B. Department of Epidemiology and Public Health, School of Medicine, Yale University, New Haven CT.

9:55 S37 MODELS FOR THE TRANSMISSION AND CONTROL OF ONCHOCERCIASIS IN WEST AFRICA. Reme J, Plaisier AP, van Ootmarssen GJ, Habbema JDF, and Alley ES. Oncocerciasis Control Programme, Burkina Faso; and Erasmus University, Rotterdam, The Netherlands.

10:20 S38 MODELING SCHISTOSOMIASIS. Cohen J. Rockefeller University, New York, NY.

10:45 S39 COMPARISON OF MODELS FOR THE LIFE CYCLE OF TICKS INVOLVED IN DISEASE TRANSMISSION. Mount GA and Haile DG. Insects Affecting Man and Animals Laboratory, Gainesville, FL.

11:10 S40 THE ECOLOGICAL INTERACTIONS INVOLVED IN THE TRANSMISSION OF LYME DISEASE. Sandberg S and Awerbuch T. Framingham State College, Framingham, MA; and Harvard School of Public Health, Boston, MA.

11:35 S41 BRIDGING HEALTH AND AGRICULTURE: EPIDEMIOLOGY OF INSECT TRANSMITTED PLANT PATHOGENS IN TROPICAL AMERICA. Anderson P and Levens R. Universidad Nacional Agaria, Managua, Nicaragua; and Harvard School of Public Health, Boston, MA.

## SYMPOSIUM:
## CELL SURFACE CYSTEINE-RICH PROTEINS AND THE PATHOGENESIS OF TROPICAL DISEASES ‡

*Second Annual Merck, Sharpe and Dohme Symposium*

Tuesday, December 3
10:00 AM - 12:00 NN — Independence West/Center
Chairpersons: W.A. Petri, Jr. and T. Nash

10:00 S42 ADHESION RECEPTORS OF THE IMMUNE SYSTEM AND SUBVERSION BY PARASITES. Staunton D. Harvard Medical School. Boston, MA.

10:20 S43 ADHERENCE LECTIN GENE FAMILY OF *ENTAMOEBA*. Mann B. University of Virginia, Charlottesville, VA.

10:40 S44 CYSTEINE-RICH PROTEINS OF *GIARDIA LAMBLIA*. Nash T. National Institutes of Health, Bethesda, MD.

11:00 S45 STRUCTURAL AND FUNCTIONAL STUDIES OF THE HIV gp 120 AND TRYPANOSOME VARIANT SURFACE GLYCOPROTEINS. Wiley D. Harvard University, Cambridge, MA.

11:20 S46 CONSERVATION OF THE CYSTEINE-RICH DOMAIN IN THE CARBOXYTERMINAL REGION OF THE MAJOR MEROZOITE SURFACE ANTIGEN OF *PLASMODIA*. Long C. Hahnemann University, Philadelphia, PA.

11:40 S47 GAMETE CYSTEINE-RICH SURFACE PROTEINS OF *PLASMODIA*. Kaslow D. National Institutes of Health, Bethesda, MD.

‡ Supported by Merck, Sharpe and Dohme, Inc.

## SCIENTIFIC SESSION G:
## MALARIA BIOLOGY AND MOLECULAR BIOLOGY

Tuesday, December 3
9:30 AM - 12:00 NN — Commonwealth
Chairpersons: M. Sedegah and F. Zavala

9:30 150 INGESTION OF *PLASMODIUM FALCIPARUM* SPOROZOITES DURING TRANSMISSION BY ANOPHELINE MOSQUITOES. Beier MS*, Davis JR, Pumpuni CB, Noden BH, and Beier JB. Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD; and Department of Immunology and Infectious Diseases, The Johns Hopkins School of Public Health, Baltimore, MD.

9:45 151 POPULATION DYNAMICS OF *PLASMODIUM FALCIPARUM* WITHIN LABORATORY-INFECTED *ANOPHELES GAMBIAE*. Vaughan JA*, Noden BH, and Beier JC. Department of Immunology and Infectious Diseases, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

10:00 152 EVALUATION OF INGESTED HUMAN ANTI-SPOROZOITE SERA ON *PLASMODIUM FALCIPARUM* SPOROGONY IN *ANOPHELES STEPHENSI*. Davis JR*, Beier MS, Beier JB, Clyde DF, and Edelman R. Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD; and Department of Immunology and Infectious Diseases, The Johns Hopkins School of Public Health, Baltimore, MD.

10:15 153 COMPARISON OF APYRASE LEVELS IN FOUR SPECIES OF *ANOPHELES*. Cupp MS*, Cupp EW, and Ramberg FB. Department of Entomology, University of Arizona, Tucson, AZ.

10:30 154 A NUTRIENT-PERMEABLE CHANNEL ON THE INTRAERYTHROCYTIC MALARIA PARASITE. Desai SA*, Krogstad DJ, and McCleskey EW. Washington University School of Medicine, St. Louis, MO; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

10:45 155 EXTRACELLULAR DEVELOPMENT *IN VITRO* OF THE ERYTHROCYTIC CYCLE OF *PLASMODIUM FALCIPARUM*. Trager W* and Williams JH. The Rockefeller University, New York, NY.

11:00 156 MOLECULAR CLONING OF THE GENES THAT ENCODE THE REPLICATIVE DNA POLYMERASES OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*. Fox BA and Bzik DJ*. Department of Microbiology, Dartmouth Medical School, Hanover, NH.

11:15 157 AN ANALYSIS OF *PFMDR1* mRNA EXPRESSION IN *PLASMODIUM FALCIPARUM*. Volkman SK*, Wilson CM, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:30 158 REGULATION OF MALATE DEHYDROGENASE-ISOENZYMES IN *PLASMODIUM FALCIPARUM*. Lang-Unnasch NE*. University of Alabama at Birmingham, Department of Medicine, Division of Geographic Medicine, Birmingham, AL.

11:45 159 PHOSPHOINOSITIDE METABOLISM IN *PLASMODIUM FALCIPARUM* GAMETOCYTES AND SIGNAL TRANSDUCTION. Ogwang RA, Mwangi JK, Roberts CR, and Martin SK*. Kenya Medical Research Institute, Nairobi, Kenya and U.S. Army Medical Research Unit, Nairobi, Kenya.

## SCIENTIFIC SESSION H:
## HELMINTH DIAGNOSIS AND EPIDEMIOLOGY

Tuesday, December 3
10:00 AM - 12:00 NN Hampton A/B
Chairpersons: R. Ruiz and G.R. Olds

10:00 160 *SCHISTOSOMA MANSONI* TROPOMYOSIN: A SPECIES-SPECIFIC IMMUNODIAGNOSTIC REAGENT. Nicholson LJ*, Xu H, Thakur AN, Rekosh DM, and LoVerde PT. Departments of Microbiology and Biochemistry, School of Medicine and Biomedical Science, State University of New York at Buffalo, NY.

## TUESDAY AM
## HELMINTH DIAGNOSIS

10:15 161 CIRCULATING SCHISTOSOMAL ANTIGEN IN DIAGNOSIS AND ASSESSMENT OF CURE IN CHILDREN INFECTED WITH *SCHISTOSOMA MANSONI*. Hassan MM* and Strand M. Department of Parasitology, Faculty of Medicine, Zagazig University, Zagazig, Egypt; and Department of Pharmacology and Molecular Sciences, The Johns Hopkins University, School of Medicine, Baltimore, MD.

10:30 162 DIAGNOSTIC ASSAY PERFORMANCE OF THE IMMUNOBLOT AND ITS IMPACT ON THE EPIDEMIOLOGY OF CYSTICERCOSIS IN PERU. Tsang VC*, Gilman R, and Pilcher JB. Cysticercosis Working Group, Immunolology and Molecular Biolology Activity, Parasitological Disease Branch, CID, Centers for Disease Control, Atlanta, GA; The Johns Hopkins University, Baltimore, MD; and Universidad Peruana Cayetano Heredia and PRISMA, Lima Peru.

10:45 163 EVALUATION OF THE LMD ELISA FOR THE DETECTION OF ANTIBODIES TO THE CYSTICERCI OF *TAENIA SOLIUM*. Rosenblatt JE*, Kagan IG, and Boodram C. Mayo Clinic, Rochester, MN; Parasitic Disease Consultants, Tucker, GA; and Provincial Laboratory of Public Health, Edmonton, Alberta, Canada.

11:00 164 COMPARISON OF THE BENTONITE FLOCCULATION TEST WITH THE LMD ELISA KIT FOR DETECTION OF ANTIBODIES TO *TRICHINELLA SPIRALIS*. Wilson M*, Ware DA, and McAuley JB. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

11:15 165 PHYSICAL GROWTH AND SCHISTOSOMIASIS JAPONICA INFECTION IN LEYTE, PHILIPPINES AND JIANGXI, CHINA. McGarvey ST*, Aligui G, Olveda R, Wu G, Zhong S, Peters P, Olds GR, and Wiest PM. Program in Geographic Medicine, The Miriam Hospital, Brown University, Providence, RI.

11:30 166 THE EPIDEMIOLOGY OF *OPISTHORCHIS VIVERRINI* AND ASSOCIATED HEPATOBILIARY DISEASES IN TWO DISTRICTS IN KHON KAEN PROVINCE, NORTHEAST THAILAND. Haswell-Elkins M*, Sithithaworn P, Mairiang E, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Elkins D. Tropical Health Program, Queensland Institute of Medical Research, Herston, Australia; Departments of Radiology, Parasitology and Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

11:45 167 OUTCOME OF CASE-CONTAINMENT STRATEGY TO ELIMINATE DRACUNCULIASIS FROM PAKISTAN IN 1990. Kappus KD*, Hopkins DR, Ruiz-Tiben E, Imtiaz R, Andersen J, Azam M, Attiq A, Hightower A. Centers for Disease Control, Center for Infections Disease, Division of Parasitic Disease; Global 2000, Inc., Atlanta, GA; and National Institute of Health, Pakistan.

## SCIENTIFIC SESSION I:
## FILARIA BIOLOGY AND MOLECULAR BIOLOGY

Tuesday, December 3
10:00 - 11:45 AM Gardner A/B
Chairpersons: N. Raghavan and T. Egwang

10:00 168 INSECT JUVENILE HORMONES AND THEIR ANALOGS PROMOTE DEVELOPMENT OF THIRD-STAGE FILARIAL LARVAE *IN VITRO*. Bodri MS* and Lok JB. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

10:15 169 ISOLATION AND PARTIAL STRUCTURAL CHARACTERIZATION OF A MAJOR IgE-INDUCING ANTIGEN IN PATIENTS WITH TROPICAL PULMONARY EOSINOPHILIA. Lobos E*, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD.

10:30 170 CHARACTERIZATION OF A CYSTEINE PROTEASE INHIBITOR FROM *DIROFILARIA IMMITIS*. Hong Y*, Limberger R, Poole CB, and McReynolds LA. New England Biolabs, Inc., Beverly, MA; and New York State Department of Health, Albany, NY.

10:45 171 MOLECULAR CLONING OF A CYSTEINE PROTEINASE INHIBITOR OF *ONCHOCERCA VOLVULUS*. Lustigman S*, Smith AB, Prince AM, and McKerrow J. Virology and Parasitology, Lindsley F. Kimball Research Institute of The New York Blood Center, New York, NY; and Department of Pathology, School of Medicine, University of California, San Francisco, CA.

11:00 172 MOLECULAR CHARACTERIZATION OF A *WUCHERERIA BANCROFTI* RECOMBINANT, WBN43 RECOGNIZED BY PUTATIVELY IMMUNE INDIVIDUALS. Raghavan N*, Freedman DO, Tuan RS, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Department of Orthopaedic Surgery & Microbiology, Thomas Jefferson University, Philadelphia, PA.

11:15 173 A HIGHLY SENSITIVE, SPECIES-SPECIFIC DNA PROBE FOR THE DETECTION OF EGYPTIAN *WUCHERERIA BANCROFTI*. Williams SA*, Chan TY, Ramzy R, Weil G, Gad A, and Hamburger J. Department of Biological Sciences, Smith College, Northampton, MA.; Research and Training Center on Vectors of Disease, Ain Shams University, Cairo, Egypt.; Washington University School of Medicine, St. Louis, MO; Hebrew University, Hadassah Medical School, Israel.

11:30 174 CLONING AND CHARACTERIZATION OF A *LOA LOA* SPECIFIC REPETITIVE DNA. Egwang TG*, Akue JP, and Pinder M. International Medical Research Center of Franceville (CIRMF), Franceville, Gabon.

## SCIENTIFIC SESSION J: ARBOVIRUS IMMUNOLOGY

Tuesday, December 3
10:00 AM - 12:00 NN Fairfax A
Chairpersons: A. Schmaljohn and J. Roehrig

10:00 175 ASSAYS OF CELL-MEDIATED IMMUNITY IN RECIPIENTS OF A LIVE, ATTENUATED JUNIN VIRUS VACCINE. Peters CJ*, Kenyon RH, Barrera-Oro JG, McKee KT, and MacDonald C. Disease Assessment Division, U.S.Army Medical Research Institute of Infectious Diseases, Frederick, MD; and Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD.

10:15 176 CYTOTOXIC T CELL EPITOPES CONSERVED AMONG MOPEIA, MOBALA, LASSA, AND LYMPHOCYTIC CHORIOMENINGITIS VIRUSES. Higgins YK*, Schmaljohn AL and Peters CJ. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD.

10:30 177 BIOCHEMICAL PARAMETERS OF FLAVIVIRUS SYNTHETIC PEPTIDE T-CELL EPITOPES. Roehrig JT*, Johnson AJ, Mathews JH, Hunt AR, and Beaty BJ. Division of Vector-Borne Infectious Diseases, CID, CDC, PHS, DHHS, Fort Collins, CO. and Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

10:45 178 ANTIGENIC COMPARISON OF AFRICAN AND INDIAN STRAINS OF WEST NILE VIRUS BY WESTERN BLOT ANALYSIS. Summers PL, Martinez BC*, Dubois DR, Silor DL, Barvir DA, Timchak RL, and Eckels KH. Walter Reed Army Institute of Research, Washington DC.

11:00 179 MONOCLONAL ANTIBODY CHARACTERIZATION OF JAMESTOWN CANYON (CALIFORNIA SEROGROUP) VIRUS TOPOTYPES ISOLATED IN CANADA. Artsob H*, Spence L, Brodeur B, and Th'ng C. National Laboratory for Special Pathogens, Laboratory Centre for Disease Control, Ottawa, Canada; Department of Microbiology, University of Toronto, Toronto, Canada; and National Laboratory for Immunology, Laboratory Centre for Disease Control, Ottawa, Canada.

11:15 180 STUDIES OF RABIES VIRUS PATHOGENESIS USING ANTI-IDIOTYPIC MONOCLONAL ANTIBODIES. Hanham CA*, Zhao F, Tignor GH. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

11:30 181 THE FC PORTION OF MONOCLONAL ANTIBODY TO YELLOW FEVER VIRUS NS1 IS A DETERMINANT OF PROTECTION AGAINST YF ENCEPHALITIS IN MICE. Schlesinger JJ* and Foltzer M. The Rochester General Hospital and the University of Rochester School of Medicine and Dentistry, Rochester NY.

11:45 182 INHIBITION OF BUNYA- AND FLAVIVIRUSES *IN VITRO* BY ANTICARBOHYDRATE MONOCLONAL ANTIBODIES. Blough HA*, Kefauver D, Clausen H, and Hansen JS. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD; and Department of Infectious Diseases, Hvidovre Hospital, Denmark.

## SCIENTIFIC SESSION K: SAND FLIES AND *LEISHMANIA*

Tuesday, December 3
10:00 AM - 11:45 AM Fairfax B
Chairpersons: P.V. Perkins and M. Wilson

10:00 183 HOST FEEDING PREFERENCE OF *PHLEBOTOMUS GUGGISBERGI*, THE VECTOR OF *LEISHMANIA TROPICA* IN KENYA. Johnson RN, Ngumbi P, Mwanyumba P, Makasa J, Roberts CR. United States Army Medical Research Unit, Kenya and Kenya Medical Research Institute, Kenya.

10:15 184 CHEMICAL ATTRACTANTS OF PHLEBOTOMINE SAND FLIES. Tesh RB*, Guzman H, and Wilson ML. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT

10:30 185 THE EFFECT OF *LEISHMANIA* INFECTION ON THE LONGEVITY AND FECUNDITY OF THEIR SAND FLY VECTORS. El Sattar SA*, Shehata MG, El Sawaf BM. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

10:45 186 EXPERIMENTAL DUAL INFECTIONS OF *LEISHMANIA* IN *PHLEBOTOMUS LANGERONI*. Shehata MG*, El-Sattar SA, Morsy TA, and El-Sawaf BM. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

11:00 187 REFRACTORY BARRIERS IN THE SAND FLY, *PHLEBOTOMUS PAPATASI* TO INFECTION WITH *LEISHMANIA PANAMENSIS*. Walters LL*, Irons KP, Modi GB, and Tesh RB. Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK; and Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

11:15 188 BIOCHEMICAL SEPARATION OF FOUR *PHLEBOTOMUS* SPECIES BELONGING TO THE SUBGENERA *LARROUSSIUS* AND *PHLEBOTOMUS* (DIPTERA: PSYCHODIDAE). Kassem HA* and Fryauff DJ. Research and Training Center on Vectors of Diseases, Faculty of Science, Ain Shams University, Cairo, Egypt; and Medical Zoology Division, US Naval Medical Research Unit No. 3, Cairo, Egypt.

11:30 189 ADULT DIET AS A FACTOR AFFECTING BIOLOGY OF THE SAND FLY *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE). El Kordy E*, El Shafai A, El Said A, Kenawy MA, Shoukry M, and El Sawaf BM. Research and Training Center on Vectors of Diseases, Ain Shams University, Abassia, Cairo Egypt.

## FILM ON LYME DISEASE

Tuesday, December 3
1:00 - 1:45 PM — Fairfax A
Commentator: A. Spielman

*"WOOD TICKS AND LYME DISEASE: AN EMERGING THREAT"*

This 30 min film produced by IWF, a German scientific film maker, focuses on the mode of transmission of Lyme disease an the environmental factors that underlie transmission. Clinical features of the disease are also shown. (Note: the film will be shown again during the lunch hour on Wednesday.)

## UPDATE: GLOBAL IMMUNIZATION

Tuesday, December 3 — Independence W/C
1:00 - 1:30 PM

*THE CHILDREN'S VACCINE INITIATIVE*

Philip K. Russell
National Vaccine Program, Washington, DC

## COMMEMORATIVE FUND LECTURE

Tuesday, December 3
1:00 - 1:45 PM

Grand Ballroom

*DENGUE HEMORRHAGIC FEVER, SCOURGE OF SOUTHEAST ASIAN CHILDREN*

Suchitra Nimmannitya
Director, Childrens Hospital, Bangkok,Thailand.

## ASTMH PRESIDENTIAL ADDRESS

Tuesday, December 3
2:00 PM - 3:00 PM — Grand Ballroom

*" ... AND HYGIENE?" OUR ONCE AND FUTURE MISSION*

Scott B. Halstead
Health Sciences Division, The Rockefeller Foundation, New York, NY

## CONGRESSIONAL OUTLOOK

Tuesday, December 3
3:00 - 3:15 PM Grand Ballroom

*THE FUNDING OUTLOOK FOR BIOMEDICAL RESEARCH IN TROPICAL INFECTIONS AND OTHER DISEASES*

Representative Joseph D. Earley (D-MA)
Member, House Appropriations Committee

## ASTMH AWARDS CEREMONY

Tuesday, December 3
3:45 - 4:15 PM Grand Ballroom
Chairperson: S.B. Halstead

## ANNUAL BUSINESS MEETING

Tuesday, December 3
4:15 - 5:15 PM Grand Ballroom
Chairpersons: S.B. Halstead and J.I. Ravdin

## PUBLIC POLICY WORKSHOP
## HOW TO PARTICIPATE IN THE PUBLIC POLICY PROCESS

Tuesday, December 3
5:15 - 6:15 PM Grand Ballroom
Chairperson: S. Sagabiel

5:15 REVIEW OF THE 1991 LEGISLATION AFFECTING TROPICAL MEDICINE. Capital Associates.

5:25 PUBLIC POLICY 101: INFLUENCING GOVERNMENT POLICY AT THE GRASSROOTS LEVEL. Capital Associates.

5:35 ASTMH PUBLIC POLICY NETWORK -- HOW TO PARTICIPATE IN THE ASTMH'S LEGISLATIVE & POLICY CAMPAIGNS. John David. ASTMH Committee for Public Affairs.

5:45 RECRUITING THE GENERAL PUBLIC TO SUPPORT OUR GOALS. Representatives of RESEARCH!AMERICA and the Science Writers Association.

6:05 Discussion

# POSTER SESSION II

Wednesday, December 3
7:30 - 10:00 AM Liberty

## ARBOVIRUSES AND HEPATITIS

190 USE OF POLYVALENT IMMUNE ASCITIC FLUIDS AND INDIRECT IMMUNOFLOURESCENCE IN IDENTIFYING ARBOVIRUS ISOLATES FROM AFRICAN MOSQUITOES. Digoutte JP*, Calvo-Wilson MA, and Mondo M. Institut Pasteur, Dakar, Senegal.

191 DENGUE VIRUS INFECTED B CELLS DISSEMINATE INFECTION BEFORE VIREMIA. King AD*, Myint KS, Kalayanarooj S, Pattanapanyasat K, Smith CD, Nisalak A, and Innis BL. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Children's Hospital, Bangkok, Thailand.

192 IMMUNOFLUORESCENT STUDIES IN CELLS, INFECTED WITH EYACH VIRUS, A EUROPEAN ISOLATE OF COLORADO TICK FEVER VIRUS GROUP. Dobler GJ* and Meier-Ewert H. Abteilung fur Virologie, Technische Universitat, Munchen, Germany.

193 DISTRIBUTION AND REPLICATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS (CCHF) IN EXPERIMENTALLY INFECTED *HYALOMMA TRUNCATUM* TICKS. Dickson DL* and Turell MJ. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

194 RAPID MEMBRANE BASED IMMUNOBINDING ASSAY FOR THE DETECTION OF DENGUE VIRUS IN TISSUE CULTURE. Simmons M*, Dubois DR, Oprandy JJ. Diagnostic Technology, Naval Medical Research Institute, Bethesda, MD; and Communicable Diseases and Immunology, Walter Reed Army Institute of Research Washington D.C.

195 SIX HOUR LABORATORY CONFIRMATION OF DENGUE: ANTIGEN DETECTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS BY IMMUNOHISTOCHEMISTRY. Myint KS*, Nisalak A, Kalayanarooj S, Nimmannitya S, and Innis BL. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Children's Hospital, Bangkok, Thailand.

196 COMPARATIVE SEQUENCE ANALYSIS OF THE S SEGMENT RNA FROM SEVEN STRAINS OF CCHF VIRUS AND DEVELOPMENT OF A PCR-BASED DIAGNOSTIC SYSTEM. Lofts RS*, Hodgson LA, Ksiazek TG, and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious diseases, Ft. Detrick, MD; and Disease Assesment Division, U.S Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

197 ENZYME IMMUNOSORBENT ASSAYS FOR EBOLA VIRUS IgG AND IgM ANTIBODIES. Ksiazek TG*, Rollin PE, Jahrling PB, and Peters CJ. Disease Assessment Division, U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Institute Pasteur, Paris, France.

198 IMPROVEMENT OF AN ALKALINE PHOSPHATASE BASED NON-RADIOACTIVE DETECTION SYSTEM TO DETECT RIFT VALLEY FEVER VIRUS RNA BY FILTER HYBRIDIZATION. Knauert FK* and Parrish BA. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD

199 DIRECT AMPLIFICATION OF HANTAVIRUSES FROM HUMAN SERUM BY POLYMERASE CHAIN REACTION Xiao SY* and LeDuc JW. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

200 COMPARATIVE AGGLUTINABILITY OF ERYTHROCYTES FROM DIFFERENT SPECIES OF ANIMALS BY HEPATITIS A VIRUS. Silor DL*, Dubois DR, Eckels KH, and Summers Walter Reed Army Institute of Research, Washington DC.

201 LACK OF CROSS-REACTIVITY BETWEEN ANTIBODIES TO MALARIAL ANTIGENS AND HUMAN T-LYMPHOTROPIC VIRUS TYPE-I/II. Lal RB*, Sulzer A, Shi YP, Sinha S, Alpers M, Povoa M, Roberts C, and Lal AA. Retrovirus Diseases Branch, DVRD, DPD, Centers for Disease Control, Atlanta, GA; Malaria Branch, DPD, Centers for Disease Control, Atlanta, GA.; Papua New Guinea Institute of Medical Sciences, Goroka, New Guinea; Instituto Evandro Chagas, Belem, Brazil; and Department of Diagnostic Retrovirology, Walter Reed Army Institute of Research, Washington, DC.

202 DENGUE INFECTION OF HUMAN STROMAL CELLS IN CULTURES OF HUMAN BONE MARROW. LaRussa V, Putnak R*, and Knight R. Department of Hematology and Department of Viral Diseases, Walter Reed Army Institute of Research, Washington, DC.

203 INHIBITION OF EBOLA VIRUS *IN VITRO* AND IN A SCID MOUSE MODEL BY S-ADENOSYLHOMOCYSTEINE HYDROLASE INHIBITORS 3-DEAZAADENOSINE AND 3-DEAZANEPLANOCIN A. Huggins JW*, Zhang ZX, and Monath TP. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

204 BACULOVIRUS EXPRESSION OF TRUNCATED HANTAAN VIRUS SEGMENT GENES: EPITOPE MAPPING USING MONOCLONAL ANTIBODIES TO THE G1 AND G2 PROTEINS. Pennock DG* and Schmaljohn C. Virology Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

205 SEQUENCE AND GENETIC ORGANIZATION OF THE S RNA SEGMENT OF CRIMEAN-CONGO HEMORRHAGIC FEVER (CCHF) VIRUS. Smith JF*, Hodgson LA, and Lofts RS. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

206 THE NON-STRUCTURAL GENOME OF DENGUE-1 VIRUS CV1636/77: COMPARISON OF THE NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES WITH THE OTHER DENGUE SEROTYPES. Chu MC*, Putvatana R, and Trent DW. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

207 RNA POLYMERASE, TYPE II, ACTIVITY IN BRAIN NUCLEI OF RATS INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS de Lopez ET*, Rangel P, Belloso J, Benitez Y, and Martinez M. Instituto de Investigaciones Clinicas, Universidad del Zulia, Maracaibo, Venezuela.

208 ANTIBODY ENHANCEMENT OF DENGUE-3 VIRUSES. Kuno G*. Centers for Disease Control, Division of Vector-Borne Infectious Diseases, Dengue Branch, San Juan, PR.

## KINETOPLASTIDA

209 THE FIRST CONFIRMED ISOLATION OF *LEISHMANIA MAJOR* FROM SOUTHERN SINAI, EGYPT. Kamal H*, Shehata M, Osman A, Doha A, El Hoosany S, and Schnur LF. Ain Shams University Research and Training Centre on Vectors of Diseases, Cairo, Egypt; and Kuvin Centre of Infectious and Tropical Diseases, Hebrew University Medical School, Jerusalem, Israel.

210 SPECIES-SPECIFIC DETECTION OF *LEISHMANIA MEXICANA* PROMASTIGOTES WITHIN SAND FLY HOMOGENATES ON NYLON BLOTS USING A RECOMBINANT KINETOPLAST DNA PROBE. Stiteler JM*, Bruckner PR, and Perkins PV. Walter Reed Army Institute of Research, Department of Entomology, Washington, DC

211 LEISHMANIASIS IN DESERT SHIELD/STORM. Grogl M*, Mendez J, Milhous WK, Nuzum EO, Martin RK, Berman JD, Schuster BG, and Oster CN. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC and Department of Infectious Diseases, Walter Reed Army Medical Center, Washington, DC.

212 VENEZUELAN KALA AZAR AND *LEISHMANIA COLOMBIENSIS N. SP.* Delgado O, Castes M, White AC*, and Kreutzer RD. Instituto de Medicina Tropical,Universidad Central de Venezuela, Caracas,Venezuela; Instituto de Biomedicina, Caracas, Venezuela; Department of Medicine, Baylor College of Medicine, Houston, TX; and Biology Department, Youngstown State University, Youngstown, OH.

213 THE EFFECT OF *LUTZOMYIA LONGIPALPIS* SALIVARY GLAND MATERIAL ON THE ABILITY OF MACROPHAGES TO PRESENT ANTIGEN TO *LEISHMANIA MAJOR*-SPECIFIC T CELLS. Theodos CM* and Titus RG. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

214 THE EFFECT OF SAND FLY SALIVA ON MACROPHAGE FUNCTION IN *LEISHMANIA MAJOR* INFECTION. Hall LR* and Titus RG. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

215 INTERACTIONS OF MURINE AIDS AND CUTANEOUS LEISHMANIASIS IN C57BL/6 MICE. Barral-Netto M*, Barral A, Silva JS, and Reed SG. Seattle Biomedical Research Institute; Seattle, WA; Faculdade de Medicina-Universidade Federal da Bahia; Salvador-Bahia Brazil; and Faculdade de Medicina, USP, Ribeirao Preto-SP, Brazil.

216 ANALYSIS OF *LEISHMANIA MAJOR* SPECIFIC T CELLS GENERATED *IN VITRO* FROM NONSENSITIZED MICE Shankar AH* and Titus RG. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

217 *TRYPANOSOMA CRUZI* INFECTION IN β-2-MICROGLOBULIN DEFICIENT MICE Tarleton RL*, Postan M, Koller BH, Latour A, and Smithies O. Department of Zoology, University of Georgia, Athens, GA; and Department of Pathology, University of North Carolina, Chapel Hill, NC.

218 CELLULAR IMMUNITY AS A DETERMINANT OF CHRONIC PATHOLOGY IN HUMAN *TRYPANOSOMA CRUZI* INFECTION IN NORTHEAST BRAZIL. Cetron MS*, Basilio FP, Moraes AP, Sousa AQ, Paes JN, and Van Voorhis WC. Department of Medicine, Infectious Disease, University of Washington, Seattle, Washington; HEMOCE Division of Serology; Department of Infectious Disease, Hospital Sao Jose, Brazil; and Department of Cardiology, Federal University of Ceara, Fortaleza-Ceara, Brazil.

## FILARIA

219 DIAGNOSTIC POTENTIAL OF RECOMBINANT EXCRETORY/SECRETORY PRODUCTS OF MALE ADULT WORMS OF *BRUGIA MALAYI*. Philipp M*, Bakeer M, Lillibridge CD, and Jayaraman K. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA; and Centre for Biotechnology, Anna University, Madras, India.

220 A MODEL FOR THE PROTEOLYTIC PROCESSING OF THE *DIROFILARIA IMMITIS* ANTIGEN, DI5. Poole CB*, Benner J, Grandea A, and McReynolds LA. New England Biolabs, Inc., Beverly, MA.

221 *CARDIOFILARIA KALIMANTANI* N. SP. (FILARIOIDEA: ONCHOCERCIDAE) FROM NATURAL AND EXPERIMENTAL HOSTS, LIFE CYCLE AND EPIDEMIOLOGIC SIGNIFICANCE. Purnomo, Atmosoedjono S, and Bangs MJ*. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.

222 $CD8^+$ T LYMPHOCYTES ARE NOT REQUIRED FOR MURINE RESISTANCE TO THE HUMAN FILARIAL PARASITE, *BRUGIA MALAYI* Rajan TV*, Nelson FK, Koller BH, Shultz LD, and Greiner DL. Department of Pathology, University of Connecticut Health Center, Farmington CT; Department of Pathology, University of North Carolina, Chapel Hill, NC; Department of Medicine, University of Massachusetts Medical Center, Worcester, MA; and The Jackson Laboratory, Bar Harbor, ME.

223 CHARACTERIZATION OF *BRUGIA MALAYI* PARASITE ANTIGENS USING BIOTINYLATED LECTIN PROBES. Rao UR, Vickery AC*, Kwa BH, and Nayar JK. College of Public Health, University of South Florida, Tampa, FL; and Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL.

224 NUMBER AND DISTRIBUTION OF *HHA I* REPEAT CLUSTERS IN THE GENOME OF THE FILARIAL WORM *BRUGIA MALAYI*. Sachar R*, Li Z, Spiegelman M, and Williams SA. Department of Biological Sciences, Smith College, Northampton, MA.

225 DEVELOPMENT OF *DIROFILARIA IMMITIS* IN UNITED STATES STRAINS OF *AEDES ALBOPICTUS*. Scoles GA* and Craig GB. Vector Biology Labs, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

226 CLONING MF1, A TRANSMISSION-BLOCKING ANTIGEN FROM *BRUGIA MALAYI*. Southworth MW*, Fuhrman JA, and Perler FB. New England Biolabs, Inc., Beverly, MA; Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

227 CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF MONOCLONAL ANTIBODIES SPECIFIC TO NEMATODE TUBULIN. Bughio NI*, Faubert GM, and Prichard RK. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

228 THE DEVELOPMENTALLY REGULATED EPITOPE RECOGNIZED BY MAB MF2 IS LOCATED IN A FILARIAL ISOACTIN. Sritharan M* and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

229 IMMUNITY IN ONCHOCERCIASIS: ANTIGEN INDUCED IL-2 PRODUCTION BY PUTATIVELY IMMUNE INDIVIDUALS IS REFLECTED BY INCREASED IL-5 SYNTHESIS. Steel C*, Abrams JS, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; DNAX Research Institute, Palo Alto, CA.

230 AN EXAMINATION OF THE IMMUNE RESPONSE TO CLONED *ONCHOCERCA VOLVULUS* ANTIGENS OI3 AND OI5. Strang G*, Southworth MW, and Perler FB. New England Biolabs, Inc. Beverly, MA

231 USING THE POLYMERASE CHAIN REACTION TO AMPLIFY AND SEQUENCE DNA FROM MUSEUM SPECIMENS OF *BRUGIA MALAYI* AND *BRUGIA PAHANGI*. Xie H*, Bain O, and Williams SA. Molecular and Cellular Biology Program, University of Massachusetts at Amherst, MA; Laboratoire de Zoologie-Vers, associe au CNRS, Museum National d'Histoire Naturelle, Paris; and Department of Biological Sciences, Smith College, Northampton, MA.

232 GTP-BINDING *RAS* P21 PROTEINS OF FILARIAL PARASITES HAVE A UNIQUE C-TERMINAL SEQUENCE. Xu M*, Dissanayake S, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

233 INTRINSIC ANTIMALARIAL ACTIVITY OF CIPROFLOXACIN ALONE OR IN COMBINATION WITH CHLOROQUINE OR MEFLOQUINE. Coyne PE*, Gerena L, and Milhous WK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

## MALARIA CHEMOTHERAPY

234 THE EFFECTS OF ARTEMISININ (QINGHAOSU) ON THE RED CELL CYTOSKELETON. Yang Y* and Meshnick SR. Department of Microbiology, City University of New York Medical School, New York, NY.

235 CHINESE HERBAL ANTIOXIDANTS IN MALARIA CHEMOTHERAPY. Meshnick SR*, Hong YL, Scott MD, Yang YZ, Ranz A, and Pan HZ. Department of Microbiology, City University of New York Medical School, New York, NY; Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China; and Childrens' Hospital Oakland Research Institute, Oakland, CA.

236 ABSOLUTE STEREOCHEMISTRY OF THE ENANTIOMERS OF MEFLOQUINE *HYDROCHLORIDE IN RELATION TO THEIR ANTIMALARIAL ACTIVITY*. *Karle JM*, Karle IL*, Gerena L, and Milhous WK. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC.; Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC.; and Department of Parasitology, Walter Reed Army Institute of Research, Washington, DC.

237 QUANTITATIVE ISOBOLOGRAPHIC ANALYSIS OF ANTIMALARIAL DRUG INTERACTIONS. Brueckner RP*, Milhous WK, and Canfield CJ. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Pharmaceutical Systems, Gaithersburg, MD.

238 IDENTIFICATION OF NEW REVERSAL MODULATORS FOR *PLASMODIUM FALCIPARUM*. Bass GT*, Sr., Gerena L, Milhous WK, Andersen SL, Oduola AM, Kyle DK, and Martin RK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; University of Ibadan, Ibadan, Nigeria; and Department of Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

239 REVERSAL OF CHLOROQUINE RESISTANCE IN THE ISOLATES OF *PLASMODIUM FALCIPARUM* FROM AFRICA. Basco LK and Le Bras J*. Laboratoire de Parasitologie, Hopital Bichat-Claude Bernard, Paris, France.

240 TRANSPORT AND INCORPORATION OF [$^3$H-] P-AMINOBENZOIC ACID BY *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES. Zhang Y*, Merali S, and Meshnick SR. Department of Microbiology, City University of NY Medical School, New York, NY.

241 THE RELATIONSHIP OF *PF-MDR1* TO MEFLOQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM*. Wilson CM*, Thaithong S, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA and WHO Collaborating Centre, Chulalongkorn University, Bangkok, Thailand.

242 ACCESSIBILITY AND COMPLIANCE IN THE USAGE OF MEFLOQUINE IN ITS FIRST YEAR OF AVAILABILITY AT A US TRAVEL CLINIC. Eaton M* and Kozarsky P. Emory University School of Medicine, Atlanta, GA.

243 RECRUDESCENCE OF FALCIPARUM MALARIA OCCURRING FIVE YEARS AFTER EXPOSURE IN WEST AFRICA. Furlong WB*, Gerena L, Waltersdorff RL, Oduola AM, and Milhous WK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Washington Adventist Hospital, Takoma Park, MD.

244 EPIDEMIOLOGIC AND ENTOMOLOGIC CONDITIONS RESPONSIBLE FOR STABLE HYPERENDEMIC TRANSMISSION OF MALARIA IN THE CENTRAL HIGHLANDS OF IRIAN JAYA. Bangs MJ*, Hamzah N, Purnomo, Basri H, and Anthony RL. US Naval Medical Research Unit, No.2, Jakarta, Indonesia; and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

245 MALARIA SURVEILLANCE IN 15 SOUTHERN PROVINCES OF VIETNAM, 1976-1989. Nguyen Long G*, Phan Dinh L, Nguyen Van K, and Nguyen-Dinh P. Sub-Institute of Malariology, Parasitology and Entomology (Sub-IMPE) and Cho Ray Hospital, Ho Chi Minh City, Vietnam; and Malaria Branch, Centers for Disease Control, Atlanta, GA.

246 USE OF BED NETS IMPREGNATED WITH PERMETHRIN FOR MALARIA CONTROL IN EASTERN GUATEMALA. Richards FO*, Zea Flores RM, Klein RE, Sexton JD, and Gatica Palacios MR. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; Centro para Investigaciones en Enfermedades Tropicales, Universidad del Valle de Guatemala, Guatemala; and Division de Malaria, Ministerio de Salud Publica, Guatemala.

247 FORMALDEHYDE/DETERGENT SOLUTION PREVENTS BLOOD BORNE TRANSMISSION OF *PLASMODIUM* INFECTION IN A MOUSE MODEL. Ager AL*, Andersen SL, Louderback AL, May R, and Milhous WK. Center for Tropical Parasitic Diseases, University of Miami, Miami, FL; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Clinical Chemistry Consultants, Inc., Arcadia, CA.

248 THE RELATIVE FITNESS OF PYRIMETHAMINE-RESISTANT AND SUSCEPTIBLE LINES OF *PLASMODIUM BERGHEI* IN THE SPOROGONIC CYCLE IN *ANOPHELES STEPHENSI*. Shinondo CJ*, Lanners HN, Lowrie, Jr. RC, and Wiser MF. Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane Univiversity, New Orleans, LA; and Parasitology Department, Tulane Regional Primate Research Center, Covington, LA.

249 LACK OF CAUSAL PROPHYLACTIC ACTIVITY OF PROGUANIL PLUS SULFAMETHOXAZOLE AGAINST *PLASMODIUM CYNOMOLGI BASTIANELLII* IN RHESUS MONKEYS. Edstein MD*, Shanks GD, Smith CD, Corcoran KD, Chedester AL, Sattabongkot J, Ngampochjana M, Hansukjariya P, and Webster HK. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

250 FIELD USE OF THE MICRO *IN VITRO* TEST FOR PREDICTING CHLOROQUINE SENSITIVITY IN *PLASMODIUM VIVAX*. Andersen EM*, Purnomo, Masbar S, Murphy GS, and Bangs MJ. U.S. Navy Medical Research Unit No. 2, Jakarta, Indonesia.

WEDNESDAY AM
POSTER SESSION II

## ENTAMOEBA AND GIARDIA

251 *GIARDIA LAMBLIA* ENCYSTMENT IN THE MONGOLIAN GERBIL. Campbell JD*, Mohammed SR, Faubert GM. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

252 THE EFFECTS OF AXENISATION ON THE ZYMODEME STATUS OF *ENTAMOEBA HISTOLYTICA*. Gathiram V*, Jackson TG, Suparsad S, and Anderson CB. Research Institute for Diseases in a Tropical Environment, Congella, Durban, Republic of South Africa; and Medical School, University of Natal, Congella, Durban, Republic of South Africa.

253 ASSOCIATION OF SALIVARY IgA SPECIFIC FOR THE 260KD ADHERENCE PROTEIN OF *E. HISTOLYTICA* WITH THE PRESENCE OF AMEBIC LIVER ABSCESS. Kelsall BL*, Jackson TG, Pearson RD, and Ravdin JI. University of Virginia, Charlottesville, Virginia; RIDTE, Durban, Republic of South Africa; Case Western Reserve University, Cleveland, OH; and the Cleveland VA Medical Center, Cleveland, OH.

254 KILLING OF *ENTAMOEBA HISTOLYTICA* TROPHOZOITES BY MACROPHAGES IS MEDIATED BY NITRIC OXIDE FROM L-ARGININE. Lin JY* and Chadee K. Institute of Parasitology of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

255 *ENTAMOEBA HISTOLYTICA* ELICITS SECRETION OF NEUTRAL AND ACIDIC MUCINS IN RAT COLON AND COLONIC ADENOCARCINOMA CELLS *IN VITRO*. Tse S*, Keller K, and Chadee K. Institute of Parasitology of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

256 RIBOSOMAL PROTEINS OF *GIARDIA LAMBLIA*: ISOLATION AND NOMENCLATURE. Montanez C*, Depardon F, Sinker S, and Ortega-Pierres MG. Department of Genetics and Molecular Biology, Center for Research and Advanced Studies of IPN, Mexico, DF, Mexico.

## SCHISTOMA IMMUNOLOGY

257 HISTOPATHOLOGIC STUDY OF SCHISTOSOMAL PERIOVULAR GRANULOMAS IN CHALLENGED MICE PREVIOUSLY EXPOSED TO EITHER MODERATELY OR HIGHLY IRRADIATED CERCARIAE. Ramos EA*, Reynolds SR, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, FIOCRUZ, Bahia, Brazil.

258 LARVICIDAL PROPERTIES OF MACROPHAGES INDUCED BY CLONED MURINE SCHISTOSOMAL EGG ANTIGEN-SPECIFIC CD4 POSITIVE T HELPER LYMPHOCYTES. Kanazawa T* and Stadecker MJ. Tufts University School of Medicine, Boston, MA.

259 IMMOBILIZATION OF *SCHISTOSOMA MANSONI* MIRACIDIA BY ACTIVATION OF THE ALTERNATE PATHWAY OF COMPLEMENT AT EXTREMELY HIGH DILUTIONS OF NORMAL SERA. Knopf PM* and McLaren DJ. Division of Biology and Medicine, Brown University, Providence, RI; and National Institute for Medical Research, Mill Hill, London, UK .

260 THE ROLE OF MACROPHAGES IN MURINE *SCHISTOSOMA JAPONICUM* INFECTION Laxer MJ* and Tuazon CU. Division of Infectious Diseases, Department of Medicine, George Washington University, Washington, DC.

261 FURTHER CHARACTERIZATION OF THE MAJOR LYMPHO-STIMULATORY ACIDIC COMPONENTS OF SCHISTOSOME EGGS RECOGNIZED BY SPECIFIC MURINE TH-1 TYPE CLONES. Chikunguwo SM*, Quinn JJ, Harn DA, and Stadecker MJ. Tufts University School of Medicine, Boston, MA; and Harvard School of Public Health, Boston, MA

262 CROSS-REACTIVE IDIOTYPES ON RABBIT ANTI-SEA ANTIBODIES STIMULATE ANTI-IDIOTYPIC LYMPHOCYTE RESPONSES OF MICE INFECTED WITH *SCHISTOSOMA MANSONI*. Amano T*, Nakazawa M, Oshima T, Bosshardt S, and Colley D. Department of Parasitology, Yokohama City University School of Medicine, Yokohama, Japan; Veterans Administration Medical Center, Nashville, TN; and Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN.

263 CLONAL HAEMATOPOIETIC RESPONSE IN MURINE SCHISTOSOMAL INFECTION. Lenzi HL*, Lenzi JA, and Mota EM. Departmento Pathologia, IOC-Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

264 OMENTUM MILKY SPOTS BEHAVE AS LYMPHOHEMATOPOIETIC ORGANS IN MURINE SCHISTOSOMIASIS CAUSED BY *SCHISTOSOMA MANSONI*. Lenzi JA*, Borojevic R, Oliveira DN, and Lenzi HL. Departmento Pathologia, IOC-Fiocruz, Rio de Janeiro, Brazil; and Departmento Bioquimica, Instituto Bioquimica, UFRJ, Rio de Janeiro, Brazil.

265 *IN VIVO* TREATMENT WITH ANTI-IL-2 ANTIBODIES INHIBITS GRANULOMA FORMATION, FIBROSIS AND IL-5 PRODUCTION IN MURINE SCHISTOSOMIASIS MANSONI. Hieny S*, Cheever AW, Caspar P, Macedonia J, Finkelman F, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, Bethesda, MD; and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.

266 ROLE OF PARAMYOSIN IN THE HOST-PARASITE RELATIONSHIP. Laclette JP*, Nicholson-Weller A, Richter D, Pante N, Cohen C, Bing D, and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.; Department of Immunology, Instituto de Investigaciones Biomedicas, UNAM, Mexico D.F., Mexico; Infectious Diseases Division, Beth Israel Hospital, Boston, MA; Center for Blood Research, Boston, MA; and Rosensteil Center, Waltham, MA.

267 NITROGEN OXIDES: DIFFERENTIAL EFFECTS ON *SCHISTOSOMA MANSONI* AND DOWN-REGULATION OF MURINE MACROPHAGES. Smith JM* and Prichard RK. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

268 PROGRESS AND REFINEMENT OF A MURINE MODEL FOR KATAYAMA FEVER. Weina PJ*. Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

269 *SCHISTOSOMA MANSONI*: ANTIGENS RELEVANT TO DIAGNOSIS OF PREPATENT INFECTION. Mikhail MM*, Mansour MM, Farid Z, and Harrison RA. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

270 MOLECULAR BASIS OF GRANULOMA FORMATION IN SCHISTOSOMIASIS. Phillips SM*, Perrin PJ, Gaafar T, and Wahba SN. Allergy and Immunology Section, University of Pennsylvania, Philadelphia, PA; Navy Medical Research Institute, Bethesda, MD; University of Cairo, Cairo, Egypt; and Dr. Osman Hospital, Maadi, Cairo, Egypt.

271 COMPARISON OF SEA FRACTIONS-ELICITED LYMPHOKINE PRODUCTION BY SPLENIC AND GRANULOMA LYMPHOCYTES OF *S. MANSONI*-INFECTED MICE. Lukacs NW and Boros DL*. Department of Immunology/Microbiology, Wayne State University School of Medicine, Detroit, MI.

## SYMPOSIUM: RECENT ADVANCES IN THE EXOERYTHROCYTIC STAGES OF THE MALARIA PARASITE

*In Honor of Professor P.C.C. Garnham*

Wednesday, December 4
9:45 AM - 12:00 NN Independence West/Center
Chairpersons: M.R. Hollingdale and M. Aikawa

9:45 S48 *IN VITRO* CULTIVATION AND RODENT EE SPECIFIC ANTIGENS. Sinden RE*, O'Dowd C, Suhrbier A, Winger LA, and Couchman A. Imperial College, London, England.

10:00 S49 SPECIFIC HUMAN HEPATOCYTE RECEPTORS FOR SPOROZOITE INVASION AND EXPRESSION OF *PLASMODIUM FALCIPARUM* EE ANTIGENS. Hollingdale MR*, Zhu J, Sina B, Sakhuja K, van Pelt J, and Shoemaker J. Biomedical Research Institute, Rockville, MD; and the University of Nijmegen, Nigmegen, The Netherlands.

10:15 S50 AMINO ACID SEQUENCE HOMOLOGY BETWEEN THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX* AND THE MCH CLASS I ANTIGEN: CHANCE OR DESIGN. Millet P*, Chizzolini C, Pieniazek NJ, Charoenvit Y, Jones TR, Hoffman SL, and Collins WE. Centers for Disease Control, Atlanta, GA; and Naval Medical Research Institute, Bethesda, MD.

10:30 S51 NATURAL IMMUNITY TO EE STAGES. Vanderberg JP*. New York University, New York, NY.

10:45 S52 INHIBITION OF MALARIA LIVER STAGES BY CD8+ T CELL CLONES. Rodrigues MM, Nussenzweig RS, and Zavala F*. New York University, New York, NY.

11:00 S53 IDENTIFICATION OF T CELLS RECOGNIZING PREERYTHROCYTIC AND EXOERYTHROCYTIC STAGE ANTIGENS IN HUMAN VOLUNTEERS IMMUNIZED WITH IRRADIATED SPOROZOITES. Krzych U*, Jareed T, Seguin M, Lyon J, Hollingdale MR, and Ballou WR. Walter Reed Army Institute of Research, Washington, DC; and Biomedical Research Institute, Rockville, MD.

11:15 S54 CHARACTERIZATION OF AN INHIBITORY MONOCLONAL ANTIBODY AGAINST *PLASMODIUM YOELII* LIVER STAGE PARASITES. Charoenvit Y*, Mellouk S, Sedegah M, Leef MF, de la Vega P, and Hoffman SL. Naval Medical Research Institute, Bethesda, MD.

11:30 S55 ULTRASTRUCTURE OF EE PARASITES USING ELECTRON AND CONFOCAL LASER SCANNING MICROSCOPY. Aikawa M*, Atkinson CT, and Hollindale MR. Case Western Reserve University, Cleveland, OH; and Biomedical Research Institute, Rockville, MD.

11:45 S56 EXOREYTHROCYTIC DEVELOPMENT OF FALCIPARUM MALARIA EE PARASITES IN A SCID MOUSE MODEL. Sacci JB, Jr, Schriefer ME, Resau JH, Wirtz RA, Detolla LJ Jr, Markham R, and Azad AF. University of Maryland School of Medicine, Baltimore, MD; Walter Reed Army Institute of Research, Washington, DC; and Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

## SCIENTIFIC SESSION L: LYME DISEASE

Annual Meeting: American Society of Tropical Veterinary Medicine

Wednesday, December 4
10:00 AM - 12:30 PM Commonwealth
Chairpersons: A. Spielman and J.C. Williams

10:00 272 NATIONAL LYME DISEASE UPDATE. Dennis DT*, Paul WS, Campbell GL, and Craven RB. Centers for Disease Control, Division of Vector-Borne Infectious Diseases, Fort Collins, CO.

10:15 273 SPATIAL VARIATION IN THE PROPORTION OF TICKS INFECTED WITH THE LYME DISEASE SPIROCHETE IN NORTHERN CALIFORNIA. Kimsey RB*. Department of Entomology, University of California, Davis, CA.

10:30 274 CRITICAL ABUNDANCE OF HOSTS PERPETUATING THE TICK THAT TRANSMITS THE AGENT OF LYME DISEASE. Awerbuch T*, Sandberg S, and Spielman A. Department of Biostatistics, Harvard School of Public Health, Boston, MA; Department of Mathematics, Framingham State College, Framingham, MA; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

10:45 275 MODEL SYSTEM FOR TESTING THE INFECTIVITY OF *BORRELIA BURGDORFERI* TO TICKS. Piesman J*. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

11:00 276 NEST-ASSOCIATED FEEDING OF RUBIDIUM-MARKED DEER TICKS (*IXODES DAMMINI*). Ratanatham S, Kimsey RB, Hamlin D*, Pollack RJ, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Entomology, University of California, Davis, CA.

11:15 277 CLEARANCE OF *BORRELIA BURGDORFERI* FROM GUTS OF TICKS ENGORGING UPON RECOMBINANT OSP-A VACCINATED MICE. Telford SR*, Fikrig E, Barthold SW, Flavell RA, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Section of Immunobiology, Yale University School of Medicine, New Haven, CT; and Section of Comparative Medicine,Yale University School of Medicine, New Haven, CT.

11:30 278 PROTECTION OF MICE AGAINST LYME DISEASE INFECTION BY EAR PUNCH BIOPSY. Shih CM*, Pollack RJ, Telford SR III, and Spielman A. Dept of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:45 279 EFFECT OF ORAL OR SUBCUTANEOUS ADMINISTRATION OF IVERMECTIN IN HAMSTERS ON SUBADULT DEER TICK *IXODES DAMMINI* FEEDING AND SURVIVAL. Korch, GW, Jr.* and Miller JA. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD; and U.S. Livestock Insects Research Laboratory, U.S. Department of Agriculture, Kerrville, TX.

12:00 Business Meeting, ASTVM

## SCIENTIFIC SESSION M:
## KINETOPLASTIDA CHEMOTHERAPY AND EPIDEMIOLOGY

Wednesday, December 4
10:00 AM - 12:00 NN Independence East
Chairpersons: J. Berman and B. Herwaldt

10:00 280 S-ADENOSYLMETHIONINE SYNTHETASE IN *TRYPANOSOMA BRUCEI*. Bacchi CJ*, Yarlett N, Garofalo J, Ciminelli M, and Goldberg B. Haskins Laboratories and Department of Biology, Pace University, New York, NY.

10:15 281 ANTIMONTAL TREATMENT IN HAMSTERS AS A MODEL FOR ASSESSING PARASITOLOGICAL CURE IN TEGUMENTARY LEISHMANIASIS. Travi BL*, Martinez JE, Zea A. Fundacion CIDEIM, Cali, Colombia and Departmento de Microbiologia, Universidad del Valle, Cali, Colombia

10:30 282 DIFFERENTIAL SENSITIVITY TO THE PENTAVALENT ANTIMONIALS, PENTOSTAM AND GLUCANTIME, OF SOME LEISHMANIAL ISOLATES. Jackson JE* and Tally JD. Experimental Therapeutics Division, Walter Reed Army Institute of Research, Washington, DC.

10:45 283 ANTILEISHMANIAL ACTIVITY OF MEDICINAL PLANTS USED IN NIGERIAN TRADITIONAL MEDICINE. Iwu MM*, Jackson JE, Tally JD, and Klayman DL. Experimental Therapeutics Division, Walter Reed Army Institute of Research, Washington, DC.

11:00 284 SCHIZODEME AND ZYMODEME CHARACTERIZATION OF *LEISHMANIA* IN THE INVESTIGATION OF A FOCUS OF INFANTILE VISCERAL LEISHMANIASIS IN ALEXANDRIA, EGYPT. Karim AM, Osman AM*, Al Gauhari AI, and Shehata MG. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

11:15 285 *LEISHMANIA INFANTUM* MON-98 ISOLATED FROM NATURALLY INFECTED *PHLEBOTOMUS LANGERONI* (DIPTERA: PSYCHODIDAE) IN EL AGAMY, EGYPT. Doha S* and Shehata M. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

11:30 286 CUTANEOUS (CL) AND VISCERAL (VL) LEISHMANIASES INDIGENOUS TO ISRAEL AND EGYPT : REAPPRAISAL, CURRENT STATUS AND ANOMALIES. Schnur LF*, Youssef M, Wahba MM, and Shehata M. Department of Parasitology, Hebrew University - Hadassah Medical School, Jerusalem, Israel; and Ain Shams University Research and Training Centre on Vectors of Diseases, Cairo, Egypt.

11:45 287 A QUANTITATIVE STUDY OF LEISHMANIASIS TRANSMISSION AT A SITE IN THE NORTHEASTERN SINAI DESERT OF EGYPT DURING 1990. Fryauff DJ*, Modi GB, Mansour NS, Mikhail EM, Youssef FG, and Wassif KM. Medical Zoology and Basic Sciences Divisions, US Naval Medical Research Unit Number Three, Cairo, Egypt; and Zoology Department, Faculty of Science, Ain Shame University, Cairo, Egypt.

## SCIENTIFIC SESSION N: INTESTINAL PROTOZOA

Wednesday, December 4
10:00 AM - 12:00 NN Hampton A/B
Chairpersons: G. Faubert and S. Aley

10:00 288 PREVALENCE OF *BLASTOCYTIS HOMINIS* AMONG ASYMPTOMATIC AND SYMPTOMATIC INDIVIDUALS. Markell EK and Udkow MP*. Department of Internal Medicine, Kaiser Permanente Medical Center, Oakland, CA.

10:15 289 INDUCTION OF STRESS RESPONSES IN *TRICHOMONAS VAGINALIS*. Davis SR* and Lushbaugh WB. Parasitology Division, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS.

10:30 290 CHARACTERIZATION OF A *GIARDIA LAMBLIA* VARIANT-SPECIFIC SURFACE PROTEIN (VSP) GENE FROM ISOLATE GS/M AND ESTIMATION OF THE VSP GENE REPERTOIRE SIZE. Nash TE* and Mowatt MR. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, Md.

10:45 291 EXPRESSION OF ANTIGENS RECOGNIZED BY ANTIBODIES TO CONSERVED HEAT SHOCK PROTEINS DURING ENCYSTATION OF *GIARDIA LAMBLIA*. Reiner DS*, Aley SB, and Gillin FD. Department of Pathology, University of California, San Diego, San Diego, CA.

11:00 292 EXPERIMENTAL GIARDIASIS IN THE PROTEIN MALNOURISHED HOST. Leitch GJ*, Udezulu IA, and Visvesvara GS. Department of Physiology, Morehouse School of Medicine, Atlanta, GA; and Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

11:15 293 PULSED FIELD GEL ELECTROPHORESIS OF *GIARDIA DUODENALIS* ISOLATES FROM A WATERBORNE OUTBREAK. Sarafis K*, Shahriari H, Isaac-Renton JL. Division of Medical Microbiology, University of British Columbia, Vancouver, British Columbia, Canada.

11:30 294 PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF SEVERAL CLONES OF *ENTAMOEBA HISTOLYTICA*. Orozzco E*, Lazard D, Gamboa Y, Sanchez T, Valdes J, and Hernandez F. Department of Experimental Pathology, CINVESTAV I.P.N. Mexico D.F. Mexico.

11:45 295 DIFFERENTIATION OF *ENTAMOEBA HISTOLYTICA* STRAINS BY OLIGONUCLEOTIDE PROBES. Burch DJ, Li E, Kuntz-Jenkins CR, and Stanley SL, Jr. Department of Medicine, Washington University School of Medicine, St. Louis, MO; and Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO.

## SCIENTIFIC SESSION O: FILARIASIS PATHOLOGY & DIAGNOSIS

Wednesday, December 4
10:00 AM - 12:00 NN Gardner A/B
Chairpersons: T.V. Rajan and R.M.R. Ramzy

10:00 296 *ONCHOCERCA VOLVULUS* DNA PROBE CLASSIFICATION CORRELATES WITH EPIDEMIOLOGICAL PATTERNS OF BLINDNESS. Zimmerman PA, Dadzie KY, De Sole G, Remme J, Soumbey Alley E, and Unnasch TR*. Onchocerciasis Control Programme in West Africa, Ouagadougou, Burkina Faso; and Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

10:15 297 PATHOGENIC ANTIGENS OF *ONCHOCERCA VOLVULUS* CAUSING SCLEROSING KERATITIS IN A GUINEA PIG MODEL. Chakravarti B*, Lass J, Diaconu E, Herring TA, Chakravarti DN, and Greene BM. Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; and Division of Ophthalmology, Department of Surgery, Case Western Reserve University, Cleveland, OH.

10:30 298 MONOCLONAL ANTIBODIES TO CIRCULATING *ONCHOCERCA VOLVULUS* ANTIGENS. Chandrashekar R*, Ogunrinade AF, and Weil GJ. Washington University School of Medicine, St. Louis, MO; and University of Ibadan, Nigeria.

10:45 299 EFFICIENT ASSESSMENT OF FILARIASIS ENDEMICITY BY SCREENING FOR FILARIAL ANTIGENEMIA IN A SENTINEL POPULATION. Ramzy RM*, Hafez ON, Gad AM, Faris R, Buck AA, and Weil GJ. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

11:00 300 COMMUNITY DIAGNOSIS OF LYMPHATIC FILARIASIS IN EGYPT: A COMPREHENSIVE APPROACH. Faris R*, Ramzy RM, Gad AM, Weil GJ, and Buck AA. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington UnivSchool of Medicine, St. Louis, MO.

11:15 301 DO ELEPHANTIASIS AND HYDROCELE REPRESENT DISTINCT PARASITOLOGIC AND IMMUNOLOGIC OUTCOMES OF FILARIAL INFECTION? Lammie PJ*, Addiss DG, and Eberhard ML. Parasitic Diseases Branch, Centers for Disease Control, Atlanta GA.

11:30 302 PATHOPHYSIOLOGICAL AND IMMUNOLOGICAL CHANGES IN INGUINAL LYMPH NODES FROM RHESUS MONKEYS WITH EXPERIMENTAL *BRUGIA MALAYI* INFECTION. Dennis VA*, Lasater BL, Blanchard JL, Lowrie, Jr. RC, and Campeau RJ. Parasitology Department, Tulane Regional Primate Research Center, Covington, LA; Veterinary Sciences Department, Tulane Regional Primate Research Center, Covington, LA; and Radiology Department, Tulane University School of Medicine, New Orleans, LA.

11:45 303 AFFECTS OF PROTECTIVE RESISTANCE ON THE DEVELOPMENT OF LYMPHATIC LESIONS AND GRANULOMATIOUS HYPERSENSITY IN *BRUGIA*-INFECTED JIRDS. Petit TA, Klei TR*, Enright FM, Coleman SU, and Jones K. Louisiana State University, Baton Rouge, LA.

# SCIENTIFIC SESSION P: VIRAL VACCINES

Wednesday, December 4
10:00 - 11:45 AM Fairfax A
Chairpersons: P.K. Russell and K. Eckels

10:00 304 PRECLINICAL TESTING OF A RECOMBINANT VACCINIA VIRUS VACCINE CANDIDATE FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME. Hasty SE*, Dalrymple JM, Malinoski FJ, Schmaljohn AL, and Schmaljohn CS. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

10:15 305 STUDIES ON A MUTAGEN-ATTENUATED RIFT VALLEY FEVER VACCINE CANDIDATE (MP12) IN NON-HUMAN PRIMATES. Morrill JC* and Peters CJ. Disease Assessment Division, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

10:30 306 THE MUTAGEN-ATTENUATED RIFT VALLEY FEVER VACCINE (MP12): POTENTIAL FOR A MUCOSAL VACCINE. Pitt ML* and Morrill JC. Pathophysiology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

10:45 307 INTERFERENCE BETWEEN ALPHAVIRUS VACCINES. Malinoski FJ*, Ksiazek T, Schmaljohn A, Ramsburg HH, and Monath TP. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

11:00 308 EXPERIENCE WITH AN INACTIVATED HEPATITIS A VACCINE. Nalin DR*, Calandra G, Ryan J, Lewis J, Miller W, Clements M, and Shouval D. Merck, Sharp and Dohme Research Labs, West Point, PA; Johns Hopkins Medical College, Baltimore, MD; and Hadassah Hospital, Jerusalem, Israel.

11:15 309 SAFETY OF HEPATITIS A VACCINE (HM175, INACTIVATED-SMITHKLINE, BEECHAM) IN U.S. SOLDIERS: COMPARISON OF IMMUNIZATION SCHEDULES Kanjarpane DD*, DeFraites RF, Hoke CH, Sanchez JL, Malis DJ, Gelnett JM, Fleming JL, Krauss MR, Egan JE, Sjogren M, Moonsammy G, and Krause D. Walter Reed Army Institute of Research, Washington, DC; Madigan Army Medical Center, Tacoma, WA; and SmithKline Beecham Pharmaceuticals, Inc., Philadelphia, PA.

11:30 310 LIMITED REPLICATIVE CAPACITY OF A LIVE-ATTENUATED HEPATITIS A VACCINE. Sjogren MH*, Purcell RH, McKee K, Binn LN, Macarthy P, Lackovic M, Ticehurst JR, Hoke CH, Feinstone SM, Bancroft WH, and D'Hondt E. Walter Reed Army Institute of Research, Washington, DC; National Institutes of Health, Bethesda, MD; US Army Medical Research Institute of Infectious Diseases, Frederick, MD; Food and Drug Administration, Bethesda, MD; and SmithKline Beecham, Belgium.

## FILM ON LYME DISEASE

Wednesday, December 4
1:00 PM - 1:30 PM — Grand Ballroom
Commentator: A. Spielman

*"WOOD TICKS AND LYME DISEASE: AN EMERGING THREAT"*

## AMERICAN COMMITTEE ON CLINICAL TROPICAL MEDICINE AND TRAVELERS' HEALTH

1:30 PM - 5:45 PM — Independence West/Center
Chairperson: L. Marcus

1:30

## VINCENZO MARCOLONGO MEMORIAL LECTURE

*TROPICAL DERMATOLOGY*

Anthony Bryceson
Hospital for Tropical Diseases
London School of Hygiene and Tropical Medicine, London, England

2:30 CLINICAL PROBLEMS. Felsenstein D and Wilson M.

3:00 Coffee Break

3:30 MALARIA UPDATE. Centers for Disease Control.

4:00 CHALLENGE THE AUDIENCE. (Slide Presentations, Unknowns). Bryceson A.

4:45 Business Meeting.

## SCIENTIFIC SESSION Q: MALARIA IMMUNOLOGY I

Wednesday, December 4
1:30 - 5:00 PM Fairfax A/B
Chairpersons: F. Klotz and H. Shear

1:30 311 HYPNOZOITES OF *PLASMODIUM SIMIOVALE*. Cogswell FB*, Collins WE, Krotoski WA, and Lowrie RC. Parasitology Department, Tulane Regional Primate Research Center, Covington, LA ; Malaria Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Gillis W. Long Hansen's Disease Center, Carville, LA.

1:45 312 IMMUNOGENICITY OF MULTIPLE ANTIGEN PEPTIDE (MAPS) CONTAINING T AND B CELL EPITOPES OF THE REPEAT REGION OF THE *PLASMODIUM FALCIPARUM* CS PROTEIN. Munesinghe DY, Clavijo PJ, Calvo Calle JM, Nussenzweig RS, and Nardin E*. Department of Zoology, University of Colombo, Sri Lanka; and Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

2:00 313 CYTOTOXIC CD4+ T CELLS FROM A SPOROZOITE-IMMUNIZED VOLUNTEER RECOGNIZE THE *PLASMODIUM FALCIPARUM* CS PROTEIN. Moreno A*, Clavijo P, Edelman R, Davis J, Sztein M, Herrington D, Nardin E, Nussenzweig RS. Department of Medical and Molecular Parasitology. New York University School of Medicine, New York, NY; Center for Vaccine Development, University of Maryland, Baltimore, MD; and Bowman-Gray School of Medicine, Wake-Forest University, Winston-Salem, NC.

2:15 314 FURTHER CHARACTERIZATION OF THE *PLASMODIUM FALCIPARUM* SPOROZOITE GENE CSP-2. Sina BJ*, Sakhuja K, Anders J, and Hollingdale MR. Biomedical Research Institute, Rockville, MD; and Walter Reed Army Institute of Research, Washington, DC.

2:30 315 INDUCTION OF CYTOTOTOXIC T LYMPHOCYTES AGAINST THE *PLASMODIUM FALCIPARUM* CS PROTEIN BY IMMUNIZATION WITH SOLUBLE PROTEIN WITHOUT ADJUVANT. Malik A*, Gross M, Ulrich T, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Ribi Immunochemcial Research Inc., Hamilton, MT; and Smith Kline Beecham, King of Prussia, PA.

2:45 316 SYNERGY BETWEEN ANTIBODIES TO *PLASMODIUM FALCIPARUM* SPOROZOITE ANTIGENS ENHANCE NEUTRALIZING ACTIVITY. Appiah A* and Hollingdale MR. Biomedical Research Institute, Rockville, MD.

3:00 Coffee Break

3:30 317 PROTECTION AGAINST *PLASMODIUM YOELII* BY CD8+ CYTOTOXIC T LYMPHOCYTE CLONE THAT RECOGNIZES AN EPITOPE ON SPOROZOITE SURFACE PROTEIN 2. Khusmith S*, Sedegah M, Mellouk S, Houghten RA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Torrey Pines Institute for Molecular Studies, San Diego, CA.

3:45 318 A MURINE T CELL CLONE WHICH PROTECTS AGAINST INFECTION BY BOTH *PLASMODIUM YOELII* AND *P. BERGHEI* SPOROZOITES. Weiss WR*, Houghten R, Sedegah M, Berzofsky JA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Torrey Pines Institute for Molecular Studies, San Diego, CA; and Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

4:00 319 PFS40: A CALCIUM BINDING SEXUAL STAGE PROTEIN PREDICTED TO BE A MALARIA TRANSMISSION BLOCKING TARGET ANTIGEN BY AN IMMUNOGENETIC APPROACH. Rawlings DJ*, Keister DB, and Kaslow DC. Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

4:15 320 EXPRESSION-PCR STRUCTURE-FUNCTION ANALYSIS OF AN ERYTHROCYTE BINDING ANTIGEN (EBA-175) AND A CYTOADHERENCE RECEPTOR (CD36) FOR *PLASMODIUM FALCIPARUM*. Kain KC*, Ockenhouse CF, Orlandi PA, and Lanar DE. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

4:30 321 THE INFECTED ERYTHROCYTE RECEPTOR(S) FOR CD36 AND THROMBOSPONDIN ARE RESTRICTED TO KNOBS. Nakamura K*, Howard R, Hasler T, and Aikawa M. Case Western Reserve University, Institute of Pathology, Cleveland, OH; and DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA.

4:45 322 MACROPHAGE PRODUCTION OF $NO_2^-$ DURING *PLASMODIUM CHABAUDI* AS INFECTION IN C57BL/6 MICE. Nowotarski ME* and Stevenson MM. Centre for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada; and The Montreal General Hospital Research Institute, Montreal, Quebec.

## SCIENTIFIC SESSION R: HELMINTH IMMUNOLOGY

Wednesday, December 4
1:30 - 5:00 PM Commonwealth
Chairpersons: D. Harn and S. James

1:30 323 COMPARISON OF ANTIGENS RECOGNIZED BY MICE VACCINATED WITH MODERATE OR HIGH DOSE IRRADIATED CERCARIAE OF *SCHISTOSOMA MANSONI*. Richter D* and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA

1:45 324 PROTECTIVE ACTIVITY OF T CELL LINES AND CLONES FROM MICE VACCINATED AGAINST *SCHISTOSOMA MANSONI*. Williams M*, Caspar P, Hieny S, Sher A, and James S. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

2:00 325 *IN VIVO* ROLE OF EOSINOPHILS IN *SCHISTOSOMA MANSONI* IMMUNITY IN MURINE MODEL OF IRRADIATED VACCINE. Othman MI* and Higashi GI. University of Michigan, Department of Epidemiology, Ann Arbor, MI.

2:15 326 LYMPHOPROLIFERATIVE RESPONSE OF MURINE SPLENIC LYMPHOCYTES TO THE MAJOR LYMPHOSTIMULATORY PEAK OF SOLUBLE EGG ANTIGEN. Quinn JJ*, Secor WE, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

2:30 327 SCHISTOSOMAL EGG ANTIGEN-SPECIFIC CLONED MURINE CD4 POSITIVE TH-1 TYPE LYMPHOCYTES MEDIATE LOCAL DTH REACTIONS AS WELL AS GRANULOMA FORMATION *IN VIVO*. Stadecker MJ and Chikunguwo SM*. Tufts University School of Medicine, Boston, MA.

2:45 328 CHARACTERIZATION OF IMMUNE RESPONSES OF BRAZILIAN SCHISTOSOMIASIS MANSONI PATIENTS TO PARTIALLY PURIFIED EGG ANTIGENS. Secor WE*, Reis MG, Reis E, Quinn JJ, David RA, David JR, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, FIOCRUZ, Bahia, Brazil.

3:00 Coffee Break

3:30 329 SEA REACTIVE HUMAN T CELL CLONES: ANALYSIS OF GRANULOMA FORMATION AND LYMPHOKINE PRODUCTION. Moyes RB*, Kennedy DA, Cao M, and Doughty BL. Department of Veterinary Pathobiology, College of Vet. Medicine, Texas A&M University, College Station, TX.

3:45 330 CONSEQUENCES OF NEONATAL IDIOTYPIC MANIPULATIONS ON SUBSEQUENT SCHISTOSOME INFECTIONS IN MALE AND FEMALE CBA/J MICE. Eloi-Santos S*, Harrell H, Bosshardt S, Nix N, Correa-Oliveira R, and Colley D. Department of Microbiology and Immunology, Vanderbilt University School of Medicine, and Veterans Administration Medical Center, Nashville, TN; Centro de Pesquisas, "Rene Rachou", FIOCRUZ, and Department Bioquimica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; and Meharry Medical College, Nashville, TN.

4:00 331 IMMUNOLOGICAL PROFILE OF INDIVIDUALS RESIDING IN AN ENDEMIC AREA FOR SCHISTOSOMIASIS MANSONI. Alves-Oliveira LF, Silveira AM, Souza A, Filho J, Parra JC, Doughty BL, Colley DG, Correa-Oliveira R, and Gazzinelli G. Faculdade de Odontologia de Governador Valdares, Brazil; Fundacao Servico Hospitalar de Governador Valadares, Brazil; College of Veterinary Medicine, Texas A & M University, College Station, TX; and Veterans Administration Medical Center; Vanderbilt University, Nashville, TN; and Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, Brazil.

4:15 332 HUMAN IDIOTYPE AND ANTI-IDIOTYPE MOLECULES IN SCHISTOSOMIASIS JAPONICA. Wisnewski A*, Cheever LW, Chireau M, Olds GR, and Kresina TF. Brown University, Providence, RI.

4:30 333 ANALYSIS OF CELLULAR IMMUNITY TO FRACTIONATED *FASCIOLA HEPATICA* ANTIGENS IN A HYPERIMMUNE BOVINE MODEL. Nodland KI, Hasan S, Moyes RB, Suderman MT, and Doughty BL. Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

4:45 334 PRESENCE OF IgE OR IgE-LIKE RECEPTOR IN THE NEMATODE PARASITE OF MICE *HELIGMOSOMOIDES POLYGYRUS*. Enriquez FJ*, Bradley-Dunlop D, and Boggavarapu J. Hybridoma Technology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ.

## SCIENTIFIC SESSION S: OPPORTUNISTIC INFECTIONS

Wednesday, December 4
1:30 - 5:00 PM Hampton A/B
Chairpersons: J. Schwartzman and C. Weikel

1:30 335 EVALUATION OF AN IMMUNOASSAY FOR THE DETECTION OF *CRYPTOSPORIDIUM* IN STOOL SPECIMENS. Sloan LM* and Rosenblatt JE. Mayo Clinic and Foundation, Rochester, MN.

1:45 336 DETECTION OF *CRYPTOSPORIDIUM PARVUM* DNA IN FIXED, PARAFFIN-EMBEDDED TISSUE BY THE POLYMERASE CHAIN REACTION. Laxer MA*, D'Nicuola ME, Patel RJ. Department of Infectious and Parasitic Disease Pathology, Armed Forces Institute of Pathology, Washington, DC; and American Registry of Pathology, Armed Forces Institute of Pathology, Washington, DC.

2:00 337 DETECTION OF *CRYPTOSPORIDIUM* BY PCR AMPLIFICATION OF SMALL SUBUNIT RIBOSOMAL RNA. Pieniazek NJ*, Arrowood MJ, Mathews HM, and Slemenda SB. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

2:15 338 EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN THE NORTHEAST OF BRAZIL. Newman RD*, Lima AM, Castro MX, Guerrant RL, and Weikel CS. Johns Hopkins University School of Medicine, Baltimore, MD; University of Virginia School of Medicine, Charlottesville, Va; and Universidade Federal do Ceara, Fortaleza, Brazil.

2:30 339 CRYPTOSPORIDIOSIS AMONG PATIENTS WITH THE ACQUIRED IMMUNODEFICIENCY SYNDROME IN VENEZUELA. Bonilla LC*, Guanipa N, Raleigh X, Cano G, and Quijada L. Instituto de Investigaciones Clinicas. Universidad del Zulia, Maracaibo, Venezuela.

2:45 340 PROLIFERATIVE RESPONSIVENESS OF LYMPHOCYTES FROM *CRYPTOSPORIDIUM PARVUM* EXPOSED MICE TO TWO SEPARATE ANTIGEN FRACTIONS FROM OOCYSTS. Moss DM* and Lammie PJ. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

3:00 Coffee Break

3:30 341 GROWTH CHARACTERISTICS, ELECTRON MICROSCOPY, AND ANTIGENIC ANALYSIS OF THE MICROSPORIDIAN ISOLATED FROM THE URINE OF A PATIENT WITH AIDS. Visvesvara GS*, Leitch GJ, Moura HM, Wallace S, Weber R, and Bryan RT. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Morehouse School of Medicine, Atlanta, GA.

3:45 342 PRESENTATION OF *TOXOPLASMA GONDII* ANTIGENS TO CD8+ T LYMPHOCYTES INVOLVES A RESTRICTED SET OF ANTIGENIC POLYPEPTIDES Denkers EY*, Gazzinelli RT, Hieny S, and Sher A. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

4:00 343 ASSOCIATION OF CD8+ LYMPHOCYTE SUBSETS WITH IgM RESPONSES IN *TOXOPLASMA* INFECTED MACAQUES. Warren JJ*, Morton WR, and Fritsche TR. Department of Laboratory Medicine, University of Washington, Seattle,WA; and Regional Primate Research Center, University of Washington, Seattle, WA.

4:15 344 A MUTANT STRAIN OF *TOXOPLASMA GONDII* RESISTANT TO BENZIMIDAZOLES. Schwartzman JD*, Roberts K, and Tirrell R. Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA.

4:30 345 CLOSE ASSOCIATION OF *PNEUMOCYSTIS CARINII* TROPHIC FORMS WITH CULTURE CELLS AS SHOWN BY IMMUNE SPECIFIC STAINING AND ELECTRON MICROSCOPY. Bartlett MS*, Goheen MP, Queener SF, Durkin MM, Shaw MM, and Smith JW. Indiana University School of Medicine, Indianapolis, IN.

4:45 346 AGE-RELATED DISTRIBUTION AND SEVERITY OF HUMAN BABESIAL INFECTION. Pollack RJ*, Telford SR III , Krause PJ, Ryan R, Zemel L, Brassard P, Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; University of Connecticut Health Center, Hartford, CT; and Block Island Clinic, Block Island, RI.

## AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

Wednesday, December 4
1:30 - 5:45 PM Gardner A/B

1:30 BUSINESS MEETING. J. Dalrymple.

2:30 SCIENTIFIC MEETING. ARENAVIRUS DISCOVERIES—NEW VACCINES AND NEW DISEASE

Chairpersons: C.J. Peters and HR Paublini

2:30 S57 VENEZUELAN HEMORRHAGIC FEVER: A NEW ARENAVIRAL DISEASE OF HUMANS. Manzione M, Betancourt A, Godoy O, Salas R, Pineda E, and Paublini HR*. Ministerio de Sanidad y Asistencia Social and Instituto Nacional de Higiene, Caracas, Venezuela.

2:45 S58 VENEZUELAN HEMORRHAGIC FEVER: A SEVERE MULTISYSTEM HEMORRHAGIC ILLNESS CAUSED BY A NEWLY RECOGNIZED ARENAVIRUS. Salas R, Manzione M, Tesh RB*, Rico-Hesse R, Pacheco ME, Ramos B, Taibo ME, Lorenzo B, Bruzual R, and Shope RE. Ministerio de Sanidad y Asistencia Social and Instituto Nacional de Higiene, Caracas, Venezuela; Yale University School of Medicine, New Haven, CT; Universidad Central de Venezuela, Caracas; and Disease Assessment Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, MD.

3:05 Coffee break

3:35 S59 DEVELOPMENT OF ANIMAL MODELS FOR VENEZUELAN HEMORRHAGIC FEVER USING GUINEA PIGS AND RHESUS MONKEYS. Jahrling PB, Geisbert TW, Hall WC, and Salas R. Disease Assessment Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, MD; Pathology Associates, Inc., Frederick, MD; and Instituto Nacional de Higiene, Caracas, Venezuela.

3:55 S60 ARGENTINE HEMORRHAGIC FEVER: VACCINE DEVELOPMENT FOR AN OLD ARENAVIRAL DISEASE OF MAN. Peters CJ. Disease Assessment Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, MD.

AMERICAN COMMITTEE ON ARTHOPOD-BORNE VIRUSES

4:05 S61 A LONGITUDINAL STUDY OF JUNIN VIRUS ACTIVITY IN RODENTS FROM THE EPIDEMIC AREA OF ARGENTINE HEMORRHAGIC FEVER. Mills JN, Ellis BA, Calderon GE, Maiztegui JI, Ksiazek TM, McKee KT Jr, Peters CJ, and Childs JE. Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD; Instituto Nacional de Estudios sobre Virosis Hemorragicas, Pergamino, Argentina; and Disease Assessment and Medical Divisions, USAMRIID, Frederick, MD.

4:20 S62 SUCCESSFUL DEVELOPMENT OF A VACCINE AGAINST ARGENTINE HEMORRHAGIC FEVER. Barrera-Oro JG, McKee KT Jr, and Maiztegui JI. Salk Institute, Swiftwater, PA; Instituto Nacional de Estudios sobre Virosis Hemorragicas, Pergamino, Argentina; Disease Assessment and Medical Divisions, USAMRIID, Ft. Detrick, Frederick, MD

5:00 EPIDEMIC REPORTS.

## SCIENTIFIC SESSION T:
## FILARIAL IMMUNOREGULATION AND PROTECTIVE IMMUNITY

Wednesday, December 4
1:30 - 5:30 PM Independence East
Chairpersons: C.L. King and M. Gallin

1:30 347 PREFERENTIAL INDUCTION BY PARASITE ANTIGEN OF IL-4 AND IL-5 SECRETING LYMPHOCYTES IN HUMAN HELMINTH INFECTION: A PRECURSOR FREQUENCY ANALYSIS. *Mahanty S*, King CL, Abrams JS, and Nutman TB.* Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and DNAX Research Institute, Palo Alto, CA.

1:45 348 IgE PRODUCTION IN HUMAN HELMINTH INFECTION: RECIPROCAL INTERRELATIONSHIP BETWEEN INTERLEUKIN-4 AND INTERFERON-γ. King CL*, Low CC, Mahanty S, Abrams JS, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and DNAX Research Institute, Palo Alto, CA.

2:00 349 THE REGULATION OF EOSINOPHILIA IN ONCHOCERCIASIS: THE ROLE OF EOSINOPHILOPOIETIC CYTOKINES AND CHANGES WITH IVERMECTIN TREATMENT. Nutman TB*, Steel C, Lujan-Trangay A, and Abrams JS. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; SNEM, Ministry of Public Health, Guatemala City, Guatemala; and DNAX Research Institute, Palo Alto, CA.

2:15 350 SPECIFICITY OF EOSINOPHIL AND IL5 RESPONSES TO *BRUGIA* ANTIGENS IN MICE. Pearlman E*, Kroeze WK, Hazlett, Jr. FE, Mawhorter SJ, Boom WH, and Kazura JW. Case Western Reserve University, Cleveland, OH.

2:30 351 CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN EXPRESSION BY *IN VIVO* ACTIVATED MURINE EOSINOPHILS. Mawhorter S*, Kazura J, Pearlman E, and Boom H. Case Western Reserve University, Cleveland, OH.

2:45 352 IL-2 RESTORATION OF LYMPH NODE CELL PROLIFERATIVE RESPONSES TO BRUGIA ANTIGENS *INHIBITED DURING ONSET OF PATENCY IN DOGS.* Schreuer D*, Orton S, and Hammerberg B. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

3:00 Coffee Break

3:30 353 ANTI-FILARIAL IMMUNE RESPONSIVENESS OF CORD LYMPHOCYTES. Hitch WL*, Eberhard ML, Hightower AW, and Lammie PJ. Emory University, Atlanta; GA; and Parasitic Diseases Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

3:45 354 EVIDENCE FOR HUMORAL MECHANISMS CONTROLLING *LOA LOA* MICROFILAREMIA IN ENDEMIC RESIDENTS. Pinder M*, Martin-Prevel Y, Everaere S, and Egwang TG. International Medical Research Center of Franceville, Franceville, Gabon.

4:00 355 ANTIBODY RESPONSES TO *BRUGIA MALAYI* $L_3$ ANTIGENS IN JIRDS PROTECTED BY IMMUNIZATION WITH IRRADIATED $L_3$. Li BW*, Chandrashekar R, Liftis F, and Weil GJ. Washington University School of Medicine, St. Louis, MO.

4:15 356 PREFERENTIAL RECOGNITION OF ANTIGENS OF *ONCHOCERCA VOLVULUS* BY PUTATIVELY IMMUNE INDIVIDUALS. Gallin M*, Adams AZ, Schumacher M, and Erttmann KD. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Federal Republic of Germany.

4:30 357 DIFFERENTIAL ANTIBODY RESPONSES TO *ONCHOCERCA VOLVULUS* IN ECUADOR. Guderian R, Chico M*, Cordova X, Kron MA, Mackenzie CD, and Sisley B. Division of Clinical Investigation, Community Development Services, Hospital Vozandes, Quito, Ecuador; Department of Pathology, Michigan State University, East Lansing, MI; and Dept of Immunology, London School of Hygiene and Tropical Medicine, London, England.

4:45 358 MECHANISM OF IMMUNE-MEDIATED KILLING OF LARVAL *ONCHOCERCA VOLVULUS* IN A MOUSE MODEL. Lange A*, Yutanawiboonchai W, and Abraham D. Dept of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

5:00 359 IMMUNIZATION OF CHIMPANZEES WITH X-IRRADIATED *ONCHOCERCA VOLVULUS* THIRD STAGE (L3) LARVAE. Prince AM*, Brotman B, Johnson EH Jr., Smith AB, Pascual D, and Lustigman S. Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia; and Department of Virology and Parasitology, The Linsley F. Kimball Research Institute of the New York Blood Center, New York, NY.

5:15 360 ANALYSIS OF ISOTYPE-SPECIFIC ANTIBODY RESPONSES TO A SOLUBLE *BRUGIA PAHANGI* $L_3$ ANTIGEN IN A HAITIAN POPULATION. Bailey JW, II*, Lammie PJ, Hightower AW, Hitch WL, Walker EM, and Eberhard ML. Parasitic Disease Branch, Centers for Disease Control, Atlanta, GA; and Statistics Division, Centers for Disease Control, Atlanta, GA.

## LATE BREAKERS IN BIOLOGY AND MOLECULAR BIOLOGY

Thursday, December 5
8:00 - 11:00 AM Commonwealth
Chairpersons: M. Hollingdale and S. Meshnick

## SCIENTIFIC SESSION U: MALARIA CHEMOTHERAPY I

Thursday, December 5
8:15 AM - 10:30 AM Independence Center
Chairpersons: G.E. Chi.ds and E.F. Boudreau

8:15 361 INVITED LECTURE. INVOLVEMENT OF REACTIVE OXYGEN SPECIES IN THE DESTRUCTION OF *PLASMODIUM FALCIPARUM*. Golenser J*, Marva E, and Chevion M. Department of Parasitology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; and Department of Cellular Biochemistry, the Hebrew University-Hadassah Medical School, Jerusalem, Israel.

8:45 362 1,2,4,5-TETRAOXANES; A NEW GROUP OF PEROXIDE ANTIMALARIAL DRUGS WITH POTENTIAL FOR CLINICAL UTILITY. Andersen SL*, Vennerstrom JL, Hong-Ning F, Ellis WY, Ager AL, Gerena L, and Milhous WK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; College of Pharmacy, University of Nebraska Medical Center, Omaha, NE; and Center for Tropical Parasitic Diseases, University of Miami, Miami, FL.

9:00 363 SYNTHETIC 1,2,4-TRIOXANES, A NEW CLASS OF ANTIMALARIALS. Jefford CW*, Kohmoto S, Rossier JC, Peters W, and Milhous W. Department of Organic Chemistry, University of Geneva, Geneva, Switzerland; Department of Medicinal Protozoology, London School of Hygiene and Tropical Medicine, London, UK; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

9:15 364 AN EFFECTIVE TOPICAL TREATMENT OF *PLASMODIUM BERGHEI*-INFECTED MICE WITH ARTELINIC ACID. Klayman DL*, Ager, Jr. AL, Fleckenstein LL, and Lin AJ. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Center for Tropical Parasitic Diseases, University of Miami, Miami, FL.

9:30 365 BISQUINOLINES. 1. ANTIMALARIALS WITH POTENTIAL AGAINST CHLOROQUINE-RESISTANT MALARIA. Vennerstrom JL*, Ellis WY, Ager AL, Andersen SL, Gerena L, and Milhous WK. College of Pharmacy, University of Nebraska Medical Center, Omaha, NE; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Center for Tropical Parasitic Diseases, University of Miami School of Medicine, Miami.

9:45 366 NEW ALTERNATIVES TO CYCLOGUANIL AND PYRIMETHAMINE. Milhous WK*, Peterson DS, Wellems TE, Lehnert EK, Gerena L, Andersen SL, and Schuster BG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

10:00 367 EFFLUX OF $^{3}$H-DIHYDROQUININE FROM CHLOROQUINE-RESISTANT *PLASMODIUM FALCIPARUM*. Krogstad DJ*, Gluzman IY, Wellems TE, and Schlesinger PH. Washington University School of Medicine, St. Louis, MO; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

10:15 368 PRELIMINARY LOCALIZATION OF THE EFFLUX PROCESS IN *PLASMODIUM FALCIPARUM*: PERSISTENCE OF EFFLUX AFTER LYSIS OF THE HOST RED CELL. Gluzman IY* and Krogstad DJ. Washington University School of Medicine, St. Louis, MO; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

## SCIENTIFIC SESSION V:
## SCHISTOSOMIASIS MOLECULAR BIOLOGY AND BIOCHEMISTRY

Thursday, December 5
8:15 - 10:30 AM Independence East
Chairpersons: R. Blanton and C. Shoemaker

8:15 369 UTILIZATION OF FLUORESCENT FATTY ACID AND PHOSPHOLIPID ANALOGS BY SCHISTOSOMULA OF *SCHISTOSOMA MANSONI*. Furlong ST* and Thibault KS. Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA; and Department of Medicine, Harvard Medical School, Boston, MA.

8:30 370 INHIBITION OF THE PHOSPHOINOSITIDE RESPONSE WITH PRAZIQUANTEL IN *SCHISTOSOMA MANSONI*. Wiest PM*, Li Y, Burnham D, Olds GR, and Bowen WD. Department of Medicine, The Miriam Hospital, Brown University, Providence, RI.

8:45 371 THE NOT-SO-APPARENT ACTIVE SITE OF *SCHISTOSOMA MANSONI* CYSTEINE PROTEINASE, CP1. Chappell CL* and Rege AA. Department of Family Medicine, Baylor College of Medicine, Houston, TX; Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, TX.

9:00 372 MAMMALIAN AND BACTERIAL EXPRESSION OF RECOMBINANT SM23 - IMMUNE RECOGNITION AND EPITOPE MAPPING. Reynolds SR*, Shoemaker CB, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

9:15 373 ALTERNATE SPLICING GENERATES mRNA ENCODING TRUNCATED PRODUCTS OF THE *SCHISTOSOMA MANSONI* EGF RECEPTOR HOMOLOGUE Ramachandran H*, Landa A, Stein L, and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Instituto de Investigaciones Biomedicas, Universidad National Autonoma de Mexico, Mexico City, Mexico.

9:30 374 LOCALIZATION STUDIES ON THE *SCHISTOSOMA MANSONI* HOMOLOGUES OF P-GLYCOPROTEIN. Bosch I* and Shoemaker CB. Dept of Tropical Public Health, Harvard School of Public Health, Boston, MA.

9:45 375 CHARACTERIZATION OF THE TRIOSE PHOSPHATE ISOMERASE GENE FROM *SCHISTOSOMA MANSONI*. Reis MG*, Gross A, Harn D, and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz FIOCRUZ/UFBa, Salvador, Bahia, Brazil.

10:00 376 CHARACTERIZATION OF CDNA CLONES ENCODING GLUCOSE TRANSPORTERS FROM *SCHISTOSOMA MANSONI*. Skelly PJ* and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

10:15 377 IDENTIFICATION AND CHARACTERIZATION OF GENES ENCODING *SCHISTOSOMA HAEMATOBIUM*-SPECIFIC ANTIGENS. Blanton RE*, and Aman R. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH and Institute of Primate Research, National Museums of Kenya, Nairobi, Kenya

## SCIENTIFIC SESSION W:
## MALARIA EPIDEMIOLOGY AND FIELD STUDIES

Thursday, December 5
8:00 - 10:45 AM Fairfax A
Chairpersons: J. Beier and K. Lee

8:00 INVITED LECTURE: FALCIPARUM MALARIA: PARASITEMIA AND CLINICAL DISEASE IN A HOLOENDEMIC AREA. Gilles H. Liverpool School of Tropical Medicine. Liverpool, England.

8:30 378 LOW FREQUENCY OF ANTI-*PLASMODIUM FALCIPARUM* CS REPEAT ANTIBODIES AND HIGH MALARIA TRANSMISSION RATE IN ENDEMIC AREAS OF RONDONIA STATE - NORTHWESTERN BRAZIL . Oliveira-Ferreira J*, Teva A, and Daniel-Ribeiro C. Department of Immunology, WHO Collaborating Center for Research and Training in Immunology of Parasitic Diseases, IOC - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

8:45 379 POLYMORPHISM IN THE CS PROTEIN OF FIELD ISOLATES OF *PLASMODIUM FALCIPARUM* FROM MADANG, PAPUA NEW GUINEA. Shi YP*, Alpers M, and Lal AA. Divof Parasitic Disease, CDC, Atlanta, GA and Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea.

9:00 380 MALARIA ENDEMIC SERA IDENTIFY B CELL EPITOPES WITHIN NON REPEAT REGIONS OF THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM*. Calvo Calle JM*, Cochrane A, Clavijo PJ, Collins W, Herrington DA, Boudin C, Stuber D, Tam JP, Nussenzweig RS, and Nardin E. New York University School of Medicine, New York, NY.; Centers for Disease Control, Atlanta, GA.; Center for Vaccine Development, University of Maryland, Baltimore, MD.; Department of Parasitology, University of Grenoble, Grenoble, France; F. Hoffman-La Roche, Basel, Switzerland; and The Rockefeller University, New York, NY.

9:15 381 POLYMORPHISM OF THE ALLELES OF THE MEROZOITE SURFACE ANTIGENS MSA1 AND MSA2 IN *PLASMODIUM FALCIPARUM* WILD ISOLATES FROM COLOMBIA. Snewin VA, Herrera M, Sanchez G, Scherf A, Langsley, and Herrera S*. Unite de parasitologie Experimentale, Departement d' Immunologie Institut, Paris, France; and Department of Microbiology, Sc[illegible] Health, Universidad del Valle, Cali, Colombia.

9:30 382 HUMAN CYTOTOXIC T LYMPHOCYTES AGAINST THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN AFTER NATURAL EXPOSURE TO MALARIA. Sedegah M*, Sim KL, Malik A, Mason CA, Sherwood J, Koech D, Ware B, Roberts C, and Hoffman SL. Naval Medical Research Institute, Bethesda, MD; Pan American Health Orangization, Washington, DC; Walter Reed Army Institute of Research, Washington, DC; US Army Medical Research Unit, Nairobi, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

9:45 383 IDENTIFICATION OF *PLASMODIUM FALCIPARUM* PARASITES USING THE POLYMERASE CHAIN REACTION (PCR). Courval JM*, Barker, Jr. RH, Banchongaksorn T, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Malaria Center, Region 2, Chieng Mai, Thailand.

10:00 384 DETECTION OF *PLASMODIUM FALCIPARUM* PARASITEMIAS USING AN ANTIGEN-TARGETED ELISA AND A DNA PROBE IN THE GAMBIA. Schaeffler BA*, Taylor DW, Parra ME, Voller A, Bidwell D, Tam MR, Hassan-King B, Greenwood B, Subramanian S, and McLaughlin GL. Program for Appropriate Technology in Health, Seattle, WA; Georgetown University, Washington, DC; Institute of Zoology, London, UK; Medical Research Council Laboratories, The Gambia; and University of Illinois, Champaign, IL.

10:15 385 DEVELOPMENT OF A TWO-SITE ELISA USING MONOCLONAL ANTIBODY AGAINST A 50-KDA CATABOLITE FROM *PLASMODIUM FALCIPARUM* MEROZOITES FOR THE DIAGNOSIS OF MALARIA INFECTION. Ferreira-da-Cruz MF*, Machado-Paso R, Fortier B, and Daniel-Ribeiro CT. Department of Immunology, IOC - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; and INSERM U-42, Villeneuve d'Ascq, France.

10:30 386 VILLAGE-WIDE TRIAL OF INSECTICIDE-IMPREGNATED BED NETS AND CURTAINS: EPIDEMIOLOGICAL AND ENTOMOLOGICAL RESULTS FOR YEAR ONE. Beach RF*, Ruebush TK, Sexton JD, and Oloo AJ. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Vector Biology and Control Research Centre, Kenya Medical Research Institute, Kisumu, Kenya.

## SCIENTIFIC SESSION X: AMEBIASIS

Thursday, December 5
8:00 AM - 10:30 AM Hampton A/B
Chairpersons: G. Bailey and S. Stanley

8:00 387 P-GLYCOPROTEIN GENES OF *ENTAMOEBA HISTOLYTICA*. Samuelson J*, Descoteaux S, Ayala P, and Orozco E. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Experimental Pathology, CINESTAV-IPN, Mexico D.F., Mexico.

8:15 388 PCR METHOD FOR THE IDENTIFICATION OF *E. HISTOLYTICA* AND OTHER ENTERIC PATHOGENS IN STOOL SAMPLES. Acuna-Soto R*, Samuelson J, De Girolami P, Schoolnick G, and Wirth D. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Clinical Laboratory, Deaconess Hospital, Boston, MA; and Division of Geographic Medicine, Stanford University, Stanford, CA.

8:30 389 PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF SEVERAL CLONES OF *ENTAMOEBA HISTOLYTICA*. Lazard D, Gamboa Y, Valdes J, Hernandez F, Sanchez T, and Orozco E*. Department of Experimental Pathology, CINVESTAV I.P.N., Mexico D.F., Mexico.

8:45 390 REGULATION OF CHITIN SYNTHETASE ACTIVITY IN ENCYSTING *ENTAMOEBA INVADENS*. Das S, Aley SB*, and Gillin FD. Department of Pathology, University of California, San Diego, CA.

9:00 391 ISOLATION AND PARTIAL CHARACTERIZATION OF A PORE-FORMING PROTEIN OF PATHOGENIC *ENTAMOEBA HISTOLYTICA*. Leippe M*, Ebel S, Horstmann RD, and Muller-Eberhard HJ. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Federal Republic of Germany.

THURSDAY AM
X: AMEBIASIS

9:15 392 A MONOCLONAL ANTIBODY TO THE 170 KDA SUBUNIT OF THE GAL-ADHESIN ABROGATES THE RESISTANCE OF *E. HISTOLYTICA* TO LYSIS BY HUMAN COMPLEMENT C5B-9. Braga L*, Ninomiya H, Wiedmer T, McCoy J, Sims P, and Petri W, Jr. Department of Medicine, University of Virginia, Charlottesville, VA; and Oklahoma Medical Research Foundation, Oklahoma City, OK.

9:30 393 ISOLATION OF AN UNIQUE *ENTAMOEBA HISTOLYTICA* CDNA CLONE ENCODING A PROTEIN WITH A ZINC FINGER DOMAIN. Stanley SL, Jr., Li E. Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO.

9:45 394 APPLICATION OF TWO-DIMENSIONAL ELECTROPHORESIS TO CHARACTERIZE PROTEINS OF *ENTAMOEBA HISTOLYTICA*. Shen P, Nokkaew C, McCoomer NE, Pohl J, and Bailey GB*. Department of Biochemistry, Morehouse School of Medicine and Microchemical Facility, Emory University, Atlanta, GA.

10:00 395 MODULATION OF ARACHIDONIC ACID (AA) METABOLISM IN MACROPHAGES BY *ENTAMOEBA HISTOLYTICA*. Wang W*, Chadee K. Institute of Parasitology of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

10:15 396 METRONIDAZOLE THERAPY IN AMEBIC LIVER ABCESS (ALA) AND RECOGNITION OF A CARRIER STATE. Jackson TG*, Irusen EM, and Simjee AE. Research Institute for Diseases in a Tropical Environment, Congella, Durban, Republic of South Africa.

## SCIENTIFIC SESSION Y:
## ARBOVIRUS EPIDEMIOLOGY

Thursday, December 5
8:00 - 10:30 AM Independence West
Chairpersons: B. Innis and J. LeDuc

8:00 397 EPIDEMIOLOGY OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN AN AREA OF RURAL PEOPLE'S REPUBLIC OF CHINA (PRC) WITH MIXED APODEMUS AND RATTUS DERIVED INFECTIONS. Fisher-Hoch SP*, Ruo S, Li YL, Tang YW, Xu ZY, Liu ZL, Tong Z, Ma QR, and McCormick JB. Special Pathogens Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA; Department of Epidemiology, Shanghai Medical University, Shanghai, PRC; and Jiande County Antiepidemic Station, Jiande, Zhejiang Province, People's Republic of China.

8:15 398 FILOVIRUS OUTBREAK AMONG PHILIPPINE NONHUMAN PRIMATES IN SOUTH TEXAS. Hendricks KA*, Taylor JP, Pearson SL, Simpson DM, Jahrling PB, and Fisher-Hoch SP. Bureau of Disease Control & Epidemiology, Texas Department of Health, Austin, TX; Infectious Diseases Program, Epidemiology Division, Texas Department of Health, Austin, TX; Texas Primate Center, Hazleton Research Products, Inc., Alice, TX; Associateship for Disease Prevention, Texas Department of Health, Austin, TX; Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Division of Viral and Rickettsial Diseases, Special Pathogens Branch, Centers for Disease Control, Atlanta, GA.

8:30 399 A CASE-CONTROL STUDY OF RISK FACTORS FOR KOREAN HEMORRHAGIC FEVER AMONG SOLDIERS IN THE REPUBLIC OF KOREA. Dixon KE, Nang RN*, Kim DH, Huh J, Park J, and Hwang Y. United States Army Medical Research Unit-Republic of Korea, APO San Francisco, CA.

8:45 400 CORRELATES OF HANTAVIRAL INFECTION IN PATIENTS FROM BALTIMORE, MD, USA. Gurri Glass GE*, Childs JE, Watson AJ, LeDuc JW. Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD; Division of Nephrology, Johns Hopkins University, School of Medicine, Baltimore, MD; and Disease Assessment Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick MD.

9:00 401 SEVERE DENGUE INFECTIONS AMONG CHILDREN IN METROPOLITAN BANGKOK 1973-90. Nisalak A*, Nimmannitya S, and Innis BL. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Children's Hospital, Bangkok, Thailand.

9:15 402 FOCALITY OF DENGUE TRANSMISSION IN RURAL THAI VILLAGES AND PROPOSALS FOR COMMUNITY-BASED VECTOR CONTROL. Strickman D*, Kittayapong P, Innis BL, and Wannapong R. Department of Entomology, Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand; Walter Reed Army Institute of Research, Washington, DC; Department of Virology, Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand; and Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand.

9:30 403 A MODEL OF DENGUE FEVER TRANSMISSION, WITH AN EVALUATION OF THE PROBABLE IMPACT OF ULTRA LOW VOLUME (ULV) ADULTICIDING MEASURES ON DENGUE EPIDEMICS. Newton EA* and Reiter P. Caribbean Epidemiology Center, Port-of-Spain, Trinidad, West Indies; and Dengue Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, San Juan, PR.

9:45 404 RIFT VALLEY FEVER VIRUS ACTIVITY IN WEST AFRICA, 1989-1991. Zeller HG*, Wilson ML, Schmidt EA, Thiongane Y, Cornet JP, Camicas JL, Gonzalez JP, Bessin R, Teou K, Kpomassi M, Formenty P, Digoutte JP, and Akakpo JA. Institut Pasteur, Dakar, Senegal; Yale University School of Medicine, New Haven, CT; Institut Senegalais de Recherches Agricoles, Dakar, Senegal; ORSTOM, Dakar, Seneal; Laboratoire National d'Elevage, Ouagadougou, Burkina Faso; Ecole inter-Etats des Sciences et Medecine Veterinaires, Dakar, Senegal; and Laboratoire Central de Pathologie Animale, Bingerville, Cote d'Ivoire.

10:00 405 RIFT VALLEY FEVER VIRUS TRANSMISSION IN RURAL NORTHERN SENEGAL: HUMAN RISK FACTORS AND POTENTIAL VECTORS. Wilson ML*, Chapman LE, Hall DB, Dykstra EA, Ba K, Zeller HG, Traore-Lamizana M, Hervy JP, and Linthicum KJ. Institut Pasteur, Dakar, Senegal; Centers for Disease Control, Atlanta, GA; ORSTOM, Dakar, Senegal; Yale University School of Medicine, New Haven, CT; and U.S. Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

10:15 406 SPATIAL ANALYSIS OF RIFT VALLEY FEVER VIRUS VECTOR HABITATS BY INTEGRATION OF SENSOR DATA FROM SPOT, LANDSAT TM, AND AIRBORNE IMAGING RADAR. Freier JE*, Linthicum KJ, and Angleberger DR. Virology Division, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

## SCIENTIFIC SESSION Z: FILARIASIS CHEMOTHERAPY

Thursday, December 5
8:30 - 10:30 AM Gardner A/B
Chairpersons: M. Eberland and A. Klion

8:30 407 *BRUGIA MALAYI* AND *ACANTHOCHEILONEMA VITEAE*: ANTIFILARIAL ACTIVITY OF TRANSGLUTAMINASE INHIBITORS *IN VITRO*. Rao UR*, Mehta K, Subrahmanyam D, and Vickery AC. College of Public Health, University of South Florida, Tampa, FL; University of Texas, MD Anderson Cancer Center, Houston, TX; and Ciba-Geigy Ltd. and Swiss Tropical Institute, Basel, Switzerland.

8:45 408 DOSE RESPONSE OF THE BENZIMIDAZOLE CARBAMATE, UMF-078, AGAINST *BRUGIA PAHANGI* IN BEAGLES WITH INDUCED LYMPHATIC INFECTIONS. Dzimianski MT*, McCall JW, Elslager EF, Townsend LB, and Wise DS. Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA; Elslager Associates, Ann Arbor, MI; and College of Pharmacy, University of Michigan, Ann Arbor, MI.

9:00 409 DIETHYLCARBAMAZINE AUGMENTS PLATELET-ACTIVATING FACTOR SYNTHESIS AND NEUTROPHIL ADHERENCE TO ENDOTHELIUM. Kanesa-hasan N*, Douglas J, and Kazura J. Case Western Reserve University, Cleveland, OH.

9:15 410 LONG-TERM OUTCOME OF TREATMENT WITH DEC OR IVERMECTIN ON BANCROFTIAN FILARIASIS IN HAITI. Eberhard ML*, McNeeley DF, Addiss DG, Spencer HC, and Lammie PJ. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and St. Croix Hospital, Leogane, Haiti.

9:30 411 CHANGES IN CIRCULATING PARASITE ANTIGEN LEVELS AFTER DIETHYLCARBAMAZINE AND IVERMECTIN TREATMENT OF BANCROFTIAN FILARIASIS. Weil GJ*, Lammie PJ, Richards FO, Jr. and Eberhard ML. Washington University School of Medicine, St. Louis, MO; and Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

9:45 412 COMPARATIVE TRIAL OF IVERMECTIN AND DIETHYLCARBAMAZINE GIVEN IN A CLEARING DOSE AND A SINGLE HIGH DOSE FOR TREATMENT OF LYMPHATIC FILARIASIS, HAITI. Addiss DG*, Eberhard ML, Lammie PJ, McNeeley MB, Lee SH, and Spencer HC. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; Ste. Croix Hospital, Leogane, Haiti; and Division of Field Epidemiology, Epidemiology Program Office, Centers for Disease Control, Atlanta, GA

10:00 413 EFFICACY AND TOLERANCE OF SINGLE DOSE DIETHYLCARBAMAZINE VS. IVERMECTIN IN BANCROFTIAN FILARIASIS IN PAPUA NEW GUINEA. Greenberg J, Day K, Alpers M, and Kazura J*. Case Western Reserve University, Cleveland, OH; Papua New Guinea Institute of Medical Research, Goroka; and Imperial College, London, UK.

10:15 414 TOLERANCE, SAFETY AND EFFICACY OF ALBENDAZOLE FOR HUMAN LOIASIS: RESULTS OF A DOUBLE-BLIND, PLACEBO CONTROLLED TRIAL. Klion AD*, Massougbodji A, Horton J, Ekoue S, Lanmasso T, Ahouissou LN, Yetongnon J, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Faculte des Sciences de la Sante, Universite Nationale du Benin, Cotonou, Republique du Benin; and SmithKline Beecham Pharmaceuticals, Hertfordshire, England.

## SCIENTIFIC SESSION AA: ENTOMOLOGY

Thursday, December 5
8:00 - 10:30 AM Fairfax B
Chairpersons: J. Freier and R. Reiter

8:00 415 HOST-SEEKING BEHAVIOR IN THE YELLOW FEVER MOSQUITO INHIBITED BY A NEUROPEPTIDE. Brown MR*, Klowden MJ, and Lea AO. Department of Entomology, University of Georgia, Athens, GA; and Division of Entomology, University of Idaho, Moscow, ID.

8:15 416 DETECTION OF ADENINE NUCLEOTIDES BY THE LABRAL APICAL RECEPTORS OF MOSQUITOES. Liscia A and Galun R*. The Department of Physiology, University of Cagliari, Cagliari, Italy; and Kuvin Centre for Infectious and Tropical Diseases, Hebrew University Medical School, Jerusalem, Israel.

8:30 417 INGESTION OF VARIOUS BLOOD CELLS BY MOSQUITOES. Vardimon-Friedman H*, Frankenberg S, and Galun R. Kuvin Centre for Infectious and Tropical Diseases, Hebrew University Medical School, Jerusalem, Israel.

8:45 418 SALIVARY GLAND PROTEIN DEPLETION DURING BLOOD FEEDING IN ANOPHELINE MALARIA VECTORS. Klein TA* and Golenda CF. Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.

9:00 419 CHARACTERIZATION OF A PREFERENTIALLY EXPRESSED POLYPEPTIDE FROM THE HEMOLYMPH OF IMMUNE-ACTIVATED *AEDES AEGYPTI*. Boerntsen BT* and Christensen BM. Department of Veterinary Science, University of Wisconsin, Madison, WI.

9:15 420 ISOLATION OF *ANOPHELES GAMBIAE* RFLP FOR USE IN GENETIC AND CYTOGENETIC MAPPING. Romans PA*, Bhattacharyya RK, Colavita AC, D'cunha C, Graziosi C, Kew Y, and Seeley DC. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; and Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

9:30 421 GENETIC MAP OF *AEDES AEGYPTI* WITH RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND MORPHOLOGICAL MARKERS. Severson DW*, Mori A, Helke DM, and Christensen BM. Department of Veterinary Science, University of Wisconsin, Madison, WI.

9:45 422 DNA PROBES FOR THE MEMBERS OF THE *ANOPHELES PUNCTULATUS* COMPLEX IN AUSTRALIA AND COASTAL PAPUA NEW GUINEA. Burkot TR*, Beebe NW, Cooper L, Foley D, Bryan J, Cooper RD, Hii J, and Packer M. Tropical Health Program, Queensland Institute of Medical Research, Bramston Terrace, Herston Q 4006, Australia; Tropical Health Program, Department of Entomology, University of Queensland, St. Lucia Q, Australia; Army Malaria Research Unit, Ingleburn NSW, Australia; and Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea.

10:00 423 ESTIMATION OF THE LONGEVITY OF ADULT *AEDES AEGYPTI*, BASED ON THEIR RECUPERATION RATE AFTER APPLICATION OF ULTRA LOW VOLUME (ULV) INSECTICIDES. Reiter P* and Newton EA. Dengue Branch, Division Vector-borne Infectious Diseases, Centers for Disease Control, San Juan, PR; and Caribbean Epidemiology Center, Port-of-Spain, Trinidad, West Indies.

THURSDAY AM
SOPER LECTURE

10:15 424 EPIDEMIOLOGIC EVALUATION OF TICK-BORNE INFECTIONS AMONG MILITARY PERSONNEL CONDUCTING FIELD TRAINING IN ARKANSAS Sanchez JL*, Yevich SJ, DeFraites RF, Fishbein DB, Greene NR, Dawson JE, Uhaa IJ, and Johnson BJ. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC; Viral and Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA; and Division of Vector-Borne Infectious Diseases, Fort Collins, CO.

## FRED L. SOPER MEMORIAL LECTURE

Thursday, December 5
11:00 AM - 12:00 NN Grand Ballroom

*HOMING IN ON HELMINTHS*

D. Hopkins
Global 2000, Chicago, IL

## SYMPOSIUM :
## DISEASE CONTROL IN OPERATION DESERT SHIELD/STORM

Thursday, December 5
1:30 PM - 5:00 PM Fairfax A/B
Chairpersons: P.W. Kelley and L.C. Caudle

1:30 S63 THE THREAT OF DISEASE AND NON BATTLE INJURY ON DESERT SHIELD/STORM. Petruccelli B. Walter Reed Army Institute of Research, Washington, DC.

1:50 S64 DISEASE CONTROL FROM THE PERSPECTIVE OF GENERAL SCHWARTZKOPF'S OFFICE. Tynan B. Central Command, Tampa, FL.

2:10 S65 MEDICAL SURVEILLANCE IN NAVY AND MARINE FORCES . Hanson K. Navy Environmental and Preventive Medicine Unit # 6, Pearl Harbor, HI.

2:30 S66 STATE OF THE ART DIAGNOSTICS IN THE DESERT: THE NAVY FORWARD LAB. Hyams C, Bourgeois AL, and Burans J. Navy Medical Research Institute, Bethesda, MD.

2:50 Coffee Break

3:20 S67 THE U.S. RESPONSE TO IRAQ'S BIOLOGICAL WARFARE THREAT. Caudle L . United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

3:40 S68 THE U.S. RESPONSE TO IRAQ'S CHEMICAL WARFARE THREAT. Dunn M. Kimbrough Army Hospital, Fort Meade, MD.

4:00 S69 IMPORTANT INFECTIOUS DISEASE PROBLEMS SEEN IN RETURNING TROOPS Magill A. Walter Reed Army Medical Center, Washington, DC.

4:20 S70 THE AFTERMATH: MILITARY MEDICAL ASSISTANCE TO KURDISH REFUGEES. Erickson RL. United States Army Special Operations Command, Fort Bragg, NC.

4:40 Discussion.

## SCIENTIFIC SESSION BB: MALARIA IMMUNOLOGY II

Thursday, December 5
1:30 - 5:15 PM Constitution
Chairpersons: B. Sina and R. Sinden

1:30 425 EVIDENCE FOR FUNCTIONAL REGIONS ON THE 175 K *PLASMODIUM FALCIPARUM* ERYTHROCYTE BINDING ANTIGEN. Sim KL*, Ware L, and Gross M. Walter Reed Army Institute of Research, Washington DC; Johns Hopkins School of Public Health, Baltimore, MD; and Smith Kline Beecham, King of Prussia, PA.

1:45 426 SIALIC ACID INDEPENDENT BINDING OF SERA ANTIGEN OF *PLASMODIUM FALCIPARUM* TO HUMAN ERYTHROCYTES. Ziefer AA* and Perkins, ME. The Rockefeller University, New York, NY.

2:00 427 CROSS-REACTIONS BETWEEN IDIOTYPES, *PLASMODIUM FALCIPARUM* DERIVED PEPTIDES, DINITROPHENYL AND β(2→6) POLYFRUCTOSAN Daniel-Ribeiro C*, Deslandes D, and Ferreira-da-Cruz MF. Department of Immunology, WHO Collaborating Center for Research and Training in Immunology of Parasitic Diseases, IOC - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

2:15 428 HUMAN ANTIBODY LEVELS TO R32LR CORRELATE WITH LYMPHOCYTE PROLIFERATIVE RESPONSES TO POLYMORPHIC *PLASMODIUM FALCIPARUM* CS PROTEIN T CELL EPITOPES. Mason CJ*, Adoyo MA, Klotz FW, Coyne PE, Holland CA, Icayan LO, Sherwood JA, Copeland RS, Koech DL, Strickland GT, Chulay JD, and Hoffman SL. Kenya Medical Research Institute, Nairobi, Kenya; Malaria Program, Naval Medical Research Institute, Bethesda, MD; Walter Reed Army Institute of Research, Washington, DC; US Army Medical Research Unit, Nairobi, Kenya; Walter Reed Army Medical Center, Washington, DC; US Army Medical Research Institute for Infectious Diseases, Ft Detrick, MD; and University of Maryland School of Medicine, Baltimore, MD.

2:30 429 PROTECTIVE IMMUNITY OF RECOMBINANT *PLASMODIUM FALCIPARUM* SERA ANTIGEN EXPRESSED IN *SACCHAROMYCES CEREVISIAE*. Bathurst IC*, Inselburg J, Rossan RN, Kansopon J, Barr PJ. Chiron Corporation, Emeryville, CA.; Department of Microbiology, Dartmouth Medical School, Hanover, NH.; and Gorgas Memorial Laboratory, Panama City, Panama.

2:45 430 FAILURE OF A SYNTHETIC VACCINE TO PROTECT *AOTUS LEMURINOS* AGAINST ASEXUAL BLOOD STAGES OF *PLASMODIUM FALCIPARUM*. Herrera S*, Herrera M, Corredor A, Rosero F, Clavijo C, and Guerrero R. Department of Microbiology, School of Health, Universidad del Valle, Cali, Colombia; and Parasitology Section, National Institute of Health, Bogata, Colombia.

THURSDAY PM
BB: MALARIA IMMUNOLOGY II

3:00 Coffee Break

3:30 431 PROTECTION AGAINST MALARIA IN *AOTUS* MONKEYS IMMUNIZED WITH REC. BLOOD ANTIGEN FUSED TO A "UNIVERSAL" T CELL EPITOPE: IFN SERUM LEVELS AND PROTECTION. Herrera MA*, Rosero F, Herrera S, Caspers P, Rotmann D, Sinigaglia F, and Certa U. Department of Microbiology, School of Health, Universidad del Valle, Cali, Colombia; and Pharma Research Technology, F. Hoffmann-La Roche Ltd., Basel, Switzerland.

3:45 432 IMMUNOLOGICAL STUDIES OF RECOMBINANT POLYPEPTIDES BASED ON THE C-TERMINAL PROCESSING FRAGMENT OF GP195 EXPRESSED IN YEAST AND BACULOVIRUS SYSTEMS. Chang SP*, Hui GS, Gibson HL, Lee Ng CT, Yokota B, and Barr PL. University of Hawaii, Department of Tropical Medicine and Medical Microbiology, Honolulu, HI; Chiron Corporation, Emeryville, CA.

4:00 433 ANTIGENIC VARIATION IN *PLASMODIUM FALCIPARUM*. Brown GV*, Gooze L, Wycherley K, Woolish W, Southwell B, Leech JH, and Biggs BA. Immunoparasitology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; The Medical Service, San Francisco General Hospital and Dept of Medicine, University of California at San Francisco CA; and Division of Clinical Pharmacology and Experimental Therapeutics, University of California at San Francisco General Hospital, San Francisco, CA.

4:15 434 ULTRASTRUCTURAL LOCALIZATION OF THE 145/102 KD ANTIGENS IN ASEXUAL BLOOD STAGES OF *PLASMODIUM FALCIPARUM*-INFECTED HUMAN ERYTHROCYTES. Wu LJ*, Liu EX, Li WL, and Miao WM. Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, China; Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China; and Department of Parasitology, Second Military Medical University, Shanghai, China.

4:30 435 TRANSGENIC MICE EXPRESSING HUMAN $B^S$ HEMOGLOBIN ARE PARTIALLY RESISTANT TO RODENT MALARIA. Shear HL*, Roth EF, Fabry ME, Costantini F, Pachnis A, and Nagel RL. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY; Department of Genetics, Columbia University, New York, NY; and Division of Hematology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.

4:45 436 T CELL REGULATION OF SPLENIC IMMUNITY IN BLOOD STAGE *PLASMODIUM VINCKEI VINCKEI* MALARIA. Vinetz JM*, Kumar S, Torbett BE, Mosier DE, and Miller LH. Medical Biology Institute, La Jolla, CA; and Malaria Section, Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD.

5:00 437 NOVEL GENE ENCODING A LARGE *PLASMODIUM FALCIPARUM* SEXUAL STAGE SPECIFIC ANTIGEN CLONED BY EXPRESSION IN EUCARYOTIC (COS7) CELLS. Elliott JF*, Alano P, Carter R, Smith DK, Reed DG, and Bruce MC. Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada; Laboratorio di Biologia Cellulare, Istituto Superiore di Sanita, Roma, Italia; Division of Biological Sciences, University of Edinburgh, Edinburgh, UK; and Department of Immunology, University of Alberta, Edmonton, Alberta, Canada.

## SCIENTIFIC SESSION CC: MALARIA CHEMOTHERAPY II

Thursday, December 5
1:30 PM - 5:00 PM Gardner A/B
Chairpersons: M. Edstein and K. Rieckmann

1:30 438 ARE CURRENT MALARIA CONTROL STRATEGIES SUCCEEDING IN AFRICA? EXPERIENCES IN EVALUATION FROM TOGO Breman JG*, Gayibor A, Fitzgibbon B, Toole MJ, Glikpo AK, Murphy KB, Sudre P, Bussell KE, and Karsa T. Malaria Branch, Centers for Disease Control, Atlanta, GA; International Health Program Office, Centers for Disease Control, Atlanta, GA; and Service du Paludisme, Service des Statistiques et Division d'Epidemiologie, Ministry of Health, Lome, Togo.

1:45 439 QUININE TREATMENT OF SEVERE FALCIPARUM MALARIA IN AFRICAN CHILDREN. Pasvol G*, Newton CR, Winstanley PA, Watkins WM, White NJ, Elford BC, Marsh K, Peshu NM, Were JB, and Warrell DA . Kenya Medical Research Unit, Kilifi, Kenya; Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford UK; St Mary's Hospital Medical School, London, UK.

2:00 440 PEDIATRIC ANEMIA IN WESTERN KENYA: CAN EFFECTIVE MALARIA THERAPY REDUCE THE RISK OF ANEMIA AND BLOOD TRANSFUSION IN AFRICA? Lackritz EM*, Campbell CC, Ruebush TK, Adungosi J, and Were JB. Malaria Branch, Centers for Disease Control, Atlanta, GA; Siaya District Hospital, Siaya, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

2:15 441 *HALOFANTRINE: AN OVERVIEW OF ITS USE IN CHILDREN. Boudreau EF*, Canfield* CJ, and Horton RJ. Pharmaceutical Systems Incorporated, Gaithersburg, MD; and Smith Kline Beecham, UK.

2:30 442 INTRAVENOUS IMMUNE GLOBULIN AS ADJUNCT TREATMENT IN SEVERE PEDIATRIC MALARIA. Taylor TE*, Molyneux ME, and Wirima JJ. College of Osteopathic Medicine, Michigan State University, East Lansing, MI; Liverpool School of Tropical Medicine, Liverpool, UK; and College of Medicine, University of Malawi, Blantyre, Malawi.

2:45 443 ADJUNCTIVE IRON CHELATION THERAPY SHORTENS DURATION OF COMA IN CHILDREN WITH CEREBRAL MALARIA. Gordeuk VR*, Thuma PE, Biemba G, Parry D, and Brittenham GM. Department of Medicine, MetroHealth Medical Center, Case Western Reserve University, Cleveland OH; Department of Pediatrics, Hershey Medical Center, Pennsylvania State University, Hershey PA; and Macha Hospital, Choma, Zambia.

3:00 Coffee Break

3:30 444 EFFECTIVENESS AND TOLERANCE OF LONG-TERM MALARIA PROPHYLAXIS WITH MEFLOQUINE AMONG PEACE CORPS VOLUNTEERS. Lobel HO*, Bernard KW, Eng TR, Hightower AW, and Campbell CC. Malaria Branch, Centers for Disease Control, Atlanta, GA; Office of International Health, Department of Health and Human Services, Washington, DC; and Peace Corps Office of Medical Services, Washington, DC.

3:45 445 EMERGENCE OF MEFLOQUINE RESISTANT *PLASMODIUM FALCIPARUM* IN THAILAND: *IN VITRO* TRACKING. Wongsrichanalai C*, Webster HK, Wimonwattrawatee T, Sookto P, Chuanak N, Wernsdorfer WH, and Thimasarn K. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Institute of Specific Prophylaxis and Tropical Medicine, University of Vienna, Austria; and Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Bangkok, Thailand.

4:00 446 PROGUANIL/DAPSONE FOR MALARIA CHEMOPROPHYLAXIS ON THE THAI-CAMBODIAN BORDER. Shanks GD*, Suriyamongkol V, Timsaad S, Edstein MD, and Webster HK. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Royal Thai Navy Medical Department, Bangkok, Thailand.

4:15 447 CIPROFLOXACIN TREATMENT OF DRUG-RESISTANT FALCIPARUM MALARIA. Watt G*, Shanks GD, Edstein MD, Pavanand K, Webster HK, and Wechgritaya S. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Surasinghanart Army Hospital, Aranyaprathet, Thailand.

4:30 448 PROSPECTIVE TREATMENT TRIAL OF VIVAX MALARIA USING 25 MG/KG CHLOROQUINE BASE DEMONSTRATES RESISTANCE IN 10 (22%) CASES IN IRIAN JAYA. Murphy GS*, Basri H, Purnomo, Andersen EM, Bangs MJ, Gorden J, Arbani RP, Harjosuwarno S, Mount DL, Hoffman SL, and Sorensen K. US Naval Medical Research Unit, No.2, Jakarta, Indonesia; Malaria Division, National Institute of Health, Research and Development, Jakarta, Indonesia; Provincial Health Office, Irian Jaya, Indonesia; Malaria Branch, Centers for Disease Control, Atlanta, GA; and U.S. Naval Medical Research Institute, Bethesda, MD.

4:45 449 EFFICACY OF ARTEMISININ (QINGHAOSU) FOR THE TREATMENT OF NON-COMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN COLOMBIA Nicholls RS, Guerra MP, and Corredor A. Grupo de Parasitologia, Instituto Nacional de Salud, Bogota D.E., Colombia.

## SCIENTIFIC SESSION DD: ARBOVIRUS VECTOR STUDIES

Thursday, December 5
1:30 PM - 4:15 PM Hampton A/B
Chairpersons: K. Linthicum and P. Nuttall

1:30 450 STABILIZED LA CROSSE VIRUS INFECTION IN *AEDES ALBOPICTUS*. Streit TG, Grimstad PR, and Craig GB, Jr. Vector Biology Laboratory, University of Notre Dame, Notre Dame, IN.

1:45 451 GENETIC SELECTION OF A *FLAVIVIRUS* REFRACTORY STRAIN OF THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*. Miller BR* and Mitchell CJ. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, CO.

2:00 452 NGARI VIRUS (*BUNYAVIRUS*, BUNYAVIRIDAE): REPLICATION IN AND TRANSMISSION BY *AEDES AEGYPTI*. Tammariello RF* and Linthicum KJ. Virology Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

2:15 453 ROLE OF TICK SALIVA IN VIRUS TRANSMISSION. Labuda M*, Nuttall PA, and Jones LD. Institute of Virology, Bratislava, Czechoslovakia; and NERC Institute of Virology and Environmental Microbiology, Oxford, UK.

2:30 454 HOST RESPONSE TO TICK SALIVA-ACTIVATED TRANSMISSION OF THOGOTO VIRUS. Jones LD and Nuttall PA*. NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, UK.

2:45 455 COMPARATIVE INFECTIONS OF EPIZOOTIC AND ENZOOTIC STRAINS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS IN *AMBLYOMMA CAJENNENSE* TICKS. Linthicum KJ*, Gordon SW, and Monath TP. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

3:00 Coffee Break

3:30 456 VIRUS ISOLATIONS FROM MOSQUITOES CAPTURED IN TWO PERUVIAN AMAZON LOCATIONS; IQUITOS AND ANDOAS. Need JT* and Phillips I. Entomology Department, U.S. Naval Medical Research Institute Detachment, Lima, Peru and Virology Department, U.S. Naval Medical Research Institute Detachment, Lima, Peru.

3:45 457 WEST NILE AND SINDBIS ANTIBODIES IN BIRDS AND RODENTS IN THE NILE DELTA REGION OF EGYPT Nashed NW*, Main AJ, Tewfik SA, Metwally SA, and Hanafi HA. Medical Zoology Div., US Naval Medical Research Unit Number 3, Cairo, Egypt.

4:00 458 DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND *CULICOIDES VARIIPENNIS* VECTOR COMPETENCE FOR BLUETONGUE VIRUS. Tabachnick WJ* and Robertson MA. Arthropod-Borne Animal Diseases Research Laboratory, USDA-ARS, Laramie, WY.

## SCIENTIFIC SESSION EE: KINETOPLASTIDA IMMUNOLOGY

Thursday, December 5
1:30 PM - 4:30 PM Commonwealth
Chairpersons: H. Tanowitz and D. Wyler

1:30 459 PROPOSED MECHANISM OF ACTION FOR NORMAL HUMAN SERUM ON AFRICAN TRYPANOSOMES. Ortiz JC* and Seed JR. Department Parasitology, School Public Health, University North Carolina, Chapel Hill, NC; and Department of Epidemiology, School Public Health, University North Carolina, Chapel Hill, NC.

1:45 460 A MEMBRANE 60 KDA GLYCOPROTEIN OF INVASIVE FORMS OF *TRYPANOSOMA CRUZI* PRESENTS PUTATIVE LYMPHOCYTE EPITOPES. Villalta F*, Howard SA, and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

2:00 461 HUMAN PBMC PROLIFERATE AND SECRETE CYTOKINES IN RESPONSE TO *TRYPANOSOMA CRUZI* INFECTION *IN VITRO* Van Voorhis WC* and Barrett LK. Division of Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA.

THURSDAY PM
EE: KINETOPLASTIDA IMMUNOLOGY

2:15 462 EPITOPE IDENTIFICATION OF INFECTION-ENHANCING ANTIBODIES AGAINST THE *TRYPANOSOMA CRUZI* NEURAMINIDASE. Prioli RP*, Ortega-Barria E, Mejia JM, and Pereira M. Division of Geographic Medicine, New England Medical Center Hospitals, Boston, MA.

2:30 463 IMMUNOGENETICS AND IMMUNE MECHANISMS IN RESISTANCE TO ACUTE *TRYPANOSOMA CRUZI* INFECTION IN MICE. Powell MR*, Theodos CM, and Wassom DL. Department of Zoology and Biomedical Sciences, Ohio University, Athens, OH; Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Pathobiological Science, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

2:45 464 LIPOPHOSPHOGLYCAN MAY BE A TRANSMISSION BLOCKING VACCINE FOR LEISHMANIASIS. Sacks DL*, Warburg A, Pimenta PF, Perkins PV, and Lawyer P. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.

3:00 Coffee Break

3:30 465 IDENTIFICATION OF *LEISHMANIA DONOVANI* ANTIGENS EXPRESSED ON OR RELEASED FROM THE SURFACE OF INFECTED MACROPHAGES. Melby PC* and Darnell B. Department of Medicine, The University of Texas Health Sciences Center, San Antonio, TX.

3:45 466 INHIBITION OF NITRIC OXIDE SYNTHESIS FROM L-ARGININE RESULTS IN DECREASED HOST RESISTANCE TO *LEISHMANIA MAJOR* AND SEVERE CACHEXIA. Evans TG*, Thai L, and Hibbs JB. Division of Infectious Diseases, University of Utah, Salt Lake City, UT.

4:00 467 CD4+ T LYMPHOCYTES EXPRESSING CELL SURFACE TNF CAN MEDIATE CONTACT-DEPENDENT MACROPHAGE ACTIVATION FOR ANTILEISHMANIAL DEFENSE. Sypek JP* and Wyler DJ. Division of Geographic Medicine and Infectious Diseases, Tufts University School of Medicine, New England Medical Center Hospitals, Boston, MA.

4:15 468 THE KINETICS OF MITOGENIC AND ANTIGENIC RESPONSES IN EXPERIMENTAL MURINE LEISHMANIASIS. ASSOCIATION BETWEEN LYMPHOPROLIFERATION, TNF PRODUCTION, AND RESOLUTION OF THE DISEASE. Karagouni EE and Dotsika EN*. Hellenic Pasteur Institute, Athens, Greece.

# ABSTRACTS

## A: CLINICAL TROPICAL MEDICINE

1 ANTHRAX OUTBREAK IN CENTRAL JAVA, INDONESIA. Sianturi L, Koesharyono C, Soerjosembodo S, Suharyono W, Ezzell J, Ksiazek T, and Jennings G*. Ministry of Health, Jakarta, Indonesia; U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD; and U.S. Medical Research Unit No. 2, Jakarta, Indonesia.

An anthrax outbreak, which occurred in a Central Java dairy project in January 1990, resulted in the sudden death of approximately 3% of the cows in a herd of 12,000 cattle. The bovine disease was controlled subsequently through a vaccination and education program. A serological survey of 51 local residents who worked on the farms or had contact with the animals, found seventeen persons (33%) with serum antibodies to the lethal factor and/or protective antigen of anthrax as measured by ELISA. Positive individuals were predominantly men over the age of 20. Eleven of the seventeen (65%) had cutaneous anthrax lesions indicating possible asymptomatic anthrax infection in the remaining six. An acute disease affecting 32 children with 10 deaths occurred in July at one of the villages located in the dairy project regency. Children, primarily between the ages of 2 and 14 years, presented with symptoms of fever and abdominal distension. A retrospective analysis of sera from 12 village children found seven with ELISA antibody titers to anthrax. Acute and convalescent samples available from two of those children demonstrated diagnostic rising antibody titers to the lethal factor and protective antigen of anthrax. The results demonstrate the threat of anthrax infection to humans in a bovine outbreak and show the usefulness of the ELISA in diagnosing anthrax, particularly cases without the obvious cutaneous lesions.

2 THE USE OF TUBERCULIN SCREENING IN SUSPECTED TUBERCULOUS MENINGITIS PATIENTS. Girgis NI*, Kilpatrick ME, Farid Z, Erian M, and Corwin A. US Navy Medical Research Unit No. 3, Cairo, Egypt.

Concerns about the utility of Purified Protein Derivative (PPD) as a diagnostic aid in tuberculous meningitis (TM) prompted a review of data collected from 180 patients diagnosed between 1987-1989 in Cairo, Egypt with TM (83 by CSF culture and 79 by clinical diagnostic criteria). PPD was applied intradermally to all patients on admission and the reaction was read after 48 hours. Induration >10mm was recorded as positive. All surviving patients (95) had a repeat PPD after 60 days. On admission 17% (31/180) of TM patients were PPD positive compared to 14% (7/50) of 50 non-tuberculous controls. PPD positive results in culture-positive TM patients were not significantly different from those in clinically diagnosed TM patients. PPD-positive results were significantly greater (36%) in TM patients who were alert on admission compared to those who were comatose (12%, $p=0.01$). The mean ages of patients were also higher in alert patients compared to comatose patients. The PPD test was positive after 60 days in 65% (61/95) of TM survivors, significantly higher than on admission (17%) ($p<0.001$). Thirty of the 62 (48%) PPD positives were PPD convertors. PPD as a diagnostic aid for TM is of limited usefulness because of disease severity and/or age of these patients in Egypt.

3 OCCURRENCE AND SELF-TREATMENT OF TRAVELER'S DIARRHEA IN A LARGE GROUP OF AMERICAN TRAVELERS. Hill DR*. International Travelers' Medical Service, University of Connecticut School of Medicine, Farmington, CT.

There is little information on either the occurrence or self-treatment of traveler's diarrhea in a large number of Americans who differ widely in age and travel destination. This study examines these two issues in persons traveling to the developing world for ≤90 days. Each traveler was instructed on preventing diarrhea and given anti-diarrheal prescriptions. After their trip they returned a health questionnaire. From 6/89 to 6/90, 567 patients were enrolled; 515 (91%) were followed-up. Their mean

age was 44 yrs. (range 1 to 85), the median duration of travel was 21 d., and 110 countries were visited. Diarrhea was reported by 241 (47%). Eighty-nine (Group A) had diarrhea strictly defined as ≥3 unformed stools/day, plus cramping, vomiting, fever or blood; the remaining 152 (Group B) did not meet this definition. For Group A, diarrhea began after a mean of 13 days, was associated with 6 stools/day, cramps in 75%, vomiting in 40%, fever in 38% and blood in 3%. It lasted 5 d.; 62% altered their activity and 12% sought medical care. Diarrhea in Group B was significantly milder in terms of stools/day (3), duration (4 d.) and symptoms. Both Groups A and B took anti-diarrheals with similar frequency, 85% and 76% respectively. However, Group A took antimotility agents alone less than Group B (28% vs. 46%, $P<0.01$), and took antibiotics more often (53% vs. 26%, $P<0.01$). Although diarrhea occurred frequently during travel, only 17% met a strict definition. Nevertheless, most travelers used anti-diarrheals whether or not they met this definition. This illustrates the need for continued pre-travel teaching on preventive measures and instructions for treatment of traveler's diarrhea.

4 RABIES VACCINE IN TRAVELERS: A DECISION ANALYSIS. Wilson ME* and Fineberg HV. Mt. Auburn Hospital, Cambridge, MA; and Harvard Medical School, Harvard School of Public Health, Boston, MA.

Confusion and uncertainty cloud decisions about the use of rabies vaccine prior to travel. Some elements that complicate the decision are the generally low risk of rabies in travelers, high cost of vaccine, need for multiple doses requiring repeated medical encounters, lethality of established infection, and high visibility of human rabies deaths when they occur. At the same time, travelers and medical personnel at travel clinics frequently have no knowledge of the likelihood of a potentially rabies-transmitting exposure in another country and little firm data about destination-specific rabies risk (which animals are infected; prevalence of infection in various animals). The decision depends in part on the size of the at-risk animal population as well as the percentage of animals infected. Also pertinent are the availability, safety, and efficacy of post-exposure rabies immune globulin and vaccine in countries to be visited and the availability of sterile needles for administration. This paper will present a decision model integrating currently available data to help clinicians make decisions about the use of pre-exposure rabies vaccine. It will incorporate data from completed studies, identify the kinds of data and additional studies that are needed to improve decision-making, and reveal which factors are critical in making the decision.

5 *BLASTOCYSTIS HOMINIS*: ERADICATIVE THERAPY FOR A PROBABLE PATHOGEN. El-Masry NA*, Bassily SB, Farid Z, Mansour NS, Sabry AG, and Kilpatrick ME. US Naval Medical Research Unit No. 3, Cairo, Egypt.

*Blastocystis hominis* has been reported as the only agent identified in fecal samples from symptomatic individuals. This report describes the effectiveness of four drugs in eradicating *B. hominis* from the feces in both symptomatic and asymptomatic patients. Six patients were symptomatic with only *B. hominis*. Forty-six other symptomatic patients had helminthic and protozoan infections as well. They were first treated for the helminths, then for the protozoa. Thirty-three asymptomatic patients had mixed parasitic infections including *B. hominis*. Results of treatment are shown below:

| | Symptomatic | | Asymptomatic | | Total | |
|---|---|---|---|---|---|---|
| | No | % Success | No | % Success | No | % Success |
| Diidohydroxyquinoline | 5 | 40 | 4 | 25 | 9 | 33 |
| Tinidazole | 34 | 41 | 15 | 53 | 49 | 45 |
| Ornidazole | 6 | 83 | 13 | 62 | 19 | 68 |
| Furazol | 7 | 86 | 1 | 0 | 8 | 75 |

Success of treatment was defined as eradication of *B. hominis* from the feces. Ornidazole and possibly furazol appear to be effective for eradication of *B. hominis* from feces. Definitive studies are still required to determine if *B. hominis* is a pathogen.

6 UPDATE ON DISTRIBUTION, CHARACTERISTICS, AND MANAGEMENT OF THE POLYCYSTIC HYDATID DISEASE IN MAN. D'Alessandro A*. Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA

We now know of 62 human cases of polycystic hydatid disease (PHD), (22 *Echinoccus vogeli*, 1 *E. oligarthrus*, 39 undetermined). Clinical information available on 34 patients showed lesions distributed in the upper abdomen, (lesions of liver, gall bladder and biliary system); in the lower abdomen, (mesenteric tumors); in the chest, (polycystic tumors or abscesses); and in the orbit (eye proctosis). The diagnosis of PHD was based on the demonstration of polycystic masses by: palpation of progressively enlarging hard abdominal masses, less frequently intermittent or progressive jaundice or signs of portal hypertension; CAT scanning, simple x-ray (showing irregular calcifications), ultrasound or MRI, in persons from rural tropical areas. The IHA test (CDC) was positive in 7 of 9 persons and arc 5 in IEP was present in 4 of 9. Diagnosis was confirmed by the distinct hooklet size and shape. PHD is generally a chronic, relatively well-tolerated infection. Albendazole was reported useful in some cases. Surgery should be used to drain biliary obstruction but complications were lethal as was hepatectomy for radical cure. Paliative surgery of restricted lesions located in accessible areas should be attempted if albendazole is not effective and the patient requests exeresis of lesion producing troublesome clinical manifestations.

7 THE NATURAL HISTORY OF CUTANEOUS LEISHMANIASIS, GUATEMALA. Herwaldt BL*, Arana BA, and Navin TR. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Medical Entomology Research and Training Unit, Guatemala City, Guatemala.

Therapies for American cutaneous leishmaniasis (ACL) are difficult to evaluate because of the paucity of published data on the course of untreated infection. We studied the natural history of ACL in Guatemala by following the courses of skin lesions of untreated participants in randomized, placebo-controlled treatment trials. Forty-eight men (median age of19 years) had a total of 57 lesions; 32 were caused by *L. braziliensis* (*Lb*) and 25 by *L. mexicana* (*Lm*). When first seen in the clinic, *Lb* and *Lm* lesions had comparable median ages of 51 and 50 days, respectively, but *Lb* lesions were twice as big as *Lm* lesions, with median ulcer areas of 1.3 and 0.6 $cm^2$, respectively. Eighty-eight percent (22/25) of *Lm* lesions reepithelialized by a median lesion age of 14 weeks; 68% (17/25) were classified as cured (no wound inflammation or reulceration during one year of follow-up). In contrast, 22% (7/32) of *Lb* lesions reepithelialized by a median lesion age of 13 weeks, and only 6% (2/32) cured. Fifty-three percent (17/32) of *Lb* lesions at least doubled in size by a median lesion age of 9 weeks. These data demonstrate that the species of *Leishmania* is the primary determinant of the course of untreated ACL and underscore the need for field-applicable techniques for rapidly speciating the parasite as a guide to therapy.

8 BUBONIC LEISHMANIASIS: A NEW FORM OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION IN CEARA STATE, BRAZIL. Sousa AQ*, Pompeu MM, Gomes TN, Coelho Filho JM, Oliveira EG, Lima FF, Vasconcelos IA, Parise ME, Maguire JH, Vasconcelos AW, and David JR. Hospital Sao Jose, Ceara State, Brazil; Nucleo de Medicina Tropical, Federal University of Ceara, Brazil; Departamento de Patologia e Medicina Legal, Federal University of Ceara, Brazil; Departamento de Medicina Clinica, Federal University of Ceara, Brazil; Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

In 1987-88, we saw 4 patients with cutaneous leishmaniasis (CL) and extensive regional lymphadenopathy that preceded the cutaneous lesions by days to several weeks. In order to determine the frequency of this occurrence and describe its clinical and pathological features, we undertook a prospective study of 2448 persons living in the mountainous region of Baturite where these cases had originated. During an 18-month period 155 persons (6.3%) developed active skin lesions, and 70% reported enlarged regional lymph nodes. Careful clinical examination showed lymph nodes >2.0 cm in diameter in 28 of 92 patients (30%) and >4 cm in 6 (7%). Lymphadenopathy preceded the skin lesions in 65% of cases, appeared concurrently in 18%, and followed the emergence of skin lesions in 17%. Leishmania were isolated from skin biopsies or lymph node aspirates in 64 cases. Biopsies of enlarged nodes from 33 patients showed lymphadenitis, histicytosis and plasmacytosis in all cases, and perilymphadenitis, paracortical and follicular hyperplasia, necrosis and fibrosis in many cases. The histological picture resembled that of tuberculosis, fungal infection, toxoplasmosis, or cat scratch disease in some cases. Amastigotes were detected in 79% of the nodes, and the isoenzyme pattern of all 12 isolates studied was that of *L.(V). braziliensis*.

9 RECOMBINANT INTERFERON-γ IN COMBINATION WITH PENTAVALENT ANTIMONY IN THE THERAPY OF LEISHMANIASIS. Badaro R*, Barral-Netto M, Carvalho EM, Teixeira R, Rocha H, Johnson, W., Jr. Department of Medicine, Federal University of Bahia, Brazil; and Division of International Medicine, Cornell University College, New York, NY.

Leishmaniasis is a broad spectrum of diseases caused by obligate intracellular protozoa of the genus *Leishmania*. Two of these diseases express profound immunological abnormalities during the acute illness; visceral leishmaniasis (VL) or kala-azar and diffuse cutaneous leishmaniasis (DCL). Specific defects include: a) failure of peripheral blood T cells to respond to leishmania antigen; b) absence of production of interleukin-2 (IL-2) and interferon-γ (IFN-γ); c) defects in macrophage function after *in vitro* infection with leishmania decrease (IL-1 production, low expression of Class II MHC and increase in the generation of prostaglandin E2); d) negative delayed hypersentitivity reactions. The rationale for the use of recombinant interferon-γ in combination with pentavalent antimony to treat such patients is based on the observation that interferon-γ reverses some of these immunological defects. Forty-five leishmaniasis patients have received this combination therapy (IFN-γ plus $Sb^V$): 39 VL patients and six DCL cases. The overall clinical response of the VL patients was 89% after 10-40 days of therapy. Relapses were noted in 10 patients (26%) after 3 to 6 months of follow-up. Failure was noted in 6 patients (15%) and all were previously resistant cases. In the DCL cases, five are still receiving maintenance therapy after a dramatic regression of the skin lesions. The one DCL patient who finished treatment was completely cured with reversion of his skin test to positive. 23% of the patients treated with the combination therapy had mild adverse reactions. In addition two patients had to stop the therapy because of severe pancytopenia. Our preliminary experience with these 45 patients indicates that IFN-γ should be recommended as adjunct therapy for severely ill and resistant cases of leishmaniasis.

10 RELATIONSHIP BETWEEN INTENSITY OF *OPISTHORCHIS VIVERRINI* INFECTION AND HEPATOBILIARY DISEASE DETECTED BY ULTRASONOGRAPHY. Elkins DB*, Mairiang E, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Posri S, Sithithaworn P, Haswell-Elkins M. Tropical Health Program, Queensland Institute of Medical Research, Herston, Queensland, Australia; and Departments of Radiology, Parasitology and Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

This study was undertaken to identify hepatobiliary abnormalities associated with *Opisthorchis viverrini* infection in Northeast Thailand. Four groups of 24 locality, age, and sex-matched village residents with no, light, moderate and heavy liver fluke infection, as estimated by eggs per gram of faeces, were examined by ultrasonography. Highly significant differences were observed between the groups in the

size of the left lobe of the liver (relative to total body weight) and the fasting and post-meal size of the gall bladder. Parasite-specific serum IgG levels also correlated with gall bladder size. In addition, indistinct gall bladder wall, the presence gall bladder sludge and strongly enhanced portal vein redicle echoes were most frequently observed in the heavily infected group. Two suspected cases of early cholangiocarcinoma were identified from the heavily inspected group. The results demonstrate the importance of intensity of infection on the frequency and severity of fluke-associated heptobiliary disease.

11 IVERMECTIN IN ONCHOCERCIASIS AND IN CONCOMITANT *ONCOCERCA VOLVULUS, LOA LOA,* AND *MANSONELLA STREPTOCERCUS*. Grigsby ME*, Olopoenia L, and Sanusi S. Infectious Diseases Division, Department of Medicine, Howard University Hospital, Washington, DC.

This study reports the efficacy of ivermectin in treatment of a patient with onchocerciasis and concomitant *Loa loa* and *Mansonella streptocerca* infection and one patient with onchocerciasis alone. Two male African immigrants from Cameroon, West Africa were found to have filarial infections by skin snips, blood films and removal of an onchocercal nodule. The patients each received a single oral dose of 150 mg/kg body weight. Neither patient demonstrated adverse reactions. The patient with *O. volvulus* alone had a decrease of microfilarial levels to 1.3% of pre-treatment levels by day seven post-treatment and to zero by day 26. The patient with multifilarial infections did not return for day 7 but at day 30 and at six months and nine months post-treatment, no microfilariae were detected in skin snips or blood. Two immigrants from a highly endemic area of West Africa presented with single or multifilarial infections. Ivermectin was effective and well tolerated.

12 CLINICAL DIFFERENCES BETWEEN BLACK AND CHACHI AMERICAN ONCHOCERCIASIS PATIENTS LIVING TOGETHER IN ECUADOR. Proano R*, Mackenzie CD, Guderian RH, and O'Day J. Department of Pathology, Michigan State University, East Lansing, MI.

Two-hundred and eighty-five individuals (135 Blacks of African descent and 150 Chachi Amerindians) were clinically examined for onchocercal pathology; slit lamp and fundoscopy for ocular lesions and detailed dermatological examinations. The parasitological and clinical findings indicate that there are significant levels of onchocercal disease in these people, and there was no significant difference between these two cohabiting racial groups in terms of microfilarial load, anterior ocular disease and other eye diseases. However, there was a significantly greater degree of retinal pathology in the Black population. We concluded from this study that the disease present in coastal Ecuador was more similar to that classified in African disease as "rain forest form," although we exercise caution in making direct comparisons between South American onchocerciasis and the African disease.

13 ANEMIA AND BLOOD TRANSFUSION PRACTICES IN KENYAN WOMEN. Zucker JR*, Lackritz EM, Ruebush TK, Adungosi J, Were JB, and Campbell CC. Malaria Branch, Centers for Disease Control, Atlanta, GA; Siaya District Hospital, Siaya, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

Despite the risk of HIV transmission, blood transfusions are regularly given to anemic women in sub-Saharan Africa, though the impact of transfusion on survival is unknown. We studied anemia and itsrelationship to transfusion practices in hospitalized women in rural western Kenya. For all women admitted to the medical and maternity wards of Siaya District Hospital from December 1990 through February1991, age, malaria parasitemia, blood transfusion ordered and/or received, hemoglobin (Hb), reproductive history, and outcome of hospitalization were documented. The mean Hb of the 1127 women surveyed was 10.4 g/dl; 690 (61.2%) were pregnant. Severe anemia (Hb<8.0 g/dl)was not

associated with *Plasmodium falciparum* parasitemia. Blood transfusion was ordered for 93 (8.3%) women, of whom 47 were transfused.The mean Hb of women with transfusion ordered was 6.3 g/dl; 24 (29%) had Hb>8.0 g/dl. The mortality rate (MR) among severely anemic women with transfusion ordered but not received was 7.6% (7 women), while no severely anemic women who received transfusion died ($p<.05$). The lower MR among severely anemic women who were transfused suggests blood transfusion improves survival. Identifying the principal etiologies of severe anemia in women, and developing strategies to prevent anemia, are essential to reduce the risk of transfusion and HIV infection.

## B: ARBOVIRUS MOLECULAR BIOLOGY

14 CORRELATION OF AN ANTIGENIC SUBTYPE OF EASTERN EQUINE ENCEPHALITIS VIRUS WITH ALTERATIONS IN THE E2 GLYCOPROTEIN. Repik PM*, Strizki JM, and Calisher CH. The Medical College of Pennsylvania, Philadelphia, PA; and Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, CO.

Previous studies have indicated that an isolate of eastern equine encephalitis (EEE) virus, designated 4789 and recovered from a fatal human infection, represents the first example of an antigenic subtype of North American EEE virus (Calisher *et al.*, 1990). To characterize this virus genetically, and to determine whether antigenic variablity could be correlated with a particular structural protein, fingerprinting of genomic RNA, and peptide mapping of individual structural proteins were undertaken. Oligonucleotide mapping could not distinguish 4789 from other North American viruses. However, it was readily apparent by SDS-PAGE analysis of virion proteins that 4789 exhibited a markedly slower migrating E2 glycoprotein when compared will all other North American strains. V8 protease digestion patterns confirmed that the antigenic variability resides within the E2 protein. Although many E2 peptide bands of 4789 were identical to those of other North American strains, distinctive differences were easily demonstrated among several of the smaller (14-20 Kd) peptides. These peptides reacted with polyclonal antibodies to both North and South American EEE viruses in western blot assays. The E2 peptide profile of 4789 was not similar to those of South American viruses. In contrast to the results obtained with E2, peptide profiles of the E1 glycoprotein and nucleocapsid of 4789 were identical to those of other North American EEE viruses. These data suggest that the variation in antigenicity resulted from an alteration in the peptide structure of the E2 glycoprotein, which is known to play a major role in eliciting neutralizing antibody formation. Whether or not this alteration could affect viral pathogenicity is unknown.

15 DETECTION OF RIFT VALLEY FEVER VIRUS NUCLEIC ACID IN MOSQUITOES BY IN SITU HYBRIDIZATION WITH A DIGOXIGENIN CDNA PROBE Patrican LA*, Hoover TA, Dohm DJ, Lerdthusnee K, and Romoser WS. Department of Entomology, Cornell University, Ithaca, NY; Bacteriology Division, U. S. Army Medical Research of Infectious Diseases, Ft. Detrick, MD; Virology Division, U. S. Army Medical Research of Infectious Diseases, Ft. Detrick, MD; and Geographical Disease Institute, Ohio University, Athens, OH

Rift Valley fever virus (RVFV) isolated from 9/543 pools of *Aedes mcintoshi* collected as larvae during an interepizootic period in Kenya suggested transovarial transmission. Our inability to colonize *A. mcintoshi* precludes laboratory confirmation of vertical transmission. To determine whether or not gene expression occurs in the reproductive tissues of mosquitoes a digoxigenin-labeled cDNA probe for RVFV is being used to detect viral nucleic acid in experimentally infected *A. mcintoshi* and *Culex pipiens*, an epizootic vector. Mosquitoes fed to repletion on a viremic hamster on day 0 (5.1 $Log_{10}$ PFU/mosquito (*A. mcintoshi*) ingested and 6.0 $Log_{10}$ PFU/mosquito (*C. pipiens*) ingested ) and on day 7 refed en masse on a nonviremic hamster. Mosquitoes were fixed for 2 hr in 4% paraformaldehyde on days 8, 11, and 14 postinfectious bloodmeal and embedded in paraffin using standard histological procedures. Serial sagittal sections of infected and uninfected (control) mosquitoes are currently being examined for

evidence of RNA-cDNA hybrids by enzyme-linked immunoassay using an anti-digoxigenin alkaline phosphatase conjugate and enzyme catalyzed color producing substrate. Stain in various tissues and cells with respect to specificity and time will be compared with the avidin-biotin complex procedure used to locate RVFV antigen in infected *A. mcintoshi* and *C. pipiens*. This technique will hopefully provide insight into the capacity of *A. mcintoshi* to vertically transmit RVFV at the genomic level.

16 MOLECULAR ANALYSIS OF YELLOW FEVER VACCINE VIRUSES. Galler R*, Post PR, Santos CN, and Carvalho R. Departmento de Bioquimica e Biologia Molecular, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

As part of a broader approach to determine the molecular basis of flavivirus attenuation using yellow fever (YF) as a model we analyzed the envelop (E) protein of two YF strains: the 17D D virus (produced at FIO CRUZ for almost 50 years for human vaccination) and the 17D-213 virus (a tissue culture vaccine strain previously tested for monkey neurovirulence). Immunoprecipitation of radiolabelled protein extracts of virus-infected Vero cells followed by polyacrylamide gel electrophoresis separation revealed that both E proteins had higher molecular weight. The difference was shown to be due to N-linked glycosylation by digestion with endoglycosydases F and H. Nucleotide sequencing confirmed the existence of such glycosylation sites in the E genes of both YF virus strains. Glycosylated E protein was also present in extracellular virions which constitute the vaccine. The importance of carbohydrate moieties to the vaccine phenotype can now be tested with the availability of a YF infectious cDNA.

17 DETECTION OF WEST NILE VIRUS BY THE POLYMERASE CHAIN REACTION AND ANALYSIS OF STRAIN VARIATION. Porter KR*, Oprandy JJ, Dubois DR, Summers P, Nelson WM, Henschal EA, and Hayes CG. Infectious Diseases Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD; and Virology Division, Walter Reed Army Institute for Research, Washington, DC.

West Nile virus currently is best detected and characterized by standard tissue culture techniques and monoclonal or polyclonal antibodies. We developed a polymerase chain reaction (PCR) technique for the detection and identification of West Nile virus (WNV). Oligonucleotide primers to the NV4 nonstructural gene were designed based on the Nigerian strain published sequence. RNA was isolated from 7 strains of WNV taken from 6 geographic regions by guanidine isothiocyanate extraction method. Complimentary DNA strands were reverse-transcribed from approximately 1pg of viral RNA and the DNA used in PCR. Amplified DNA was detected by gel electrophoresis and ethidium bromide staining or southern hybridization. The sensitivity of the WNV PCR was determined by a membrane-based RNA isolation method using normal human serum spiked with known concentrations of WNV. RNA from human leukocytes, C6/36 cells, dengue virus, St Louis Encephalitis virus, Yellow Fever virus, and Kunjin virus (KV) was used as controls. All 7 strains of WNV produced bands of the predicted size. All control specimens failed to show appropriate sized bands with the exception of KV. As few as 6.5 pfu/100ul human serum could be detected by PCR. The DNA sequence of the PCR product from the Ethiopian and Egyptian strains both differed from the Nigerian strain sequence by 20%. The Ugandan strain showed only a 0.5% difference. The Egyptian strain differed by only 3% from the Ethiopian strain. These data suggest that this PCR assay may be useful for detecting and characterizing West Nile virus independent of its geographic location. Primers to distinguish WNV from KV are presently being developed.

18 USE OF POLYMERASE CHAIN REACTION (PCR) FOR THE SENSITIVE DETECTION OF ST. LOUIS ENCEPHALITIS VIRAL RNA. Vodkin MH*, Howe DK, Novak RJ, Shope RE, and McLaughlin GL. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; Illinois Natural History Survey, Center for Economic Entomology, Champaign, IL; and Department of Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT.

St. Louis encephalitis (SLE) is one of the major mosquito-borne viral diseases affecting humans in the United States and Canada. SLE is thought to be distributed in the Central U.S. by early spring, migratory birds. Cases occur sporadically, or as epidemics in about ten year cycles. Control of SLE depends on an early warning of viral activity followed by mosquito abatement. Viral detection from field or clinical isolates is typically accomplished through intracranial inoculation of suckling mice or by infection of cell cultures. Prototype polymerase chain reaction (PCR) assays were developed to provide more rapid and sensitive detection of SLE virus. Three primer pairs were selected from the capsid, envelope, and membrane-associated protein genes,respectively, of the MSI-7 strain. The first enzymatic step of the assay, reverse transcription of RNA, was primed more effectively with random hexamers than with the specific oligomeric pairs. Each primer pair specifically amplified the expected size fragment from RNA extracts of infected (Parton SLE strain) *Aedes albopictus* cells but not from RNA extracts of uninfected control cultures. The technique detected about 100 copies of SLE RNA added to one µg of brain tissue. These PCR assays may be adaptable to detect SLE virus in environmental (mosquitos and birds) and clinical (cerebrospinal fluid and brain) samples.

19 CLONING AND SEQUENCE ANALYSIS OF THE GENES ENCODING THE STRUCTURAL AND NONSTRUCTURAL PROTEINS OF LANGAT VIRUS TP-21. Iacono-Connors L*. Virology Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

The virus family Flaviviridae has been organized into antigenic complexes based on cross-neutralization properties. One complex, the tick-borne encephalitis complex, contains at least eight antigenically related flaviviruses, most of which are highly pathogenic for humans. Langat virus TP-21 (LGT) is a member of this antigenic complex, but is not known to be associated with a naturally occurring human disease. LGT and LGT-like viruses have been evaluated in Russia and Czechoslovakia as naturally occurring, attenuated vaccines against tick-borne encephalitis (TBE) virus. In an effort to define the genetic relationship between LGT and more virulent viruses in the same antigenic complex, LGT genome sequences that encode structural proteins and nonstructural proteins have been cloned and sequenced. Comparison of deduced amino acid sequences of individual LGT proteins and those known for other tick-borne viruses will permit evaluation of conserved and unique antigenic regions. In addition, evaluating sequences from the noncoding 5' and 3' portion of the genome will allow comparison of potentially important structural properties of the nucleic acids of virulent and avirulent tick-borne viruses.

20 IDENTIFICATION OF A PUTATIVE RECEPTOR PROTEIN FOR VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS FROM *AEDES ALBOPICTUS* CELLS. Ludwig GV* and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Venezuelan equine encephalomyelitis virus (VEE), a New-World alphavirus, is responsible for epidemic disease in North, Central, and South America in recent decades. Although much is known about the biology, epidemiology, and replication of VEE, little is known of the initial event of infection, attachment of virus to cells. We have used direct binding studies to characterize the attachment of virus to mosquito cells. Virus binding to C6/36 cells is saturable and can be competed with homologous virus, suggesting a specific virus-receptor interaction. To identify such receptors, we developed an assay in which intrinsically labeled VEE virus was bound to SDS-PAGE-separated, immobilized cell membrane polypeptides, and visualized by autoradiography. Results indicate that virus bound with greatest affinity to a 34-kDa band from C6/36 cell membranes. The binding of virus to this band was saturable and could be competed with homologous virus. Mouse polyclonal antibody directed against C6/36 membranes immunoprecipitated a 34-kDa band from the surface of cells, bound to the same polypeptide immobilized on nitrocellulose, and reduced virus binding to cells. Taken together, these data suggest that this 34-kDa polypeptide is present on the surface of cells and may serve as a receptor for virus attachment.

21 GENOMIC STRUCTURE, RNA POLYMERASE AND VIRULENCE OF LEISHMANIA VIRUS. Patterson JL*, Widmer G, Cadd T, Titus R, Keenan M, and Armstrong T. Department of Microbiology and Molecular Genetics, Harvard Medical School; Division of Infectious Diseases, Children's Hospital; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Viral particles infecting some stocks of the protozoan parasite *Leishmania braziliensis* contain a double-stranded RNA genome of approximately 5 kb and are associated with an RNA-dependent RNA polymerase which synthesizes *in vitro* double-stranded and single-stranded genome length transcripts. The putative replicase generates double-stranded RNA by synthesizing the opposite strand on a pre-existing RNA template (Widmer and Patterson, in press, 1991). We have recently begun to use an antibody to the viral capsid protein for subcellular localization of the virus. Preliminary data would suggest that the virus replicates entirely in the cytoplasm. Virus-infected promastigotes were inoculated in footpads of Balb/c mice. As with non-infected controls, swelling of the footpads were observed. Re-isolating of the parasites demonstrated that the viral infection persisted throughout the infective cycle of the mouse.

## C: HEPATITIS AND RETROVIRUS EPIDEMIOLOGY

22 PHYLOGENY OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I: INSIGHTS FROM SEQUENCE ANALYSES OF VIRUS STRAINS ISOLATED FROM REMOTE MELANESIAN POPULATIONS. Yanagihara R*, Gessain A, Sherman MP, Franchini G, and Poiesz BJ. National Institutes of Health, Bethesda, MD and Department of Medicine, SUNY Health Science Center at Syracuse, Syracuse, NY.

The recent isolation of human T-lymphotropic virus type I (HTLV-I) from remote populations in Papua New Guinea and the Solomon Islands puts to rest the controversy over the existence of this retrovirus in Melanesia. To clarify the molecular genetic relationship between these Melanesian strains of HTLV-I and prototype HTLV-I, we amplified by polymerase chain reaction, then cloned and sequenced regions of the HTLV-I *pol*, *env*, and *tax* genes in peripheral blood mononuclear cells and T-cell lines derived from HTLV-I-seropositive inhabitants of Papua New Guinea and the Solomon Islands, including a Solomon Islander with HTLV-I myeloneuropathy. Nucleotide sequence analyses indicated the existence of novel molecular variants of HTLV-I in Melanesia, which exhibited only 92% sequence homology with prototype HTLV-I (ATK-1). The HTLV-I variants from Papua New Guineans, in turn, differed by nearly 4% from the HTLV-I variants from Melanesian Solomon Islanders. The nucleotide sequence of proviral DNA from a Solomon Islander with HTLV-I myeloneuropathy also diverged markedly from that of prototype HTLV-I, suggesting that these variant viruses are capable of causing disease. By contrast, HTLV-I strains from inhabitants of Bellona Island, a Polynesian Outlier within the Solomon Islands, were closely related to prototype HTLV-I (≥97% sequence identity), suggesting recent introduction, possibly during this century. The discovery of novel sequence variants of HTLV-I in Papua New Guinea and the Solomon Islands is consistent with a proto-Melanesian HTLV-I strain which evolved independently of contemporary cosmopolitan strains.

23 PREVALENCE OF HIV INFECTION AND AIDS IN EGYPT OVER FOUR YEARS OF SURVEILLANCE (1986-1990). Watts DM*, Constantine NT, Sheba MF, Kamal M, Callahan JD, and Kilpatrick ME. US Naval Medical Research Unit No. 3, Cairo, Egypt (A WHO Collaborating Center for AIDS); University of Maryland School of Medicine, Baltimore, MD; and Ministry of Health, Cairo, Egypt

Serosurveys were conducted from April 1986 through March 1990 to determine the prevalence of HIV-1 infections among Egyptians and foreigners. Sera from 29, 261 high risk individuals and blood donors in Egypt, and from10,326 foreigners were tested for HIV-1 antibodies by a recombinant HIV-1 and a

recombinant combination HIV-1/HIV-2 enzyme immunoassay (EIA). All repeatedly reactive sera were tested by Western blot as a confirmation of antibody positive sera. The overall prevalence of HIV-1 infection among Egyptians was 0.18% (54/29,261) and included 4.8% (25/582) of blood recipients, 0.15% (3/1961) drug addicts, 0.18% (3/1650) of FUO patients, 0.23% (6/2602) STD patients, 1.9% (5/269) of HIV-1 contacts, 0.07% (7/9778) of international travelers, and 0.001% (2/12,070) of blood/product donors. The prevalence of HIV-1 antibody among foreigners was 0.97% (100/10,326); who were mainly (94%) from other African countries. Evidence of HIV-1 infection was not demonstrated among 349 prostitutes. Of the total 154 HIV infected individuals, 20 Egyptians and one foreigner were diagnosed with AIDS; at least 12 (57%) have died. While the AIDS cases have tripled over the latter 18 months, the overall actual prevalence of HIV infections has decreased since 1988, and endemic transmission has not been documented in Egypt.

24 PREVALENCE OF SERUM ANTIBODIES TO HTLV-1 IN AN ISOLATED COMMUNITY IN THE HIGHLANDS OF IRIAN JAYA, INDONESIA. Jennings GB*, Bangs MJ, Sie A, and Anthony RL. U.S. Naval Medical Research Unit No.2, Jakarta, Indonesia and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

There have been several recent reports on the high prevalence of serum antibodies to HTLV-1 in isolated populations residing in the highland valleys of Papua New Guinea. In the absence of significant cases of clinical disease, it has been surmised that this reactivity might be the consequence of serologic recognition of yet undefined human retroviruses or parasite antigens. These observations prompted an investigation of the prevalence of anti-HTLV-1 antibodies in the inhabitants of Oksibil; a secluded highland valley in the eastern Jayawijaya Mountains of Irian Jaya (Western Papua New Guinea). Of 157 participants, most of whom were between the ages of 20 and 40, 51 (32%) were positive for serum antibodies to HTLV-1 in an indirect ELISA. In order to increase specificity, positivity was defined as a ratio of>2.4, between the optical density of the test sample and that of the positive control, in lieu of the normal 1.0 cut-off. Of these 51 sera, all produced an indeterminate pattern in the confirmatory HTLV-1 western blot with reactivity seen to p19, p24, p26, p28, p32, and/or p36. Although all of the adult residents of Oksibil have high titers of antibodies reactive with undefined erythrocytic stage antigens of *Plasmodium falciparum*, a correlation between levels of anti-malaria antibodies and HTLV-1 reactivity could not be established.

25 COMPARISON OF TRANSMISSION RATES OF HIV-1 AND HIV-2 IN A COHORT OF PROSTITUTES IN SENEGAL. Donnelly CA*, Leisenring WM, Sandberg S, Kanki PJ, and Awerbuch T. Department of Biostatistics, Harvard School of Public Health, Boston, MA; Department of Mathematics, Framingham State College, Framingham, MA; and Department of Cancer Biology, Harvard School of Public Health, Boston, MA.

HIV-2 infection is seen mainly in western Africa, occurring primarily in the urban areas of Senegal, Cape Verde Islands,Guinea-Bissau, Burkina Faso, The Ivory Coast and Benin. Although HIV-2 attacks and weakens the immune system as does HIV-1 resulting in similar symptoms, HIV-2 has a longer latency period and is biologically somewhat different from HIV-1. We were interested in whether the biological differences were also reflected in the level of infectivity via sexual transmission. Thus, we modeled the probability of being infected with either HIV virus as a function of the number of sexual partners (N), the prevalence of the virus (p), and the infectivity per contact (r). Using maximum likelihood estimation theory and data from a prospective study of registered prostitutes in Dakar, Senegal, we estimated and compared the infectivities of HIV-1 and HIV-2. We found that HIV-1 is significantly more infectious than HIV-2. This finding is consistent with other data from laboratory and epidemiologic studies comparing the biology of HIV-1and HIV-2.

26 EXPERIMENTAL HEPATITIS E FOLLOWS THE HEPATIC REPLICATION OF HEV AND COINCIDES WITH THE APPEARANCE OF SERUM ANTI-HEV. Longer CF*, Denny S, Asher LV, Myint KS, LeDuc JW, Binn LN, Krawczynski K, and Ticehurst JR. Department of Viral Diseases, Walter Reed Army Institute of Research, Washington, DC; Animal Resources Division, US Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; Division of Pathology, Walter Reed Army Institute of Research, Washington, DC; Department of Virology, USA Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Disease Assessment Division, US Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Hepatitis Branch, Centers for Disease Control, Atlanta, GA.

Hepatitis E virus (HEV) has been detected by immune electron microscopy in the feces of patients and in bile and feces of experimentally infected animals. Viral antigen (HEV Ag) has been detected in infected liver by immunofluorescence microscopy. We sought to elucidate the course of experimental hepatitis E by correlating the presence of HEV and anti-HEV with evidence of hepatitis. Six cynomolgus macaques were inoculated intravenously with bile containing HEV. HEV Ag was present in liver biopsies collected between 14 and 25 days after inoculation but not before or after this period. Concomitant bile samples contained HEV particles. Anti-HEV and serum transaminase elevations developed after HEV Ag was detected. Microscopic evaluation of liver specimens early in the infection showed hepatocyte swelling and Kupffer cell activation. Later specimens, after hepatic HEV Ag had waned and anti-HEV appeared, showed inflammatory cells and focal necrosis. Our findings indicated that HEV replicated in the liver and was shed into the bile 2 to 3 weeks after inoculation. Inflammation associated with the presence of HEV early in the infection was minimal but became more severe as anti-HEV appeared.

27 SPORADIC ACUTE HEPATITIS E INFECTIONS IN EGYPTIAN CHILDREN DIAGNOSED BY IgM AND IgG SEROLOGIC TESTS. Goldsmith R*, Yarbough PO, Reyes GR, Gabor KA, Kamel M, and Gaffar Y. Department of Epidemiology and Biostatistics, University of Calif, San Francisco, CA; Molecular Virology Department, Genelabs, Inc.; Department of Clinical Pathology, University of Cairo; and Department of Medicine, Ains Shams University Cairo, Egypt.

Hepatitis E virus (HEV), recently molecularly cloned, has been etiologically linked with enterically transmitted non-A, non-B hepatitis (ET-NANBH). Although no convenient diagnostic test is available for HEV, ET-NANBH has been recognized in many developing countries (not in Egypt) as waterborne outbreaks that primarily affect young to middle-age adults. Sporadic ET-NANBH cases or overt infections in children have been diagnosed infrequently. We report development and application at Genelabs of a prototype ELISA and IgM and IgG western blot assays to detect antibody to HEV. In a prospective study (1988) in rural Egypt (Benha) to determine acute hepatitis HAV, HBV, HDV, EBV, and CMV. Sera taken at 3-month intervals to 9 or 12 months were available from 36 of the 65 children. Of the 36, 15 (42%) were positive for anti-HEV-IgG. When tested for anti-HEV-IgM, 8 of 9 of the 36 children were positive but none of the controls was positive. We report the first convenient solid-phase immunoassay for HEV antibody and the first anti-HEV-IgM test. These results indicated that clinical infections occur in children and many cases occur sporadically.

28 DETECTION OF ANTIBODIES TO HEPATITIS E VIRUS IN EPIDEMIC AND IMPORTED CASES OF ENTERICALLY-TRANSMITTED NANB HEPATITIS. Yarbough PO*, Gabor KA, Reyes GR, Flower AJ, and Skidmore SJ. Genelabs Inc. Redwood City, CA; Public Health Laboratory, Leicester, England; and Regional Virus Laboratory, East Birmingham Hospital, Birmingham, England.

Outbreaks of enterically-transmitted non-A, non-B hepatitis (ET-NANBH) have been reported in developing countries where fecally contaminated drinking water has been implicated as the source of the epidemics. The molecular characterization of hepatitis E virus (HEV), the agent responsible for ET-

NANBH, has been recently reported . By serologic screening, two cDNA epitope clones were shown to react specifically with antisera collected from different geographic ET-NANBH epidemics. These HEV gene segments have been shown to be useful in the diagnosis of ET-NANBH. This paper reports the development of immunoassays to detect antibody to HEV infection in India. By ELISA, 22/33 patients diagnosed with viral hepatitis were positive for anti-HEV, whereas contact cases of ET-NANBH were limited to 1/23. Anti-HEV were also detected by ELISA in two patients from the United Kingdom who had returned from the Indian subcontinent with acute non-A, non-B hepatitis. An acute HEV infection was confirmed by an anti-HEV-IgM western blot assay. Our results demonstrate usefulness of these anti-HEV specific immunoassays to diagnose ET-NANB hepatitis.

## D: KINETOPLASTIDA: BIOLOGY AND MOLECULAR BIOLOGY

29 ARE THERE NATURALLY OCCURRING LEISHMANIAL HYBRIDS? Kreutzer RD*, Tesh RB, Grogl M, Neva FA, Yemma JJ, and Iacoliangelo T. Biology Department, Youngstown State University, Youngstown, OH; Yale Arbovirus Research Unit,Yale Medical School, New Haven, CT; Leishmaniasis Section,Walter Reed Army Institute of Research, Washington, DC; and Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD.

*Leishmania* can be identified by electrophoretic methods from single bands they produce for such enzymes as MPI, 6PGDH and LP. Ten human cases of simple cutaneous leaishmaniasis (SCL) isolates (4 from Honduras, '84 and '89; 4 from Colombia, '85,'86, '87; 2 from Panama, '88 and '89) were initially typed as mixed cultures of *L. braziliensis* (LBB)/*L. panamensis* (LBP), because they produced multiple bands for these and other enzymes. Clones (22) of 2 Colombia isolates produced identical bands as the parent strains. The varied histories and handling plus the clone data indicate the multiple bands are produced by a single cell line that is polymorphic for the enzymes tested rather than a mixed culture of 2 cell lines, LBB + LBP. Single cell lines which produce multiple enzyme bands suggest a diploid condition (one as LBB and one as LBP) or the nuclei of both species have combined to form a "hybrid"; or the isolate contains sections (which include the enzyme loci being studied) of DNA from both species or heterocaryon cells. Cells from 8 isolates and controls (4LBB, 2 LBP) are being examined both microscopically and with a microspectrophotometer (MSPM) which measures the amount of DNA in the nucleus. Preliminary results indicate the "hybrid" cells are not heterocaryons. Enzyme and MSPM data will be presented and discussed.

30 IDENTIFICATION AND CHARACTERIZATION OF EPIDERMAL GROWTH FACTOR RECEPTORS IN *TRYPANOSOMA CRUZI*. Freeman-Junior P* and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

*Trypanosoma cruzi*, the causative agent of Chagas' disease, requires an intracellular location to multiply in mammalian hosts. There is a profound lack of knowledge on regulatory mechanisms controlling parasite growth and development. An understanding of these mechanisms at the molecular level would be necessary to develop novel ways to prevent parasite development. In this work we have found that epidermal growth factor (EGF) increases $^{3}$H-thymidine uptake of amastigote forms of *T. cruzi* grown in defined media. The increase in DNA synthesis is growth factor concentration dependent. Immunofluorescence studies reveal that amastigotes but not trypomastigotes present receptors for EGF. Western blots and immunoprecipitation studies performed with two monoclonal antibodies against the human EGF receptor show that a surface protein of 90 kD is recognized in amastigote forms of the parasite. Two dimensional gel electrophoresis of the immunoprecipitates indicates that the parasite's receptor is composed of three polypeptides of pI values 6.3,6.28 and 6.22. The specificity of these reactions was confirmed by up to 90% inhibition of immunoprecipitation reactions with an 11 amino

acid peptide derived from the nucleotide sequence of the human EGF receptor. These studies will help clarify the mechanisms used by an obligate intracellular parasite to multiply in its mammalian host.

31 TYROSINE KINASE ACTIVITY IN *TRYPANOSOMA BRUCEI*. Wheeler-Alm E and Shapiro SZ*. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL.

Phosphorylation of proteins at tyrosine is an important mechanism for regulating cell growth and proliferation in metazoan organisms. We report that *Trypanosoma brucei*, an evolutionarily primitive protozoan parasite, possesses a tyrosine-specific kinase that plays a role in regulation of proliferation of this protozoan. Genistein, a tyrosine kinase inhibitor, prevented multiplication of the parasite. An *in vitro* kinase assay demonstrated the presence of a kinase capable of phosphorylating an exogenous substrate at tyrosine, and genistein was able to reduce trypanosome-mediated phosphorylation of this substrate.Two-dimensional thin layer electrophoresis of alkali hydrolyzed [$^{32}$P]-labeled total trypanosome protein revealed the presence of phosphotyrosine. An alkali digestion of [$^{32}$P]-labeled trypanosome proteins resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated several proteins which may be phosphorylated at tyrosine. Anti-phosphotyrosine antibodies were used to detect tyrosine-phosphorylated parasite proteins on western blots. These results indicate that *T. brucei* has a tyrosine kinase that functions *in vivo* and is involved in proliferation or growth regulation of the parasite, and provide further evidence for the possibility of growth factor regulation and signal transduction in trypanosomes.

32 DIFFERENTIAL EXPRESSION OF THE MITOCHONDRIAL OLIGOMYCIN-SENSITIVE ATPASE IN BLOODSTREAM FORMS OF *TRYPANOSOMA BRUCEI*. Bienen EJ* and Shaw MK. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya.

Trypanosomes of the *brucei* subgroup undergo a series of biochemical and physiological changes during differentiation through their life cycle. We have previously shown, using rhodamine 123, that pleomorphic bloodstream forms regulate their ability to support a mitochondrial electron-motive force. We now show that the $F_1F_0$ ATPase is differentially expressed. Using a strain of *Trypanosoma brucei* which gives reproducible pleomorphic populations, we assayed for ATPase activity enzymatically in a crude mitochondrial fraction and by electron microscope histochemistry in whole cells. Oligomycin-sensitive specific activity increased 5-fold as the cells differentiated from the long slender forms, characteristic of rapidly proliferating cells in early infections, into the vector-infective intermediate/short stumpy forms. This increase was concomitant with a doubling of the total ATPase specific activity. As the cells continued to differentiate into procyclic (= insect midgut) forms, there was a further 3-fold increase in oligomycin-sensitive ATPase activity as total ATPase specific activity again doubled. Oligomycin-insensitive activity remained relatively constant for all life cycle stages. Differential ATPase activities were confirmed by electron microscope histochemistry using lead deposition to visualize enzyme activity.

33 CDNA CLONING OF A NOVEL TRYPANOSOME PROCYCLIC STAGE SURFACE ANTIGEN BY EXPRESSION IN MAMMALIAN (COS7) CELLS. Jackson DG*, Smith DK, Luo G, and Elliott JF. Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, U.K.; Department of Immunology, University of Alberta, Edmonton, Alberta, Canada; and Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada.

Molecular characterization of the surface proteins found on the bloodstream and procyclic (insect) forms of trypanosomes is critical to understanding host-parasite interactions and parasite defense mechanisms.

These molecules allow the parasite to interact with its environment and mediate vital functions such as transport. However, except for the most predominant surface antigens (eg. VSG), to date very few additional cell surface proteins from *T. brucei* have been cloned. We have recently used eucaryotic expression to directly clone both known and new trypanosome cell surface glycoproteins. A cDNA library of $2.2 \times 10^6$ recombinants was constructed from *T. brucei* procyclic stage mRNA by the RNAse H method, using a BstXI linker strategy to ligate into the vector pCDM8. The library was transfected into COS7 cells and rabbit antiserum raised against whole procyclic trypanosomes was used to select by 'panning' those COS cells which were transiently expressing parasite surface antigens. After three successive cycles of antibody selection a number of clones were highly enriched. The two most abundant clones have been sequenced. One is procyclin, the major surface antigen of procyclic trypanosomes. The second clone encodes a previously unidentified 412 amino acid protein with the structure of a conventional (type I) integral membrane protein. Unlike most surface glycoproteins of *T. brucei* characterized to date, this novel sequence contains a long (121 amino acid) proline rich cytoplasmic tail in place of the usual glycophosphatidylinositol anchor. The method of COS cell expression and antibody panning should be useful for cloning many of the trypanosome cell surface glycoproteins.

34 PENETRIN, A *T. CRUZI* HEPARIN BINDING PROTEIN THAT PROMOTES TRYPOMASTIGOTE PENETRATION INTO MAMMALIAN CELLS AND CONFERS INVASIVENESS TO *E. COLI*. Ortega-Barria E* and Pereira M. Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospitals, Boston, MA.

In order to survive and complete its life cycle, *T. cruzi* must attach to and enter appropriate host cells by mechanisms that appear to be dependent on specific receptor-ligand interactions. We have found that infective, live trypomastigotes attach selectively to plastic immobilized extracellular matrix proteins, namely, the proteoglycans heparin and heparan sulfate, and the fibrous protein collagen. Heparin and heparan sulfate were very effective in inhibiting attachment of trypomastigotes to glutaraldehyde-fixed fibroblasts, whereas other glycosaminoglycans such as chondroitin sulfate and hyaluronic acid, as well as glycoproteins were ineffective. Attachment is by means of a surface 60 Kd protein, that also binds to host fibroblasts in a saturable and proteoglycan dependent manner. When adsorbed to plastic, the isolated protein promotes adhesion and spreading of fibroblasts, as does the recombinant protein expressed in *E. coli*. The endogenous protein is extremely effective in neutralizing *T. cruzi* infection of fibroblasts *in vitro*, as are its ligands, heparin and heparan sulfate. When expressed in *E. coli*, the 60 Kd protein, but not the *T. cruzi* neuraminidase, induces the bacteria to penetrate non-phagocytic Vero cells in a proteoglycan- and collagen-dependent manner. This molecule, named penetrin, appears to promote recognition and invasion of mammalian cells, presumably by reacting with heparin-like structures present on their surfaces.

35 *LEISHMANIA* DNA SEQUENCE RESPONSIBLE FOR TUNICAMYCIN-RESISTANCE. Liu X* and Chang KP. Department of Microbiology/Immunology, University of Health Sciences, Chicago Medical School, North Chicago, IL.

Tunicamycin-resistant *Leishmania spp.* have been found to contain amplified DNA, which apparently mediates drug-resistance. The DNA molecules originate from a common chromosomal region in different species and exist as multiple copies of a large extrachromosomal circle.The circular amplicon (63 kb) was cloned previously from *L. amazonensis* as four BamHI fragments in pBR322. To determine the relevance of the amplified DNA to tunicamycin-resistance (TMR), wildtype cells were transfected with the cloned DNA fragments by electroporation followed by selection with tunicamycin. This approach led to the finding that a BamHI fragment of 15 kb conferred TMR to the wildtype cells as well as the intact 63 kb circle. A library of the 15 kb fragment was subsequently prepared in pUC19 at PstI sites and used to transfect wildtype cells. TMR transfectants thus obtained were found to contain a pUC19 clone with a 4.6 kb insert. The clone with this fragment was further isolated from the library and found to confer TMR

when used to transfect wildtype cells. Clones with other regions of the amplified DNA are ineffective. Thus, TMR gene(s) exist within the 4.6 kb region of the 63 kb circle. This region is being sequenced to near completion for further analysis.

36 GENE TARGETING IN *LEISHMANIA ENRIETTII*. Tobin JF* and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

We have previously demonstrated that *Leishmania enriettii* contains the enzymatic machinery to mediate efficient interplasmidic homologous recombination. In this report we show that the neomycin resistance ($neo^r$) can be targeted into the *Leishmania* genome by homologous recombination. A pBluescript-derived vector, pALT-Neo-Tub, containing the $neo^r$ gene flanked by the alpha-tubulin intergenic and alpha-tubulin coding sequences was used to integrate the $neo^r$ gene into either of the alpha-tubulin containing chromosomes. Two types of recombination events were observed. If prior to transfection pALT-Neo-Tub was linearized by cutting it once within the alpha-tubulin coding region then recombinants were obtained containing the entire plasmid integrated into the alpha-tubulin loci. If, however, the plasmid was cut twice within the pBluescript sequences flanking the insert then recombinants were obtained containing only the $neo^r$ gene integrated. The utility of this vector for stably integrating any DNA sequence into the Leishmania genome will be discussed. In addition, this technology may prove useful in producing attenuated Leishmania for use in vaccine development.

37 GENE EXPRESSION IN *LEISHMANIA*: IDENTIFICATION OF ESSENTIAL DNA SIGNALS. Lafaille MA*, Laban A, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The development of transfection systems for parasites has opened the possibility of studying these organisms by means of molecular genetics. Manipulation of the parasite genome requires an understanding of the signals and mechanisms used to control gene expression. We have previously shown that the intergenic region of alpha-tubulin could provide essential sequences for the expression of foreign genes in this organism. Using stable transfection we have now characterized a DNA element that can mimic the effect of the alpha-tubulin intergenic region in the expression of the bacterial gene CAT. By studying transcription in the transfected cell lines we have determined that the effect of the identified sequence is post-transcriptional.

38 TRANSFECTION OF *LEISHMANIA* WITH GP63 METALLOPROTEINASE GENE AND ITS EXPRESSION IN THE TRANSFECTANTS. Chang KP*, Liu X, and Du YB. Department of Microbiology/Immunology, Univ of Health Sciences, Chicago Medical School, North Chicago, IL.

Pathogenic species of *Leishmania* possess a major surface glycoprotein of 63 kDa (gp63), which is a metalloprotease and is thought to be a virulence factor. The gene encoding gp63 was first cloned by Button and McMaster from *L. major* in Bluescript at the EcoRI site (BS10Rb.1).This plasmid was used to prepare two different constructs suitable for transfection of *Leishmania*: (1) A 15 kb DNA cloned from the 63 kb circle in tunicamycin-resistant *Leishmania* was ligated to BS10Rb.1 at the BamHI site; (2) A 2.2 kb gp63 ORF of the BS10Rb.1 was inserted at the EcoRI site in pALT from Dr. D.F. Wirth (Bluescript with NEO-R gene flanked by *Leishmania* tubulin intergenic regions). *Leishmania* cells with low level of gp63 expression were transfected with these plasmids by electroporation. Transfectants with plasmids 1 and 2 were successfully selected for tunicamycin- and G418-resistance, respectively. Plasmids were recovered in their original form from the transfectants as extrachromosomal circles. More gp63 transcripts, gp63 protein and surface metalloprotease activity were expressed by the transfectants with plasmid 1 than

their parental wildtype cells. Studies are underway to characterize further the transfectants and the expression of their gp63.

39 *LEISHMANIA MAJOR* SECRETES CHITINASE THAT FUNCTIONS IN THE SANDFLY VECTOR. Schlein Y, Jacobson RL*, and Shlomai J. Department of Parasitology, Hebrew University - Hadassah Medical School, Jerusalem, Israel.

*Leishmania major* parasites ingested with host blood by the sand fly *Phlebotomus papatasi* multiply confined within the peritrophic membrane. This membrane consists of a chitin framework and a protein carbohydrate matrix and it is secreted around the food by the insect midgut. Histological sections of infected flies show lysis of the chitin layer of the peritrophic membrane that permits the essential forward migration of the parasites. The nature of this disintegration is specific to infected flies. We have found that chitinase and N-acetylglucosaminidase are secreted by cultured *L. major* promastigotes, but not by sand fly guts. Hence, lysis of the chitin layer of the peritrophic membrane could be hydrolyzed by these enzymes of the parasites. Activity of both enzymes was also observed in other trypanosomatids, including *L. donovani, L. infantum, L. braziliensis, Leptomonas seymouri, Crithidia fasciculata and Trypanosoma lewisi.*

40 REVERSAL OF ADRIAMYCIN RESISTANCE IN *LEISHMANIA AMAZONENSIS* BY THE $Ca^{2+}$ ANTAGONIST VERAPAMIL. Lopes UG*, Gueiros-Filho F, Viola JB, Gomes FA, and Campos CB. Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

*In vitro* resistance to adriamycin was developed in *Leishmania amazonensis* by a stepwise selection process. The $EC_{50}$ of the *Leishmania* wild type was 0.3 μM . The $EC_{50}$ of the resistant cell line, RA 2000 was 2.4 μM. The ability of the $Ca^{2+}$ antagonist verapamil to reverse the resistant phenotype was investigated. When a dose of verapamil, which itself did not inhibit *Leishmania*, was added to the resistant cell line RA 2000, inhibition of growth was 86% with 2.0 μM adriamycin. This was comparable to the inhibition (98%) found when the *Leishmania* wild type was treated with 2.0 μM adriamycin. However, the inhibition of the resistant cell line RA2000 by adriamycin without verapamil was 62%. Multidrug resistance mediated by P-glycoprotein is also reverted by verapamil in mammalian cells . A similar process of resistance could be suggested in the adriamycin resistance in *Leishmania*. By PCR amplification using degenerated oligonucleotides, we could clone and sequence *Leishmania enrietti* product, which is homologous to the mammalian mdr1 gene. In addition, a full size mdr 1-related gene was selected from a genomic library of *Leishmania braziliensis*. The characterization and expression of mdr-related sequences in resistant leishmanias are under analysis .

## E: BACTERIOLOGY AND RICKETTSIOLOGY

41 A FOODBORNE OUTBREAK OF TYPE E BOTULISM IN CAIRO, EGYPT, APRIL 1991. Hibbs RG*, Mishu B, Darwish A, Weber JT, Hatheway CL, El-Sharkawy S, and Corwin A. US Naval Medical Research Unit No. 3, Cairo, Egypt; Centers for Disease Control, Atlanta, GA; and Infectious Disease Department, Ministry of Health, Cairo, Egypt .

A foodborne outbreak of type E botulism in Cairo, Egypt in April 1991 involving 91 hospitalized patients and 20 deaths (18 reported to MOH) was investigated. Fifty percent (46/91) of the patients were interviewed. Symptoms were compatible with botulism. Fifty-nine percent (27/46) were treated in ICU, 28% (13/46) required ventilation and 85% (39/46) received botulism antitoxin. Reported duration of hospitalization did not exceed one month for any of the 91 patients. Ungutted, salted, uncooked grey mullet fish called "faseikh" was implicated as the source of intoxication. All hospitalized patients interviewed purchased the fish from the same store and consumed it on the same holiday. The case-

fatality rate associated with this outbreak was 22%. The mean incubation period was 13.6 hours, ranging from 2 to 36 hours. A case-control study of 5 families involving 42 persons provided an estimated overall attack rate of 62%. This outbreak documents the first cases of type E botulism in Egypt. The four fish samples obtained from affected cases were found to contain type E botulinum toxin. To date, no toxin has been detected in control fish, while testing of sera and stool specimens (10/16 positive for *Clostridium botlinum*) continues. There were no persistent signs or symptoms generally associated with type E botulism among the cases.

42 POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF *FRANCISELLA TULARENSIS*. Long GW*, Narayanan RB, Fortier AH, Nacy CA, and Oprandy JJ. Infectious Disease Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD and Cellular Immunology Department, Walter Reed Army Institute of Research, Washington, DC.

A polymerase chain reaction assay was developed for *Francisella tularensis* based on the nucleotide sequence of the 17-kDa membrane protein (TUL4). Two primer pairs were identified which generated either a 232 or 630 base pair product from both *F. tularensis* subsp. *tularensis* (Type A) and subsp. *palaearctica* (type B). PCR of tenfold dilutions of *F. tularensis* DNA followed by agarose gel electrophoresis and ethidium bromide staining resulted in visible product at the lowest dilution tested (35 fg). This is equivalent to fewer than ten gene copies. Internal primers were utilized to generate digoxigenin labelled probes which were used in Southern blots and dot blots to confirm the identity of the PCR product. We have used this technique to detect *F. tularensis* live vaccine strain (LVS) in the blood of infected mice after i.v. inoculation. *F. tularensis* was detected in the cellular fraction of blood for a period of one hour after inoculation, and again between 24 and 48 hours. This time is coincident with metastatic hematogenous spread to the lungs of infected animals. Bacteria were detected in the plasma of infected animals 48 hours after inoculation. This assay is useful for analysis of tularemia in animal models. Further work is underway to validate the assay for use in environmental sampling and epidemiologic studies.

43 IgG CLASS ANTIBODIES AGAINST GROUP B *NEISSERIA MENINGITIDIS* OUTER MEMBRANE ANTIGENS OF DIFFERENT SEROTYPES PREVALENT IN BRAZIL De Gaspari EN*, Tavares AV, Ribeiro CL, Farhat CK. Immunology Section, Adolfo Lutz Institute; and Pediatrics Division, Emilio Ribas Hospital, Sao Paulo, Brazil.

An epidemic of meningococcal disease of serogroup B is occurring in Brazil since 1988. Recently, it was shown that in Brazil there is great diversity of group B serotypes. In our study we are analyzing the homologous and heterologous immune response against outer membrane antigens of different serotypes. Serum of a group of patients taken before vaccine administration were clinically and serological selected and the bacteria isolated typed. The groups of serotypes prevalent were B:4:P1.15, B:4:nt, B:nt.nt and B:nt:P1.15. The outer membrane antigens were extracted with 0.2 M LiCl, analyzed by SDS-PAGE and then transferred to nitrocellulose. The levels of IgG were determined by Dot-ELISA. Immunoblot with homologous serum showed that polypeptides of 28, 33, 38, 41, 46, 50 and 62 KDa were recognized. However, the patterns were not the same. Interestingly, some homologous sera presented a high degree of reactivity with heterologous strains. The antigens of 28, 33, 38 and 50 KDa presented cross reactivity in the serotypes analyzed. Our approach using convalescence serum is a good way to study polypeptides relevant in the development of infection. It is important to have in mind that other factors like bacterial strains should be considered for the analysis of major antigens for obtaining vaccines with several protective specificities.

44 THE EFFECTS OF ANTIMALARIAL CHEMOPROPHYLACTIC AGENTS ON THE VIABILITY OF THE TY21A TYPHOID VACCINE STRAIN. Brachman JP*, Metchock B, and Kozarsky P. Emory University School of Medicine, Atlanta, GA.

Since 1990 in the US, the live attenuated oral typhoid vaccine (Vivorif, Berna) has replaced the parenteral heat-phenol inactivated vaccine for many travelers because of its comparable efficacy and few side effects. Many travelers also need antimalarial chemoprophylaxis. Some antimalarials have antibacterial activity and, because the Ty21a strain requires replication in the GI tract, concurrent chloroquine or mefloquine administration may interfere with the immune response to the typhoid vaccine. Published data are scant in this area and recent literature suggests 1 wk from administration of Ty21a vaccine to antimalarial prophylaxis. We thus undertook an *in vitro* study to determine the activity of chloroquine and mefloquine against the Ty21a strain and 2 other *S. typhi* strains, S-6 and S-7. Organisms were grown in serial broth dilutions of the drugs in triplicate. Ty21a, S-6 and S-7 strains grew at concentrations of chloroquine, 0.125-256 μg/ml. Mefloquine, however, was bacteristatic, MIC=16 for TY21a, and MICs 64 and 32 for S-6 and S-7, respectively. Mefloquine was bacteriocidal with an MBC 32 for Ty21a. According to the manufacturer, the T-1/2 of mefloquine in the GI tract is 36 min-2 hr, with levels 18 hr post-ingestion of <5-10 μg/ml in the terminal ileum. Steady state serum concentrations of mefloquine are 0.4-1.0 μg/ml. We conclude that although mefloquine is bacteriostatic for strain Ty21a, the MIC and MBC are higher than achievable drug levels in the gut at 24 hr and in the serum at any time. Concurrent administration could affect Ty21a replication, but the 1 wk interval is probably not necessary. Definitive recommendations await antibody testing.

45 DIVERGENCE OF SPOTTED FEVER AND TYPHUS GROUP RICKETTSIAE BY SEQUENCING THE P34-17KD COMMON PROTEIN ANTIGEN GENE REGION. Dasch GA* and Swinson KL. Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

Polymerase chain reaction amplification (PCR) and sequencing of the P34-17 KD common protein antigen gene region (1695 bp) of typhus and spotted fever rickettsiae was used to determine evolutionary relationships among these rickettsiae. Five forward and seven reverse primers were selected from different regions of the P34-17 KD antigen gene of *R. rickettsii* and used in PCR with DNA from different species of typhus and spotted fever rickettsiae. One P34-17KD primer pair efficiently amplified a 1016 bp fragment with DNA from most spotted fever rickettsiae and *R. canada* but not *R. typhi* or *R. prowazekii*. PCR of a 434 bp region internal to the 17KD antigen gene could be used to detect nearly all typhus (10/11) and spotted fever group (74/83) rickettsiae tested but not *R. tsutsugamushi* or *Ehrlichia*. Because restriction fragment length polymorphisms (RFLP) in the 434 bp fragment useful for distinguishing some spotted fever species were not detected empirically, we analyzed biotinylated17 KD PCR products on an automated DNA sequencer. Relationships determined by partial 17 KD gene sequence data obtained on 20 strains of rickettsiae are in good agreement with RFLP data obtained by others either by genomic analysis with random cloned DNA probes or by digestion of PCR fragments of the citrate synthase or SPA genes. However, the present sequence data permit a more precise measure of rickettsial evolution than can be determined by RFLP analysis. We are extending our sequence data to include segments of the P34 gene and the intervening P34-17 KD spacer region to determine if they are more satisfactory for RFLP typing and for measuring rickettsial divergence.

46 OCCURRENCE OF A TYPHUS-LIKE RICKETTSIA ASSOCIATED WITH OPOSSUMS AND THEIR FLEAS IN LOS ANGELES COUNTY. Williams SG*, Sacci Jr. JB, Fujioka K, Sorvillo FJ, Barr RA, and Azad AF. Department of Microbiology, University of Maryland School of Medicine, Baltimore, MD.; Los Angeles County Department of Health, Los Angeles, CA; and University of California, Los Angeles, CA.

The recent discovery of colonized cat fleas, *Ctenocephalides felis*, infected with a typhus-like Rickettsia (designated as El agent) raises the question of whether similar rickettsial infections exist in wild cat flea populations. We have verified the presence of this as yet undescribed Rickettsia as well as *R. typhi* in the urban and suburban areas of Los Angeles. Opossum trapped in close proximity to the residences of human murine typhus cases in Los Angeles county, and other areas within the city of Los Angeles were tested for the presence of typhus group rickettsiae using polymerase chain reaction (PCR). The presence of rickettsiae in the spleen tissues of three opossums (N=9), and in 13 cat fleas (N=183) were determined by PCR. Further analysis of the amplified PCR products generated by a series of primer pairs derived from either17 kD antigen gene or citrate synthase gene revealed that both *R. typhi* and El agent were present in the tested samples. Dual infection was not noted in the samples, however the fleas were either infected with *R. typhi* or El agent. The presence of El agent in the cat flea populations may have important implications for public health. Whether this agent is responsible for the mild cases of human murine typhus in urban and suburban areas of Los Angeles, or in other endemic foci remains to be determined.

## F. MEDICAL MALACOLOGY

47 A MAJOR REPETITIVE ELEMENT IN THE *SCHISTOSOMA MANSONI* SNAIL HOST *BIOMPHALARIA GLABRATA* IS RELATED TO LINE-1 TRANSPOSONS. Knight M*, Miller A, Richards C, and Lewis F. Biomedical Research Institute, Rockville, MD.

As part of an ongoing molecular approach to studying the genome of *B. glabrata*, we were interested in determining the molecular composition of a major non-ribosomal 2.0Kb repetitive element which was discernable by ethidium bromide staining in BamH1 digested DNA. Several clones which hybridized to the [$^{32}$P]-labeled 2Kb BamH1 fragment (purified from preparative gels) were isolated from a lambda Dash *B. glabrata* genomic library. Four clones (insert sizes ranging from 7.5Kb to 15Kb) were selected for further analysis. Cross-hybridization studies of BamH1 digested DNA isolated from these clones demonstrated the presence of the full-length 2.0 Kb repetitive element. Results from nucleotide sequence analysis demonstrated that this repetitive element is homologous at both DNA and protein levels to LINE-1 transposable elements which have been well characterized among several eucaryotes but not snails. In common with the LINE-1 transposons a single-large open reading frame in the snail homologue shares considerable homologies with reverse transcriptase. The distribution of this element and molecular characterization in *B. glabrata* and other *Biomphalaria* species will be discussed.

48 OBSERVED LINKAGE BETWEEN SUSCEPTIBILITY TO *SCHISTOSOMA MANSONI* INFECTION AND ABNORMAL EGG PRODUCTION IN *BIOMPHALARIA GLABRATA* SNAILS. Cooper L*, Richards CS, Lewis F, Cousin C, Minchella DJ. University of Maryland, Department of Entmology, College Park, MD; Biomedical Research Institute, Rockville, MD; University of the District of Columbia, Washington, DC; and Purdue University, West Lafayette, IN.

*Schistosoma mansoni* transmission in endemic areas is related to the abundance, distribution, and susceptibility of the intermediate snail hosts. In our laboratory stocks of *B. glabrata* we have observed linkage between snail susceptibility to infection and abnormal egg production (by selfing snails) characterized by greatly reduced fecundity and altered egg clutch morphology. Three to 20% of F1 offspring from outbred NMRI populations show abnormal egg production, and 3 to 15% are nonsusceptible (NS). However, after 6 generations of selection, 87% were NS and 90% laid abnormal eggs. In the next generation 97% were NS and 96% produced abnormal eggs. 65% of F6's were able to cross fertilize and produce normal eggs. When reisolated, egg production in these snails reverted to abnormal in 5 to 10 weeks. This indicates a definite reproductive cost to these snails associated with their ability to avoid parasitism and pass on, undiluted, the genes responsible. However, two mutated snails stocks (B-85,TLC) also exhibit a high degree of abnormal egg production and are highly

susceptible, suggesting either chromosomal linkage of the two traits or the action of a common physiologic/metabolic pathway(s). Studies are currently underway to determine the genetic basis for the observed linkage and its possible role in maintaining the genes for susceptibility in field populations of snails which are subject to parasite pressure.

49 IS *THIARA (TAREBIA) GRANIFERA* (LAMARCK) AN INTERMEDIATE HOST OF *PARAGONIMUS WESTERMANI* (KERBERT)? Sodeman WA*. Division of Gastroenterology, Department of Medicine, Medical College of Ohio, Toledo, OH.

*Thiara (Tarebia) granifera* (Lamarck) is regularly identified in the malacologic and parasitologic literature as an intermediate host for the human lung fluke *Paragonimus westermani* (Kerbert). This identification was an error made in 1917 by Nakagawa. He quickly realized his error and published a correction in 1918. No subsequent identification of *T. (T.) granifera* as an intermediate host for *P. westermani* has been reported. This association has become so deeply embedded in parasitologic literature that it has survived for 73 years. *T. (T.) granifera* has now come to general attention as an effective, environmentally safe agent for the control of *Biomphalaria glabrata*, an intermediate host for *Schistosoma mansoni* in the Antilles and South America. The ethics and the safety of the spread of *T. (T.) granifera* by its use in *Biomphalaria* control has come into question and has impeded the study and use of this control agent. This paper readdresses the history of this problem and its resolution.

50 A GEOGRAPHIC INFORMATION SYSTEM FOR HABITAT OF THE LYMNAEID SNAIL INTERMEDIATE HOST OF *FASCIOLA HEPATICA* ON LOUISIANA COASTAL MARSH RANGELAND. Zukowski SH*, Wilkerson GW, Jones FW, and Malone JB. Deptartment of Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA and Computer-Aided Design and Geographic Information Systems Laboratory, Louisiana State University, Baton Rouge, LA.

Severity of fascioliasis hepatica in cattle varies markedly between farms on chenier plain coastal marsh in southwest Louisiana. A geographic information (GIS) model for 1 farm, based on a soil map, suggested that habitat of the snail intermediate host, *Fossaria bulimoides*, clustered about interfaces of cheniers (relict beaches) with marsh. The habitat model was extrapolated and tested on12 additional farms. The association of habitat with interfaces held, but was limited to interface along broad cheniers (>100m); soils on narrower cheniers were eliminated from the model. Additional soils on broad cheniers were incorporated. Results suggest a sensitivity of >0.9 and a specificity of >0.75 for the revised model. Similar results held when the revised model was applied back to the initial study farm. The proportion of farm comprised of habitat correlated with the proportion comprised of model habitat ($n=12$, $r^2=0.58$, $p<0.05$). Anthelmintics interfered with *F. hepatica* egg shedding. However, eggs of *Paramphistomum microbothrioides*, a rumen fluke also carried by *F. bulimoides*, were shed in numbers correlated with a soil model index [SMI = proportion of farm comprised of model habitat* stocking rate] ($n=9$, Spearman's $r=0.7$, $p=0.05$). This GIS shows promise for modeling habitat-related risk for a vector-borne disease.

## POSTER I: CLINICAL TROPICAL MEDICINE

51 MORTALITY IN A PROSPECTIVE COHORT OF NEWBORNS IN MALAWI, 1987-1990. Bloland PB*, Steketee RW, Wirima JJ, Breman JG. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Ministry of Health, Lilongwe, Malawi.

Causes and rates of mortality among children in Africa have generally been estimated from health facility statistics, which under estimate overall community mortality. Location and age-specific causes of mortality must be determined to target interventions to high-risk groups. A cohort of children in rural

Malawi was enrolled at birth and monitored for 2 to 3 years. Cause of death was determined from th eassociated signs and symptoms given by the mother during interview (verbal autopsy). Of 3,770 singleton, live births enrolled, 725 (19.2%) children died; 456 (62.9%) died at home, 237 (32.7%) in hospital, and 32(4.4%) at an unknown location. 81% of deaths at home and 67.1% of deaths in hospital occurred >= 28 days of age. Age-specific clustering in mortality was observed: diarrhea-associated deaths (29.6% overall) peaked at 7-12 months and pneumonia (10.5%) at 2-6 months. Malaria(17.2%) and measles (10.1%) deaths clustered in older children, with 32% of malaria and 48% of measles deaths occurring in children >12 mos. By overrepresenting less severe disease or diseases with a prolonged time course, a facility-based estimate would potentially have given a 3-fold underestimate of overall childhood mortality. Consequently, systems capable of measuring age- and cause-specific mortality, especially acutely occurring deaths at the community level, are required.

52 HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA DONOVANI* S.L. IN KENYA. Mebrahtu YB*, Van Eys GJ, Lawyer PG, Pamba H, Koech DK, Roberts CR, Perkins PV, Were JB, and Hendricks LD. United States Army Research Unit and Kenya Medical Research Institute, Nairobi, Kenya; Royal Dutch Tropical Research Institute, Amsterdam, Holland; Walter Reed Army Research Institute, Washington DC; Medical School, University of Nairobi, Nairobi, Kenya; Kenya Medical Research Institute, Nairobi, Kenya; and Trotting Horse Lane, Missoula, MT.

Our laboratory has an ongoing study to characterize all *Leishmania* stabilates and isolates from active leishmaniasis cases. Smears and cultures from aspirates made on different dates from a single lesion on the bridge of the nose of an 18 year-old Kenyan male from Nyandarua District were positive for *Leishmania*. The isolates, NLB-271 and NLB-271-IA, were characterized by cellulose acetate electrophoresis (CAE) using 20 enzyme systems and by genomic DNA Southern blotting filter hybridization using two DNA probes (pDK10 and pDK20) from a *L. major*, Dakar strain (MHOM/SN/00/DK1) and a third probe, ITMAP-263 (p7-059) from a *L. infantum* strain, respectively. Digestion of the two *Leishmania* DNAs with *HindIII* and *PstI* endonucleases, followed by hybridization with the three probes revealed DNA fragment banding patterns indistinguishable from the *L. donovani* species complex. However, the CAE isoenzyme profile of the two isolates from Kenya were closer to the WHO *L. infantum* s.l. reference strain from Tunisia. Excluding post-kala-azar dermal leishmaniasis, this constitutes the first human cutaneous leishmaniasis caused by *L. donovani* s.l. in Kenya. Previously, cutaneous leishmaniasis cases in Kenya had been due only to *L. aethiopica, L. major* and *L. tropica*. This finding may have epidemiological significance for cutaneous leishmaniasis in East Africa.

53 GLUTAMINE SUPPLEMENTED ORS IS SUPERIOR TO STANDARD CITRATE GLUCOSE ORS FOR THE MAINTENANCE THERAPY OF ADULT CHOLERA PATIENTS IN JAKARTA. Punjabi NH*, Kumala S, Rasidi C, Witham ND, Pulungsih SP, Rivai AR, Sukri N, Burr DH, Lesmana M, Hisham MA, and Simanjuntak CH. U.S. Naval Medical Research Unit No.2, Jakarta, Indonesia; Infectious Diseases Hospital of Jakarta, Indonesia; Subdirectorate Diarrhea, CDC, Jakarta, Indonesia; and National Institute of Health, Research and Development, Jakarta, Indonesia.

The value of glutamine fortification of oral rehydration solution (ORS) was evaluated by comparing this solution with standard citrate-glucose ORS (S-ORS) for maintenance and hydration therapy in 181 adult, hospitalized, cholera patients. The study was conducted at the Infectious Diseases Hospital of Jakarta from February to August 1990. After initial rehydration with Ringer's Lactate (RL) solution, the patients were assigned to receive one of the ORS. The 92 patients who received Glutamine supplemented ORS (G-ORS) and 89 patients who received S-ORS were comparable in clinical characteristics on admission for age, sex ratio and mean body weight. Duration and episodes of diarrhea (12.6+9.7 vs. 12.0+8.3 hrs and 16.0+18.7 vs. 19.9+23.9 episodes of diarrhea) as well as vomiting (10.1+14.3 vs. 7.6+5.3 hours and 9.6+17.2 vs. 8.2+14.5 episodes) prior to admission were not significantly different. Mean intravenous fluid requirement prior to ORS therapy were also comparable with 8,385+1,464 ml in G-ORS vs. 8,613+1,

601 ml in S-ORS. Due to severe ongoing fluid loses, 30 patients (16.6%) required intravenous rehydration. The results showed that number of patients who required reinfusion (9 vs. 21 patients), mean volume of fluid reinfused (592+2,095 vs. 1,893+ 3,890 ml), mean total ORS intake (9,069+4,489 vs.11,117+7,203 ml), volume stool output (5,075+3,351 vs. 7,290+4,908 ml,volume stool output per kg BW (111.7+73.6 vs. 160.5+101.1 ml/kg) all were significantly lower in the G-ORS compared to S-ORS. The duration of diarrhea was also lower in the G-ORS group compared to S-ORS (28.8+11.2 vs. 33.2+13.7 hours); however, the difference did not reach statistical significance (p=0.514). Glutamine supplemented ORS appears to be a better solution for maintenance therapy of cholera patients compared to standard citrate ORS in the hospital after intravenous rehydration.

54 THE INTERRELATIONSHIP BETWEEN DIARRHEA AND VITAMIN A DEFICIENCY IN CHILDREN UNDER FIVE YEARS OF AGE IN THE SUDAN. El Bushra HE*, Ash LR, Coulson AH, and Neumann CG. Department of Community Medicine, Faculty of Medicine, University of Khartoum, Khartoum. Sudan; Department of Epidemiology, UCLA School of Public Health, Los Angeles, California.; and Department of Health Sciences, UCLA School of Public Health, Los Angeles.

Recent Indonesian studies suggested that vitamin A deficiency could be a risk factor for diarrheal diseases. Conversely, other studies argue that repeated diarrheal episodes could result in vitamin A deficiency. No firm evidence for either argument has been provided so far. The main objectives of this study were to investigate the interrelationship between vitamin A deficiency and diarrheal diseases in children under five years of age. A cross-sectional study, a follow-up study, and an evaluation of the impact of community-based distribution of vitamin A were conducted in Omdurman (Sudan) between November, 1988 and March, 1989. Children under five years of age (n=1441) participated in the survey which established baseline values for plasma retinol-binding protein (RBP), anthropometric measurements. They also completed a questionnaire. Incident cases of diarrhea (n=290) were followed up, each over a period of two weeks. Three measurements of plasma RBP were made: on the first day the child presented with diarrhea, 3-4, and 11-15 days later. Low levels of plasma RBP proved to be a risk factor for diarrhea especially in girls. The relative risk (RR) increased after the second year of life. Children who received vitamin A supplementation prior to the commencement of the study had lower incidence of diarrhea. The protective effect of vitamin A supplementation was greater in girls. The plasma RBP level in children who presented with diarrhea showed a slight drop (day 3-4) followed by a significant increase (day 11-15). The significant rise in the plasma RBP in the second week after diarrhea suggests that it is most likely that diarrheal episodes mobilize the body stores of vitamin A.

55 INTESTINAL PARASITOSES AMONG PATIENTS AND STAFF OF AN INSTITUTION FOR THE MENTALLY RETARDED. Ferrer I* and Kozek WJ. Medical Sciences Campus, University of Puerto Rico, Rio Piedras, PR.

Lack of current data on the prevalence of intestinal parasitoses in institutionalized populations in Puerto Rico prompted us to survey which intestinal parasites were endemic in an institution for the mentally retarded. The study also included a comparison of the traditional diagnostic methods for giardiasis with a new immunological *Giardia* ELISA Direct Detection Kit. A single fecal sample obtained from each of 86 patients (49 males, 37 females) and 43 staff members (6 males, 37 females) were examined by direct smear, Zinc sulfate flotation- concentration method ($ZnSO_4$ FC), and by Harada-Mori culture method. Results of the $ZnSO_4$ FC disclosed that 54% of the patients (65% of males and 38% of females) and 14% of the staff members (7% of males and 16.7% of females) harbored intestinal parasites. Forty-five percent of the patients had *Trichura trichiura*, 1% hookworm, 7% *Entamoeba coli*, 1% *Endolimax nana*, and 2% *Dientamoeba fragilis*; 9% of staff members harbored *T. trichiura* and 7% *E. coli*. Very light *Giardia* infection was detected by $ZnSO_4$ FC in two patients, but their samples were negative in the ELISA test, although 5

heavy control *Giardia* infections were detected by the ELISA test. These results indicated that the prevalence of intestinal parasitoses in the institutionalized population examined was much higher than in the general population of Puerto Rico, and suggest that the diagnostic efficacy of the *Giardia* ELISA Direct Detection Kit should be thoroughly evaluated, since it apparently detects moderate and heavy, but not light *Giardia* infections.

56 SYPHILIS AND MALARIA DURING PREGNANCY IN MALAWI. Steketee RW, McDermott JM*, and Wirima JJ. Malaria Branch, Centers for Disease Control, Atlanta GA; Malaria Branch, Centers for Disease Control, Atlanta GA; and Ministry of Health, Malawi.

The prevention of malaria and congenital syphilis have been recommended as important components in antenatal care programs in Africa. We investigated perinatal and neonatal survival in infants born to women who were examined for placental malaria infection and syphilis serology (VDRL and MHA-TP). Among 794 women with singleton births, 27 (3.4%) women had reactive VDRL (titre >1:8) and reactive MHA-TP, 194 (24.4%) had evidence of placental *P. falciparum* infection, and 8 (1%) of these had both reactive serology and placental malaria. A reactive syphilis serology was associated with second or third trimester fetal loss (RR=8.5, 95% CI 2.3, 26.2), neonatal death (RR=3.7, 95% CI 1.2,11.6), and postneonatal death (RR=2.4, 95% CI 1.1, 5.5). Placental malaria was not associated with perinatal or neonatal mortality; the relative risk for postneonatal death was 2.9 (95% CI 1.8,4.7). The population attributable risk (AR) percent for syphilis was 18% for perinatal mortality, 8% for neonatal mortality, and 5% for postneonatal mortality; the AR for placental malaria and postneonatal mortality was 32%. Thus, syphilis and malaria during pregnancy contribute to infant mortality at different ages. Antenatal care programs must address the detection and treatment of syphilis in areas with high prevalence to reduce perinatal mortality.

## POSTER I: MEDICAL ENTOMOLOGY

57 VITELOGENESIS IN THE IXODID TICK, *HYALOMMA DROMEDARI: IN VIVO* AND *IN VITRO* ANALYSIS. Schriefer ME* and Sonenshine DE. School of Medicine, Departments Immunology and Microbiology, University of Maryland at Baltimore, Baltimore, MD; and Department of Biology, Old Dominion University, Norfolk, VA.

Polyclonal antibody against PAGE purified yolk protein from freshly oviposited *Hyalomma dromedarii* eggs was utilized in Western Blots and ELISA formats in order to qualitatively and quantitatively monitor vitellogenin (VG) and vitellin (VN) as a function of reproductive development in adult ticks. Levels of VG in adult females rose from 0.15% to 11.29% of the hemolymph protein between the stages of part fed virgin and four days after mating and repletion. Similarly, levels of VN and VG in the ovary and the fat body, respectively, rose from 0. 39 % to 55% and from 0.15% to 3.00% during the same developmental period. Minimal levels of VG, which were not consistent with synthetic activity, were observed in other tissues (salivary gland, midgut, and muscle). *De novo* synthesis of VG *in vitro*, was monitored by incorporation of radiolabeled amino acids into immunoprecipitable protein. Of the above tissues from replete females, only fat body was capable of VG synthetic activity *in vitro*. Although fat body from partially fed virgin females synthesized only trace levels of VG *in vitro*, this tissue was stimulated (35 fold over controls) to produce levels of VG comparable (on a tissue protein basis) to those by replete female fat body upon coincubation with synganglion extracts from replete females. Replete female synganglion extracts also stimulated VG synthesis in fed virgin females *in vivo*.

58 LABORATORY EVALUATION OF PREDATORY CAPACITY OF CYCLOPOID COPEPODS ON *AEDES AEGYPTI* LARVAE IN PUERTO RICO. Suarez MF*, Clark GG, and Marten G. International Health, Johns Hopkins University, San Juan, PR; San Juan Laboratories, Division of

Vector-Borne Infectious Diseases, Centers for Disease Control, San Juan, PR; and Tulane University, New Orleans, LA.

Cyclopoid copepods (planktonic microcrustaceans) have recently been identified as a promising new form of biological control for container breeding mosquito species, such as *Aedes aegypti*. We present results of studies with Puerto Rican copepods in stage I of the WHO scheme for screening and evaluating the efficacy of biocontrol agents for disease vectors. We have found 20 cyclopoid species in Puerto Rico, in a variety of aquatic habitats, including lakes, ponds, reservoirs,roadside ditches, and natural (bromeliads) and artificial (flowerpots and discarded tires) containers. We evaluated the functional response, i.e., the relationship between feeding rate and prey density, which is the most widely studied response of aquatic insect predators to prey density. We determined the predatory capacity of the largest species on first instar *Ae. aegypti* larvae. The most promising species were *Mesocyclops aspericornis*, *Macrocyclops albidus*, and *Mesocyclops* nsp. Large quantities of all three species have been cultured in the laboratory and in outdoor wading pools.

59 DISPERSAL OF THE SAND FLY *LUTZOMYIA LONGIPALPIS* IN AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN COLOMBIA. Ferro C, Morrison AC*, Morales A, Tesh RB, and Wilson ML. Instituto Nacional de Salud, Bogota. Colombia; Yale University School of Medicine, Deparment of Epidemiology and Public Health, New Haven, CT.

American visceral leishmaniasis (AVL) is a potentially fatal disease which is endemic in many dry regions of Tropical America. *Lutzomyia longipalpis*, has been implicated as the major vector of AVL throughout its distribution. Little information is available on the longevity, dispersal or flight range of this important vector species. During 1990-91 mark, release and recapture studies of *Lu. longipalpis* were carried out in El Callejon, Colombia, a highly endemic focus of AVL. Groups of wild caught (N=1539) and laboratory reared F1 sandflies (N=2208) were marked with fluorescent dusts and released. Recaptures took place for 20 days following release at daytime resting sites, on animal bait, and in CDC light traps. Eleven species of *Lutzomyia* were captured, 92.7% of which were *Lu. longipalpis*. Recapture rates of the latter ranged from 2-13% per group, differing between the sexes (7.6% M, 1.7% F). Half of the *Lu. longipalpis* were captured at 50m, 48% within 110-320 m, and nearly 3% at > 1/2 km from the release site. Sex differences in recapture site, distance, and direction were observed and their potential implications will be discussed. Our results suggest that the behavior of peridomestic *Lu. longipalpis* differs from that described before for neotropical sylvatic phlebotomines.

## POSTER I: KINETOPLASTIDA

60 MAXADILAN, A POTENT VASODILATOR FROM SAND FLY SALIVARY GLANDS. Lerner EA*, Vibbard DE, and Shoemaker CB. Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, MA; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Maxadilan, a vasodilator peptide present in salivary glands of the sand fly *Lutzomyia longipalpis*, aids this vector of leishmaniasis in obtaining a blood meal. This peptide was recently isolated and its corresponding gene cloned. The gene for this potent vasodilator has now been expressed in *E. coli* in order to obtain material for functional studies. We are in the process of obtaining the homologous gene from other sand flies and of making mutant recombinant maxadilans in order to study structure-function relationships. It appears that the cysteine residues near the N-terminus may be less important than the amino acids near the C-terminus of the peptide. Laser-doppler blood flow studies of the erythema induced by full-length recombinant maxadilan following injection into the skin of a volunteer reveals that is is active at the five femptomole level, confirming that maxadilan is the most potent vasodilator peptide known. It is possible that this peptide and other pharmacologically vasoactive molecules from

arthropod salivary glands may have therapeutic uses. In addition, as sand fly salivary gland extracts have been shown to be important in the transmission of leishmaniasis, the possibility of using maxadilan as a vaccine against this disease can now be evaluated.

61 TRANSFORMATION FACTORS INDUCING *LEISHMANIA* AMASTIGOTE FORMATION IN A CELL-FREE MEDIUM. Mohareb EW*, Mikhail EM, and Mansour NS. US Naval Medical Research Unit No. 3, Cairo, Egypt.

Environmental cues for transformation of *Leishmania* promastigotes to amastigotes are not fully understood. Although elevated temperatures induce transformation, resulting amastigotes fail to survive and multiply in culture media. A cell-free cultivation system was devised to investigate other determinants of amastigote transformation. Promastigotes of *L. major* were inoculated into paired sets (1-4) of flasks containing RPMI 1640 with 10% fetal calf serum: set 1 contained no additional supplements; set 2 was supplemented with a mixture of ATP, glutathione (reduced), cysteine-HCl and ascorbic acid; set 3 was supplemented with U 937 macrophage cell line lysate; and set 4 was supplemented with the macrophage spent medium. One flask of each set was incubated at 24°C while the other was incubated at 37°C. Two days post-inoculation, promastigotes appeared less slender and had shorter flagella in flasks 2 and 3 incubated at 24°C, and in all flasks incubated at 37°C. Six days later, transformation to amastigotes with typical morphology (oval, ≤ 5 μm in length, aflagellated) was complete in all flasks. Factors other than or in addition to temperature may play an important role in the transformation process. Work is in progress to propagate the transformed amastigotes axenically to produce pure antigens for diagnostic purposes.

62 STUDIES ON THE INTRACELLULAR CALCIUM HOMEOSTASIS IN *TRYPANOSOMA BRUCEI BRUCEI*. Vercesi AE*, Docampo RE, and Moreno SN. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; and Departamento de Bioquimica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil

The permeabilization of *Trypanosoma brucei* procyclic trypomastigotes with digitonin allowed the study of $Ca^{2+}$ fluxes between intracellular organelles in situ and the determination of the mitochondrial membrane potential using safranine O. The cytosolic $Ca^{2+}$ concentration in the intact cells was determined using fura-2. When these cells were permeabilized in a reaction medium containing MgATP, succinate and 3.5 μM $Ca^{2+}$, they lowered the medium $Ca^{2+}$ concentration to the submicromolar level (0.05-0.1 μM), a range which correlates favorably with that detected in the intact cells with fura-2. The presence of 1 μM FCCP decreased by about 60% the initial rate of $Ca^{2+}$ sequestration by these permeabilized cells but did not change the $Ca^{2+}$ set point. This FCCP-insensitive calcium uptake, certainly represented by the endoplasmic reticulum, was completely inhibited by 500 μM vanadate. On the other hand, when vanadate instead of FCCP was present, the initial rate of $Ca^{2+}$ accumulation was decreased by about 40% and the $Ca^{2+}$ set point was increased to 0.5-0.6 μM. The succinate dependence and FCCP-sensitivity of the later calcium uptake indicate that it may be exerted by the mitochondria. This interpretation was further supported by the decrease in mitochondrial membrane potential caused by $Ca^{2+}$ additions. The combined use of FCCP and the calcium ionophore A23187 indicated that mitochondria in situ contained virtually no $Ca^{2+}$ while the nonmitochondrial pool contained a high Ca2+ concentration (about 61 nmol $Ca^{2+}$/mg protein). Despite the presence of inositol phosphates in these protozoa, as determined by [$^3$H]inositol incorporation, no IP3-sensitive $Ca^{2+}$ pool could be detected.

63 IMMUNOTHERAPY AND CHEMOTHERAPY OF MUCOCUTANEOUS AND DISSEMINATED CUTANEOUS LEISHMANIASIS IN A HORSE IN BRAZIL. Barbosa-Santos EG*, Marzochi MC, Urtado W, Queiros F, and Chicarino J. Biological Sciences Department, National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; Equine Therapeutic Clinic, Evandro Chagas Hospital, Rio de Janeiro, Brazil.

Immunotherapy and chemotherapy for mucocutaneous leishmaniasis were conducted in a pregnant horse which had disseminated leishmanial ulcers and cutaneous nodules on both hind footpads and on genital and nasal mucosa. Infection was naturally acquired in a rural area of Brazil (Sapucaia, Rio de Janeiro). Leishmaniasis was confirmed by nodule and ulcer biopsy. The horse was IFA/IgG (1:160) and skin test positive. After birth immunotherapy (as proposed by Convit et al.) was initiated. A solution of 4.0 mg of BCG plus 2.0 mg of particulated antigen of *Leishmania braziliensis* was injected i.d. at 5 points on the ventral abdomen in two series with 30 days interval between series. By the second series, the infection had disseminated by superficial lymphatics and additional nodules were noted. A chronic inflammatory infiltrated with granulomas, necrotic foci, and numerous amastigotes forms were observed in sections. The antibody titer increased to 1:320. Because of the apparent failure of the immunotherapy, treatment was changed to the WHO recommendation to human mucocutaneous lesions. The animal received two series of i.m. 20 mg $Sb^5$ of N-methyl-glucamine (Glucantime, Rhodia)/kg/day for 35 days. Following the first series the lesion involuted. Histopathology of ulcers and nodules showed marked reduction of the inflammatory infiltrate. The indirect immunoperoxidase reaction was positive only in the area around blood vessels. No amastigote were observed. By the completion of the second series all ulcers had regressed except for several small ones on the nasal mucosa. The colt was serologically postive at birth (1:40) and titers rose to 1:80 during its first month. This may be maternal antibody. However, both animals were evaluated at one year and no lesions were noted. Although this represents a single case in a horse, it suggests that immunotherapy may not be as effective as chemotherapy for the treatment of disseminated leishmaniasis.

64 PARTIAL PURIFICATION AND CHARACTERIZATION OF S-ADENOSYLMETHIONINE SYNTHETASE OF LEISHMANIA. Nolan LL*, Tang S and Sufrin JR. School of Public Health, University of Massachusetts, Amherst, MA; School of Public Health, University of Massachusetts, Amherst, MA and Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, NY.

Leishmaniasis is considered one of the major public health problems currently affecting humanity. The purpose of this study is to aid in the development of an effective, non-toxic treatment. The involvement of S-adenosylmethionine (AdoMet) as a methyl donor in transmethylation reactions, and its critical role as an aminopropyl donor in polyamine biosynthesis combined with other regulatory functions make it a pivotal molecule in cell metabolism. Since our previous studies have shown that AdoMet analogs are potent inhibitors of parasite growth, we have partially purified and characterized the AdoMet synthetase for the purpose of chemotherapeutic exploitation. Two isozymes of AdoMet synthetase have been isolated and partially purified from *Leishmania mexicana*. The purification procedures involve ammonium sulfate fractionation, DEAE cellulose chromatography and Sephacryl S-200 gel filtration. Thermal stability and lability of the isozymes have been studied. The apparent molecular masses, estimated by Sephacryl S-200 are 91 kDa for isozyme a and 44 kDa for b. The inhibitory effect of dimethylsulfoxide and some amino acid analogs on the activities of both a and b isozymes were studied. Differences between the inhibitory properties of parasite and host enzymes supports the rationale that selective inhibitors of the AdoMet synthetase might have chemotherapeutic utility.

65 ANTI-LEISHMANIAL ACTIVITY OF ETHER ANALOGUES OF LYSOPHOSPHOLIPIDS. Fiavey NP and Ngwenya BZ*. Hahnemann University School of Medicine, Philadelphia, PA.

The inflamed lesions of normal and parasitized tissues release decomposed products of membranous lipids, lysophospholipids and alkylglycerols, which have been found to be potent macrophage activating agents and cytotoxic to certain tumor cells and bacteria. These findings and the elucidation of the lipid composition and metabolic pathway of lipid metabolism in *Leishmania* parasites, has prompted us to examine the anti-leishmanial effects of alkyl-lysophospholipid compounds. When promastigotes of three strains of *Leishmania, L. major, L. mexicana mexicana,* and *L. braziliensis panamensis* were treated with 5-10.0 μg/ml of the Sn conformation of ET-18-$OCH^3$-choline (Sn-1-octadecyl-2-methyl -glycero-3-phosphocholine) a 90-100% leishmanicidal effect was observed at 3 days of treatment as determined by microscopic quantification using typan blue exclusion test. The leishmanicidal mechanism appeared to be via the suppression of DNA and protein synthesis as demonstrated by significant inhibition of $^3H$-thymidine and L-leucine incorporation by the organisms. In addition, promastigote killing was accompanied by distinct morphological changes characterized by conversion from their slender to round forms with reduction in the length of the flagella. Furthermore, macrophages activated by these agents acquired the potential to significantly reduce intracellular multiplication of amastigotes of *L. major* and *L. donovani*. These preliminary results suggest that alkyl-lysophospholipids are potential chemotherapeutic and immunotherapeutic agents for the treatment of leishmaniasis.

66 AN ENZYME IMMUNOASSAY AND CONFIRMATORY TEST FOR THE SPECIFIC AND SENSITIVE DETECTION OF ANTIBODY TO *TRYPANOSOMA CRUZI*. Pan AA*, Brashear RJ, Winkler MA, and Lee H. Transfusion Diagnostics, Abbott Laboratories, North Chicago, IL.

A first generation EIA was developed for Latin America to detect antibody to *Trypanosoma cruzi*. This 90 minute total room temperature assay has an initial reactive rate of 1.3% and repeat reactive rate of 1.2%. A second generation test, targeted for use in low endemic regions of North America, requires a 10- to 40-fold improvement in specificity. The repeat reactive rate is to be reduced to about 0.03% while maintaining close to 100% sensitivity. To optimize for specificity varying conditions of antigen concentration, specimen dilution, incubation time/temperature, and goat anti-human IgG/HRPO conjugate concentration reduced false positive samples. Preliminary data from a low risk US population (N=1135) yields: The Latin America protocol had 22 repeat reactive samples (specificity 98.06%) compared to an optimized protocol with 1 borderline repeat reactive sample (specificity 99.91%). Sensitivity remained at 100% in a positive population (N=115). For the development of a confirmatory assay, two candidate antigens have been selected. One antigen, from the amastigote stage of the organism, was purified to homogeneity by affinity and HPLC anion-exchange chromatography. The second antigen, from the epimastigote stage, was also purified by affinity chromatography. Both antigens have a sensitivity of 100% on xenodiagnosed positive samples and show no cross-reactivity with sera from patients having malaria, toxoplasmosis, leishmaniasis, or schistosomiasis. A radioimmunoprecipitation test will also be used to confirm suspect positive samples.

67 EVALUATION OF A RAPID DOT ELISA FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS. Thai L, Geronimo SE, Filho JH, Ponce E, Ponce CA, Evans TG*, and Wright JD. Division of Infectious Diseases, University of Utah, Salt Lake City, UT; Univ. Fed. de Natal, Natal, RGN, Brazil; Univ. Fed. do Ceara, Fortaleza, CE, Brazil; Lab. Central, Min. de Salud, Tegucigalpa, Honduras; and Gull Foundation for Medical Research, Salt Lake City, UT.

Although serological tests for visceral leishmaniasis (VL) have markedly improved in sensitivity and specificity in the past decade, a user-friendly, field-oriented, rapid test is still not available. We have adapted a commercial dot ELISA (DetectaDot®) which uses optimized and pre-diluted, color coded 1) diluent, 2) wash solution, 3) anti-human IgG-peroxidase, and 4) insoluble substrate. Ten μg of protein of a sonicated, 20,000 g supernatant of *Leishmania donovani chagasi* is placed on nitrocellulose and backed

with an absorbent material inside a plastic 35 mm slide frame. After serum is obtained the test takes a maximum of five minutes to run, requires only visual reading, and interpretation is facilitated with the use of negative and positive control wells run on the same module. These kits were evaluated on various normals, disease controls, and biopsy-proven cases of VL in Honduras and two locations in Brazil. Sensitivity was 69/72 (96%) in proven VL. Serum from only 2/40 normal controls from endemic areas were read as equivocal or weak positive reactions (but less reactive than VL cases) by the technician. In Brazil only 2/10 (20%) of cutaneous cases were positive, but 32/45 (71.1 %) were positive in Honduras. All 7/7 cases of mucocutaneous leishmaniasis were positive. There was light color development in 3/8 patients with proven Chagas disease, and in 4/17 patients (24%) with potential cross-reactive diseases (tuberculosis, leprosy, and malaria). The dot ELISA (DetectaDot) format holds promise as a simple screening device for field use by non-technologists in the diagnosis of visceral leishmaniasis.

68 CANINE LEISHMANIASIS IN A U. S. MILITARY WORKING DOG. Nuzum EO*, Wempe JM, Wilber J, Grogl M. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D. C.; U. S. Army Regional Veterinary Laboratory, Ft Sam Houston, TX; Kirtland Air Force Base Veterinary Services, and William Beaumont Army Medical Center, El Paso, TX.

Canine leishmaniasis is often regarded a lethal, incurable disease. In some countries where this disease is endemic, infected dogs are euthanized because they may represent a reservoir for human infection. Because of their value and the public health implications, Military Working Dogs (MWD) returning from Desert Storm/Shield have been closely monitored. Protocols were developed to systematically screen dogs by indirect immunofluorescence to compare Leishmania antibody titers in paired pre- and post-deployment sera. Dogs with seroconversion (a four-fold or greater titer increase) were further analyzed by Polymerase Chain Reaction (PCR), Direct Immunofluorescent Monoclonal Antibody assay (DIFMA), and culture for diagnostic confirmation. Of 53 dogs tested serologically, two were positive. Further studies of liver and skin biopsies from one of these dogs using DIFMA, *in vitro* isolation of the parasite, and non-radioactive, immunochemiluminescent PCR confirmed the presence of parasites in this dog. Identification by isoenzyme electrophoresis indicated that the parasite was *L. tropica*. Except for a draining skin lesion on the leg, no clinical signs have been present. This study demonstrates that serology can be a powerful epidemiological and diagnostic technique for leishmaniasis when paired sera are available. A clinical history and the results of treatment with a developmental drug (WR- 6026) will be presented. It is hoped that this combination of early diagnosis quickly followed by novel chemotherapeutic intervention will show that canine leishmaniasis is a curable disease.

## POSTER I: FILARIA

69 "TRICKLE" INFECTIONS IN JIRDS: CELLULAR AND HUMORAL RESPONSES TO *BRUGIA PAHANGI*. Chisholm ES* and Lammie PJ. Parasitic Disease Branch, Centers for Disease Control, Atlanta, GA.

Humans residing in areas endemic for lymphatic filariasis are exposed to infective stage larvae in small numbers over long periods of time.To develop an animal model reproducing this exposure pattern, pilot studies were initiated using inbred jirds (*Meriones unguiculatus*) inoculated subcutaneously with 5 *Brugia pahangi* infective stage larvae (L3) every 2-3 weeks over a 353 day period. Blood was drawn from each jird at 12-14 day intervals by retro-orbital puncture to check for the presence of microfilariae (mf), and antibodies using ELISA, FAST ELISA, and immunoblot. Lymphocyte blastogenesis was periodically performed to monitor cellular responses. Adult worms were detected by microscopic examinations of heart, lung, and lymphatics of the testes. Microfilaremia developed slowly with only 20% of jirds demonstrating *mf* through 75 L3 and 9 months post infection. However, 100% of the animals exhibited mf after receiving 90 L3 at 13 months post infection. Antibody responses in the ELISA and FAST ELISA were detectable in greater than half the animals after 10 L3 were administered, plateaued at 20 L3, and

then were essentially unchanged. Proliferative responses were detected at a dosage of 20 L3 and peaked at 65 L3. These initial findings indicate that this model may be useful in studying the evolution of antifilarial immune responses and their regulation.

70 *ONCHOCERCA VOLVULUS* PARAMYOSIN: CHARACTERISATION OF A FULL-LENGTH cDNA, MOLECULAR CLONING AND EXPRESSION. Dahmen A*, Gallin M, Schumacher M, and Erttmann KD. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Federal Republic of Germany.

Paramyosin is a filamentous muscle protein of invertebrates which is immunogenic in filarial infections and is a potential vaccine candidate in murine infections with *Schistosoma mansoni* and *Brugia malayi*. We have isolated a full-length cDNA clone from an *Onchocerca volvulus* lambda ZAP expression library containing a 2712 bp insert. Analysis of the 5` terminus of the clone, obtained by primer extension of total *O. volvulus* RNA, tailing and PCR amplification, revealed a 22 nucleotides spliced leader sequence followed by a 36 nucleotides untranslated region and the coding region. Expression of the full-length cDNA clone as a fusion protein with beta-galactosidase and further purification yielded a 97 kD protein which reacts with rabbit antisera to *O. volvulus* as well as *S. mansoni* paramyosin in immunoblots. Sera from persons with generalised onchocerciasis or sowda showed reactivity to the recombinant protein. These results indicate that *O. volvulus* paramyosin may undergo splicing and that the protein is immunogenic in human onchocerciasis.

71 DAYTIME IDENTIFICATION OF PATIENTS WITH PATENT BANCROFTIAN FILARIASIS WITH A RECOMBINANT FILARIAL ANTIGEN. Dissanayake S*, Xu M, Zheng HJ, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Guizhou Provincial Institute of Parasitic Diseases, Guiyang, People's Republic of China.

In an attempt to develop a diagnostic test for lymphatic filariasis that eliminates the need to collect night blood samples to detect microfilaremic persons, we have cloned and bacterially expressed a filarial antigen (SXP-1) that is preferentially recognized by sera from patients with microfilaremia due to *Wuchereria bancrofti*. Sera from 28/38 microfilaremic patients and from 3/40 amicrofilaremic donors with other manifestations of lymphatic filariasis reacted with the SXP-1 antigen. Anti-SXP-1 antibodies were restricted to the IgG4 subclass. Seropositivity was age-related: only 50% of microfilaremic children below the age of 12 were positive compared to >90% microfilaremic adults. Anti-SXP-1 antibody levels correlated with antibody titers to crude *B. malayi* ES antigens and with levels of circulating HC11 antigens. Levels of anti-SXP-1 antibodies declined in most patients who became amicrofilaremic after treatment with DEC or Ivermcetin. We conclude that a diagnostic test based on the SXP-1 antigen may be a replacement for night blood filming to detect bancroftian microfilaremia during population-based surveys. The test is superior to antigen detection assays in terms of cost, speed and reproducibility and can be performed with daytime serum samples.

72 A HIGHLY ENDEMIC FOCUS OF BANCROFTIAN FILARIASIS IN THE NILE DELTA: ENVIRONMENTAL AND ENTOMOLOGICAL STUDIES. Gad AM*, Sabry Z, Reiad IB, Ramzy RM, Weil GJ, Buck AA, and Faris R. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

Kafr Tahoria is a village with high filariasis endemicity 30 km NE of Cairo. Survey methods and basic epidemiological findings in this village are described in a companion abstract. Preliminary examination of survey results indicated that filariasis was not uniformly distributed in the village. Therefore, the objective of this study was to identify risk factors for filariasis that might account for this finding. Filariasis (infection and disease) was much more common in individuals residing in houses adjacent to

agricultural land or vacant areas (periphery) than in those living in core areas of the village (prevalence rates 34.6% in periphery vs. 18.3% in core, difference significant by Chi square, P<.001, odds ratio 2.8). This finding is independent of age and family size. Entomological studies are in progress. To date, data from 18 outdoor and indoor sampling sites collected over 28 weeks have been analyzed. Mosquito densities, infection, and infectivity rates for *Culex pipiens*, the main filaria vector in the village, were all significantly higher in the periphery than in core sites. Additional studies are needed to further define environmental factors that might explain these entomological findings and the interesting distribution of filariasis in this village.

73 A MODEL FOR THE SEASONAL TRANSMISSION OF DRACUNCULIASIS IN A RURAL COMMUNITY. Heuschkel C* and Awerbuch T. MPH Program, Harvard School of Public Health, Boston, MA; and Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Dracunculiasis is one of the most easily preventable parasitic diseases, and eradication programs are under way in Asia and Africa. However, in West Africa, the disease is still common. The worm generally breaks through the skin during the rainy season, the most labor intensive season of the year for farming communities. Superinfection of the resulting wounds handicaps a person for months, and the morbidity is greatest among adults, having a strong negative impact on crop production and the economic standing of the family. Transmission of the disease has never been studied longitudinally in time, and little is known about transmission parameters and the importance of temporal immunity to reinfection. Accordingly, a model has been developed that predicts incidence and prevalence of disease in a community as a function of seasonal variability in the number of infective larvae ingested and the probability of one ingested larva developing into an adult worm and causing a skin lesion. Transmission parameters were estimated by fitting the model to published data from West Africa. Implications of the model concerning temporal immunity to reinfection are discussed. The model will be used to predict transmission in villages where control measures are substandard.

74 *ONCHOCERCA* MICROFILARIAE STIMULATE SUPEROXIDE PRODUCTION BY EOSINOPHILS AND SECRETE SUPEROXIDE DISMUTASE *IN VITRO*. James ER*, Tuxworth WJ, Callahan HL, and Crouch RK. Department of Ophthalmology, Medical University of South Carolina, Charleston, SC; and Department of Biological Chemistry, Harvard University Medical School, Boston, MA.

*Onchocerca* microfilariae (mf) stimulate marked tissue eosinophilia in naturally infected humans and large animals and in experimentally infected mice. *In vitro*, eosinophils have been shown to adhere to mf and to degranulate in an antibody dependent cellular cytotoxicity (ADCC ) reaction at a high cell to target ratio. This host cell reaction is unusual *in vivo* and mf appear to be able to tolerate the presence of tissue eosinophils. Eosinophils are capable of respiratory burst generation of superoxide radical. The enzyme superoxide dismutase is present at a relatively high concentration in mf crude homogenate (approx 112 U $mg^{-1}$ protein). This study aimed to investigate the oxidant-antioxidant interaction between mf and eosinophils. *O. cervicalis* mf were harvested from the skin of naturally infected horses by standard methods and cleaned on a DE 52 minicolumn. Eosinophils from mf+ horses were purified from peripheral blood by percoll centrifugation. $12 \times 10^6$ mf were cultured in RPMI medium for 4 sequential 1 hour periods with media changes, centrifugation and microscopic viability assessment at each hr. Viability remained >97%. Mf-conditioned medium was centrifuged to remove any mf, concentrated by ultrafiltration and assayed for superoxide dismutase (SOD) activity. SOD was released throughout the 4 hr period by mf but total protein release was below detection by Bradford. Superoxide production by eosinophils was assayed by iodonitrozolium violet reduction with an ELISA reader. Superoxide production was maximal in the presence of mf with infected-horse sera and increased linearly with time

over 8 hr. Addition of endogenous SOD and other antioxidants/radical scavengers did not affect reaction OD.

75 ANTIGENICITY AND B CELL EPITOPES OF A PROTECTIVE FILARIAL ANTIGEN IN HUMAN BANCROFTIAN FILARIASIS. Kazura J*, Hazlett F, Nilsen T, Alpers M, and Day K. Case Western Reserve University, Cleveland, OH; Papua New Guinea Institute of Medical Research, Goroka, New Guinea; and Imperial College, London, UK.

Previous work indicates that a fusion protein (fp) corresponding to amino acids (aa) 1-479 of a 62-kD Ag expressed by *Brugia malayi* microfilariae (mf) and adult worms induces partial resistance to mf challenge in mice. The objective of the current study was to evaluate the B cell antigenicity of this recombinant protein in human Bancroftian filariasis. Sera pooled from 100 residents of an endemic area of Papua New Guinea contained high levels of IgG3 Ab (1/1280 dilution) to the aa1-479 fp, low levels of IgG1 and IgG4 (1/40 - 1/80), and no Ag-specific IgG2 or IgE. No differences ($p>0.05$) in the levels of fp-specific IgG3 were observed between a group of 14 mf-/low phosphocholine antigenemic persons vs 11 mf+/high phosphocholine antigenemic persons (mean ± SE 54 ± 9 vs. 64 ± 8 units IgG3/ml, respectively). B cell epitopeswere mapped using fps truncated from the C-terminus of the filarial Ag. Relative to Ab reactivity to aa 1-479, IgG3 to aa 1-437 was higher (ratio of reactivity to aa 1-479: aa 1-437 = 1.37, $p<0.001$) >90% of IgG3 Ab bound to constructs including aa 1-302 and 1-248, consistent with epitope predictions by Chou-Fasman analysis. These results indicate that this recombinant polypeptide is antigenic in *W. bancrofti*-infected humans and that persons categorized according to parasitologic status do not react differentially to the molecule interms of humoral immunity.

76 A RAPID DNA ASSAY FOR THE NON-RADIOACTIVE DETECTION OF *BRUGIA* MICROFILARIAE IN BLOOD SAMPLES Lizotte MR*, Poole CB, and Williams SA. Department of Biological Sciences, Smith College, Northampton, MA; and New England Bioloabs, Beverly, MA.

Lymphatic filariasis caused by *Brugia* nematode worms is one of the most pervasive diseases in tropical regions of the world. There is an obvious need for rapid, species-specific diagnosis of infected patients, since traditional methods are often slow and tedious. The microfilarial stage of the parasite can be readily obtained by taking a small blood sample (finger-stick) from infected individuals, and is therefore an ideal target for diagnosis. A species-specific *B. malayi* DNA probe was developed and synthesized by S. Williams, C. Poole, and D. Landry with an extended biotin tail. This probe can be used in a non-radioactive assay to detect *Brugia* microfilariae in blood samples. To test the new assay, mouse blood samples were mixed with known numbers of microfilariae and digested with a proteinase K cocktail. The samples were centrifuged and the supernatant spotted onto nitrocellulose filters using a dot blot apparatus. Using a biotin-streptavidin alkaline phosphotase detection system, the oligonucleotide probe is used to detect the microfilariae DNA on the filter. The DNA is denatured, neutralized and baked onto the filter. Results indicate that this non-radioactive method is fast, species-specific, and sensitive for the diagnosis of *Brugia* infections. As few as ten microfilariae can be detected easily without PCR. With PCR, samples with a single worm can be detected. We are currently using this assay to detect microfilariae in human blood samples collected in the field. This study demonstrates the development of an effective, non-radioactive detection system which is simple, fast, inexpensive, and species-specific. In the coming months, this assay will also be tested on blood samples collected in Egypt using newly developed *W. bancrofti* specific probes.

77 POTENT MACROFILARICIDAL ACTIVITY OF THE BENZIMIDAZOLE CARBAMATE, UMF-078, AGAINST *BRUGIA PAHANGI* AND *ACANTHOCHEILONEMA VITEAE* IN JIRDS. McCall JW*, Dzimianski MT, Elslager EF, Townsend LB, Wise DS, Jun JJ, and Supakorndej P. Department of

Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA ; Elslager & Associates, Ann Arbor, MI; and College of Pharmacy, University of Michigan, Ann Arbor, MI.

For detection and early evaluation of macrofilaricidal compounds for human onchocerciasis and lymphatic filariasis, we use either single or dual infections of intraperitoneally (IP) transplanted *B. pahangi* (BP) adult worms and subcutaneously (SC) transplanted *A. viteae* (AV) adult worms in jirds. UMF-078 and its dihydrochloride monohydrate salt, UMF-289, have emerged as potentially valuable compounds. When given SC for 5 days (x5) at dosages of 12.5 mg/kg/day (MKD) or higher, UMF-078 was 100% effective against BP; at 6.25 MKD, it killed 92%; it was ineffective at lower dosages. It was 88% effective against AV when given SC at 25.0 MKD x 5. UMF-289 was 100% effective against BP when given SC at dosages of 12.5 MKD x 5 or higher. At 6.25 MKD x 5 (SC), it killed 96% of the BP and 100% of the AV. At 3.13 MKD x 5 (SC), it was 84% effective against BP and 69% effective against AV; it was ineffective at lower dosages. A single dose of 25.0 mg/kg (SC) was curative for BP and killed 96% of the AV. When given orally at 75 or100 MKD x 5, UMF-289 killed 30-32% of the BP and 80-83% of the AV. Against lymphatic infections of BP in jirds, UMF-289 was 73% effective when given SC at 12.5 MKD x 5.

78 SIGNIFICANCE OF TRANSGLUTAMINASE-CATALYZED REACTIONS IN *BRUGIA MALAYI* FILARIAL PARASITES. Mehta K*, Rao UR, Vickery AC, Fesus L. University of Texas MD Anderson Cancer Center, Houston, TX; College of Public Health, University of South Florida, Tampa, FL; and University Medical School of Debrecen, Debrecen, Hungary.

Identification of biochemical pathways or enzymes that are parasite-specific and vital for their growth and survival will be useful to pinpoint the possible sites of attack for novel inhibitors. Recently we described the presence of a novel protein cross-linking enzyme, transglutaminase (pTGase), in adult worms of filarial parasites. The enzyme activity seemed to be essential for growth, development and survival of the parasites. Partial purification by immunoaffinity column chromatography, confirmed that pTGase is about 25 kDa protein and unlike other mammalian transglutaminases, does not require $Ca^{+2}$ for its catalytic activity. Analysis of adult worm extracts by HPLC for e-(g-glutamyl) lysine bonds, a marker for physiologically active enzyme, revealed the presence of a significant level of the isopeptide. At least six proteins in adult worms and five proteins in peritoneal exudates from jirds, the site where adult worms reside, were identified that could serve as -acyl donor substrates for pTGase. Enzyme-catalyzed covalent conjugation of these substrate proteins was observed in adult female worms which were subsequently incorporated into the sheath/cuticle of the *in utero* developing microfilariae as determined by puls - and chase of $^{35}S$-labelled proteins in live worms followed by an autoradiography. From these results we conclude that pTGase-mediated covalent conjugation of host/parasite proteins may be an essential step for assimilation of proteins into newly formed sheaths/cuticles during *in utero* development of microfilariae and that pTGase-catalyzed incorporation of the host's proteins into the sheath/cuticle may be an important mechanism of immune invasion by the parasites.

79 INTRACELLULAR DEVELOPMENT OF FILARIAE INFLUENCED BY MOSQUITO HOST TISSUE. Nayar JK*, Bradley TJ, LeFevre LC, and Knight JW. Institute of Food and Agricultural Sciences - University of Florida, Medical Entomology Lab, Vero Beach, FL; and Department of Ecology and Evolutionary Biology, University of California, Irvine, CA.

Filarial larvae develop intracellularly in specific tissues of their mosquito hosts. Earlier, we demonstrated that the factors which control development of *Dirofilaria immitis* microfilariae to the infective stage are uniquely associated with the Malpighian tubules (host tissue) of genetically selected susceptible and refractory strains of *Aedes aegypti*. Subsequently, we demonstrated that one-day old intracellularly lodged larvae of *Brugia malayi* and *B. pahangi* develop *in vitro* in excised thoraces (host tissue) of susceptible mosquitoes. We then reasoned that if the factor controlling refractoriness is dependent on a

source outside the thoracic muscles, then thoraces maintained *in vitro* should not retain their refractory characteristics. In the present study, we simultaneously *in vitro* cultured *B. malayi* infected excised thoraces (24 hr after infection) of selected susceptible and refractory strains of *Ae. aegypti* and *Anopheles quadrimaculatus* and demonstrated that microfilariae develop to the infective stage in excised thoraces of susceptible mosquitoes. These results gave additional evidence to the premise that the factors which control development of microfilariae of *B. malayi* to the infective stage larvae are present in thoracic muscles of genetically selected susceptible and refractory strains of their mosquito hosts. Intracellular melanization of $L_1$ larvae or *An. quadrimaculatus* previously demonstrated *in vivo* did not occur on $L_1$ larvae *in vitro* in mosquito thoraces excised 24 hr post-infection. These studies suggest that mechanisms which control refractoriness and melanization of *B. malayi* larvae in thoraces of *An. quadrimaculatus* are two distinctly separate phenomena. Ultrastructure studies of *in vivo* and *in vitro* reared *B. malayi* larvae in mosquito hosts are being conducted to examine these phenomena.

80 STRAIN DEPENDENT DIFFERENCES IN LYMPHATIC DILATATION DUE TO BRUGIAN INFECTION IN THE MOUSE. Nelson FK*, Shultz LD, Greiner DL, and Rajan TV. Department of Pathology, University of Connecticut Health Center, Farmington CT; Department of Medicine, University of Massachussets Medical Center, Worcester MA; and The Jackson Laboratory, Bar Harbor, ME.

We have been working toward the establishment of immunodeficient rodent models for human filarial parasites. Vickery and co-workers and we have independently noticed that there is a difference in the parasite burden and in the amount of the early lymphatic dilatation depending on the strain of inbred mouse used. When injected with *Brugia malayi* L3 larvae, the worm burden is higher and the lymphatic dilatation is greater in C57BL/6 nude mice than in Balb/c nude mice. We have injected *B. malayi* L3 larvae into (B6 nu/nu x BALB/c nu/nu) $F_1$ mice, and are following the results to determine if the parasite burden and pathogenicity will be similar to one of the two parental strains. We have also observed that there is a difference in the pathogenicity and worm burden in male (B6 x Balb/c) $F_1$ nude animals compared to female mice. Female $F_1$ mice have a lower parasite burden and have less lymphatic dilatation than male $F_1$ mice. In addition, the adult worms produced no microfilariae in the female $F_1$ nude mice which may be a reflection of the very low adult worm burden. These observations suggest that immunodeficient mice are a suitable model for the genetic dissection of susceptibility to Brugian infection.

81 ONCHOCERCIASIS IN COLOMBIA? AN UPDATE ON THE LOPEZ DE MICAY FOCUS. Palma GI*, Travi BL, Satizabal JE, Martinez F, and Smith DS. Department of Microbiology, School of Health Sciences, Universidad del Valle, Cali, Colombia; Fundacion CIDEIM, Cali, Colombia; Department of Opthalmology, School of Health Sciences, Universidad del Valle, Cali, Colombia; and Fulbright Scholar, School of Medicine, University of Colorado, Boulder, CO.

Human onchocerciasis was first described in Colombia in 1965 in a small focus in Lopez de Micay on the Pacific Coast. Subsequent follow up of the focus 12 years later showed that infection prevalence had fallen from 15 to 7.5%. Because no patients were reported since then, the focus was considered extinguished until 1989, when a child with ocular keratitis was referred from Lopez de Micay to the University Hospital in Cali. Onchocerciasis was confirmed by skin snip examination. In July 1989 a multidisciplinary team conducted a new survey in the area. Skin biopsies were obtained from 170 individuals. Prevalence of infection detected by skin snip examination was 4.1% (7/170). Ten percent of the surveyed males and 0.9% of females had detectable microfilariae (mf) in skin. MF density in skin varied between 0.5 mf per milligram of skin to 47 mf/mg and was directly related with age of the patient. Neither palpable subcutaneous nodules nor dermal alterations attributable to the parasite were detected in any of the patients. Ocular pathology was found in two patients, consisting of bilateral

keratitis and retinal degeneration, respectively. Simuliid activity at the time of the survey was very low, and collection was not attempted. We hypothesize that active transmission may be taking place further upstream where vector activity is greater. An increase in human settlements in the area is expected due to the future construction of hydroelectric plants and connecting highways. Questions are raised on the impact of the immigration of naive population on this hypoendemic focus as well as the existence of undetected foci between the latter and the Ecuadorian onchocerciasis foci.

82 A PRELIMINARY STUDY OF THE FEMALE REPRODUCTIVE SYSTEM OF *SCHISTOSOMA JAPONICUM* AT ULTRASTRUCTURAL LEVEL. Zhou SL*, Yang MX, Kong CH, Li Y, Liang HL, Fang P, and Lei SL. Department of Parasitology, Hubei Medical College, Wuhan, People's Republic of China; and Laboratory of Electron Microscopy, Hubei Medical College, Wuhan, People's Republic of China.

A preliminary transmission ultrastructural study of reproductive system of *Schistosoma japonicum* including the oocyte, vitelline cell, oviduct and uterus is described. The morphological characteristics of meiosis in preleptotene, pachytene,and anaphase of both oocyte and nearly matured ovium are described. The common component of the cytoplasm is the presentation of the cortical granule. Further, the ultrastructure of laminated immature and mature vitelline cells and the ciliated oviduct and uterus is presented. The origin, structure, and function of the cortical granules as well as the capacity for production of the shell precursor as related to variations of the morphology of matured vitelline cell is discussed.

## POSTER I: HELMINTH BIOLOGY

83 USE OF SOIL TYPE AND LANDSAT MSS SATELLITE DATA IN A GEOGRAPHIC INFORMATION SYSTEM TO ASSESS RISK OF FASCIOLIASIS IN CATTLE IN LOUISIANA Malone JB*, Fehler DP, and Loyacano AF. Veterinary Microbiology and Parasitology, Louisiana State University, Baton Rouge, LA; Landscape Architecture, Louisiana State University, Baton Rouge, LA; and Dean Lee Research Station, Louisiana State University, Alexandria, LA.

A geographic information system (GIS) was constructed in an ERDAS environment using soil type maps from the USDA Soil Conservation Service, LANDSAT satellite multispectral scanner data (MSS), boundaries of 25 study farms and slope and hydrology shown in a 2 quadrangle (USGS, 7.5') area in the Red River Basin near Alexandria, LA. Fecal sedimentation examinations were done in the fall of 1989 and/or 1990 on 12-15 random samples from each herd. Fecal egg shedding rates for *Fasciola* ranged from 10-100% prevalence and 0.3-21.7 eggs per 2 grams (EP2G). For *Paramphistomum*, a rumen fluke also transmitted by *Fossaria bulimoides* but not affected by flukecides, egg counts ranged from 0.1-91% and 0.1-42.8 EP2G. Soil types present ranged from sandy loams to hydric, occasionally flooded clays. Herd *Paramphistomum* egg shedding rates increased with the proportion of hydric clays present, adjusted for slope and major hydrologic features. *Fasciola* infection intensity followed a similar trend, but were complicated by differing treatment practices. Results suggest that earth observation satellite data and soil maps can be used, with an existing climate forecast based on the Thornthwaite water budget, to develop a second generation model that accounts for both regional climate variation and site-specific differences in fascioliasis risk based on soils prone to snail habitat.

84 INCIDENCE AND EFFECT ON COGNITIVE FUNCTION OF SUBCLINICAL *TOXOCARA* INFECTION IN INNER-CITY CHILDREN, CINCINNATI. Schantz PM, Addiss DG*, Succop PA, Fried JA, Wilson M, Roda S, and Bornschein RL. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Institute of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH.

Although serologic evidence suggests that children in the United States are commonly infected with *Toxocara spp.*, the age-specific incidence and long-term neurobehavioral consequences of subclinical infection are poorly understood. In a prospective study of the developmental effects of low-level lead exposure, a cohort of 241 children in inner-city Cincinnati were followed for a mean of 5.3 years with cognitive function tests, physical examinations, and periodic phlebotomy. An enzyme immunoassay with *Toxocara* excretory-secretory antigen was used to test sera for the presence of antibody to *Toxocara*. Overall, 47 children (19.5%) had one or more sera with an antibody titer of >1:16, for an incidence of 8.1 infections/100 children/year. The incidence increased from 1.7 infections/100 child-years among children aged 0-4 years to 11.9 among 5-6 year-olds. No children < 2 years old were infected; in contrast, increased blood lead levels were first detected at 6 months of age. In a preliminary analysis controlling for the effects of lead and other covariates, no significant differences in cognitive function were detected between children with serologic evidence of *Toxocara* infection and uninfected children. Additional risk factors for subclinical toxocariasis and temporal relationships between *Toxocara* antibody seroconversion and neurobehavioral changes are presented.

85 PREVALENCE OF INTESTINAL PARASITES IN GUATEMALA. Gonzalez-Camargo CL*. Central Laboratory Department. Division for Surveillance and Diseases Control, Ministry of Public Health, Guatemala.

The present study is part of the Surveillance Program conducted by the Central Laboratory Department of the Ministry of Public Health of Guatemala. The information has been collected by laboratories located in all Government State Hospitals and Health Units within the country. Since 1973, the Central Laboratory Department has used as a standardized format for submission of monthly data related to the diagnosis of intestinal parasites using the direct method. Data is tabulated and analyzed to determine prevalence within each country and state. For this study, laboratories of 22 State Hospitals and 52 Health Units reported the results of an average of 100,000 fecal samples/year. The prevalence of intestinal helminths has remained stable from 1986 to 1990. For example, *A. lumbricoides* has had a prevalence which has varied from 19.9 to 27.8%. Similar patterns have been noted for *Trichuris trichiura* and *Uncinaria*. Standardization of the diagnostic procedure appears to be important. In some states the prevalence of *A. lumbricoides* was 35% with direct method, but was higher using more sensitive techniques as Kato-Kats. The prevalence of intestinal helminths in Guatemala has not changed significantly in recent years. Such data serves as a base for surveillance and control programs of intestinal helminths which could be improved with appropriate treatment and health education programs.

86 *STRONGYLOIDES STERCORALIS*: THREE DIMENSIONAL ULTRASTRUCTURAL INVESTIGATION OF NEUROSENSORY AND ASSOCIATED CEPHALIC STRUCTURES OF FILARIFORM LARVAE. Ashton FT, Bhopale VM, Volk SW, and Schad GA*. University of Pennsylvania, Philadelphia, PA.

The filariform infective larva (L3) of *S. stercoralis* is environmentally resistant, initiates skin penetration, and migrates to the intestine. To understand these processes, we have studied its sensory neuroanatomy and the oral structures presumably involved in penetration and feeding. The L3 has 6 lips around a small, open mouth. A 3-dimensional reconstruction revealed 16 labial sensilla, including 6 internal and 6 external labial sensilla. There are 4 cephalic sensilla. None of these sensilla open to the outside. Consequently, they are unlikely to be chemosensory in function and must act as touch, stretch or thermoreceptors. The amphidial pores, occuring just behind the lateral lips, are nearly, but not totally, closed. The amphids contain 13 neurons each. Although their pores are small, they are the only chemosensory organs remaining open to the exterior and are, therefore, likely to function importantly in host penetration and migration. Behind the mouth, the oral cavity contains an occulding, electron dense, plug, and like similar plugs in other environmentally resistant stages, presumably excludes toxins of

saprophytic habitats. The plug must be lysed before larvae begin feeding in the host. This may explain the 43 hrs, on average, larvae spend in the skin. Under *in vitro* conditions, feeding resumes gradually, beginning at 3hr and becoming maximal by 24hr and 96hr when, respectively, 50 and 70% feed.

87 SERUM ANTIBODY RESPONSES IN HUMAN OPISTHORCHIASIS. Akai PS*, Pungpak S, Ho M, Bunnag D, and Befus AD. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada; Department of Clinical Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada; and Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand.

*Opisthorchis viverrini* infection is a major public health problem in northeastern Thailand. To facilitate the development of immunodiagnostic methods and vaccines against these parasites, the immunology of this infection must be better characterized. Accordingly, *O. viverrini*-specific serum antibodies against whole adult worm or metacercarial extracts were measured by enzyme-linked immunosorbent assay in 67 residents of endemic areas, including 39 apparently non-infected (stool-egg-negative) individuals. Controls included 19 non-infected individuals from non-endemic areas of Thailand. All egg-positive individuals were treated with praziquantel. In those who became stool-egg-negative, there was no consistent pattern of change in antibody levels at 60 days post-treatment. These preliminary results, along with observations of high reactivity between the crude worm extracts and the controls, suggest the need for identification and careful selection of parasite-specific antigens for immunodiagnosis. Several stool-egg-negative residents of endemic areas had elevated anti-*O. viverrini* antibody levels, relative to controls. The hypothesis that this group includes individuals uniquely resistant to this usually chronic infection will be further explored, since such individuals may represent important models of protective immunity.

88 HUMORAL RESPONSE IN RABBITS INOCULATED WITH ADULT *ASCARIS* EXTRACT OR INFECTED WITH *ASCARIS* LARVAE. Barbosa JM* and Kozek WJ. Medical Sciences Campus, University of Puerto Rico, Rio Piedras, PR.

The humoral immune response of rabbits inoculated with *A. suum* whole adult worm saline extract was compared with that of rabbits infected with the embryonated eggs of *A. lumbricoides* or *A. suum* to define the pattern of humoral responses to *Ascaris* infection. Extracts of *A. suum* adults were analyzed by SDS-PAGE and tested by Western blots against homologous rabbit sera, and sera of rabbits infected with *A. lumbricoides* or *A. suum* eggs. SDS-PAGE revealed at least 42 bands (14.4-330 Kd) in the *A. suum* extract. Homologous rabbit antisera recognized at least 31 components in *A. suum* extract in Western blots, at least 30 components were detected by the sera of rabbits infected with *A. lumbricoides*, and at least 22 components by those infected with *A. suum*. A different but specific pattern of bands was produced by sera of infected rabbits during the pulmonary phase of infection; this pattern essentially disappears within 8 weeks after inoculation. These results suggest that it may be possible to identify the pulmonary phase of ascariasis by the typical pattern observed in Western blots, and the response elicited during this phase of infection may contain antibodies which cross-react with other parasitic nematodes. Further studies are being conducted on sera of humans infected with *Ascaris* to determine whether their Western blot patterns compared to those observed in rabbits during the pulmonary phase of ascariasis.

89 IMMUNOLOGIC CHARACTERIZATION OF *ANCYLOSTOMA CANINUM* ESOPHAGEAL EXTRACTS. Pedro JF* and Kozek WJ. Medical Sciences Campus, University of Puerto Rico, Rio Piedras, PR.

Saline extracts of hookworm esophagus were analyzed to determine whether critical organs of parasitic nematodes produce antigens which elicit a detectable humoral response in infected hosts. SDS-PAGE of esophageal extracts revealed at least 32 components whose molecular weight ranged from 13.5 to 160 kD; 6 prominent components were detectable in the 36-67 kD range. Immunoelectrophoresis of the esophageal extract against rabbit antisera produced at least 16 precipitin arcs. Western blots of the esophageal extracts against rabbit antiserum indicated the presence of at least 16 antigenic components; whereas reaction with sera of dogs harboring naturally acquired hookworm infections detected at least components, including the 6 prominent components detected in SDS-PAGE. Indirect immunofluorescent antibody test performed on cryostat sections of hookworm esophagus indicated that the antigenic components apparently originate in the cytoplasmic ramifications near the ducts of the esophageal glands. These results indicated that the esophagus is a source of secretory antigens which elicit an immune response in the host, and suggests that the antibodies to the esophageal secretions may constitute a significant part of host immune resistance against adult hookworms.

90 ELEVATED NITRATE EXCRETION IN HUMANS AND EXPERIMENTAL HAMSTERS ASSOCIATED WITH LIVER FLUKE INFECTION. Satarug S*, Haswell-Elkins M, Bygott J, Sithithaworn P, Mairiang E, Mairiang P, Yongvanit P, Elkins D. Departments of Biochemistry, Parasitology, Radiology and Medicine, Faculty of Medicine, Khon Kaen Univ. Thailand; Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Australia; and Department of Medicine, University of Queensland, Brisbane, Australia.

There is considerable experimental and epidemiological evidence supporting an important etiological role for *Opisthorchis viverrini* infection in the development of cholangiocarcinoma (CHCA). Although the mechanism by which infection enhances susceptibility to carcinogenesis remains unclear, the relevant carcinogen may be dietary or endogenously produced nitroso-compounds. We have tested the hypothesis that liver fluke infection is associated with enhanced endogenous nitrate production in both experimental hamsters and infected humans. A 57% increase ($p<0.01$) in nitrate excretion by hamsters experimentally infected with liver flukes over uninfected controls reared under identical conditions was recorded 4 and 5 months after exposure to metacercariae. This increase was abolished 9 days after praziquantel treatment. Human studies are also being carried out whereby nitrate levels in 24 hour urine samples are measured among 30-50 year old males drawn from endemic villages. During investigation, the subjects refrain from smoking and consume a controlled, low nitrate diet for 24 hours prior to urine collection. Preliminary results demonstrate a statistically significant difference ($p<0.05$) in nitrate excretion by infected and uninfected men (total 76 subjects). In conclusion, endogenous nitrate production appears to increase during *Opisthorchis* infection and this process may be associated with the production of endogenous nitroso-compounds; a putative carcinogen in fluke-associated carcinogenesis.

91 CLINICAL, RADIOLOGIC AND EPIDEMIOLOGIC CORRELATIONS OF ELISA AND IMMUNOBLOT ASSAYS FOR *TAENIA SOLIUM* CYSTICERCOSIS IN TWO POPULATIONS IN MEXICO. Schantz PM*, Sarti-G. E, Plancarte A, Wilson M, Roberts J, and Flisser A. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; Direccion General De Epidemiologia, Secretaria de Salud, Mexico D.F., Mexico; Departamento de Immunologia, Instituto de Investigaciones Biomedicas, Mexico DF, Mexico.

We compared ELISA and Immunoblot assays for their ability to identify cases and risk factors for human neurocysticercosis in villages in Morelos (A) and Michoacan (B) states. Seroprevalence was 2.3% (35/1552) and 2.2% (22/1005) by ELISA and10.8% (167/1552) and 5.5% (55/1005) by Immunoblot in villages A and B, respectively. Tests results were widely discordant: less than 10% of seropositive individuals were positive in both assays. Immunoblot but not ELISA was associated with convulsions: 5 of 16 persons with histories of convulsions were Immunoblot-positive whereas all were negative by ELISA ($p<.05$). Of 117 subjects who consented to examination by computed tomography (CT), brain CT

images compatible with cysticercosis were demonstrated in 23% of persons with positive immunoblots, 27% of persons with positive ELISA and 78% of persons with histories of convulsions. Risk factors significantly associated with seropositivity in both assays included a history of passing tapeworm proglottids, poor individual hygiene and deficient household hygiene.Persons positive by Immunoblot but not by ELISA were significantly clustered by household ($p<.005$). We conclude that the greater sensitivity and specificity of the Immunoblot assay favors it over ELISA as the test of choice for epidemiologic studies of cysticercosis.

92 FURTHER EVIDENCE OF 100% SPECIFICITY IN A RECENTLY DEVELOPED *TAENIA SOLIUM* (CYSTICERCOSIS) IMMUNOBLOT ASSAY. Pilcher JB*, Tsang VC, Gilman RH, Rhodes ML, and Pawlowski ZS. Immunolology and Molecular Biolology Activity, Parasitic Diseases Branch, CID, Centers for Disease Control, Atlanta, GA; The Johns Hopkins University, Baltimore, MD; USDA, ARS, Beltsville, MD; and Institute of Microbiology and Infectious Diseases, Academy of Medicine, Poznan, Poland.

After 3 years of field and laboratory application (>13,000 tests), the specificity of the enzyme-linked immunoelectrotransfer blot (EITB) for diagnosing human and pig cysticercosis *Taenia solium*, remains at 100%.The heterologous infections we previously tested for cross reactivity included *Echinococcus sp., E. granulosis, S. japonicum, S.haematobium, S. mansoni, Treponema sp., Fasciola sp.,* Amoebiasis,*Strongyloides sp., Brugia malayi, Wuchereria bancrofti, Paragonimus, Chlonorchis sp.,* Hepatitis, and *T. spiralis*. Possible cross reactivity with more closely related diseases to *T. solium* such as other Taenia species is questioned. Serum from patients infected with *T. saginata* (N=21) and *H. nana* (N=10) produced no reaction using the *T. solium* EITB. These results show a continued claim of 100% specificity using the *T. solium* immunoblot assay both for pigs and humans. However, SPF pigs that were infected with "Taiwan" *Taenia* (N=5) in the laboratory showed positive bands using the *T. solium* EITB. This may indicate that "Taiwan" *Taenia* is closely related to *T. solium*.

93 FIVE-YEAR IMPACT OF REPEATED, AGE-TARGETED DRUG THERAPY ON THE URINARY TRACT MORBIDITY ASSOCIATED WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION. King CH*, Muchiri EM, and Ouma JH. Division of Geographic Medicine, Department of Medicine, Case Western Reserve University, Cleveland, OH; and Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya.

Severity of urinary tract morbidity increases with intensity and duration of *S. haematobium* (*S.h.*) infection. We assessed the ability of yearly drug therapy to control infection intensity and reduce *S.h.*-associated disease in children 5-25 yr old (N=4207) in an endemic area of Kenya. In year 1, therapy resulted in reduced prevalence (66% to 22%, $P<0.01$) and intensity of *S.h.* infection (20 to 2 eggs/10mL urine), with reductions in prevalence of hematuria (52% to 19%, $P< 0.01$) and proteinuria (53% to 29%, $P< 0.01$). There was not, however, a significant first-year effect on urinary tract abnormalities detected by ultrasound. Repeat therapy (years 2 and 3) resulted in significant regression of hydronephrosis and bladder abnormalities (46% to 12% prevalence, $P<0.01$) and further reductions in proteinuria. Repeat therapy was also associated with decreased prevalence of infection among young children (<5 yr) entering into target age-groups. Two years after discontinuation of therapy, intensity and urinary tract morbidity remained suppressed (1988 mean egg count=2; prevalence of hydronephrosis/ bladder abnormality=6%). We conclude that annual oral therapy provides an effective strategy for control of morbidity due to *S.h.* on a population basis, both through regression of disease in treated individuals, and prevention of infection in untreated subjects.

## POSTER I: OPPORTUNISTIC INFECTIONS

94 *NAEGLERIA FOWLERI* BY SCANNING ELECTRON MICROSCOPY. John DT* and John RA. Oklahoma State University College of Osteopathic Medicine, Tulsa, OK; and Symex Corp., Tulsa, OK.

*Naegleria fowleri* is a pathogenic free-living ameba and the cause of a fatal meningoencephalitis in humans. It is also an ameboflagellate and as such is able to transform into a transient, nonfeeding, nondividing, biflagellate form. This paper presents scanning electron micrographs (SEM) of the 3 stages in the life cycle of *N. fowleri*. Cells for SEM were fixed at 23 C for 1 hour with 2.5% glutaraldehyde in Sorensen buffer (pH 7.2) containing 1% sucrose. Fixed cells were attached to polylysine (1%)-coated plastic strips. Strips were mounted on stubs and coated with gold palladium. A JEOL 35C SEM was used to examine and photograph processed specimens at 15kV. Amebae of *N. fowleri* have well-organized sucker-like structures known as amebostomes. The average number of amebostomes per ameba varied from 0.2 to 1.6 for 13 human isolates of *N. fowleri*. Incubation temperature and growth phase affected the number of amebostomes present. Amebostomes were shown to be functional by their ability to engulf yeast cells. Approximately 90 min after being placed in non-nutrient buffer, amebae began to round up and produce 2 short blunt flagella. Flagella lengthened and became pointed as preflagellates elongated to the typical pear-shape with anterior rostrum. Enflagellation was affected by temperature, phase of growth, and agitation. Encystment occurred when amebae were grown on non-nutrient agar (1.5%) with *Enterobacter cloacae*. Cysts were spherical and often clumped together. Usually they possessed fewer than 2 mucoid - plugged pores, without rims. Presumably, pores are for the escape of amebae upon excystment.

95 A SPECTRUM OF TOXOPLASMOSIS IN THE IMMUNOSUPPRESSED PATIENT Bertoli F, Espino M, Arosemena VJ, Fishback JL, and Frenkel JK*. Department of Pathology, Metropolitan Social Security Hospital Complex, Panama City, Republic of Panama; and Department of Pathology, University of Kansas Medical Center, Kansas City, KA.

Seven immunosuppressed patients who died with toxoplasmosis were encountered during epidemiologic studies of toxoplasmosis in Panama (5) and Kansas City (2). Three patients were not treated for *Toxoplasma* infections (T). One patient had tachyzoites in intestinal ulcers with antibody titers of 1:65,000 by dye test and 10+ by the capture IgM-ISAGA test; apparently an acute infection limited to the portal of entry. He died with disseminated histoplasmosis. A second HIV positive patient who died with disseminated tuberculosis had inactive chronic T. with intact cysts and a few glial nodules in the brain, interpreted as maintaining intact anti-T. immunity. A third patient died with T. encephalitis manifested by multiple foci of necrosis accompanied by tachyzoites in the brain. He was HIV negative. Four additional patients showed the effects of chemotherapy after 3, 14, 21 days and 7 months with progressive loss of tachyzoites and eventually of antigen and T. DNA. Cysts persisted for at least 21 days. Prolonged treatment, can apparently eliminate T. organisms. This argues for the employment of similarly prolonged treatment to reduce the parasite load in congenitally infected babies and in patients with relapsing retinochoroiditis, in whom because of their immunocompetence treatment can eventually be stopped.

96 SENSITIVITY OF PROTOZOAN PARASITES TO BENZIMIDAZOLES: CORRELATION WITH β-TUBULIN SEQUENCE. Edlind TD*, Li J, and Katiyar SK. Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, PA.

The high *in vitro* sensitivity ($IC_{50}$ < 0.05 μg/ml) of the protozoan *Giardia lamblia* to two anthelmintic, microtubule-targeted benzimid was recently reported. The sensitivity of *G. lamblia* to 10 additional benzimidazoles has now been determined. In terms of structure-activity relationships, there is an

apparent requirement for the 2-carbamate and specific 5-position modifications; benomyl, which lacks a 5-position group, is inactive. A similar pattern of benzimidazole sensitivity was observed for *Trichomonas vaginalis. Entamoeba histolytica* and *Leishmania major* promastigotes were resistant to all derivatives tested, with the possible exception of benomyl, resembling the pattern seen with the yeast, *Saccharomyces cerevisiae*. We are using a molecular genetic approach to analyze β-tubulin sequences responsible for benzimidazole activity. Previous and ongoing mutational studies indicate that amino acid residues at positions 165, 167, and 241 are critical for the antifungal activity of benomyl and thiabendazole. A Tyrat 167 in *T. vaginalis* β-tubulin may explain its natural resistance to benomyl. Several adjacent sequences can be correlated with the high level sensitivity of *G. lamblia* and *T. vaginalis* to other benzimidazoles; further studies are required to evaluate their role.

97 OPTIMAL DOSAGE OF PYRIMETHAMINE IN HUMAN TOXOPLASMOSIS. Furmaniuk J, Pawlowski ZS*, Ewertowska D, and Senczuk W. Clinic of Parasitic and Tropical Diseases, Department of Toxicology, University School of Medicine, Poznan, Poland.

Optimal dosage of pyrimethamine for treatment of human toxoplasmosis has not been determined. Twenty-eight adult patients were treated with pyrimethamine (50 mg daily for 3 days and 25 mg daily for 11 days) and sulfadiazine. Blood levels of pyrimethamine were measured by modified Bonini technique 4 hrs after single oral drug dose in the 1st, 3rd, 7th, 9th and 14th days of treatment. Maximal blood levels in treated patients varied between 0.2 to 4.35 mg/L on the Day 1 and between 0.91 to 6.88 mg/L subsequently. The 28 patients could be grouped according to blood levels: 11 had high, 11 had low, and 6 had intermediate levels. Factors influencing such individual variations of pyrimethamine blood levels could not be determined by routine clinical investigation. In the group with high levels a continuation of 25 mg daily supportive dose over 14 days may increase toxicity. In the group with low levels the efficacy of the treatment may be inadequate. A need of pyrimethamine blood level monitoring is recommended.

98 NEW MANIFESTATIONS & SIMPLIFIED DIAGNOSIS OF HUMAN MICROSPORIDIOSIS. Bryan RT*, Weber R, Stewart JM, Angritt P, and Visvesvara GS. Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA; and AIDS Pathology Division, Armed Forces Institute of Pathology, Washington, DC.

Microsporidia are important opportunistic pathogens that have been associated with chronic diarrhea and keratoconjunctivitis in HIV-infected patients. Other clinical manifestations have been suspected, but rarely documented. Diagnosis of microsporidiosis is often difficult due to the need for histopathologic studies. We present herein the case of an HIV-positive man with previously undescribed manifestations of this disease whose diagnosis was parasitologically confirmed by simple, noninvasive means. He had AIDS and a complex combination of potential risk factors for opportunistic infections. In brief, the patient was a 30 year old Hispanic homosexual who had a history of travel to Mexico, Italy, and western Europe. He kept both a dog and cat in his home and had raised numerous cockatiels. After an initial episode of pneumocystis pneumonia in 1985, he went on to develop cytomegalovirus colitis, disseminated mycobacterium avium-intracellulare, and pansinusitis. In late 1990, symptoms of flank pain, dysuria, and gross hematuria developed. Cystoscopic bladder biopsy was performed in early 1991 and a tentative diagnosis of microsporidiosis was reached. Diagnosis was confirmed, however, by a simple examination of stained urinary sediment. The methods and results of this diagnostic technique will be presented and clinical and epidemiologic implications will be discussed.

99 EXPERIMENTAL MICROSPORIDIOSIS IN RHESUS (*MACACA MULATTA*) MONKEY. Aldras A* and Didier ES. *Microbiology Department, Tulane Delta Regional Primate Research Center,* Covington, LA.

Microsporidia are obligate intracellular protozoan parasites that infect all classes of vertebrates. The interest in these parasites is increasing as a result of increasing microsporidial infections in AIDS patients. Several syndromes that may be caused by different species of microsporidia have been reported in AIDS patients, such as, keratoconjuntivitis due to *Encephalocytozoon hellem*, hepatitis and peritonitis due to *E. cuniculi* and most often diarrhea which has been associated with *Enterocytozoon bienusi*. The aim of this study is to identify a suitable animal model for microsporidiosis. Serum samples from 8 HIV (human immunodeficiency virus) negative human subjects, and from 16 SIV (simian immunodeficiency virus) negative Rhesus (*Macaca mulatta*) monkeys were tested for microsporidian specific antibodies by ELISA. An ELISA titer was considered to be positive if it was higher than 1:800. Of those 8 human sera tested for microsporidiosis, positive titers were detected: 3 (37.5%)for *E. cuniculi*; 4 (50.0%) for *E. hellem*; 5 (62.5%) for *Nosema algerae*; and 3 (37.5%) for *Nosema corneum*. None of the monkeys samples showed any positive titer against any tested microsporidian species. These results suggest that the monkey in this study may be unexposed to the microsporidian parasites and may serve as an experimental model to study the course of microsporidial infection in the SIV infected monkeys. Studies are in progress to analyze the anti-microsporidian immune responses to SIV-positive and negative monkeys, which have been experimentally infected with *E. cuniculi, E. hellem,* and *E. bienusi.*

100 RISK OF DISSEMINATED *MYCOBACTERIUM AVIUM-INTRACELLULARE* INFECTION (DMAI) IN HIV-POSITIVE POPULATION IN A U.S. METROPOLITAN AREA. Fisher EJ*, Hayashi H, and Carleton B. Department of Internal Medicine, Henry Ford Hospital, Detroit MI; and Department of Pathology, Henry Ford Hospital, Detroit MI.

DMAI is a major late infection in AIDS in the United States, being diagnosed in ≥25% of patients (pts) in life and up to ≥50% postmortem. Almost all cases occur when the number of #CD4 lymphocytes (T-helper) are very low, ≤50 per $10^{-6}$L. Risk of DMAI in our medical center's HIV-positive pts was assessed via retrospective chart review of pts whose #CD4 was ≤150 between 24 to 35 months earlier. Selection criteria were: (1) #CD4 ≤150 between 5/88 & 4/89 (= initial #CD4); (2) survival ≥3 mos after initial #CD4; (3) no mycobacterial infection until >1 mo after initial #CD4; (4) chart available. 134 pts met these criteria. Status of AIDS diagnoses at the time of initial #CD4 was: prior AIDS in 37%, concomitant in 13%, subsequent in 40% and none in 9%. Analysis combines living (41/31%) and deceased (93/61%) pts, with mean follow-up of 17 mo. Thirty-five pts (26%) have developed DMAI (diagnosed via culture of blood in 33, bone marrow in 1 and abdominal node in 1). No *M. tuberculosis* infections occurred. Overall DMAI risk was 1.45%/mo. As expected, outcome varied inversely with initial #CD4, with #CD4 of 75 providing a breakpoint. Comparing 94 patients with initial #CD4 ≤75 vs 40 pts with initial #CD4 >75, percent alive was 20% vs 55%, DMAI 33% vs 10%,risk DMAI/mo 2.12% vs 0.44% and mean survival 17 vs 24 mo. Studies of DMAI prophylaxis should concentrate on the HIV population with low #CD4, particularly those with #CD4 ≤75.

## POSTER I: BACTERIA AND RICKETTSIA

101 POLYMERASE CHAIN REACTION BASED DIAGNOSIS OF *MYCOBACTERIUM TUBERCULOSIS* AND *M. AVIUM* INFECTION IN PATIENTS. Sritharan V*, and Barker, Jr. RH. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

I aboratory diagnosis of mycobacterial infections is presently based upon culture of organisms from clinical samples followed by biochemical analysis to identify species. This is time consuming, and depends upon the ability to culture organisms from clinical samples. We have developed a simple PCR-based method for identification of mycobacteria in clinical samples. The method involves simple lysis of bacilli in clinical specimens by boiling in TE-Triton X-100, followed by PCR amplification. Presence of PCR products is detected by chemiluminescence using non-isotopically labelled specific DNA probes.

We are currently testing sensitivity and specificity of PCR primers specific for *M. tuberculosis, M. avium* and for the genus *Mycobacterium* on clinical samples. These probes were developed previously by us and others. The sensitivity and specificity of these probes has been tested on 200 clinical specimens which included sputa, and also blood, bone marrow, and lymph nodes. Preliminary analysis shows good correlation between culture and PCR-based diagnosis. However, in order to assess the diagnostic sensitivity and specificity, additional samples from both clinically related and unrelated cases are being analyzed. Results will be presented and discussed.

102 CHRONIC CHILDHOOD MALNUTRITION REDUCES ANTIBODY RESPONSE TO *H. INFLUENZAE* TYPE B (HIB)-PROTEIN CONJUGATE VACCINE. Oyango F, Steinhoff MC*, Mbori-Ngacha D, and Siber G. University of Nairobi, Nairobi, Kenya; Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD; and Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

Vaccines to prevent disease caused by *S. pneumoniae* and Hib have high potential to reduce morbidity and mortality in children in developing countries. Polysaccharide (PS) vaccines are more immunogenic in infants and children if the PS is conjugated to a protein carrier. However, there is little data on the effect of malnutrition on the immune response to these T-lymphocyte dependent PS-protein conjugate vaccines. We evaluated the antibody (Ab) response of 86 Kenyan children 15-20 months of age to an Hib conjugate vaccine. Each child had height and weight recorded, received a DPT booster and Hib-mutant diphtheria toxin conjugate vaccine (Praxis), and provided pre- and 1 month post-immunization serum samples. Anti-Hib PS Ab were determined by RIA. Preliminary data show that stunted children (< -2 S.D for height for age = chronic malnutrition) had significantly lower Ab GMT than non-stunted children: 0.34 vs. 0.65 mcg/ml, respectively (p=0.01 by ANCOVA with pre-existing Ab and age as covariates). The Ab GMT of 7 wasted (<-2 S.D for weight for height = acute malnutrition) children was similar to that of normals. The Ab response to tetanus toxoid is being assayed. The nature of the nutritional insult may be an important determinant of the antibody response of malnourished children to PS-conjugate vaccines.

103 DIAGNOSIS AND TREATMENT OF CRYPTOGENIC TUBERCULOSIS, CAIRO, EGYPT - 1990-91. Farid Z*, Kilpatrick ME, and Kamal M. U.S. Naval Medical Research Unit No.3, Cairo, Egypt; and Abbassia Fever Hospital, Ministry of Health, Cairo, Egypt.

During 1990, 133 patients with prolonged fever were referred for investigation. Seventy-five (56%) had infection, 22 (17%) neoplasm, and 20 (15%) collagen vascular disease. The leading systemic infections were tuberculosis (TB) (18), sepsis (15), salmonellosis (13), and brucellosis (11). Of the 18 patients with TB, all but one were extrapulmonary infections, including tuberculous adenitis (4), disseminated TB (3), tuberculous meningitis (1), tuberculous enteritis (1), and skeletal TB (1). The remaining 7 patients, 4 males and 3 females, age range 8-48 years, were classified as cryptogenic TB.Mean duration of fever was 2.3 months. In all 7 patients constitutional symptoms were non-specific: primarily fever, weight loss, night sweats, and increasing fatigue. All had normocytic hypochromic anemia, elevated ESR, and a positive tuberculin skin test. Diagnosis depended on a high index of suspicion, clinical acumen and a rapid response to therapy with isoniazid and ethambutol.Five patients were afebrile within one week of starting therapy and 2 within 3 weeks. In this study, a therapeutic trial with anti-TB chemotherapy was a mainstay in diagnosis of cryptogenic TB, emphasizing the importance of the need for rapid serodiagnostic and antigen detection methods for TB.

104 DETECTION OF STRAIN VARIATION IN *RICKETTSIA TSUTSUGAMUSHI* BY MOLECULAR ANALYSIS OF THE IMMUNODOMINANT 54-56 KD PROTEIN ANTIGEN GENE. Kelly DJ*, Swinson KL, and Dasch GA. Rickettsial Diseases Program, Naval Medical Research Institute, Bethesda, MD.

*Rickettsia tsutsugamushi*, the etiologic agent of scrub typhus, exhibits great variation in its antigenicity and virulence. We have developed a rapid method for detection of strain variants of scrub typhus by restriction fragment length polymorphism analysis (RFLP) of a 1.4 kb region of its immunodominant 54–56 KD major outer membrane protein gene. The gene fragment was generated by polymerase chain reaction amplification (PCR) with 20 bp primers selected from conserved gene regions. An automated DNA extractor was used to purify the DNA template directly from unfractionated L929 cells infected with the rickettsiae. Intact and digested PCR products were analyzed by electrophoresis in agarose or polyacrylamide gels. PCR products obtained with more highly conserved 16S rRNA and consensus tRNA primers were used as controls to ensure that adequate rickettsial DNA was present in each preparation for PCR detection. All five of the proto-serotypes of scrub typhus tested exhibited considerable RFLP variation in their 54-56 KD genes. This heterogeneity could be detected readily on either gel system with any of the enzymes *Rsa* I, *Pst* I, *Mae* I, *Mae* III, *Hinf* I, or *Mbo* II. Whether any correlation exists between RFLP types and the serotypes of *R. tsutsugamushi* determined by classical procedures is presently being determined. However, PCR-RFLP of the variable immunodominant antigen is useful for the rapid detection of variants of scrub typhus rickettsiae and will be an important tool for investigating the ecology, epidemiology, and evolution of this important pathogen.

105 CHEMILUMINESCENT SUBSTRATE BASED WESTERN BLOTTING FOR DETECTION OF URINARY IgA IN INFANTS WITH *CAMPYLOBACTER* ENTERITIS. Wu SL*, Pazzaglia G, Haberberger RL, Oprandy JJ, Sieckmann DG, and Hayes CG. Infectious Disease Threat Assessment Program, Naval Medical Research Institute, Bethesda, MD; Enteric Immunoprophylaxis Program, Naval Medical Research Institute, Bethesda, MD; and U.S. NAMRU-2 DET, Jakarta, Indonesia.

The development of a rapid and non-invasive urinary IgA assay may be useful for confirming *Campylobacter* enteritis in infants. Paired urine specimens from infants with naturally acquired *Campylobacter* enteritis were analyzed for IgA response by Western blots using sonicated whole cell preparations of *Campylobacter jejuni* and *Campylobacter coli*. The enhanced chemiluminescence (ECL) substrate was compared with chromogenic substrates for sensitivity. Different degrees of binding of *Campylobacter* common antigens (18, 43 and 62 kD) were detected among first urine specimens (taken 4 to 8 days post onset; titer varied from 1:2 to 1:8). No *Campylobacter* antigens were bound by follow-up urine specimens (taken 10 to 14 days post onset). The comparison of different peroxidase substrates for Western blotting indicated similar sensitivity between tetramethylbenzidene (TMB) and ECL; and both were more sensitive than 4-chloro-1-naphthol (4-CN). The chemiluminescent substrate is advantageous as it provides a non-fading, permament record of blotting experiments which can be quantitated using a densitometer. This non-invasive urinary IgA immunoblot assay may be useful for confirmation of *Campylobacter* infection.

## POSTER I: MALARIA

106 POLYMORPHISM OF THE GENE ENCODING *PLASMODIUM FALCIPARUM* LSA-1, A LIVER STAGE-SPECIFIC VACCINE CANDIDATE. Zhu J* and Hollingdale MR. Biomedical Research Institute, Rockville, MD.

We have identified LSA-1 as a 230kd antigen localized specifically in the parasitophorous vacuole of *P. falciparum* exoerythrocytic parasites, and cloned and sequenced the entire gene from NF54 parasites. The gene encodes a central repeat region containing 86 repeats of a 17 amino acid sequence, and N- and C-terminal non-repeat regions. Various algorithms have predicated several potential T epitopes in the N- and C-terminal regions. LSA-1 is being developed as a candidate malarial vaccine that could elicit cytotoxic T-cells (CTL) that recognize LSA-1 epitopes in association with Class I molecules on the infected hepatocyte membrane. Since T epitopes, including the CTL epitope, of CS proteins are

polymorphic, we have investigated the polymorphism of LSA-1 non-repeat regions. Using PCR and direct sequencing, we have found that the C-terminal region of LSA-1 is relatively non-variant, whereas significant polymorphism occurs in the N-terminal region. Since the C-terminal region contains several predicted T-epitopes that are undergoing investigation in proliferation assays, as well as CTL assays, with a sequence has significant homology to the CS CTL epitope, these results suggest that a LSA-1 vaccine may elicit strain-independent protection. LSA-1 is currently being expressed in Salmonella and vaccina virus for further CTL evaluation.

107 CYTOKINE PROFILES IN EXPERIMENTAL HUMAN MALARIA. Harpaz R*, Edelman R, and Sztein MB. Center for Vaccine Development, University of Maryland, Baltimore, MD.

Extensive evidence supports a role for cytokines in protection from, and the pathology of, exoerythrocytic and blood stage *P. falciparum*. We evaluated cytokine kinetics in 3 irradiated-sporozoite-immunized, and 4 non-immunized, volunteers following challenge with virulent *P. falciparum*. All 3 vaccinees were protected from infection and all 4 non-immune volunteers developed symptomatic infection at which time they were treated. Sera were evaluated at about 20 time points regularly collected during the 28-day challenge period; IL-1*a*, IL-1*b*, IL-2, γ-interferon (IFN), TNF, IL-4, IL-6, GM-CSF, and soluble receptors for IL-2, CD4 & CD8 (sIL-2R, sCD4 & sCD8, respectively) were assayed using commercial ELISA kits. CRP was assayed by radial immunodiffusion. Parasitemic subjects developed abrupt increases in CRP at the onset of fever; abrupt increases in IFN preceded those of fever and CRP by 1 day. Increases in sIL-2R and sCD8 were less abrupt. The magnitude of increases in each volunteer was correlated for these parameters. TNF levels did not change significantly. In protected vaccinees, statistically significant cytokine increases were not noted, although increases in IL-6 approached significance. Thus, changes in systemic cytokines after challenge are detectable in unvaccinated persons with malaria but not in protected vaccinees.

108 SYNTHETIC MONOPHOSPHORYL LIPID A AND ITS SUBUNIT ANALOGS AS ADJUVANTS FOR THE *PLASMODIUM FALCIPARUM* GP195 PROTEIN. Hui GS*, Chang SP, Matsuura M, and Hasegawa A. Department of Tropical Medicine, School of Medicine, University of Hawaii, Honolulu, HI; Department of Microbiology, Jichi Medical School, Tochigi-ken, Japan; and Department of Agricultural Chemistry, Gifu University, Gifu, Japan.

Lipid A's have been shown to possess potent adjuvant activity, and monophosphoryl lipid A's (MPL) are attractive candidates for vaccine adjuvants because of their low toxicity yet retaining adjuvanticity. We found that a synthetic MPL, LA-15-PH, can induce a higher antibody response to the *P. falciparum* gp195 than CFA in mice. LA-15-PH also induced a significant cell-mediated response as measured by antigen specific T cell proliferation assays. Moreover, gp195-antibodies induced by LA-15-PH strongly (>80%) inhibited parasite growth in an *in vitro* assay, while antibodies induced in mice by CFA had no effect. We also studied two lipid A subunit analogs (based on the non-reducing monosaccharide moiety of the disaccharide Lipid A backbone), GLA 59 and GLA 60, because they have dramatically lower toxicity than LA-15-PH or MPL. Results showed that GLA 59 induced gp195-specific antibodies most similar to levels obtained with LA-15-PH. These antibodies also significantly inhibited parasite growth *in vitro*. Our results suggest that synthetic MPL and its subunit analogs may be efficacious as adjuvants for a human malaria vaccine based on gp195. We have begun to evaluate these lipid A analogs in potentiating the antibody response to a recombinant circumsporozoite protein, CSP. Results of these studies will also be discussed.

109 SEQUENCE ANALYSIS OF *PLASMODIUM VIVAX* (VK247 VARIANT) FROM GLOBALLY DIVERSE REGIONS. Lanar DE*, Kain KC, and Wirtz RA. Department of Immunology, Walter

Reed Army Institute of Research, Washington, DC; and Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.

Phenotypic heterogeneity in the repetitive portion of the circumsporozoite (CS) gene of *Plasmodium vivax* isolated from Thailand (VK247) has been reported. By dot blot hybridization the existence of this variant has now been demonstrated in many *P. vivax* endemic areas around the world, including India, Peru, West Africa, Mexico and Papua New Guinea. We report here the analysis of the sequence variation in this variant from these globally dispersed samples. We PCR amplified and cloned the DNA between region I and region II in the CS gene from blood of patients with *P. vivax* parasitemia. Clones were selected by hybridization to a VK247 DNA specific repeat oligo. Sequence analysis revealed that the major repeat was ANGAGNQPG, however, minor variations were also found: ANGADDQPG, ANGAGDQPG, and ANEAGNQPG. There were between 18 and 20 of these nine residue repeats. These were followed by 1 or 2 copies of a 23 amino acid residue semiconserved region. This region was followed by a 33 amino acid residue, highly conserved region ending at the region II portion of the CS gene. Analysis of sequences of clones isolated from single individual patients revealed that they often contained different motifs of this VK247 variant as well as copies of the original VK210 variant containing the GDRA(A/D)GQPA repeat unit. This high amount of *P. vivax* genetic diversity seen within single individuals with patent parasitemia could lead to rapid genetic recombination and selection in the wild.

110 RENAL PATHOLOGY IN *SAIMIRI* MONKEYS DURING A VACCINE TRIAL USING THE RECOMBINANT CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX*. Tegoshi T*, Broderson JR, Iseki M, Oo MM, Nagatake T, Collins WE, and Aikawa M. Case Western Reserve University, Institute of Pathology, Cleveland, OH; Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, GA.

Renal specimens of squirrel monkeys (*Saimiri*) *sciureus boliviensis* were studied by light microscopy and immunohistochemistry to examine the pathologic changes during vaccine trials with 4 recombinant circumsporozoite (CS) proteins (rPvCS-1, rPvCS-2, rPvCS-3, $NS1_{81}V_{20}$) of *Plasmodium vivax*. The monkeys were vaccinated and later challenged with *P. vivax* sporozoites. Among the 33 post trial biopsies, 17 had mild to moderate mesangial proliferation and 9 had interstitial nephritis. Immunohistochemistry by the peroxidase-antiperoxidase (PAP) method revealed IgG deposits in only 3 of 24 specimens and failed to demonstrate C3 deposits and *P. vivax* antigens in their glomeruli. There was no relationship between the severity of nephropathy and intensity of parasitemia. The intensity of parasitemia was the same in the vaccinated and control groups. Vaccinated monkeys from the groups (rPvCS-1, rPvCs-2, rPvCS-3) had no differences in renal pathology from the unvaccinated controls, but one group vaccinated with $NS1_{81}V_{20}$ did not develop renal changes.

111 CSP-3: A *PLASMODIUM FALCIPARUM* SPOROZOITE-SPECIFIC MALARIAL VACCINE CANDIDATE. Anders J*, Zhu J, Aikawa M, Chen G, Sina B, Offutt S, and Hollingdale MR. Walter Reed Army Institute of Research, Washington, DC; Biomedical Research Institute, Rockville, MD; and Case Western Reserve University, Cleveland, OH.

At least three proteins have been identified on the surface of *Plasmodium falciparum* sporozoites, and each is being developed as potential malarial vaccine candidates. CS protein has been evaluated in clinical trials with encouraging results. We have identified CSP-2, a 42/54kd protein that elicits sporozoite neutralizing antibodies, and a 140kd protein that may be analogous to SSP-2, a *P. yoelii* specific sporozoite antigen that elicited protection in mice. As part of our evaluation of LSA-1, a *P. falciparum* liver stage-specific antigen, various peptides representing the repeat and non-repeat regions were tested for immunogenicity in mice. One N-terminal peptide elicited antibodies that did not recognize LSA, but

detected a novel 55/60kd *P. falciparum* sporozoite-specific antigen, CSP-3. CSP-3 was located on the sporozoite surface by immunoelectron microscopy, and anti-CSP-3 antibodies strongly neutralized sporozoite infectivity *in vitro*. We are currently screening a *P. falciparum* sporozoite cDNA library, as well as constructing peptide vaccines to further develop CSP-3 as a malarial vaccine candidate.

112 NATURAL EXPOSURE AND IMMUNIZATION WITH A SUBUNIT VACCINE DO NOT INDUCE SIGNIFICANT ANTIBODY LEVELS TO A *PLASMODIUM VIVAX* PROTECTIVE EPITOPE. Jones TR*, Yuan LF, Marwoto H, Gordon DM, Wirtz RA, and Hoffman SL. Naval Medical Research Institute, Bethesda, MD; National Institute for Health Research and Development, Jakarta, Indonesia; and Walter Reed Army Institute of Research, Washington, DC.

We evaluated the capacity of natural exposure and active immunization with $NS1_{81}V20$, a vaccine containing the entire repeat region from the circumsporozoite protein of *P. vivax*, to induce antibodies to the protective epitope AGDR. Serum samples from 176 human subjects living in an endemic area in Flores, Indonesia were tested for antibodies to AGDR and three other peptides from the repeat region of the *P. vivax* CS protein. Reactivity to $NS1_{81}V20$ was found in 53% of the population, reactivity to VIVAX-1, a peptide consisting of the repeat region and some of the adjacent flanking regions, was found in 67% of the subjects. Reactivity correlated well with increasing age. Eight subjects (4.5%) had antibody to $(ANGAGNQPG)_3$, the repeat peptide from the *P. vivax* CS protein variant VK247. Antibody to AGDR was detected in only 14% of the subjects; intensity in ELISA was substantially less than that seen with $NS1_{81}V_{20}$ and VIVAX-1. Six naive human subjects vaccinated with $NS1_{81}V_{20}$, and who produced antibodies to it, were also tested for antibodies to $AGDR_6$ but none were detected. Because the amino acid sequence AGDR is present in repeated copies in both native *P. vivax* circumsporozoite protein and in $NS1_{81}V_{20}$, we hypothesized that the failure to make antibody to AGDR is due to the presence of highly immunogenic, but nonprotective epitopes near the sequence AGDR. To test this, $(AGDR)_6$ conjugated to KLH was used to immunize two *Aotus trivirgatus* monkeys. Sera from both animals reacted strongly to $(AGDR)_6$ in ELISA but did not react with VIVAX-1. Studies are underway to develop vaccine candidates capable of consistently inducing high levels of antibodies to AGDR.

113 ESTIMATING MALARIA CHALLENGE ON INDIVIDUAL VOLUNTEERS DURING A *PLASMODIUM FALCIPARUM* VACCINE TRIAL IN WESTERN KENYA. Copeland RS*, Taylor KA, Sherwood JA, Kamanza J, Asiago C, and Roberts CR. U.S. Army Medical Research Unit-Kenya, Kenya Medical Research Institute, Nairobi, Kenya; and Centers for Disease Control, Nairobi, Kenya.

In order to account for local variation in the intensity of malaria transmission during a small-scale vaccine trial, malaria challenge was estimated for individual volunteers. Volunteers (one vaccinee and one control) lived in two-person houses. Daily 0.5 hr collections of resting mosquitoes were made in each of 38 study houses. The blood of engorged *Anopheles* females was typed using ABO markers, and the host (human or cow) was determined with a bloodmeal ELISA. Of blood fed *Anopheles gambiae s.l.* collected while resting indoors, 93.8% contained human blood while 6.2% had fed on cattle. Of females captured in window traps while entering houses, 60% had human blood, while 40% had cow blood. From these data, it was estimated that 9.3% of resting, bloodfed *A. gambiae s.l.* which contained human blood had fed outside the house in which they were collected. There was a greater than 100-fold difference between the houses with the highest and lowest challenge, as determined by *P. falciparum* sporozoite ELISA. In houses which had volunteers with different blood types, the percentage of bites taken from the most frequently bitten volunteer ranged from 50.4% to 87.0%. In 45% of houses, one volunteer received 60% or more of mosquito bites. These data underscore the importance of accounting for individual variation in malaria challenge when assessing vaccine efficacy in small-scale vaccine trials.

114 FIELD TRIAL OF A MALARIA SPOROZOITE VACCINE. Brown AE*, Singharaj P, Webster HK, Pipithkul J, Gordon DM, Boslego JW, Krinchai K, Su-archawaratana P, Wongsrichanalai C, Cryz SJ, and Sadoff JC. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Walter Reed Army Institute of Research, Washington, DC; and Swiss Serum and Vaccine Institute, Berne, Switzerland.

A controlled field trial of a falciparum sporozoite vaccine was carried out in Thailand to assess safety, immunogenicity and efficacy (Phase IB, IIB) of "R32Tox-A". The vaccine molecule consisted of a recombinant protein derived from the central repeat region of the circumsporozoite (CS) protein of *Plasmodium falciparum* (PF) conjugated to exotoxin A (detoxified) of *Pseudomonas aeruginosa*. Three doses of R32Tox-A or a control preparation were given to 191 Thai soldiers at 0, 2 and 4 months in a malaria-free area. Following immunization, the soldiers were deployed as part of their regular duties to a malarious area on the Thai-Cambodian border. The soldiers were closely monitored and malaria parasitemias promptly treated. The vaccine was well tolerated. Immunogenicity will be determined in terms of specific antibody (IgG and IgM) and cellular immune responses. Assessment of efficacy will include a comparison of the incidence of PF infection in subjects grouped by serum concentration of specific antibody. These results will be presented and discussed in terms of continued efforts for malaria sporozoite vaccine development.

115 COMPARISON OF DISSECTION AND ELISA ANALYSIS OF *ANOPHELES DARLINGI* EXPERIMENTALLY INFECTED WITH *PLASMODIUM VIVAX*. Miller RE, Klein TA, Milstrey EG*, Bento JL, Pereira TR, McGreevy PB, and Wirtz RA. US Army Medical Research Unit, Rio de Janerio, Brazil; Division of Communicable Disease and Immunology, Entomology Branch, Walter Reed Army Institute of Research, Washington, DC; and Division of Experimental Therapeutics, Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, DC.

Laboratory-reared *Anopheles darlingi*, the primary malaria vector in South America were fed on *Plasmodium vivax* infected patients. From day 4 to 12 post infection, dissection was compared to ELISA using anti-circumsporozoite monoclonals for detection of malaria infections. ELISA analysis was done on four body parts: anterior, posterior, salivary glands and stomach. Data indicated that ELISA was unreliable for detecting an dissection-confirmed infection until day 9 (95% confidence). The earliest date that ELISA could detect infections was 6 (5.2%). As infection time increased so did ELISA detection: day 7 (76.4%) day 8 (67.4), day 9 (104.9%), 10 (106.9%), 11 (108.2%), 12 (117.8%). There was also a reduction in detectability of malaria infections by dissection later on possibly due to mosquito immune response and healing of the oocysts. ELISA rarely detected circumsporozoite proteins on oocyte-infected stomachs (5.8%). ELISA analyses of the body parts confirmed that there is a 1 to 2 day period where the body is infected but the salivary glands have not been invaded.

116 EFFECT OF ADJUVANTS ON THE SPECIFICITY OF ANTIBODY TO WHOLE BLOOD STAGE *PLASMODIUM YOELII*. ten Hagen T, Sulzer A, Lal AA, and Hunter RL*. Department of Pathology, Emory Unversity, Atlanta, GA; and Malaria Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

Immunization of mice with killed blood-stage *P. yoelii* parasites has been reported to induce protection from infectious challenge, but the adjuvants were toxic. We recently reported that new copolymer adjuvants are more effective and less toxic than previous preparations and may act synergistically with several immunomodulators. In this study we report the effect of some of these copolymer adjuvants on the specificity of antibody and protection. Sixteen groups of ICR outbred mice (6-10 mice per group) were immunized repeatedly with 100 μg of killed *P. yoelii* parasite antigen in selected adjuvant formulations. Mice were challenged with $10^4$ infected red blood cells. Groups were graded as protected

if their parasitemia was below 10% at day 10, unprotected if the parasitemia was greater than 10% and intermediate if only some individuals were protected. Five of six groups immunized with oil-in-water emulsion or formulations without oil were protected. Effective adjuvants were saponin, pertussis, emulsions of L180.5 and L180.5 with detoxified Ra LPS. In contrast, 0 of 9 groups immunized with water-in-oil emulsions were protected even though high titer antibodies were elicited by all of these formulations. Ineffective adjuvants included Freund's Complete and water- in-squalene emulsions with copolymer L141, LPS, DDA, CWS, and silica. Antibody was measured by an ELISA using disrupted parasites and IFA using intact parasites. Protection was strongly associated with antibody of the IgG2a isotype against the epitopes located on the parasite surface (IFA), weakly with IgG2b, and not at all with IgG1.

117 PERSISTENCE OF IRRADIATED *PLASMODIUM BERGHEI* EXOERYTHROCYTIC MALARIA PARASITES. Scheller LF*, Wirtz RA, and Azad AF. University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore, MD.; and Walter Reed Army Institute of Research, Washington, DC.

It has been shown that gamma-irradiated sporozoites (IRR-SPZ) are living organisms, which invade hepatocytes, transform into trophozoites, and depending upon the radiation dose may undergo few rounds of replication. IRR-SPZ (10-12krads) renders the recipient animals immune to subsequent challenge with homologous parasites. We have determined that the protective immunity against a sporozoite challenge in IRR-SPZ immunized mice continues for more than a year. The possibility that such a long lasting immunity to challenge infection is the result of host stimulation with the living parasite within hepatocytes was examined. Because of their small size, IRRAD-SPZ are difficult to visualize by Giemsa staining and by immunofluorescence microscopy. We applied a DNA *in situ* hybridization technique in which a biotinylated 2.3kb probe specific for the subtelomeric region of the *P. berghei* chromosome was used. Outbred mice were immunized with hundreds of weekly bites from infected irradiated mosquitoes (12 krads from a $^{137}Cs$ source) for a period of 8 weeks. Mice were challenged 5-12 months after receiving a last boost by the bite of 5-20 infected mosquitoes. Thirty six out of thirty seven mice (97%) were protected. Liver resections were performed on two mice which were immunized nine months prior with IRR-SPZ, paraffin sections were made, and DNA *in situ* hybridization was applied. The results utilizing this technique indicate that IRR-SPZ do persist in the livers of mice immunized with irradiated-attenuated sporozoites. The significance of this finding in the context of host protection to sporozoite challenge will be discussed.

118 IMMUNE RESPONSE TO PLASMODIAL ANTIGENS AND SPLENOMEGALY IN A HIGHLY ENDEMIC AMERINDIAN COMMUNITY OF VENEZUELA. Torres JR*, Zisman A, DiJohn D, and Sulzer AJ. Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas; Department of Parasitology, University of New York, New York, NY; Centers for Disease Control, Department of Parasitology, Malaria Branch, Atlanta, GA

A clinical and sero-epidemiological, community-oriented survey, was carried out in several isolated Yanomami hamlets of the Upper Orinoco basin, during a three-year period, in order to correlate humoral immune response to various *P. falciparum* antigens and the occurrence of splenomegaly (S) and/or hyperreactive malarial splenomegaly syndrome (HMSS), in a population with high endemicity and naturally acquired immunity to malaria. Initial evaluations in 110 individuals revealed an unusually high serological prevalence of *P. falciparum* at elevated titers (100% positivity for total anti-*P. falciparum* by IFA and ELISA; GMT1:8,079); as well as a high global rate of S (44.4%) and of HMSS(23.5%). Anti-RESA was detected at high titers (GMT 1:806.3) by IFA in all of 58 samples assayed. Titers showed a tendency to increase with age. HMSS patients showed significantly higher anti-RESA titers (GMT1:1,856) than those with S only (GMT 1:568.4) ($p < 0.01$). Antibodies against a circumsporozoite (NANP)50

peptide, were measured by ELISA in 390 individuals for a global prevalence of 15.3%. None of 6 patients with HMSS were positive, in contrast to 13.8% of 167 patients with S,and 16.6% of 217 individuals without S ($p < 0.0005$ in both cases). Our results indicate that anti-RESA instead of anti-circumsporozoite antibodies, might play a significant role in the development of acquired clinical immunity to malaria in this setting. Moreover, the absence of antibodies against sporozoites, along with the occurrence of significantly higher titers of anti-RESA in patients with HMSS, suggests that they continue to experience repeated active, although subpatent, infections which resolve at the early erythrocytic stage.

119 CHARACTERIZATION OF ANTI-*PLASMODIUM FALCIPARUM* ANTIBODIES IN HUMAN BREAST MILK. Leke R*, Ndansi R, Southerland NJ, Quakyi IA, and Taylor DW*. University Centre for Health Sciences, University of Yaounde, Yaounde, Cameroun; and Department of Biology, Georgetown University, Washington, DC.

Malaria infection is rare in neonates. It has been postulated that some immunity may be passively transferred during nursing, but antimalarial antibodies (Abs) have not been detected in human milk. Accordingly, paired plasma and milk samples were collected 2-14 days after parturition from women at the Hospital Maternity, Yaounde. For comparison, milk samples were also collected from women residing in the U.S. They were analyzed for: i) total IgG and IgA by radial immunodiffusion; ii) protein composition by SDS-PAGE; iii) antimalarial antibodies using an isotype-specific IFA assay; and iv) the ability to immunoprecipitate *P. falciparum* antigens metabolically-labeled with $^{35}$S-MET. Results showed that the level of Ig was higher in the milk of Camerounian women than U.S. residents, but that the protein composition was similar. Antimalarial Ab of both the IgG and IgA class were detected in the milk of Camerounian women by IFA. Immunoprecipitation studies demonstrated that: i) Abs in milk reacted with numerous asexual-stage Ags; ii) there was no direct correlation between amount of Ab in serum and in milk; and iii) Abs were present in most milk samples. Thus, antimalarial Abs are present in human breast milk. Their relevance in malarial immunity remains to be determined.

120 ANOPHELES MIDGUT ANTIGEN BASED MALARIA TRANSMISSION BLOCKING IMMUNITY. Lal AA*, Schriefer M, Qari SH, Goldman IF, Azad AF, and Collins WE. Division of Parasitic Disease, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Department of Microbiology and Immunology, University of Maryland, Baltimore, MD.

In view of the success of vector-based tick vaccines, we have explored the ability of antibodies to mosquito midgut proteins to affect malaria parasite development within the mosquito midgut. Outbred (ICR) mice immunized with *An. stephensi* midgut antigen extracts developed high titer antimidgut protein antibodies that were stable for at least 3 months and reacted with midgut antigens of two other species, *An. freeborni* and *An. gambiae*. No differences in mortality between mosquitoes fed on An. stephensi midgut-immunized mice and those fed on control mice were observed. To determine the effect of antibodies directed against the midgut antigens, we allowed *An. stephensi* mosquitoes to take infectious (*Plasmodium berghei*) blood meals from midgut- immunized or control mice. These mosquitoes were subsequently allowed to feed on uninfected but midgut-immunized or unimmunized mice at days 2, 5, and 8. We found that the mosquitoes that ingested antimidgut antibodies along with infectious parasites had 3.9 (range 0-19) average oocysts, compared with 26.4 (range 0- 180) average oocysts per infected mosquito in the control group. We also observed a significant reduction in sporozoite infections in the salivary glands of mosquitoes that fed on immunized; one of 13 mosquitoes in the immune blood-fed group had salivary gland sporozoites compared with 10 of 15 in the control blood-fed group. The advantages of vector based intervention in malaria are 1) transmission of all 4 human malaria parasites may be affected; 2) the midgut antigens may not exhibit polymorphism; and 3) the midgut antigen-based transmission blocking may also be applicable in other mosquito-borne infectious diseases.

121 AN EVALUATION OF IMMUNE RESPONSES OF *AOTUS* MONKEYS. Hickey MA*, Johnson AH, Araujo HA, Mercolino T, Lyon J, and Taylor DW. Department of Biology, Georgetown University, Washington, DC; Department of Pediatrics, Georgetown University Hospital, Washington, DC; Walter Reed Army Institute of Research, Washington, DC; and Becton-Dickinson Immunocytometry Systems, San Jose, CA.

*Aotus* monkeys serve as experimental hosts for human *Plasmodium falciparum* malaria. Unfortunately, little is known about the immune system of this New World primate. Accordingly, a series of experiments were undertaken to identify i) IgG isotypes, ii) lymphocyte subsets, and iii) Major Histocompatibility Complex (AoLA) antigens in *Aotus* monkeys. Normal *Aotus* plasma was separated by Protein A chromatography using a linear pH gradient. Immunoelectrophoresis revealed that *Aotus* have only 2 major IgG isotypes, of approximate equal concentrations which are tentatively referred to as $IgG_1$ and $IgG_2$. Commercial anti-human IgG recognized only 1 of the 2 isotypes. Using flow-cytometry, 23 fluorochrome-labeled monoclonal antibodies (mAbs) to human T and B cell surface markers were tested on *Aotus* lymphocytes. Only 3 surface markers, namely HLA-DR, CD4, and CD16, were detected. Approximately 50 mAbs to human Class I determinants were assessed. To date, the only reactions were to A9, A10, Bw6 and B27,B7. Anti-human Class II-specific mAb were tested and reactivity with DRw6 was detected. Interestingly, DRw13, which correlates with immunoresponsiveness to CSP, is a member of the DR6 family. Results indicate that *Aotus* -specific reagents need to be developed to adequately evaluate malaria immunity.

122 ANTIBODY RESPONSE TO THE NONREPEAT REGIONS OF *PLASMODIUM FALCIPARUM* CS PROTEIN IN PERSONS LIVING IN MALARIA ENDEMIC REGIONS. Goldman IF*, Shi YP, Alpers M, Marinette P, Gross M, and Lal AA. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.; Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea; Instituto Evandro Chagas, Belem, Brazil; and Smith Kline Beecham, King of Prussia, PA.

Three regions of the CS proteins of the evolutionarily related malaria parasites, *Plasmodium falciparum* and *P. reichenowi* have amino acid sequences that are invariant within *P. falciparum* but not between species. As part of a large study to test whether these between- species divergent regions of the CS protein or other parasite proteins that interface with host cellular receptors are essential for structural and/or biologic (host-parasite) functions, we investigated whether human serum from malaria infected persons would recognize peptides representing these regions. Employing overlapping synthetic peptides spanning the divergent domains and plasma samples (n=92) from malaria-endemic regions of Papua New Guinea and Brazil, we found that plasma from nearly 24% of the infected persons had antibodies that recognized NANPx32 sequences. The reactivity of the plasma antibodies with overlapping nonrepeat based peptides, however, was variable, ranging from a low of 3 % (CANPNKNNQGNGQGHNMPNDPNRNVDY) to a high of 24% (CHKKLKQPGDGNPDPNANPNVDP and CNGQGHNMPNDPNRNVDENANANNAVY). We have also demonstrated that these peptides elicit antibodies in mice that recognize both peptides and native proteins on the surface of the sporozoites. These studies demonstrate that the non-repetitive sequences of the CS protein of *P. falciparum* are immunogenic in natural infections.

123 IMMUNOENZYMATIC LABELLING OF MULTIPLE PLASMODIAL SALIVARY GLAND SPOROZOITES IN A SINGLE TEST. Golenda CF*, Hall T, Wirtz RA, and Schneider I. Department of Entomology, Walter Reed Army Institute of Research, Washington, DC and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

A direct, double and triple staining immunoenzymatic method detected and differentiated by color sporozoites in *Anopheles stephensi* salivary glands and in mixed sporozoite slide preparations. A double staining method utilized galactosidase and alkaline phosphatase labelled monoclonal antibodies to the circumsporozoite (CS) proteins of*Plasmodium berghei* and *Plasmodium falciparum* in mosquito salivary glands. The CS proteins were distinguished clearly by the blue-green and red substrate products of beta-galactosidase and alkaline phosphatase, respectively. A triple staining method differentiated by color among a mixture of *Plasmodium falciparum* and two strains of *Plasmodium vivax* sporozoites. Monoclonal antibodies to the CS proteins conjugated to galactosidase (*P. falciparum*), alkaline phosphatase (*P. vivax* variant), and horseradish peroxidase (*P. vivax* predominant) readily color-differentiated among sporozoites by the blue-green, purple-blue and orange-brown substrate products, respectively.

124 PREVALENCE OF ANTIBODY TO THE VARIANT REPEAT OF THE CS PROTEIN OF *PLASMODIUM VIVAX* IN PERU. Franke ED*, Lucas CM, Cachay M, Covenas H, and Wirtz RA. U.S. Naval Medical Research Institute Detachment - Lima, Peru; and Walter Reed Army Institute of Research, Washington, DC.

A variant repeat in the circumsporozoite (CS) protein of *Plasmodium vivax* was recently reported from Thailand. Serological evidence suggests that the variant CS protein is present in Brazil, India and Thailand. Sera obtained from two populations in Peru with different exposure histories to *P. vivax* were tested for IgG antibodies to antigens representing the variant circumsporozoite (CS) protein (Pvk247) and the predominant CS protein ($NS1_{81}V20$). The populations studied were: (1) children and adults living in an area endemic for vivax malaria in northern Peru and (2) non-immune adults who had patent vivax infections acquired in central Peru. In the residents of the malaria endemic area the prevalence of antibodies to each of the antigens increased with age. In the >16 year age group, 21% had antibodies to $NS1_{81}V20$ and 21% had antibodies to Pvk247, but only 6% had antibodies to both antigens. Fifteen percent of the non-immune adults with vivax infections had antibodies to $NS1_{-81}V20$ and 6% had antibodies to Pvk247. These data suggest that the variant as well as the predominant repeat sequence of the vivax CS protein should be included in candidate sporozoite vaccines.

125 IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* BLOOD-STAGE ANTIGENS AMONG VENEZUELAN YANOMAMI INDIANS. Di John D*, Torres JR, Murillo J, Murphy JR, and Levine MM. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY; Inst de Medicina Tropical, Univ Central, Caracas, Venezuela; Centro Medico, Caracas, Venezuela; and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD.

Prior seroepidemiologic studies of the Venezuelan Yanomami showed age-related increases in antibody to circumsporozoite protein (CSP) of *P. falciparum* and of splenomegaly, despite a virtual absence of patent parasitemia. The low incidence of clinical malaria in this population was thought to reflect acquired immunity. We measured serum antibodies against surface antigens expressed on cultured *P. falciparum*-infected erythrocytes by immunofluorescence to indicate how immunity varied with age. Specimens from 102 Yanomami who resided in Amazonas, Venezuela were analyzed. Geometric mean titers (GMTs) corresponded to the last of serial 2-fold dilutions that displayed fluorescence.

Blood-Stage Antibody GMTs

| Age (years) | 3-5 | 6-10 | 11-15 | 16-20 | 21-30 | >30 |
|---|---|---|---|---|---|---|
| N = | 7 | 10 | 18 | 13 | 24 | 30 |
| GMTs | 535 | 1047 | 1468 | 3709 | 6683 | 7943 |

Antibodies to blood-stage antigens were low in those less than 5 years old and increased markedly with age. These increased titers parallel increases with age in titers to CSP, indicate that repeated injections of sporozoites and at least low-level parasitemia continue throughout the lifetime of these individuals, and may collectively contribute to the widespread immunity of this population.

126 STUDY OF THE HUMORAL RESPONSE AGAINST THREE DEFINED *PLASMODIUM FALCIPARUM* ANTIGENS IN DIFFERENT POPULATIONS USING SYNTHETIC PEPTIDES IN AN IMMUNOENZYMATIC ASSAY. Deslandes D, Ferreira-da-Cruz MF, Oliveira-Ferreira J, Druilhe P, and Daniel-Ribeiro C*. Department of Immunology, IOC - FIOCRUZ, Rio de Janeiro, Brazil; and Institut Pasteur, Paris, France.

To study the natural immune response to the defined plasmodial antigens (Ag), we tested the presence of antibodies (Ab) for the Ag expressed on the *P. falciparum* sporozoites $(NANP)_4$, hepatic schizonts (307) and blood merozoites (70 kDa) by ELISA. Sera from individuals with transfusional malaria (8), nonimmune migrants to an endemic area (Rondonia-Brazil/187), Indians from Amazon (Xingu/235), hyperimmune individuals from Africa (Garetango-Bourkina-Faso/54), and control individuals that have never been in an endemic area of malaria (15) were tested. None of the transfusional malaria sera presented Ab to sporozoite stage Ag although 50% of them presented IgG Ab to the blood stage peptide. Indians from Xingu and nonimmune migrants presented low percentages of Ab against hepatic (36% and 17%) and sporozoite (21% and 12%) peptides although a greater frequency of Ab for blood stage peptide (58% and 49%) was observed in both cases. Only hyperimmune African individuals presented high percentages of Ab to hepatic Ag (85%) contrasting with the low frequency of Ab for the circumsporozoite (CS) repeat Ag (20%). The low frequency of Ab to the hepatic stages presented not only by nonimmune individuals but also by indigenous individuals living in endemic areas in Xingu contrasting with the high frequency observed in hyperimmune African individuals may reflect a polymorphism of the hepatic Ag of *P. falciparum*. We have also observed low frequencies of anti-CS $(NANP)_4$ repeat Ab as compared to the relatively high prevalence of Ab for blood stage Ag found in all studied populations and even to the high frequency of Ab directed to the hepatic Ag recorded among African. In view of these data it may be tempting to conclude that either individuals of the present study were not exposed to enough CS repeat Ab to develop Ab or that these or other cross-reacting Ag that are responsible for the induction of anti-$(NANP)_4$ - Ab are not represented in the strains infecting the studied populations.

127 CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PFMDR2 GENE. Zalis MG*, Wilson CM, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Previous work in our laboratory has demonstrated that *P. falciparum* contains at least two genes related to the mammalian multiple drug resistant (mdr) genes. One of these genes, Pfmdr2, was partially cloned by inverse PCR from W2 clone of Indochina I genomic DNA. Nucleotide sequence analysis of a 2.2 kbp fragment demonstrated an open reading frame of 2.1 kbp. The predicted amino acid sequence is consistent with the structure of known P-glycoproteins. Southern analysis showed a single copy of the Pfmdr2 gene in all *P. falciparum* strains analyzed. Northern analysis with this fragment showed a single band of 6.0 kb in size and in the Poly A+ enriched fraction. A LacZ fusion protein was constructed using a sequenced DNA fragment from the Pfmdr2 gene and pUR291 expression vector. Indirect immunofluorescence analysis utilizing rabbit antiserum to this fusion protein localized an antigen on the parasite within the red blood cell. Preliminary results indicate trophozoite stage specific expression of both the mRNA and the protein.

128 GLUCOSAMINE-6-PHOSPHATE DEAMINASE AND N-ACETYL-GLUCOSAMINE-6-PHOSPHATE DEACETYLASE FROM NORMAL AND *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES. Weidanz JA*, Campbell P, Roden L, and Vezza AC. Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; and Laboratory of Metabolic Diseases, University of Alabama at Birmingham, Birmingham, AL.

The enzymes involved in hexosamine catabolism during asexual erythrocytic development of *Plasmodium falciparum* have not been reported previously. The role parasite and host enzymes perform in sugar metabolism was investigated. Several amino sugar catabolic enzymes were identified and partially characterized from uninfected and infected erythrocytes. N-acetyl-glucosamine-6-phosphate deacetylase (Glcnac-6-P),which converts n-acetyl-glucosamine-6 phosphate to glucosamine-6-phosphate with the release of acetate was assayed in both parasite and uninfected erythrocyte derived extracts. A 150-fold purification was achieved using DE52 ionic exchange chromatography. Results indicate that the parasite lacks Glcnac-6-P deacetylase activity while the uninfected erythrocyte control extract had a specific activity of 200,000 cpm/mg/hr. Glucosamine-6-phosphate deaminase activity, which results in the deamination of glucosamine-6-phosphate to form fructose- 6 phosphate was present in both parasite and uninfected erythrocyte extracts; however,the parasite exhibited a 30-fold greater specific activity than extracts prepared from uninfected erythrocytes. Phospho- hexose kinase isomerase activity was also present in both uninfected erythrocyte and parasite extracts. These findings suggest that the parasite may utilize and be dependent upon the human erythrocyte deacetylase during amino sugar metabolism.

129 THE *PLASMODIUM FALCIPARUM* G6PD GENE: CLONING, SEQUENCE, AND EXPRESSION Shahabuddin M* and Kaslow DC. Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

The pentose phosphate pathway, of which glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme, has two main functions: production of pentose (ribose), and reduction of NADP. Previously it has been reported that when cultured in G6PD deficient RBCs, *P. falciparum* grow this initially reduced, but following an adaptation period, the growth again approximates *in vivo* rates. Production of parasite encoded G6PD(PfG6PD) following a lag phase seemed to fully explain this recovery of normal growth rate. However, we have subsequently observed that PfG6PD is expressed constitutively, even in normal RBCs. The mechanism by which the parasite recovers to normal growth within a few cell cycles, and the mechanism that confers relative protection in females heterozygous for G6PD deficiency, despite expression of PfG6PD, now is an even more perplexing enigma. Further characterization of PfG6PD may provide clues, and to this end we have cloned the PfG6PD gene. Comparison of the deduced amino acid sequence of the PfG6PD with that of mammalian G6PD revealed significant differences. Although the amino acid sequence near the reactive lysyl residue at the G6P binding site appeared similar, the sequence at the proposed NADP binding site did not. The differences between the parasite and the human proteins may be exploited for designing new chemotherapeutic agents.

130 WIDE GEOGRAPHIC DISTRIBUTION OF THE VARIANT FORM OF THE HUMAN MALARIA PARASITE *PLASMODIUM VIVAX*. Qari SH*, Goldman IF, Mellit P, Alpers M, Marinette P, Collins WE, and Lal AA. Division of Parasitic Disease, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.; Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea; and Instituto Evandro Chagas, Belem, Brazil.

We have found polymorphism in the repetitive and nonrepetitive regions of the sporozoite vaccine antigen, the CS protein, in *Plasmodium vivax* malaria parasites from Papua New Guinea (PNG) and Brazil. We used hybridization procedures and nucleotide sequencing to analyze 14 and 30 isolates from PNG and Brazil, respectively. From *P. vivax* isolates from PNG, we found that one patient had parasites with GDRA(D/A)GQPA CS repeat sequence, one had mixed infections with GDRA(D/A)GQPA and the

variant CS repeat sequence ANGA(G/D)(N/D)QPG bearing parasites, and the remainder of the patients were infected with parasites whose CS protein had the ANGA(G/D)(N/D)QPA repeat sequence. In contrast, of the 30 isolates analyzed in Brazil, 2 had *P. vivax* with mixtures of ANGA(G/D)(N/D)QPG and GDRA(D/A)GQPA repeat-bearing parasites, and the remaining 28 had parasites of the GDRA(D/A)GQPA CS repeat sequence. Like the *P. falciparum* CS protein, we have found that the *P. vivax* CS proteins are also polymorphic outside of the repeat regions. One of the three polymorphic regions is the recently identified proliferative T-cell site VTCGVGVRVRRRVNA(A/T)NKKP. We have also found that the CS protein sequence of *P. simium*, a monkey malaria parasite closely related to *P. vivax* (but not infectious to humans by sporozoite), is the same as the CS protein of *P. vivax*. CS polymorphism in *P. vivax* has implications for vaccine development, and identical CS proteins in *P. simium* and *P. vivax* suggests that this protein may not enforce host restriction, or that the host-parasite restrictions are enforced post sporozoite stage.

131 ABSENCE OF PYRIDOXAL KINASE ACTIVITY IN *PLASMODIUM FALCIPARUM* AND *P. BERGHEI*. Inyama JS, Mulaya N, Ofulla OA, Roberts CR, and Martin SK*. Department of General Biology, Kenyatta University, Nairobi, Kenya; Vector Biology Research Center, Kisian, Kenya; and U.S. Army Medical Research Unit, Nairobi, Kenya.

Pyridoxal kinase (PLK) phosphorylates vitamin $B_6$ into the active vitamer pyridoxal phosphate (PLP) which then acts as a cofactor in several key biochemical reactions in the cell. The enzyme is coded for by a high and low activity allele and the low activity gene is expressed phenotypically as decreased red blood cell PLK (RBC-PLK) activity. Afro-americans have a uniquely high frequency of the low enzyme activity phenotype. Nigerian children at risk of death from falciparum malaria were found to have significantly higher RBC-PLK levels than their matched controls. It has, therefore, been postulated that *P. falciparum* may lack PLK activity and be dependent on RBC-PLK for its PLP requirements. We have simultaneously measured PLK activity in the red blood cell hemolysate and corresponding parasite pellet. from: A. cultured *P. falciparum* parasites and B. pooled *P. berghei* infected mouse blood. All of the PLK activity expressed in mMPLP/L/min per $10^6$ cells was present in the RBC hemolysate. Addition of lysed parasites to RBC hemolysate had no effect on PLK activity.

| Sample | Mean PLK Act (mM PLP) | Sample | Mean PLK Act (mM PLP) |
|---|---|---|---|
| *P. falciparum* (n=8) | | *P. berghei* (n=2) | |
| Hemolysate | $1.5 \times 10^{-5}$ | Hemolysate | $1.09 \times 10^{-5}$ |
| Parasite | 0 | Parasite | 0 |

Low RBC-PLP may be an innate resistance factor against lethal malaria infections.

132 *PLASMODIUM FALCIPARUM* SPOROZOITE RELEASE OF CIRCUMSPOROZOITE PROTEIN IN THE MOSQUITO HOST. Beier JC*, Madani A, Vaughan JA, and Noden BH. Department of Immunology and Infectious Diseases, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

The release of circumsporozoite (CS) protein by *Plasmodium falciparum* sporozoites from experimentally-infected *Anopheles gambiae* was investigated to identify factors affecting this process in the mosquito host. The test system involved detecting soluble-form CS protein by ELISA in supernatants from washed, incubated, and centrifuged sporozoite samples. Relationships between the numbers of sporozoites and

the amount of CS protein released were dependent upon incubation times and temperatures. There were no differences in the amount of CS protein released by sporozoites from either the salivary glands, the hemolymph or mature oocysts, or those obtained from mosquitoes 16 or 25 days post-infection. Contact with immune sera in the mosquito host did not affect the ability of sporozoites to release CS protein. The amount of CS protein released during incubation of sporozoites from 45 individual mosquitoes was almost half of the amount of free CS protein within the salivary glands. The amount of CS protein per sporozoite, either released during incubation or free in the glands, was inversely related to the numbers of sporozoites per sample. Further experiments indicated that sporozoites regulate their production of CS protein according to background levels of soluble CS protein. Such mechanisms in the mosquito host may contribute to the viability and transmission potential of sporozoites.

133 THE QUANTITATIVE BUFFY COAT SYSTEM (QBC) FOR THE RAPID DIAGNOSIS OF *PLASMODIUM FALCIPARUM, P. VIVAX* AND *P. MALARIAE* IN A HYPERENDEMIC COMMUNITY. Anthony RL*, Purnomo, and Bangs MJ . US Naval Medical Research Unit, No. 2, Jakarta, Indonesia; and The Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

The QBC malaria diagnostic system was used for the detection and identification of malaria parasites in blood specimens collected from 322 residents of Oksibil; an isolated highland valley in the eastern Jayawijaya Mountains of Irian Jaya, Indonesia. The availability of a rechargeable centrifuge and a fiber-optic paralens, which permits visualization of the plasmodia without the need of a fluorescent microscope, enabled us to complete and interpret the assay in this remote environment. Parasites were easily identified in 53 of 59 specimens which were subsequently confirmed as positive on matched Giemsa stained thick smears (sensitivity = 89.8%). Of the 6 specimens with disparate results, 3 were from cases of *P. falciparum* showing gametocytes only, 1 was from a light *P. ovale* infection and 2 were identified as *P. vivax*. Twenty-three of 263 specimens which were negative by thick smear were positive by QBC (specificity = 91.2%). Fourteen of these specimens were interpreted as "rare *P. vivax* rings", 2 were regarded, and later reconfirmed, as *P. malariae* and 7 were identified as *P. falciparum*. Of the 53 plasmodia identified by thin smear, 26 were identified correctly by QBC; 20/24 *P. falciparum*, 4/22 x*P. vivax* and 2/7 *P. malariae*. Most of the 27 discordant results were attributed to lack of experience in distinguishing between light infections of *P. vivax* and *P. falciparum*. In spite of these difficulties, it was concluded that the QBC is an easy, sensitive and rapid method for diagnosis of malaria in the field and that it provides an additional means for the identification of those individuals who are in need of treatment.

## POSTER I: ARBOVIRUS AND HEPATITIS

134 HEPATITIS IN NORTHERN PAKISTAN. Bryan JP*, Rauf A, Ahmed A, Perine PL, Malik IA, and Legters LJ. Department of Preventive Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD; and Pakistan-U.S. Laboratory for Sero-Epidemiology (PULSE), Rawalpindi, Pakistan.

The etiology of hepatitis in 1129 men admitted to Military Hospital (MH) in Rawalpindi, Pakistan between February 1987 and November 1990 was studied. During this period, hepatitis B was the etiology in 311 (28%) while 804 (71%) were non-A, non-B cases. Hepatitis A was the etiology in only 13 (1%) cases. However, IgG anti-HAV was present in all 179 sera tested, indicating prior hepatitis A infection. Over these 4 years, the proportion of cases of NANB increased from 65% to 77% per year ($p = 0.006$) and the proportion of cases of acute hepatitis B decreased from 32% to 23% $p = 0.003$. Between 1987-89, one of 70 patients with HBsAg was positive for anti-delta antibody. Between 1987-89, HBsAg was present in 169 (26%) of 646 cases of NANB hepatitis while in 1990, at least 14% of NANB cases had HBsAg indicating convalescent or chronic carriage. The mean age of patients during these four years remained constant at 31 years. Patients with HBV or NANB were of similar age. The total bilirubin was

significantly higher in patients with acute hepatitis B on clinical presentation than non-A, non-B hepatitis (10.4 ± 0.8 vs 8.3 ± 0.6) as was the ALT (480 ± 65 vs 263 ± 16). Patients with HBV were more likely than patients with NANB to have had domiciliary or sexual contact with a patient with hepatitis (12.4% vs 4.2%; p = 0.012), to have received injections (39% vs 30%; p=0.12) or to have had dental treatment (26% vs 7.9%; p<0.001). Infections with hepatitis A, B and agents of NANB are common in the Pakistan military. Since hepatitis E has been documented in other military settings and in travelers to Pakistan, it may be a major cause of non-A, non-B hepatitis.

135 RIFT VALLEY FEVER EPIZOOTIC IN THE CENTRAL HIGHLANDS OF MADAGASCAR. Morvan J, Rollin PE*, Laventure S, Rakotoarivony I, Coudrier D, and Roux J. Institut Pasteur de Madagascar, Antananarivo, Madagascar; and Institut Pasteur, Paris, France.

Between February and April 1991, Official Veterinary Services reported an unusual number of bovine abortions around Antananarivo (central highlands, Madagascar). RVF virus isolations were made from sixteen aborted foetus and one dead calf in different foci. By monoclonal antibody characterization, the isolated viruses were found identical to the 1979 RVF strains isolated in Madagascar from mosquitoes and human laboratory infection. These strains were more related to the Egyptian RVF strains isolated during the 1977 epidemic than from any other African strains. Serological surveys in bovine and human were done by IFA, IgM capture ELISA and all positive sera were confirmed by PRNT. In the formerly negative or very low RVF antibody prevalence bovine population, we found a high prevalence of IgM antibodies in bovine (267/968, 27.5% positives). The IgM prevalence in recently aborting females varied between 40 and 91%. Among 490 human sera tested, respectively 10.4 and 6.1% were found positive by IFA and IgM ELISA. One RVF human death was confirmed by virus isolation and specific IgM. A total of 11,597 mosquitos (58% *Culex antennatus*) were collected in the epizootic areas and tested. No isolation was made. Extensive studies were conducted to determine the geographical extension and the impact of this epidemic on the highly susceptible livestock and human populations.

136 TITERS OF VESICULAR STOMATITIS VIRUS, NEW JERSEY SEROTYPE, IN MALE AND FEMALE *LUTZOMYIA SHANNONI* (DIPTERA: PSYCHODIDAE) COLLECTED IN GEORGIA. Comer JA*, Stallknecht DE, Corn JL, and Nettles VF. Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, GA.

Vesicular stomatitis viruses are important pathogens of cattle, horses, and swine in the American tropics and the United States. The New Jersey serotype of vesicular stomatitis virus (VSNJ) is enzootic on Ossabaw Island, Georgia, and *Lutzomyia shannoni*, a phlebotomine sand fly, is the suspected vector. In 1990, 7973 *L. shannoni* were collected from hollow trees on the island and processed for virus isolation in Vero cells. Four isolates of VSNJ were obtained: two from pools of female, one from a pool of male, and one from a pool of damaged, unsexed sand flies. Three pools contained between $10^{4.3}$ and $10^5$ plaque-forming units of virus per pool, suggesting that the positive flies within these pools had supported VSNJ replication. This study provides further evidence that *L. shannoni* is a biological vector of VSNJ and that transovarial transmission of the virus occurs in nature.

137 NATURAL GENETIC VARIATION AMONG JAPANESE ENCEPHALITIS VIRUS STRAINS; IDENTIFICATION OF A NEW GENOTYPIC GROUP. Chen WR*, Rico-Hesse R, and Tesh RB. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

Primer-extension sequencing of the RNA templates of polio, dengue, Rift Valley fever and Japanese encephalitis (JE) viruses has provided new information on their geographic distribution, origin and evolution. In a previous study of 46 diverse JE virus strains, we demonstrated the existence of 3 distinct

JE genotypes in Asia. We now report the occurrence of a fourth genotype. In the present study, 19 JE virus isolates, representing various geographic regions of Asia isolated over a 50 year period, were compared with each other and with Murray Valley encephalitis (MVE) and Kunjin viruses. Two hundred forty nucleotides from the C/ pre-M gene region of JE virus were used in these comparisons. Twelve of the JE strains from the Indonesian Archipelago and Philippines had not been examined before; the remainder were representatives of the 3 previously identified genotypes. Using 12% divergence as a cut-off point, the 19 JE strains fell into 4 distinct genotypic groups; maximum divergence across the comparison region was 21%. The new fourth genotype was comprised of 5 Indonesian isolates which were 7% divergent from the rest of the JE strains and were 16% divergent from a MVE strain. The JE strain as a group were only 9% divergent from a MVE strain.

138 EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE VECTOR COMPETENCE OF *AEDES TAENIORHYNCHUS* FOR VEE AND RVF VIRUSES Turell MJ*. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

We evaluated the effect of environmental temperature, both during mosquito rearing and after virus exposure, on the susceptibility of *Aedes taeniorhynchus* mosquitoes to Venezuelan equine encephalomyelitis (VEE) and Rift Valley fever (RVF) viruses. Mosquitoes reared at low temperature (19 °C) were significantly more susceptible to infection with either virus [infection rates = 237/243 (98%) and 158/226 (70%) for mosquitoes exposed to VEE and RVF viruses, respectively] than were those mosquitoes reared at standard temperature (26 °C) [infection rates =251/322 (78%) and 165/327 (50%) for mosquitoes exposed to VEE and RVF viruses, respectively], regardless of the temperature at which mosquitoes were held after virus exposure (19 or 26 °C). In contrast, in infected mosquitoes, virus disseminated from the midgut to the hemocoel more rapidly in those mosquitoes held at 26 °C than in those held at 19 °C, regardless of the rearing temperature. Thus, a combination of low rearing temperature and warm holding temperature produced the most efficient vectors for both viruses.

139 THE EFFECTS OF TEMPERATURE ON FECUNDITY AND VIRUS REPLICATION IN *AMBLYOMMA CAJENNENSE* INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS. Dohm DJ* and Linthicum KJ. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

A tick-rodent cycle may serve as a possible interepizootic maintenance mechanism for epizootic variants of Venezuelan equine encephalomyelitis(VEE) virus. Laboratory studies have indicated that *Amblyomm cajennense* (F.), an aggressive three-host tick commonly found on equines throughout the geographic range of this virus, is a potential vector of a I-A VEE variant. Transstadial transmission by infected larvae to nymphs and adults and horizontal viral transmission to mammalian hosts by nymphs occurred in this species. Seasonal variations throughout their geographical range suggest that these ticks are subject to a broad temperature range. To assess how temperature affects fecundity and viral replication, adult ticks were intracoelomically inoculated with VEE virus or diluent, divided into three groups, and held at 19, 26, or 33°C, respectively. Ticks were sampled over 36 days. Ten pairs from each group were allowed to feed upon guinea pig hosts on day 8 post inoculation. At drop off, replete females were held at 26°C until oviposition. There was no significant difference between the maximum titers for each group (student t-test, = 0.05) and each group maintained high viral titers throughout the sampling period. However, peak titers (mean = $10^{5.7}$) were attained earlier in ticks maintained at 26 or 33°C (2 days) than in those held at 19°C (8 days). Individuals held at 26°C survived better than those held at the other temperatures. It appeared that infected ticks produced more eggs per tick (mean = 5,315) than did uninfected individuals (mean =4,169); however, there was no significant difference in fecundity between groups inoculated with virus or diluent and held at the same temperature or between groups held at different temperatures. The ability of infected ticks to produce substantial numbers of eggs and maintain

high viral titers at the three tested temperatures suggests that *Amblyomm cajennense* may be a potential vector throughout its geographical range. Further testing to determine egg viability and transovarial transmission may support these results.

140 TRANSMISSION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY HEMATOPHAGOUS MITES. Durden LA*, Linthicum KJ, and Turell MJ. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Previous studies have demonstrated that various species of hematophagous mites may harbor viruses that are pathogenic to vertebrates (e.g., Cocal, Hantaan, Junin, Mayaro, Pichinde, Seoul, and eastern equine, western equine, and St. Louis encephalitis viruses). However, with the exception of St. Louis encephalitis virus, mites have not definitively demonstrated the ability to transmit these pathogens to their hosts during blood-feeding. Studies were undertaken to determine whether two species of hematophagous mites could transmit epizootic Venezuelan equine encephalomyelitis (VEE) virus to suckling mice. The chicken mite, *Dermanyssus gallinae*, mechanically transmitted this virus to previously uninfected mice up to 16 hr after taking a blood meal from a viremic animal. In contrast, the small mammal mite, *Laelaps kochi*,failed to transmit virus to mice in any feeding trials. Virus did not replicate in either species of mite but was detected in 20% of *D. gallinae* and in 12% of *L. kochi* specimens tested 48 hr after they had taken a viremic blood-meal. In mites inoculated intracoelomically with virus, the percentage of positive mites declined over time, but virus was detected up to 7 days later in 5% of *D. gallinae* and up to 3 days later in 10% of *L. kochi*. Neither vertical nor biological transmission of VEE virus was detected in either species of mite. In light of these data, we do not consider either species of mite to be an efficient vector of VEE virus or a candidate reservoir arthropod of epizootic VEE virus during interepizootic periods.

141 HUMAN SEROSURVEY FOR LACROSSE (LAC), WESTERN EQUINE (WEE), AND ST. LOUIS (SLE) ENCEPHALITIS VIRUSES IN CENTRAL AND NORTHERN MISSOURI. Frazier CL*. Department of Biology, Southeast Missouri State University, Cape Girardeau, MO.

Vectors and reservoirs are found in Missouri for LaCrosse (LAC), Western Equine (WEE) and St. Louis (SLE) Encephalitis Viruses, yet there is no continuing statewide surveillance program. To study the incidence of SLE, WEE, and LAC, residual human sera/plasma were obtained from hospitals. In St. Louis, Jewish Hospital serves an urban population from varied socioeconomic groups while Berkley Clinic serves a low socioeconomic area. Sixty-three percent of the donors from Jewish and14% from Berkley were over 58. Jefferson City is located in central Missouri. Truman Medical Center in Kansas City serves individuals, most age 20-40, in the low socioeconomic group. Hannibal is in Northeast Missouri. Forty-eight percent of the sera came from individuals over 60. Sera were tested for antibody using an ELISA. Of the 388 sera tested from Jewish, 16.5% were positive for SLE, 3.1% for WEE and 4.9% for LAC. Of the 348 sera from Berkley, 5.7% were positive for SLE, 2.0% for WEE and 6.9% for LAC. Of the 404 sera from Jefferson City, 13.1% were positive for SLE, 2.5% for WEE and 2.5% for LAC. Of the 348 sera from Kansas City, 5.5% were positive for SLE, 2.3% for WEE and 9.5% for LAC. Of the 311 sera from Hannibal, 1.9% for SLE, 1.0% for WEE and 3.5% for LAC. In all locations, at least 68% of the sera had antibody to only one virus. No SLE positive sera were found in individuals younger than 16.

142 HEMORRHAGIC FEVER WITH RENAL SYNDROME IN FRANCE: UPDATE. Rollin PE*, Courdrier D, Saluzzo JF, and Sureau P. Laboratoire des Fievres Hemorragiques Virales; Institut Pasteur, Paris, France.

Hemorrhagic fever with renal syndrome (HFRS) was first diagnosed in the northern part of France in 1977. From 1977 to 1986, 83 human cases were diagnosed and confirmed by serology (FA). Then the

following years the number of cases decreased. Ninety-one and 38 human cases were confirmed, respectively, in 1990 and during the first six months of 1991. Since the first diagnosis, HFRS cases were observed only in the northern and eastern part of France except for 2 imported cases from Finland and Romania (laboratory infection). Recently, viruses were isolated twice: one from a early serum sample of a human case. By monoclonal antibodies, both viruses belongs to the Puumala serogroup. Patients are between 11 and 79 years old, mainly male (81.8%), lived in the country and noticed rodent in the vicinity in 68% of cases. Clustering of cases in the same family occurred several times. The symptoms observed are classical of the Scandinavian nephropathia epidemica form with a nonspecific, pseudogrippal early phase followed by thrombopenia and acute renal failure with high levels of serum creatinine, proteinuria and hematuria. Only 5% of the patients were dialysed. Neither death nor sequellae (except mild hypertension in early convalescent phase of 2 cases) were observed. High titer (1024-2048) of specific antibodies against Puumala-related viruses were detected by IFA as soon as 5-7 days after the onset of the disease and remained so for several years. The incidence of HFRS is significant in France and appears to be higher than elsewhere in western Europe except for the Scandinavian countries. Physicians in the endemic areas should be aware of this renal disease.

143 REPLICATION AND PERSISTENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN TWO SPECIES OF WEST AFRICAN TICKS. Gonzalez JP*, Camicas JL, Zeller HG, Cornet JP, Some J, and Wilson ML. Institut Francais de Recherche scientifique pour le Developpement en Cooperation, Dakar, Senegal; Institut Pasteur, Dakar, Senegal; Ministere de l'Agriculture et l'Elevage, Bobo-Dioulasso, Burkina Faso; and Yale University School of Medicine, New Haven, CT.

Crimean-Congo hemorrhagic fever (CCHF) virus is a tick-borne *Nairovirus* producing severe zoonotic disease throughout much of Eurasia and Africa. Many of the >30 tick species found naturally infected may be capable of maintaining CCHF virus transmission; studies of vector competence are needed to understand this, as well as the risk of human infection. We studied CCHF virus survival and replication in *Hyalomma truncatum* and *Amblyomma variegatum*, two ticks that are abundant and widespread in Africa. Adult male and female ticks were infected by intracoelomic inoculation. Ticks were later tested for virus by suckling mouse inoculation, antigen capture ELISA, and immunoflorescent antibody. Both species became infected, although in *H. truncatum* the titer of CCHF virus was greater and was detected longer (up to 10 mo. post infection). In another experiment, hypostomectomized male *H. truncatum* that had been inoculated with CCHF virus were allowed to feed and mate with uninfected females on laboratory rabbits. Female ticks became infected, apparently during spermatophore transfer as males were unable to feed. Subsequent transovarial transmission was observed. Results are discussed in the context of CCHF epidemiology in West Africa.

144 RIFT VALLEY FEVER VIRUS ANTIBODY IN HUMAN SERA COLLECTED AFTER AN OUTBREAK IN DOMESTIC ANIMALS IN KENYA. Logan TM*, Davies FG, Linthicum KJ, and Ksiazek TG. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Veterinary Research Laboratory, Kabete, Kenya; and Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

An outbreak of Rift Valley fever (RVF) occurred among herds of domestic animals during June and July 1989, on farms along the margin of Lake Naivasha, Kenya. During October 1989, finger-prick blood samples were collected from herdsmen that worked with affected herds on the 3 farms that had RVF virus-infected herds. Rift Valley fever virus antibodies were measured using an ELISA test. Blood samples that were RVF IgG antibody positive were tested for RVF IgM antibodies. Twelve of the 30 (40%) herdsmen tested in this study had detectable RVF IgG antibody. Five of these twelve IgG-positive samples also contained RVF IgM antibodies. No human disease was seen during this outbreak and none of the herdsmen could recall being sick during the outbreak period despite an association with the RVF affected herds. Thus the 5 RVF IgM-positive herdsmen may be the only humans that encountered RVF

virus during this outbreak. Epizootics of RVF in Kenya have never led to a recognized widespread disease outbreak in the human population even though RVF virus antibodies have been found in some populations. A possible reason that human infections in RVF endemic areas in Kenya are uncommon may the greater distance that Kenyan herdsmen and their families live from their sheep and cattle as compared to other countries where the people and their animals are more closely situated.

145 OUTBREAK OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN YUGOSLAVIA ASSOCIATED WITH DOMESTIC RATS AND MICE. Diglisic G*, Gligic A, Stojanovic R, Obradovic M, Velimirovic D, Lukac V, Xiao SY, Rossi CA, and LeDuc JW. Institute of Immunology and Virology, Belgrade, Yugoslavia; Military Medical Academy, Belgrade, Yugoslavia; Medical University of Belgrade, Belgrade, Yugoslavia; and US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

Hemorrhagic fever with renal syndrome (HFRS) is an acute infectious disease caused by viruses of the Family Bunyaviridae, genus *Hantavirus*. In Yugoslavia, HFRS appears in both mild and severe forms, with lethality rate of 5-16%. In February, 1988, an outbreak of severe HFRS occurred in a family residing in Pozarevac, 60 km SE of Belgrade. Four children, aged 2-12, were infected, and the 2 year-old died. In July of the same year, an adult male residing in the same neighborhood also developed severe HFRS. Signs and symptoms of their diseases included fever, headache, sore throat, myalgia, nausea,"coffee-ground" vomit, conjunctival injection, back pain, rash, hematoma and anuria. Tests of their sera found evidence of hantaviral infection. Small mammals were trapped in and around houses of those affected in February and July, 1988 with 16 *Rattus norvegicus* and 21 *Mus musculus* captured. IFA tests on these sera found 12/12 rats and 6/20 mice with antibodies to Hantaan virus. Hantaviral antigen was found in cryostat sections of lungs from 10/16 rats and 10/21 *Mus*. Lung tissue suspensions from rats positive by IFA were inoculated into seronegative newborn and adult laboratory rats, and seroconversion was documented. Attempts to adapt these suspect isolates to growth in cell culture, and virus characterization are still in progress.

146 HANTAAN AND BORRELIA POSTIVITY IN ALPINE MOUNTAIN TROOPS. Nuti M*, Arreghini S, Peragallo M. Institute of Tropical and Infectious Diseases, First University of Rome, Italy; Health Medical Service "Cadore Alpine Brigate" Belluno, Italy ; and Preventive Medicine Service, Italian Army Medical Corps, Rome, Italy.

The Alpine Mountain Troops ("Alpini") are special troops trained to operate in mountain areas, where they could have putative exposure risks, such as animal (rodents) waste contact or infestation by *Ixodes ricinus* ticks, which in Italy appear to be the more common reservoir of *B. burgdorferi*. Several hundred Alpine Mountain soldiers camped in a military base in forested Dolomite area from June to September 1989. Two months later, a post-exercise epidemiological investigation on 299 recruits was performed. The soldiers tested could be subdivided in two groups: the first cluster included 119 recruits who participate in summer bivouac involving open field living and training exercises; the second included 180 soldiers not attending field activities. At the end of the military service, a venipuncture was routinely made for syphilis serological test. Antibodies to hantavirus were detected by the HNTA-HDPA agglutination test and an ELISA test: a titer of 1:40 and 1:160 or over were considered positive. *B. burgdorferi* antibodies (IgG and IgM, polyvalent) were measured by an ELISA test: ODRs equal or greater than 0.200 were considered positive. Among 299 Alpine soldiers 13 (4.3%) were positive for hantaviral antibody by HANTA-HDPH test; by ELISA, the prevalence was lower (0.7%), with one titer of 1:1600. Borrelia antibodies have been found in 8 out of 267 recruits encamped during the summer bivouac. Among farmers and foresters from the same zones the anti-borrelia antibody was present in 11% and 19%. Furthermore, in these high risk subjects hantaviral antibody was detectable in 4.8% and 7.1% respectively. Hantaan and borrelia seropositivity of soldiers (20 years) and in middle aged foresters (40-45 years), were significantly different ($P < 0.0001$). The lack of borrelia and Hantaan seropositivity in

soldiers who have spent the summer time in bivouacs, with the low overall positivity among Alpine soldiers studied, let us presume that borrelia and Hantaan positivity is linked to an infection previously acquired during the civilian life.

147 A POSSIBLE MOUSE MODEL FOR DENGUE HEMORRHAGIC FEVER. Feighny R*, Dubois D, Putnak R, Burrous J, Summers P, Strupczewski K, LaRussa V, Krishnamurti C, and Hase T. Departments of Virus Diseases, Biologics Research, Hematology, and Ultrastructural Pathology, Walter Reed Army Institute of Research, Washington, DC.

A possible model for dengue hemorrhagic fever was developed. Ninety percent of Balb/c mice injected by the intracranial route with a strain of dengue 4 virus developed encephalitis and severe hemorrhage within 10-12 days on the tail, feet and ears. Examination of internal organs revealed bleeding in the lungs and kidneys with atrophy of the spleen and loss of red pulp. Mice immunized by the intraperitoneal route with dengue 4 virus were protected against both lethal encephalitis and hemorrhage. Experiments are underway to measure virus titers, antibody, clotting factors, and ultrastructural changes to elucidate the cause of hemorrhage.

148 MOLECULAR CHARACTERIZATION OF VACCINIA VIRUS SURFACE ANTIGENS Harrison SA* and Schmaljohn AL. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD.

Current interest in live recombinant vaccinia viruses, candidate subunit vaccines for a variety of tropical pathogens, prompted our investigation of antigens involved in humoral immunity to vaccinia virus. We previously characterized monoclonal antibodies (MAbs) to five virion surface proteins. Estimated Mr, and typical vaccinia neutralization by MAbs to these proteins, were: 26 KDa (>99%); 34 KDa (50-95%); 14 KDa (60%); heterogeneous 15-25 KDa (60%), and 37 KDa (<50%). Two complementary approaches were taken to provide molecular identification of the proteins: 1) a lambda gt-11 expression library was constructed from 1-4 Kb fragments of sonicated vaccinia DNA, and screened for immunoreactivity with specific MAbs; and 2) a combination of detergent solubilization, affinity purification, and western blotting was used to purify individual proteins for N-terminal sequence analysis. From more than $10^5$ lambda plaques screened, one or more were found to express epitopes from either the 34 KDa, 14 KDa, 15-25 KDa, or 37 KDa proteins but not the 26 KDa target of the most efficient neutralizing MAbs. However, purification was successful with the 26 KDa as well as the 34 and 37 KDa proteins. DNA and protein sequence analyses, serving to identify molecular and structural features of these immunologically relevant proteins from among the more than 200 encoded by vaccinia virus, will be presented.

149 ISOLATION OF A HANTAVIRUS FROM A FATAL HFRS CASE IN SLOVENIA. Avsic-Zupanc T*, Likar M, Furlan P, Kaps R, Xiao SY, Rossi CA, and LeDuc JW. Institute of Microbiology, Medical Faculty of Ljubljana and General Hospital, Novo Mesto, Slovenia; and US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

Hemorrhagic fever with renal syndrome (HFRS) has been serologically confirmed in Slovenia during the last six years. Clinical picture of the disease varies from mild to severe, with a mortality rate of 4%. There is substantial evidence that two hantaviruses circulate simultaneously in Slovenia. Recently, a hantavirus was isolated from a fatal HFRS case. The patient's urine and brain homogenates were inoculated onto monolayers of Vero E-6 cells. Positive immunofluorescent reactions with reference human sera and monoclonal antibodies were first recognized after the second passage. The reaction pattern of the isolate, named U1, was similar to prototype Hantaan virus by IFA using a panel of

monoclonal antibodies. Extensive cross-reactivity between U1 and Hantaan, Fojnica and Plitvice isolates was revealed by ELISA using specific rat antisera. Furthermore, the specificity of the U1 isolate was confirmed by restriction analysis of polymerase chain reaction amplified products of this virus with eight restriction endonucleases.

## G: MALARIA BIOLOGY AND MOLECULAR BIOLOGY

150 INGESTION OF *PLASMODIUM FALCIPARUM* SPOROZOITES DURING TRANSMISSION BY ANOPHELINE MOSQUITOES. Beier MS*, Davis JR, Pumpuni CB, Noden BH, and Beier JB. Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD; and Department of Immunology and Infectious Diseases, The Johns Hopkins School of Public Health, Baltimore, MD.

We investigated mechanisms of sporozoite transmission during blood feeding of *Anopheles gambiae* and *A. stephensi* experimentally infected with *Plasmodium falciparum*. Sporozoites were detected in the midgut of 96.5% of 57 *A. gambiae* (GM=29.7; range: 3-374) and in 96.2%of 26 *A. stephensi* (GM=18.4; range: 1-345) fed 22 days post-infection on an anesthetized rat. Sporozoite loads and the number of ingested sporozoites were significantly correlated for both *A. gambiae* (r=0.37)and for *A. stephensi* (r=0.69). Subsequently, *A. gambiae* were tested for sporozoite transmission by allowing them to feed individually on a suspended capillary tube containing 10 µl of blood. A total of 88.9% of 18 infective mosquitoes transmitted a GM of 4.0 (range: 1-36) sporozoites. The same mosquitoes contained a GM of 16.1 (range: 2-165) ingested sporozoites which accounted for 66.6% of the total number of sporozoites released during feeding. The ingested sporozoites persist up to 10 hours, indicating that the number of sporozoites present in the midgut may be a useful indicator of minimal numbers released during blood-feeding. The predictive value in estimating the number transmitted to the human host is being investigated.

151 POPULATION DYNAMICS OF *PLASMODIUM FALCIPARUM* WITHIN LABORATORY-INFECTED *ANOPHELES GAMBIAE*. Vaughan JA*, Noden BH, and Beier JC. Department of Immunology and Infectious Diseases, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

We investigated the population structure of *Plasmodium falciparum* parasites during their sporogonic development in *Anopheles gambiae*. Estimates of absolute densities were determined for each life stage of the parasite. Life tables were constructed for each of 26 infections to determine stage-specific mortalities and sporozoite production. On average, only one macrogametocyte in 2,610 developed to the oocyst stage. There was no relationship between macrogametocyte and ookinete densities. Indeed, efficiency of macrogametocyte fertilization decreased with increasing macrogametocyte density. Ookinete density was the crucial factor determining the success or failure of an infection and thus, the status of an infection could be ascertained from 28 to 34h post-feeding. Oocysts never developed when ookinete densities were below 50 per mosquito. Above this threshold, there was a positive linear relationship between ookinete and oocyst densities (r=0.75). Likewise, there was a positive linear relationship between oocyst and sporozoite densities (r=0.73). Each oocyst produced a geometric mean of 1,317 salivary gland sporozoites. Sporozoite production per oocyst was not affected by oocyst density. The techniques used to quantify each major life stage of *P. falciparum* in *An. gambiae* and to identify key factors affecting vector potential through life table analysis are applicable for any vector-*Plasmodium* system.

152 EVALUATION OF INGESTED HUMAN ANTI-SPOROZOITE SERA ON *PLASMODIUM FALCIPARUM* SPOROGONY IN *ANOPHELES STEPHENSI*. Davis JR*, Beier MS, Beier JB, Clyde DF, and Edelman R. Center for Vaccine Development, University of Maryland School of Medicine,

Baltimore, MD; and Department of Immunology and Infectious Diseases, The Johns Hopkins School of Public Health, Baltimore, MD.

We investigated the effects of human anti-sporozoite antibodies on the sporogonic development of *Plasmodium falciparum* in *Anopheles stephensi*. Human sera from 1) protected, irradiated *P. falciparum* sporozoite immunized volunteers, 2) the same volunteers before immunization, or 3) Kenyans exposed to natural sporozoite transmission,were fed to cohorts of *P. falciparum* infected *A. stephensi* on either day 5, 8, or 11 post-infection blood meal. A fourth group of infected mosquitoes from the same cohort were not re-fed. In 3 experiments, the effects of anti-sporozoite antibodies were evaluated by determining the oocyst infection rate, sporozoite rate, and the number of oocysts and sporozoites per infected mosquito in each group. In contrast to previous studies, there was no evidence that anti-sporozoite antibodies had any effect on the development or intensity of *P. falciparum* infection in *A. stephensi*. Likewise, there was no evidence for enhancement or inhibition of infections due to a second blood meal without anti-sporozoite antibodies. Interestingly, salivary gland sporozoites from the two groups fed on immune sera contained bound human IgG detectable for up to 5 days, as demonstrated by IFA. The infectivity and transmission potential of human IgG-coated sporozoites is unknown.

153 COMPARISON OF APYRASE LEVELS IN FOUR SPECIES OF *ANOPHELES*. Cupp MS*, Cupp EW, and Ramberg FB. Department of Entomology, University of Arizona, Tucson, AZ.

Apyrase, an enzyme that hydrolyzes phosphate bonds of ATP and ADP to AMP, occurs in salivary glands of a large number of hematophagous arthropods. Apyrase provides an important anti-hemostatic component to saliva by preventing platelet aggregation initiated by ADP released from platelet granules. In mosquitoes, enzyme levels have been inversely correlated with length of feeding time on hosts, suggesting that selection for increased levels of apyrase, hence decreased feeding time, is a favorable behavioral trait. We compared enzyme levels in two anthropophilic malaria vectors (*Anopheles gambiae* s.s. and *An. arabiensis*) with two zoophilic species (*An. quadriannulatus* and *An. albimanus*). Salivary gland (s.g.) extracts were prepared in 0.15M NaCl, 0.05% Triton X100. Apyrase activity was defined by release of orthophosphate from ADP, pH 8.5 in the presence of s.g. extracts at 37C. *An. gambiae* and *An. arabiensis* glands contained similar levels of activity (30.7 and 32.4 mU/pair of glands) that were significantly higher than activity in *An. quadriannulatus* (15.3 mU/pair of glands), an antecedent taxon in this African sibling species complex. Apyrase activity in *An. albimanus*, a New World vector, was intermediate (21.6 mU /pair of glands) between *An. gambiae/arabiensis* and *An. quadriannulatus*. Thus enzyme activity is positively correlated with vector competence.

154 A NUTRIENT-PERMEABLE CHANNEL ON THE INTRAERYTHROCYTIC MALARIA PARASITE. Desai SA*, Krogstad DJ, and McCleskey EW. Washington University School of Medicine, St. Louis, MO; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

The patch clamp method was used to study ion transport in the asexual intraerythrocytic stages of *Plasmodium falciparum*. A major difficulty, exposing the parasitophorous vacuole membrane, was overcome with either digitonin or 200 mV electrical pulses to remove the red cell membrane. A further difficulty, forming gigaohm seals on the extremely small parasite (3-5 μm in diameter), was surmounted with exacting pipette tip formation, stringent pipette and solution cleanliness, and high $Ca^{++}$ concentrations. With these precautions, recordings revealed a 150 pS channel with a reversal potential ($E_{rev}$) of -2 mV when the pipette solution contained $Cs^+$ and $Cl^-$ as the major electrolytes. Replacing the CsCl with glycine, a neutral zwitterion, reduced the slope conductance to 85 pS without significantly changing $E_{rev}$, indicating that monovalent cations and anions are equally permeant. Lysine (a cationic amino acid) and glucuronate (an anionic monosaccharide) were also highly permeant. This channel is not demonstrably inhibited by 100 nM chloroquine and has a high open probability that decays under

the nonphysiological experimental conditions. Thus, this channel may play an important role in the parasite's acquisition of nutrients such as amino acids and monosaccharides from the red cell cytoplasm.

155 EXTRACELLULAR DEVELOPMENT *IN VITRO* OF THE ERYTHROCYTIC CYCLE OF *PLASMODIUM FALCIPARUM*. Trager W* and Williams JH. The Rockefeller University, New York, NY.

Among the protozoa that are obligate intracellular parasites are such important pathogenic agents as *Toxoplasma, Babesia, Theileria,* and the malaria parasites. We would like to know the nature of the dependence of such intracellular parasites on their living host cell. One approach to this problem is to grow the parasites extracellularly in a non-living medium. This is what we have done with the erythrocytic stages of *Plasmodium falciparum*. Merozoites of this parasite were suspended in the red cell sonicate medium with ATP and pyruvate previously developed. This was mixed with Matrigel to form a soft gel. The gel was overlaid with the red cell sonicate-ATP- pyruvate medium. This medium was replaced with fresh medium at 12, 24 and 36 hr. At these times and also at 46 hr rhodamine 123 was added to some cultures and gels were sampled for preparation of wet mounts and stained slides. Viable extracellular forms showing rhodamine fluorescence were seen: rings at 12 hr, trophozoites and early schizonts with pigment at 36 hr, and late schizonts with forming merozoites at 46 hr. These merozoites were shown to be infective to erythrocytes added to the cultures at 46 hr. We conclude that the complex process of entry, and the intactness of the host erythrocyte are not essential to the development of a merozoite through its complete asexual cycle.

156 MOLECULAR CLONING OF THE GENES THAT ENCODE THE REPLICATIVE DNA POLYMERASES OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*. Fox BA and Bzik DJ*. Department of Microbiology, Dartmouth Medical School, Hanover, NH.

Because of particularly severe difficulties associated with the biochemical isolation and characterization of DNA polymerases from *Plasmodium*, our efforts have been directed at cloning of *P. falciparum* genes that encode the replicative DNA polymerase catalytic subunits. This approach has allowed us to classify the *P. falciparum* replicative DNA polymerases based on their primary structure, and to establish a framework for the further study of their biochemical properties *in vitro*, and *in vivo*, in order to identify specific inhibitors of their activity. We will describe a general method for cloning of α-like DNA polymerase genes that was used to clone the genes that encode the *P. falciparum* DNA polymerase α and δ catalytic subunits. *P. falciparum* DNA polymerases α and δ are members of the α-like DNA polymerase family because they share six regions of amino acid sequence similarity that define members of this group. The DNA polymerase δ catalytic subunit shares five additional regions of amino acid sequence similarity with the eukaryotic DNA polymerase δ, and δ-like family. The DNA polymerase α and δ genes are single copy genes that reside on chromosome 4, and chromosome 10, respectively, and their encoded mRNA's are expressed just prior too, and when parasite chromosomal DNA synthesis is active. Interestingly, the primary structure of *P. falciparum* DNA polymerase δ was found to be similar to a group of drug sensitive δ-like viral DNA polymerases that includes the herpes virus encoded DNA polymerase family. Thus, we suggest that *P. falciparum* DNA polymerase δ, and possibly DNA polymerase α, represent interesting candidate targets for antimalarial drugs.

157 AN ANALYSIS OF *PFMDR1* mRNA EXPRESSION IN *PLASMODIUM FALCIPARUM*. Volkman SK*, Wilson CM, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The *pfmdr1* gene of *Plasmodium falciparum* encodes a protein with homology to the mammalian P-glycoprotein, the product of the mammalian *mdr1* gene. To better understand the role of this gene, an

analysis of mRNA expression was initiated. We have previously reported an increased expression of mRNA for *pfmdr1* in mefloquine resistant parasites and have confirmed this with extensive quantitative analysis of mRNA. During the course of analyzing the expression of this gene in mefloquine sensitive and resistant *P. falciparum*, we discovered that there were two mRNAs homologous to this gene, one of 8.5 kb expressed in ring and trophozoite stages and a second mRNA of 7.5 kb expressed only in trophozoites. Analysis of these mRNAs by reverse transcriptase-PCR has demonstrated them to be collinear with the DNA in the coding region. Our efforts are presently focused on examining the differences between these stage specific transcripts as well as characterizing them fully with respect to the genomic information in an attempt to further define the structural nature of the gene and its relationship to drug resistance.

158 REGULATION OF MALATE DEHYDROGENASE-ISOENZYMES IN *PLASMODIUM FALCIPARUM*. Lang-Unnasch NE*. University of Alabama at Birmingham, Department of Medicine, Division of Geographic Medicine, Birmingham, AL.

Most eukarytic cells contain two genetically distinct NAD dependent malate dehydrogenase (MDH) isoenzymes. One isoenzyme is localized in the cytoplasm and the other in the mitochondria. Both have important roles in cellular energy flow and mitochondrial function. Since *Plasmodium falciparum* mitochondria change morphologically during gametocytogenesis, it was hypothesized that the expression of at least one MDH isoenzyme would be developmentally regulated. *P. falciparum* MDH activities in trophozoite and gametocyte lysates were identified by the formazan procedure following PAGE. The trophozoite lysates contain one parasite specific MDH activity. The MDH isoenzyme responsible for this activity was purified to homogeneity. It had an apparent subunit molecular weight of 32kD, a pH optimum of 7.0, and a sharp thermal transition between 40°C and 45°C. These characteristics distinguished this *P. falciparum* MDH isoenzyme from human cytoplasmic and mitochondrial MDH isoenzymes. In addition, the resistance of the parasite MDH to substrate inhibition by oxaloacetate suggests that it is the cytoplasmic isoenzyme. Gametocyte lysates contain an additional MDH activity not found in trophozoites or uninfected blood. Together, these observations suggest that expression of the *P. falciparum* mitochondrial MDH is developmentally regulated.

159 PHOSPHOINOSITIDE METABOLISM IN *PLASMODIUM FALCIPARUM* GAMETOCYTES AND SIGNAL TRANSDUCTION. Ogwang RA, Mwangi JK, Roberts CR, and Martin SK*. Kenya Medical Research Institute, Nairobi, Kenya and U.S. Army Medical Research Unit, Nairobi, Kenya.

Mature malaria gametocytes transform into male and female gametes immediately after removal from the vertebrate bloodstream. Both second messengers, inositol trisphosphate Ins(1,4, 5)$P_3$ and diacylglycerol (DAG), associated with phosphoinositide (PI) hydrolysis have been identified in activated *P. falciparum* gametocytes. To further investigate the role of PI hydrolysis products in malaria gametogenesis, compounds that have an effect on the metabolism and biological function of these messengers were tested in an *in vitro* system. Inhibitors of Ins(1,4, 5)$P_3$ phosphatase (i.e., $Mg^{2+}$, $Zn^{2+}$ and 2,3 diphosphoglycerate); calcium ionophore - A- 23187; and a lipid soluble DAG analogue 1-oleoyl-2-acetyl-glycerol (OAG) all stimulated exflagellation in suspended animation buffer, pH 7.4. In addition, methylxanthine inhibitors (i.e., caffeine, theobromine) and aminoglycosides (e.g., gentamycin) also stimulated exflagellation. In contrast, phospholipase inhibitors (i.e., quinine and chloroquine) inhibited all the above triggers except A-23187 and OAG. Ins(1,4,5)$P_3$ receptor blocker (i.e., heparin) also blocked exflagellation. These results implicate phosphatidylinositol-4,5-bisphoshate turnover as the likely mechanism by which *P. falciparum* gametocytes are activated. Our understanding of the biochemical mechanisms for initiation of the sexual cycle of this parasite may lead to new stategies for controlling transmission of the disease.

## H: HELMINTH DIAGNOSIS AND EPIDEMIOLOGY

160 *SCHISTOSOMA MANSONI* TROPOMYOSIN: A SPECIES-SPECIFIC IMMUNODIAGNOSTIC REAGENT. Nicholson LJ*, Xu H, Thakur AN, Rekosh DM, and LoVerde PT. Departments of Microbiology and Biochemistry, School of Medicine and Biomedical Science, State University of New York at Buffalo, NY.

We have identified a protein antigen, *Schistosoma mansoni* tropomyosin (SMTM), which elicits an active antibody response during human schistosomiasis infections. The cDNA for SMTM has been isolated and characterized from a lambda gt11 library, over expressed in a pOTSNCO plasmid vector in *Escherichia coli*, and purified by a combination of 20% ammonium sulfate precipitation and FPLC ion-exchange chromatography. The purified SMTM has been tested on ELISA against the sera of schistosome and non-schistosome infected patients and shown to be disease-specific which was confirmed on western blots. Initial B-cell epitope mapping of SMTM indicates that two recombinantly generated fragments of SMTM are recognized by *S. mansoni* infected patient sera: an 88 amino terminal amino acid peptide, and a carboxy 187 amino acid peptide. Ten monoclonal antibodies produced in mice against SMTM differ in their reactivity to these SMTM fragments, recognizing at least three different epitopes.

161 CIRCULATING SCHISTOSOMAL ANTIGEN IN DIAGNOSIS AND ASSESSMENT OF CURE IN CHILDREN INFECTED WITH *SCHISTOSOMA MANSONI*. Hassan MM* and Strand M. Department of Parasitology, Faculty of Medicine, Zagazig University, Zagazig, Egypt; and Department of Pharmacology and Molecular Sciences, The Johns Hopkins University, School of Medicine, Baltimore, MD.

The effectiveness of praziquantel in treating schistosomiasis is most commonly assessed by quantitating egg production . We have developed an Mab-based antigen-capture ELISA for the serological diagnosis and have used this assay to monitor the efficacy of praziquantel therapy in 50 children with parasitologically proven schistosomiasis. The overall sensitivity of the ELISA was 78% with 100% sensitivity in patient secreting >100 eggs/g feces and 72% for those excreting <100 eggs/g feces. ELISA positivity was directly related to the fecal egg counts before treatment, but there was no correlation between antigen levels and the clinical stage of the disease. After treatment, we observed a highly significant negative correlation between the extent of antigenemia and the time elapsed since treatment. Once the ELISA-positive patients became ELISA-negative as a result of treatment, they had no recurrence of antigenemia. In contrast, the eggs disappeared and reappeared between weeks 2 and 8 in some of the treated patients. Although no eggs were detected in any of the stool specimens at week 12, the presence of antigen in the serum of 18% (7 of 39 ) of the treated patients suggested that they were still actively infected. These data suggest that quantitation of circulating antigens may be more useful than egg counts in monitoring the fate of the parasite after treatment .

162 DIAGNOSTIC ASSAY PERFORMANCE OF THE IMMUNOBLOT AND ITS IMPACT ON THE EPIDEMIOLOGY OF CYSTICERCOSIS IN PERU. Tsang VC*, Gilman R, and Pilcher JB. Cysticercosis Working Group, Immunolology and Molecular Biolology Activity, Parasitological Disease Branch, CID, Centers for Disease Control, Atlanta, GA; The Johns Hopkins University, Baltimore, MD; and Universidad Peruana Cayetano Heredia and PRISMA, Lima Peru.

We previously reported the development of an enzyme-linked immunoelectrotransfer blot (EITB) assay for *Taenia solium* cysticercosis with a 100% specificity and 98% sensitivity. After 3 years of field and laboratory applications (>13,000 tests), the specificity of the EITB remains at 100%, sensitivity for patients with a single cyst (subcutaneous and/or paranchymal) is ~60-80%, and for multiple cysts in the CNS, sensitivity is maintained at 98%. Field comparison of the EITB to other assays in conjunction with neurological, radiological, and clinical findings, indicate that the EITB is the most practical diagnostic

assay available for cysticercosis. The EITB showed that 11% of all patients admitted to a neurological ward in Lima have cysticercosis, while control patient groups hospitalized for other ailments have a prevalence of 1%. We also showed that more than half of the clinical cysticercosis cases were misdiagnosed. Epidemiological data indicate that residents of rural, endemic areas of Peru have a disease prevalence of 8%. Domesticated pigs in these areas have a prevalence of 43-52%, while that of animals from urban areas is 0%. Our data suggest that the EITB is an useful tool for sero-epidemiology and that neurological admissions in endemic areas should be routinely screened for cysticercosis with this test.

163 EVALUATION OF THE LMD ELISA FOR THE DETECTION OF ANTIBODIES TO THE CYSTICERCI OF *TAENIA SOLIUM*. Rosenblatt JE*, Kagan IG, and Boodram C. Mayo Clinic, Rochester, MN; Parasitic Disease Consultants, Tucker, GA; and Provincial Laboratory of Public Health, Edmonton, Alberta, Canada.

We evaluated a commercially available (LMD Laboratories, Inc.) ELISA for the detection of antibodies to the cysticerci of *Taenia solium*. Porcine cyst fluid is used as the antigen and Protein-A peroxidase is the indicator system. Negative ELISA results were obtained from 189 of a total of 198 serums from two different normal human serum banks (9 false positives). Positive ELISAs (titer of > 1:32) were obtained from 7 of 8 serums which were positive by a cysticercosis immunoblot assay performed at the CDC (one false negative). Positive ELISAs were also obtained from 32 of 33 serums which were positive by cysticercosis IHA tests (titer of >1:16) and from 2 of 20 negative IHAs (one false negative and two false positives). On the basis of the above data, the sensitivity of the ELISA was calculated to be 95% (39 true positives and 2 false negatives) and the specificity was also 95% (207 true negatives and 11 false positives). The positive predictive value of the ELISA was 78%. The ELISA was found to frequently cross-react with serums which were positive by IHA or CF tests for antibodies to *Echinococcus* (13 of 22 or 59%), a circumstance well known with other serological tests for cysticercosis. The LMD ELISA appears to be a rapid (20 mins.), simple (read visually), sensitive and specific test which does, however, cross-react with *Echinococcus* -positive serums.

164 COMPARISON OF THE BENTONITE FLOCCULATION TEST WITH THE LMD ELISA KIT FOR DETECTION OF ANTIBODIES TO *TRICHINELLA SPIRALIS* Wilson M*, Ware DA, and McAuley JB. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

Serum specimens from individuals involved in two recent outbreaks of trichinosis were used to compare results of the CDC bentonite flocculation (BF) test and a commercially available ELISA kit (LMD Laboratories) to determine if ELISA is more efficient at identifying acute infection than the BF. Melcher's extract of larvae was the antigen employed in the BF, while an excretory- secretory antigen from larvae was used in the ELISA. Of initial specimens from 27 patients drawn 2-8 weeks after infection, 22% were positive by both tests, 22%were positive by ELISA alone, and 56% were negative by both tests. Of 8 non-infected controls tested, one was positive by ELISA but not by BF; the rest were negative by both tests. Ten patients had specimens drawn 2-3 weeks and 2 months post-infection. Eight were negative by both tests and 2 were positive by ELISA alone in the acute sample. All 10 patients were positive by both tests in the second sample. ELISA appears to offer an increase in sensitivity in detecting acute specimens, but with perhaps a corresponding decrease in specificity.

165 PHYSICAL GROWTH AND SCHISTOSOMIASIS JAPONICA INFECTION IN LEYTE, PHILIPPINES AND JIANGXI, CHINA. McGarvey ST*, Aligui G, Olveda R, Wu G, Zhong S, Peters P, Olds GR, and Wiest PM. Program in Geographic Medicine, The Miriam Hospital, Brown University, Providence, RI.

The association between schistosomiasis infection and child growth was studied cross-sectionally in : 1) 1,581 males and females aged 4 -19.9 years residing in Leyte, Philippines; and 2) 239 males and females aged 4 -19.9 years residing in Jiangxi, China. Stature, weight, arm muscle area and fatness, assessed by sum of triceps and subscapular skinfolds, were related to presence of *S. japonicum* eggs in Kato stool smears, intensity of schistosomiasis infection, and presence of hookworm, ascaris and trichuris. Multivariable models were used to control for the effects of age, age$^2$, and polyparasitism on growth. In the Philippine males and females, intensity of schistosomiasis infection was significantly related ($p<0.001$) to retarded height, arm muscle and fatness, after adjusting for hookworm infection, and especially among those >7 years of age. The greatest age-specific differences were during adolescence in both males and females. In all female Chinese, intensity of schistosomiasis infection was significantly related ($p<0.01$) to retarded weight, arm muscle and fatness, especially in adolescence. In males only fatness was related to schistosomiasis infection intensity. These data strongly suggest that schistosomiasis japonica infection independently reduces child growth, especially in adolescence.

166 THE EPIDEMIOLOGY OF *OPISTHORCHIS VIVERRINI* AND ASSOCIATED HEPATOBILIARY DISEASES IN TWO DISTRICTS IN KHON KAEN PROVINCE, NORTHEAST THAILAND.
Haswell-Elkins M*, Sithithaworn P, Mairiang E, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Elkins D. Tropical Health Program, Queensland Institute of Medical Research, Herston, Australia; Departments of Radiology, Parasitology, and Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

A population-based study used ultrasonography in combination with mass stool examination is being carried out in Northeast Thailand, an endemic area for the liver fluke, *Opisthorchis viverrini*. The purpose is to estimate the frequency of hepatobiliary disease associated with this infection among the population. So far the program has covered 46 villages in two districts in Khon Kaen province, quantitative egg counts have been performed on stools collected from over 7,000 people and 1,500 have undergone abdominal ultrasonography. The two districts differ markedly in the frequency of heavy infections and severe hepatobiliary disease, including hydrops gall bladder, dilated ducts and cholangiocarcinoma, recorded as both suspected early cases and as a cause of death. In addition, the data demonstrate that individuals with heavy liver fluke infection are at much higher risk of these signs of severe disease than uninfected or lightly infected people. We conclude that in areas with a high intensity of *Opisthorchis* infection, hepatobiliary disease, especially cholangiocarcinoma, is a major cause of mortality among the adult population.

167 OUTCOME OF CASE-CONTAINMENT STRATEGY TO ELIMINATE DRACUNCULIASIS FROM PAKISTAN IN 1990.
Kappus KD*, Hopkins DR, Ruiz-Tiben E, Imtiaz R, Andersen J, Azam M, Attiq A, Hightower A. Center for Infections Disease, Division of Parasitic Disease, Centers for Disease Control, Atlanta, GA; Global 2000, Inc., Atlanta, GA; and National Institute of Health, Pakistan.

During the 1990 transmission season, the Guinea Worm Eradication Program in Pakistan initiated case containment. This new strategy of intensified surveillance and control measures in villages with endemic dracunculiasis was implemented to meet the national target of eliminating transmission of dracunculiasis by 1990. Information indicating how completely the operational elements of the strategy were applied to the handling of cases was collected on each of the 160 cases in 1990. The five operational elements were: identifying cases within 24 hours of worm emergence, providing medical treatment to the patient, identifying and treating water contaminated sources, and mobilizing the community in response to the case.. Intensive surveillance supplemented by cash rewards for case detection continued in Pakistan during the 1991 transmission season. Only 19 villages reported cases from January through

mid-August, 1991. These included 9 of 56 villages that had case reports in 1990, 7 villages with case reports before 1990, and 3 villages without previous case reports. Over half of the 59 cases were identified in one village. Each case in 1991 will be investigated to determine if it is imported or indigenous and if it can be linked to a 1990 case. The significance of the results for the ongoing eradication campaign will be discussed.

## I: FILARIA BIOLOGY AND MOLECULAR BIOLOGY

168 INSECT JUVENILE HORMONES AND THEIR ANALOGS PROMOTE DEVELOPMENT OF THIRD-STAGE FILARIAL LARVAE *IN VITRO*. Bodri MS* and Lok JB. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Our aim is to investigate the potential for endocrine control of molting in larval filariae. Other laboratories have demonstrated that insect juvenile hormones (JH's) are capable of promoting molting in *Caenorhabditis elegans* and *Phocanema decipiens*. We have examined the effects of these agents alone and in combination with a putative JH antagonist, precocene II, on development by third-stage larvae (L3) of *Dirofilaria immitis* and *Onchocerca lienalis in vitro*. Using a serum-free culture system, we found that in the concentration range of 0.4-40 mM, farnesol, regarded as an insect JH analog, gave significant increases in percentage molting (75 *vs* 31%) by L3 of *D. immitis* over controls. To ascertain an endogenous JH-like mechanism, we examined the effects of the anti-JH compound precocene II. Precocene II at concentrations greater than 2 and 3 mM inhibited molting relative to controls in *O. lienalis* and *D. immitis* respectively. In an effort to determine whether inhibition by precocene was due to blockade of an endogenous JH-like mechanism we found that in *O. lienalis*, 0.34 mM JH-I gave partial restoration of molting (15.9% vs 6.1% for controls) in larvae treated with 0.2 mM precocene. In view of these findings, further investigation of endocrine control of molting in larval filariae is warranted.

169 ISOLATION AND PARTIAL STRUCTURAL CHARACTERIZATION OF A MAJOR IgE-INDUCING ANTIGEN IN PATIENTS WITH TROPICAL PULMONARY EOSINOPHILIA. Lobos E*, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD.

Tropical Pulmonary Eosinophilia (TPE) is an unusual manifestation of filarial infection with either *Wuchereria bancrofti* or*Brugia malayi*. TPE is characterized by nocturnal cough, impaired lung function, hypereosinophilia in the peripheral blood and the lower respiratory tract and extremely elevated levels of antifilarial antibodies (Ab), especially IgE. To identify and characterize molecules responsible for the induction of IgE Ab, we have optimized conditions for protein separation of *B. malayi* adults for two dimensional gel electrophoresis (2-DGE). Two dimensional immunoblot analysis using serum pools from TPE patients and from other filarial infections allowed the identification of two acidic allergens with $M_r$23 and $M_r$25 (Bm23 and Bm25) that were uniquely recognized by patients with lymphatic filariasis. Tryptic peptide analysis of the *in situ* radioiodinated allergens and subsequent peptide mapping revealed homology between Bm23 and Bm25. Western blot analysis revealed that IgE and IgG4 Ab recognize both allergens in parallel. IgG4 and IgE Ab affinity-purified from sera of TPE patients on isolated Bm23 and Bm25 identified them as microfilarial proteins. Furthermore, IgE Ab present in the bronchoalveolar lavage fluid of patients with TPE recognize both allergens in a compartmentalized manner. A partially purified protein fraction containing Bm23 and Bm25 induced lymphocyte proliferation in PBMC of patients with TPE. Further characterization of these allergens could give insight into the pathology of the TPE syndrome and may help define the antigenic signals for the induction of IgE in lymphatic filariasis.

170 CHARACTERIZATION OF A CYSTEINE PROTEASE INHIBITOR FROM *DIROFILARIA IMMITIS*. Hong Y*, Limberger R, Poole CB, and McReynolds LA. New England Biolabs, Inc., Beverly, MA; and New York State Department of Health, Albany, NY.

The L4 stage of *D. immitis* is an attractive vaccine target for it is present in the host for several months. One antigen, DiCys, was isolated with anti-L4 sera, from a cDNA library. A computer search of the derived protein sequence identified a region of homology with several cystatins, cysteine protease inhibitors. DiCys was purified by fusion to maltose binding protein [MBP]. The MPB-DiCys fusion retained the ability to inhibit papain, a cysteine protease, even after SDS gel electrophoresis. The renatured fusion protein was resistant to digestion while control proteins were digested. A protein related to DiCys, has been cloned from *O. volvulus* by S. Lustigman. Comparison of the two protein sequences reveal that they have a conserved carboxy terminal region [87%] and variable amino terminal region [12%]. The organization of the gene in *D. immitis* was determined by Southern blot hybridization with two DNA probes; one to the conserved and one to the variable region of DiCys. Both DNA probes recognized overlapping sets of restriction fragments in *D. immitis* DNA demonstrating that the two regions of the protein are contigious in the genome. *B. malayi* and *B. pahangi* DNA also hybridized to both probes. Western blots with antisera raised against the recombinant DiCys identify two bands ( est. 14kD and 34 kD) in extracts of *D. immitis*, *B. malayi* and *B. pahangi*. These studies demonstrate that DiCys is a member of a gene family that is present in different filarial parasites. Further studies are underway to determine if the two different protein bands are from related genes or are created by protein processing. The target for these parasite protease inhibitors is unknown.

171 MOLECULAR CLONING OF A CYSTEINE PROTEINASE INHIBITOR OF *ONCHOCERCA VOLVULUS*. Lustigman S*, Smith AB, Prince AM, and McKerrow J. Virology and Parasitology, Lindsley F. Kimball Research Institute of The New York Blood Center, New York, NY; and Department of Pathology, School of Medicine, University of California, San Francisco, CA.

In our efforts to identify potentially protective recombinant proteins of the larval stages of *Onchocerca volvulus* we have isolated and characterized a cDNA clone designated OV7. Clone OV7 encodes a polypeptide that corresponds to a 17 kDa parasite protein that is present in L3, L4 and adult worms but not in the microfilariae. Immunoelectron microscopy revealed antigen localization in the hypodermis and the basal layer of the cuticle of L3 and female adult worms, and in the egg shell around developing microfilariae. Animo acid sequence analysis showed that OV7 has a significant homology with the cystatin superfamily of cysteine proteinase inhibitors. To establish if our recombinant protein is active as a cysteine proteinase inhibitor, we first subcloned the clone into pGEX and Mal E expression vectors, producing GST-OV7 and MBP-OV7 fusion polypeptides. 1μM GST-OV7 or 4 μM MBP-OV7 fusion polypeptide inhibited 50% of the activity of the cysteine proteinase Cathepsin B. Neither fusion polypeptides inhibited serine or metallo- proteinase activity. The inhibition constant for GST-OV7 is 170nM for Cathepsin B and 70 pM or 25 nM for two parasite cysteine proteinases purified from *E. histolytica* or *C. elegans* respectively. Thus, OV7 fusion polypeptide contains an active cystatin-like domain that inhibits the activity of cysteine proteinases at physiological concentrations. The possible function of a cysteine proteinase inhibitor during the development of the parasite, and its immunological significance, will be discussed.

172 MOLECULAR CHARACTERIZATION OF A *WUCHERERIA BANCROFTI* RECOMBINANT, WBN43 RECOGNIZED BY PUTATIVELY IMMUNE INDIVIDUALS. Raghavan N*, Freedman DO, Tuan RS, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Department of Orthopaedic Surgery & Microbiology, Thomas Jefferson University, Philadelphia, PA.

Putatively immune individuals (PI's) living in a *W. bancrofti* endemic area (Mauke, Cook Islands) were found to recognize a 43 kDa infective larval stage antigen from *Brugia malayi*. To characterize further this 'potentially protective' antigen, antiserum was raised in rabbits against the purified 43 kDa antigen isolated from 2-D gels. Using this antiserum, a recombinant clone WbN43 (2.3 kb) expressing a 162 kDa fusion product was isolated from a genomic expression library of *W. bancrofti*. The 162 kDa fusion product was recognized only by the rabbit antiserum and sera from the PI's and not from infected patients. In addition, hyperimmune rabbit serum and sera from the putatively immune individuals affinity purified on WbN43 could recognize a 43 kDa antigen from *B. malayi* L3 extracts. In contrast, affinity purified normal rabbit, normal human or infected patient sera failed to recognize this antigen. Further characterization of WbN43 was done after subcloning the 2.3 kb insert into pUC19. Southern analyses indicate that this 43 kDa antigen is present as a low copy gene. Sequence analyses indicate that the recombinant protein expressed is hydrophobic and has six highly antigenic domains. Both *in situ* hybridization and immunostaining using *B. malayi* adult tissue sections showed that this antigen is expressed to a certain degree in the intrauterine microfilariae but not in the adult tissues. This recombinant antigen is currently being overexpressed in the *Mal E* vector and pEV-vrf 1 vector for further characterization at the T and B cell level and to define its role as a potentially protective antigen.

173 A HIGHLY SENSITIVE, SPECIES-SPECIFIC DNA PROBE FOR THE DETECTION OF EGYPTIAN *WUCHERERIA BANCROFTI*. Williams SA*, Chan TY, Ramzy R, Weil G, Gad A, and Hamburger J. Department of Biological Sciences, Smith College, Northampton, MA; Research and Training Center on Vectors of Disease, Ain Shams University, Cairo, Egypt; Washington University School of Medicine, St. Louis, MO; Hebrew University, Hadassah Medical School, Israel.

We have developed a new sensitive, species-specific DNA probe for the detection of *Wuchereria bancrofti* in human blood and mosquitoes. Unlike previously described probes developed for *W. bancrofti*, this probe shows absolutely no cross-hybridization to human DNA or *Brugia malayi* DNA. This probe is based on a new repeated DNA sequence isolated from an Egyption *W. bancrofti* Eco-RI star genomic library constructed in lambda-gt 10. This library was screened with *W. bancrofti* genomic DNA and counter-screened with human and *B. malayi* DNA. Clones hybridizing strongly to *W. bancrofti* DNA but showing no hybridization to large quantities of human and *B. malayi* DNA were selected for further analysis. Upon further testing, one clone (Wb14) proved to be extremely sensitive in detecting *W. bancrofti* DNA (< 50 pg) and showed no cross-hybridization to human, *B. malayi* or *Culex pipens* DNA. The ability of this probe to detect less than 50 pg of *W. bancrofti* DNA is crucial, since this is less than the amount of DNA found in a single microfilaria or L3 larva. The lack of cross-hybridization to human and mosquito DNA is important since this probe is currently being tested on field collected human blood and mosquito (*Culex pipens*) samples from Egypt.

174 CLONING AND CHARACTERIZATION OF A *LOA LOA* SPECIFIC REPETITIVE DNA. Egwang TG*, Akue JP, and Pinder M. International Medical Research Center of Franceville (CIRMF), Franceville, Gabon.

Repetitive DNA from filarial parasites have proved to be useful diagnostic tools for detection of microfilariae in host blood and infective larvae in insect vectors. Repetitive DNA probes are species-specific and may differentiate between strains of the same species. Our objective was to identify and clone a *L. loa* repetitive DNA which might be used, not only for diagnostic purposes, but also to distinguish between simian and human *L. loa*. A *L. loa* Eco RI genomic library was screened with $^{32}$P-labelled *L. loa* DNA and one repetitive clone, LL20, was isolated. An 860 bp Rsa I fragment of LL20, which is *L. loa* specific, was subcloned into pUC19 and the recombinant plasmid was designated pRsa4. While the 3.8 kb Eco RI fragment of LL20 cross-hybridized to other filarial DNA under low stringency conditions, the 860 bp fragment of pRsa4 was *L. loa* specific under the same conditions. Further

characterization of the insert of pRsa4 was therefore carried out. Its lower limit of detection is 800 pg of *L. loa* genomic DNA, it has a low copy number (50-100) and it appears to have an interspersed distribution in the genome. The probe does not distinguish between simian and human *L. loa* DNA. Further work is in progress to determine the sequence of this repetitive DNA and its usefulness for detection of *L. loa* infective larvae in *Chrysops* vectors.

## J: ARBOVIRUS IMMUNOLOGY

175 ASSAYS OF CELL-MEDIATED IMMUNITY IN RECIPIENTS OF A LIVE, ATTENUATED JUNIN VIRUS VACCINE. Peters CJ*, Kenyon RH, Barrera-Oro JG, McKee KT, and MacDonald C. Disease Assessment Division, U.S.Army Medical Research Institute of Infectious Diseases, Frederick, MD; and Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD.

We monitored cellular and humoral responses to a new Argentine hemorrhagic fever (Junin virus) vaccine. Antigen-specific DNA synthesis by blood lymphocytes was compared to serum plaque-reduction neutralization antibody tests (PRNT) in 322 observations made on 80 Junin virus seronegative volunteers from USAMRIID. The pre-vaccination stimulation index (SI) was less than 2.0 in 95% of those tested. The SI usually began to rise within three weeks of vaccination, reaching a median of 7.8. A decline was noted after 1 year. PRNT also became positive in the third week in many subjects, but in others its increase was delayed 1-3 weeks. There was no correlation between the magnitude of the SI and the PRNT except that a very high SI (>20) predicted a positive antibody titer. Only 3 recipients were low responders by SI, but the distribution of the PRNT test was bimodal with 18 persons having titers of 16 or less. Serological non-responders were twice as likely to have previously worked with arenaviruses as other subjects. The proliferation of lymphocytes in response to antigen measures an independent parameter of the immune system and deserves further exploration in assessment of vaccines for viruses in which cellular immunity is important.

176 CYTOTOXIC T CELL EPITOPES CONSERVED AMONG MOPEIA, MOBALA, LASSA, AND LYMPHOCYTIC CHORIOMENINGITIS VIRUSES. Higgins YK*, Schmaljohn AL and Peters CJ. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD.

As typified by the response to lymphocytic choriomeningitis virus (LCMV) in mice, T cells -- particularly cytotoxic T lymphocytes (CTL) -- play a major and often decisive role in arenavirus immunity. To better understand the significant but sometimes incomplete cross-reactive immunity among Old World arenaviruses LCM, Mopeia, Mobala, and Lassa, we examined specificities of CTL in BALB/cJ ($H2^d$), C57BL/6J ($H2^b$), and C3H/HeJ ($H2^k$) mice. H2-restricted cytotoxic cells (i.e. CTL) were easily demonstrable against LCMV in all three strains of mice, while only BALB/c and C57BL/6 made CTL against Mobala. In BALB/c, Mobala-immune CTL efficiently lysed LCMV-infected syngeneic targets and vice versa. Insusceptibility of target cells to Mopeia virus prevented clear demonstration of Mopeia-specific CTL; however, Mopeia-immune BALB/c and C57BL/6 mice exhibited modest lytic activity against both LCMV- and Mobala-infected syngeneic cells. Cross-reactivity of BALB/c CTL was shown to coincide with cytotoxic activity against a previously defined LCMV nucleoprotein peptide (RPQASGVYMGNLTAQ), and with homologous peptides from Lassa (RPLSAGVYMGNLSSQ) or Mopeia (RPLAAGVYMGNLTAQ). Patterns of cross-protection *in vivo* were consistent with CTL data: BALB/c mice previously infected with either Mopeia or Mobala were fully resistant to intracerebral challenge with LCMV, whereas C57BL/6 were partially resistant and C3H remained susceptible to LCMV.

177 BIOCHEMICAL PARAMETERS OF FLAVIVIRUS SYNTHETIC PEPTIDE T-CELL EPITOPES. Roehrig JT*, Johnson AJ, Mathews JH, Hunt AR, and Beaty BJ. Division of Vector-Borne Infectious Diseases, CID, CDC, PHS, DHHS, Fort Collins, CO. and Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO.

The observation that flaviviruses may contain as yet undefined "enhancing epitopes" that prime individuals for severe disease makes the design of a monovalent whole virus vaccine difficult. Inclusion of only type specific, protective epitopes in a vaccine could limit severe complications. It is clear that the most successful subunit vaccines will require both B- and T-cell epitopes. Using synthetic peptides which define either B- or helper T-cell epitopes of the E-glycoprotein of Murray Valley encephalitis or dengue viruses, we have begun to define the required interactions necessary for an efficient immune response. The antibody response to some of the B-cell epitope containing peptides is genetically restricted in inbred mice. Most of these restricted peptides do, however, elicit antibody in outbred animals. The genetic restriction can be overcome be mixing the B-cell peptide with a peptide containing an active T-helper cell epitope. One peptide (352-368) demonstrates T-helper activity with both viruses. This peptide is derived from an E-glycoprotein region which occurs in a predicted amphipathic alpha-helix. We have determined that the T-helper cell activity of this peptide requires crosslinking with the B-cell epitope containing peptide. We will report on the immunogenic activity of colinear syntheses of B- and T-cell epitopes.

178 ANTIGENIC COMPARISON OF AFRICAN AND INDIAN STRAINS OF WEST NILE VIRUS BY WESTERN BLOT ANALYSIS. Summers PL, Martinez BC*, Dubois DR, Silor DL, Barvir DA, Timchak RL, and Eckels KH. Walter Reed Army Institute of Research, Washington DC.

*West Nile* (WN) causes disease outbreaks in Africa, the Middle East and India. For the purpose of vaccine development, we evaluated 11 strains of WN virus from different regions for antigenic differences that may be helpful in designing a vaccine strategy for WN virus. A panel of mouse hyperimmune ascitic fluids (MHAF) prepared against the WN strains were used in this antigenic analysis. Immunoblots of WN virus-infected mosquito C6\36 cell extracts revealed the presence of 5-6 WN proteins.The MHAF of the WN strain Egypt 101 cross-reacted strongly with the flavivirus encephalitic subgroup (Japanese encephalitis (JE), St. Louis encephalitis, Murray Valley encephalitis, and Kunjin virus), moderately with yellow fever (YF) and very poorly if at all with the four serotypes of dengue (DEN) virus. Interestingly the MHAF's from JE, DEN-1, 2 and 3 cross-reacted well with Egypt 101 strain viral proteins, whereas DEN-4 and YF MHAF reacted weakly. The WN African virus strains B956, Egypt 101, Eth. An 4766, Eth. An 4767, Dak Ar B310, Ib An 7019, and 14778 cross-reacted well with all of the WN MHAF's tested as did the Madagascar strain Dak An Mg798. The Indian strain 804994 reacted well with all the MHAF's except the African B956 and Egypt 101 where only a faint reaction with a 51Kd protein could be detected. The MHAF's from the other two Indian strains IG2266 and 68856 also were cross-reactive with all WN strains, 68856 reacting the strongest and IG2266 reacting very weakly. These data suggest that despite temporal and geographical differences between the 11 WN isolates, there appears to be very little antigenic differences as detected with MHAF polyclonal antibodies with the possible exception being the Indian strain 804994.

179 MONOCLONAL ANTIBODY CHARACTERIZATION OF JAMESTOWN CANYON (CALIFORNIA SEROGROUP) VIRUS TOPOTYPES ISOLATED IN CANADA. Artsob H*, Spence L, Brodeur B, and Th'ng C. National Laboratory for Special Pathogens, Laboratory Centre for Disease Control, Ottawa, Canada; Department of Microbiology, University of Toronto, Toronto, Canada; and National Laboratory for Immunology, Laboratory Centre for Disease Control, Ottawa, Canada.

Jamestown Canyon (JC) virus of the California (CAL) serogroup has been isolated in at least 12 American states and 6 Canadian provinces. A study was undertaken to produce monoclonal antibodies to JC virus and to use these monoclones to assay for possible heterogeneity among naturally occurring JC topotypes in Canada. Monoclonal antibodies were produced to the prototype strain of JC virus using BALB/c mice. Numerous secreting monoclones were obtained. Three of these monoclones were propagated and studied. One monoclone was shown to react by fluorescent antibody staining to early antigen while the other two monoclones reacted with late antigen. All three monoclones reacted by ELISA. One monoclone cross-reacted with all members of the Melao complex, one cross-reacted only with Keystone virus while the third exhibited no cross-reactivity with other CAL serogroup viruses. One monoclone possessed neutralization and hemagglutination inhibition activities. The monoclones were tested by ELISA and neutralization against 13 JC topotypes isolated in five provinces from Newfoundland to Saskatchewan. ELISA confirmed closer identity of the Canadian topotypes to JC as opposed to the closely related South River virus. The monoclones verified all Canadian topotypes to be JC virus but revealed different patterns of reactivity between these topotypes and prototype JC virus.

180 STUDIES OF RABIES VIRUS PATHOGENESIS USING ANTI-IDIOTYPIC MONOCLONAL ANTIBODIES. Hanham CA*, Zhao F, Tignor GH. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

We have used antiidiotypic monoclonal antibodies (Mabs) to demonstrate that rabies virus binds the acetylcholine receptor (AChR). Mab B9, an IgM antiidiotypic antibody raised against virus- neutralizing Mab 7.11, binds both AChR and a membrane molecule on brain cells. B9 reactivity *in vitro* strongly suggests that this antireceptor antibody is an internal image of 7.11. The aim of this work was to investigate whether uptake of B9 into the CNS could be demonstrated *in vivo*. Intact B9 (IgM) antibody does not enter the CNS. $F(ab')_2$ fragments were made and characterized. They stained both Torpedo electric organ and mouse brain tissue. Suckling mice were inoculated with B9 and subsequently sacrificed at various intervals. B9 stained specific regions of brain tissue with peak uptake at 45 min. Longer incubation intervals resulted in diminished signal. Localization of B9 binding resembles virus during rabies infection. Our results suggest that B9 not only has value in studies of the pathogenesis of rabies or rabies-like viruses, but also has potential therapeutic use. Protection studies *in vivo* are currently underway.

181 THE FC PORTION OF MONOCLONAL ANTIBODY TO YELLOW FEVER VIRUS NS1 IS A DETERMINANT OF PROTECTION AGAINST YF ENCEPHALITIS IN MICE. Schlesinger JJ* and Foltzer M. The Rochester General Hospital and the University of Rochester School of Medicine and Dentistry, Rochester NY.

We prepared the F(ab')2 moiety of a protective IgG2a Mab, 1A5, against YF NS1 and measured its effect on YF replication in mouse brain after lethal challenge. F(ab')2 and IgG 1A5 bound to cell surface NS1 with equal avidity, and F(ab')2 1A5 abrogated IgG 1A5 sensitization ofYF-infected cells to complement-mediated cytolysis. Passive transfer of IgG, but not F(ab')2 1A5, resulted in a 10-fold ($p<0.002$) reduction in titratable brain-associated YF. An IgG1 anti-NS1 Mab, 4E3, which blocked IgG 1A5 binding and cytolytic activity, also had no effect on replication of YF in brain and did not protect. Co-administration of 4E3 and 1A5 resulted in partial ($p<0.02$) interference with the protective effect of 1A5. Protection against lethal YF challenge and diminished YF replication were also observed in 1A5-immunized mice depleted of C3 by treatment with cobra venom factor. Taken together, these results suggest that Fc-piece characteristics, as much as epitope specificity, determine the protective capacity of anti-NS1 Mab and raise the possibility that activation of early components of the complement cascade by anti-NS1 antibody may interfere with YF replication without C3-initiated lysis of infected cells.

182 INHIBITION OF BUNYA- AND FLAVIVIRUSES *IN VITRO* BY ANTICARBOHYDRATE MONOCLONAL ANTIBODIES. Blough HA*, Kefauver D, Clausen H, and Hansen JS. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD; and Department of Infectious Diseases, Hvidovre Hospital, Denmark.

The role of carbohydrates in arboviral neutralization has not been explored. These studies were undertaken to test the hypothesis that monoclonal antibodies (MAb) directed against specific linkages and/or sequences of oligosaccharides could neutralize flavi- and bunyaviruses. MAbs directed against different carbohydrate epitopes were interacted with ca 50 pfu of sand fly fever (SFS), yellow fever (YF 17-D), and Japanese encephalitis (JEV) viruses; both dialyzed hybridoma supernatants and chromatographically purified MAbs were used. Seed virus was propagated in Vero or *Aedes albopictus* (C6-36) cells; Vero, MK-2 and CEM (human lymphoblast) cells were used for challenge. Virus was harvested at 24, 48, and 72 hours and titrated on Vero cells. Two MAbs directed against alpha-Tn (Tn=GalNAc-Ser/Thr) and alpha-sialosyl-Tn (NeuNAc-GalNAc-Ser/Thr) completely neutralized SFS and YF at concentrations of 0.1 μg/pfu in MK-2 cells; virus propagated in C6-36 cells was not neutralized. Pretreatment of MK-2 cells with these MAbs had no effect; four other MAbs, directed against different carbohydrate epitopes, did not neutralize. These studies suggest that carbohydrate neoantigens are incorporated into the virions (perhaps as O-linked glycans) and could serve as potential targets for broad-spectrum immunotherapy or vaccines.

## K: SAND FLIES & *LEISHMANIA*

183 HOST FEEDING PREFERENCE OF *PHLEBOTOMUS GUGGISBERGI*, THE VECTOR OF *LEISHMANIA TROPICA* IN KENYA. Johnson RN, Ngumbi P, Mwanyumba P, Makasa J, Roberts CR. United States Army Medical Research Unit, Kenya and Kenya Medical Research Institute, Kenya.

In the 2 years since *Phlebotomus guggisbergi* was found to be a probable vector of *Leishmania tropica* in Kenya, an extensive search has been made for the reservoir host. The negative results obtained have not shed light on the identity of the reservoir. In order to discover more about the biology of the vector, a host feeding preference study was conducted using wild sand flies in their natural cave environment over a 6 month period. Solid state Army minature (SSAM) traps, without light bulb, were suspended over cages with potential hosts or an empty cage control. The animals tested included sheep, goat, dog, cat, Syrian hamster, giant rat (*Cricetomys gambianus*), crested rat (*Lophiomys imhausi*), and rock hyrax (*Procavia capensis*). All of these, except hamsters, are normally found in the vicinity of the study site. The sand fly collections from the animal baited traps were significantly higher than the control, with the exception of the rock hyrax collection, which was not significantly different than the control. The number of sand flies in the collections from the larger animals (bovids and carnivores) was significantly higher than that from the rabbit and rodents. The sex ratio also varied between collections; the larger animals attracted a larger proportion of females than did the smaller animals. The data suggest that a greater emphasis should be placed on surveying larger animals, particularly carnivores, to assess their potential to fill the role of reservoir host for *L. tropica* in Kenya.

184 CHEMICAL ATTRACTANTS OF PHLEBOTOMINE SAND FLIES. Tesh RB*, Guzman H, and Wilson ML. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT

Phlebotomine sand flies are vectors of the agents causing leishmaniasis, bartonellosis and several viral diseases, yet little is known about the field biology of these insects. Recently, we have examined a wide variety of synthetic and naturally occurring chemical compounds for their attractiveness to sand flies. The goal of this research is to identify substances which might enhance field collections or be used to attract sand flies to a poison bait. The attractiveness of the chemicals was tested by allowing lab-reared

flies to choose between colored sucrose solutions with and without the substances. The aphid alarm pheromone trans-beta-farnesene and several structurally related compounds were attractive to *Lutzomyia longipalpis* but not to other sand fly species. In contrast, the plant hormones (indole-3-acetic acid) was attractive to both sexes of a wide range of species. Recognition of these substances may help the insects to locate essential carbohydrate sources in nature. The attractiveness of plant hormones to sand flies suggests that plant fluids may be an important part of these insects' natural diets.

185 THE EFFECT OF *LEISHMANIA* INFECTION ON THE LONGEVITY AND FECUNDITY OF THEIR SAND FLY VECTORS. El Sattar SA*, Shehata MG, El Sawaf BM. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

There is evidence in the lierature from both field and laboratory observations that *Leishmania* are pathogenic to their sand fly vectors. We used two natural vector-parasite associations: *Phlebotomus papatasi* with *L. major* and *P. langeroni* with *L. infantum*. Sand flies were infected with $2 \times 10^6$ promastigotes $ml^{-3}$ in blood so that each sand fly took approximately 200 parasites per blood meal. Sand fly mortality rates, adult longevity and egg production were measured to determine the pathogenicity of *L. infantum* and *L. major* and mixed infections to both vector species. Mixed infections caused significantly higher mortality in both *P. papatasi* and *P. langeroni* than infection with a single parasite species. Single infections in turn cause significanlty higher mortality than uninfected controls, although there was no significant difference between infections with either *L. major* or *L. infantum* in their natural or surrogate vector. There was a marked effect of infection on the mean number of eggs laid per female; e.g., *P. langeroni* controls laid 53 eggs (±4.04), those with mixed infections 2l(±4.03) eggs; with *L. infatum* infections 3l (±4.35) eggs; and flies infected with *L. major* l5 eggs (±3.29).

186 EXPERIMENTAL DUAL INFECTIONS OF *LEISHMANIA* IN *PHLEBOTOMUS LANGERONI*. Shehata MG*, El-Sattar SA, Morsy TA, and El-Sawaf BM. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

There are numerous examples of sympatric *Leishmania* which could be taken up by polyphagous sandflies. One such case is in northern Egypt where *L. major* and *L. infantum* coexist with the vectors *Phlebotomus papatasi* and *P. langeroni* has been reported previously. The present study sought to determine whether two *Leishmania* species can coexist in a vector and whether there is any evidence of hybridisation between *Leishmania* under these conditions. Two sets of experiments were carried out: (a) dual infection with *L. infantum* and *L. major* and (b) infection with two zymodemes of *L. infantum*, both in *P. langeroni*. Sand flies were infected with a total of $2xl0^6$ promastigotes $ml^{-3}$ in blood (50:50 ratio) so that each sand fly took approximately 200 parasites per blood meal. Cloned parasites from six-day old sand fly infections were subsequently identified by enzyme electrophoresis. Two strains or two species were found to coexist in a single sand fly, at least until after the blood meal was digested, a critical time in the survival of parasites in the sand fly gut. In neither experiment was there any evidence of hybridisation between either the two zymodemes or the two parasite species.

187 REFRACTORY BARRIERS IN THE SAND FLY, *PHLEBOTOMUS PAPATASI* TO INFECTION WITH *LEISHMANIA PANAMENSIS*. Walters LL*, Irons KP, Modi GB, and Tesh RB. Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK; and Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

Factors influencing *Leishmania* development in the sand fly gut are largely uninvestigated. We studied the life cycle of *Le. panamensis* in *Ph. papatasi* to elucidate potential barriers limiting development in an unnatural host-parasite relationship. Flies were fed a suspension of *Le. panamensis*-infected macrophages

and human RBC, and were examined up to 16 days post-infection by light and electron microscopy. Histological examination of 401 flies showed the peritrophic membrane to be an important barrier to parasite colonization of the gut lumen. In most flies, parasites were unable to escape from the closed peritrophic sac, which was either excreted or retained intact in the midgut. After 5 days, only 31% of flies were infected; attached parasites colonized the pylorus-ileum and/or colon regions of the hindgut. Anterior migration of parasites into the midgut was rare (0.95%), suggesting the lack of appropriate stimuli. In the blood meal, 5 morphological forms developed from ingested amastigotes: stumpy, spatulate, elongate, and short nectomonad promastigotes; paramastigotes. Abnormal retention of amastigotes in macrophages and delayed development of promastigote stages was observed. The primary form attached in the hindgut was a pear-shaped haptomonad promastigote. Differentiation of *Le. panamensis* in *Ph. papatasi* appeared similar to that described in natural hosts, but parasites developed in locations unfavorable for transmission by bite. *Ph. papatasi* appears to be a refractory biological host for *Le. panamensis*.

188 BIOCHEMICAL SEPARATION OF FOUR *PHLEBOTOMUS* SPECIES BELONGING TO THE SUBGENERA *LARROUSSIUS* AND *PHLEBOTOMUS* (DIPTERA: PSYCHODIDAE). Kassem HA* and Fryauff DJ. Research and Training Center on Vectors of Diseases, Faculty of Science, Ain Shams University, Cairo, Egypt; and Medical Zoology Division, US Naval Medical Research Unit No. 3, Cairo, Egypt.

Because of the close morphological similarities, differentiation between females of some Old World sand fly species has been problematic. The objective was to develop a non-morphological procedure for identification of 3 proven and 1 suspected vectors of leishmaniasis. By means of a rapid form of enzyme electrophoresis (CAE, cellulose acetate electrophoresis), three enzymes (fumarate hydratase, isocitrate dehydrogenase-2, xanthine dehydrogenase) permitted the separation of *P. langeroni* and *P. perniciosus*. Both are vectors of *Leishmania infantum* and occasionally occur sympatrically in the Mediterranean basin. At least one other enzyme (glucose 6-phosphate dehydrogenase) has been found to separate between *P. papatasi* a vector of cutaneous leishmaniasis and phleboviruses, and *P. bergeroti*, an occasionally sympatric sand fly species suspected of having similar vector capabilities. The significant enzymic differences identified may be useful in the rapid differentiation of sibling sand fly species in endemic foci of leishmaniasis.

189 ADULT DIET AS A FACTOR AFFECTING BIOLOGY OF THE SAND FLY *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE). El Kordy E*, El Shafai A, El Said A, Kenawy MA, Shoukry M, and El Sawaf BM. Research and Training Center on Vectors of Diseases, Ain Shams University, Abassia, Cairo Egypt.

The effects of adult nutrients on egg retention, immature development, and adult survival of *P. papatasi*, the important vector of leishmaniasis in Egypt were investigated. The nutrients were distiller water, overripe fig fruits, guinea pig blood, sucrose solution and after native meals of blood and sucrose. Egg retention was observed in females irrespective to the type of offered nutrient ($r = 0.21$) but higher proportion (47%) of blood fed females had retained eggs. Duration of the life cycle was higher for the progeny of fig fed females ($P < 0.05$) and mean generation time was longer for sucrose fed females ($P < 0.05$). The mean lifetime differed significantly ($P < 0.05$) among females fed on different nutrients with the highest % (life expectancy at emergence) value ($14.98 \pm 2.75$ days) for sucrose-fed females. Males fed on distilled water, fig fruits, or sucrose solution displayed similar longeveties ($P < 0.05$). In respect to leishmaniasis transmission, the calculated expectancies for females beyond the infective age indicated that blood sucrose fed females have higher capability than those fed on sucrose blood or blood alone.

## POSTER II: ARBOVIRUSES AND HEPATITIS

190 USE OF POLYVALENT IMMUNE ASCITIC FLUIDS AND INDIRECT IMMUNOFLOURESCENCE IN IDENTIFYING ARBOVIRUS ISOLATES FROM AFRICAN MOSQUITOES. Digoutte JP*, Calvo-Wilson MA, and Mondo M. Institut Pasteur, Dakar, Senegal.

As a part of our research on the natural cycles of arthropod-borne viruses in Africa, we developed and applied a polyvalent immune ascitic fluid (IAF) system for isolating and identifying viruses from wild-caught mosquitoes. 70 monovalent IAFs were combined into 7 polyvalent IAFs (10 each) by grouping antigenically similar viruses that showed low cross-reactivity. 83 different reference arboviruses were cultured in Vero and *Aedes pseudoscutellaris* continuous cell lines and were tested using the polyvalent IAFs. Cells were tested by indirect immunoflorescence at the appearance of cytopathogenic effects or on day 8 post-inoculation. Of 73 viruses that multiplied, 64 were detected by one or more of the polyvalent IAFs. 9 viruses were undetected and 10 did not multiply in these cell lines. These polyvalent IAFs were then applied to 6133 pools of West African mosquitoes. The following viruses were isolated: Denge 2 (47 strains), Zika (10), Denge 2 + Zika (20), Bagaza (13) Wesselsbron (3), Yellow Fever (3), Rwamba (1), Middleburg (1), West Nile (68), Kedougou (6), Babanki + West Nile (1). These viruses were identified using monovalent IAFs. This technique allows rapid testing of many mosquito pools, is sensitive to unpassaged strains, permits multiple isolations from one pool, and allows for the isolation of new arboviruses through heterologous reactions.

191 DENGUE VIRUS INFECTED B CELLS DISSEMINATE INFECTION BEFORE VIREMIA. King AD*, Myint KS, Kalayanarooj S, Pattanapanyasat K, Smith CD, Nisalak A, and Innis BL. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Children's Hospital, Bangkok, Thailand.

Dengue virus has been isolated from human peripheral blood mononuclear cells (PBMC). Since mononuclear cell populations (Monocytes,T cells, B cells, and NK cells) have different circulatory patterns and physiology, the effect of each on dengue pathogenesis would be different. The objective of this study was to determine what circulating human mononuclear cells are infected by dengue virus and to determine, in an experimental animal model, the distribution of the virus. Virus was titered in human PBMC that were FACS sorted according to the presence or absence of cell surface markers, CD3, CD14, CD16 and CD20. In the animal model, rhesus monkeys were infected by mosquito bite. Tissue distribution of the virus was determined by immunocytochemistry.In human PBMC, B cells were the principal circulating mononuclear cells infected by dengue virus. The recovery of virus from B cells was unaffected by the presence or absence of an anamnestic antibody response. Little virus was isolated from the monocytes, T cells or NK cells. In the rhesus, virus was detected in lymph nodes and at the site of inoculation before serum viremia. Initial dissemination of the virus was, therefore, to lymph nodes. We infer that B cell infection is important for the early dissemination of dengue virus.

192 IMMUNOFLUORESCENT STUDIES IN CELLS, INFECTED WITH EYACH VIRUS, A EUROPEAN ISOLATE OF COLORADO TICK FEVER VIRUS GROUP. Dobler GJ* and Meier-Ewert H. Abteilung fur Virologie, Technische Universitat, Munchen, Germany.

EYACH virus was isolated 1972 from *Ixodes ricinus* in the Southwestern part of Germany. Adaption of the virus to Vero cells was tried after 36 baby mouse brain passages. After 3 blind passages a discrete cytopathic effect could be seen 48 hours p.i. Virus titers were measured in culture supernatants by virus dilution test and plaque assay. $10^5$ TCID could be found 48 hours p.i., declining to $10^4$ TCID after 72 hours p.i. Immunofluorescent studies using Colorado tick fever virus hyperimmune serum showed diffuse fluorescent pattern in infected cells first seen 22 hours p.i. Organization of diffuse fluorescent

pattern into small fluorescing vacuoles could be seen 30 hours p.i. During further time of infection enlargement of fluorescing vacuoles could be observed. Polyacrylamide gel electrophoresis (PAGE) of infected Vero cell extracts showed first virus-induced changes of protein migration pattern 24 hours p.i. Adaption of EYACH virus to other cell cultures now is tried to show whether the long latency between infection and first changes in immunofluorescence and PAGE in EYACH virus-infected Vero cells is a Vero cell-specific phenomenon or whether it is virus-specific in other cell lines.

193 DISTRIBUTION AND REPLICATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS (CCHF) IN EXPERIMENTALLY INFECTED *HYALOMMA TRUNCATUM* TICKS. Dickson DL* and Turell MJ. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

Intracoelomic inoculation of Crimean-Congo hemorrhagic fever (CCHF) virus into *Hyalomma truncatum* has been used to study this tick's ability to support CCHF virus infection. This technique was used to elucidate the mechanisms of virus dissemination, replication, and tissue tropisms. Adult male and female *H. truncatum* were inoculated intracoelomically with approximately 0.14-0.27 µl of a CCHF virus suspension (IbAr 10200) and maintained in laboratory incubation chambers (R.H. 90%, 24 ± 2°C). At scheduled time intervals (10 post-inoculation points), unfed and blood-fed ticks were processed(surface disinfected and triturated) either as whole or dissected specimens. Triturated specimens were stored at -70°C until tested for CCHF virus by plaque assay on SW-13 cell monolayers. Virus was recovered from 98% of the whole specimens tested (male 95/95; female 144/150) and virus titers were not significantly different. The numbers of virus recovered from hemolymph or hemolymph-hemocyte suspensions of either sex did not differ significantly and were unaffected by blood-feeding. Salivary glands of blood-fed females contained significantly more virus than those of unfed females, unfed males, and blood-fed males. The percentage of ovarian tissues that yielded CCHF virus was greater in blood-fed females than in unfed females (blood-fed 98%; unfed 43%). Similarly, the median titer was significantly higher in blood-fed females than in unfed females. In summary, we established a CCHF virus infection in *H. truncatum* that persisted throughout the length of this study (42 days). Furthermore, CCHF virus infection significantly increased in the salivary glands and ovaries after blood-feeding.

194 RAPID MEMBRANE BASED IMMUNOBINDING ASSAY FOR THE DETECTION OF DENGUE VIRUS IN TISSUE CULTURE. Simmons M*, Dubois DR, Oprandy JJ. Diagnostic Technology, Naval Medical Research Institute, Bethesda, MD; and Communicable Diseases and Immunology, Walter Reed Army Institute of Research Washington D.C.

Tissue culture methods for the identification of dengue viruses are presently used in conjunction with various immunoassays. These methods, however are laborious, require trained personnel and expensive equipment. We have developed a diagnostic method, which is rapid, specific, sensitive and easy to perform. Infected tissue culture cells were solubilized in SDS and the lysate filtered through a hydrophobic polyvinylidene difluoride (PVDF) membrane. Viral antigen was detected by a type-specific monoclonal antibody and a peroxidase-labeled second antibody. Addition of substrate produced a blue-colored precipitate which allowed for quantitation by densitometry. Comparison of viral titers with the dot immunoassay and a standard plaque assay indicated an excellent correlation between these two methods. This test detected viral antigen between day three and day five, depending on the concentration of initial inoculum. Positive results have been obtained which correspond to an infectivity titer as low as $10^3$ pfu/ml. This procedure can be performed in less than three hours and could be useful for detecting viruses in clinical specimens.

195 SIX HOUR LABORATORY CONFIRMATION OF DENGUE: ANTIGEN DETECTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS BY IMMUNOHISTOCHEMISTRY. Myint KS*, Nisalak A, Kalayanarooj S, Nimmannitya S, and Innis BL. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Children's Hospital, Bangkok, Thailand.

If lab diagnosis of dengue is to influence patient management, even small hospitals must have a sensitive, specific diagnostic test able to yield results in < 1 day. Presently, there is no such test. We examined the diagnostic potential of staining for dengue antigen in peripheral blood mononuclear cells (PBMC) collected from patients being evaluated for dengue. Virus isolation and serologic assays were done using standard procedures. Additionally, PBMC from 3 ml of acute blood were divided for culture in *Toxorhynchites splendans* or cytocentrifugation onto glass slides for antigen staining using polyclonal anti-dengue or 4G2 monoclonal anti-flavivirus Ig, biotinylated anti-mouse IgG and streptavidin-alkaline phosphatase conjugates. Specimen preparation took 1 hour, staining took less than 5 hours. Staining was specific; cells from normal donors were negative for antigen. Sensitivity appeared to be greater for immunocytochemical staining than culture. Among 27 patients with dengue examined to date, 19 (70%) were antigen positive whereas only 11 (41%) were culture positive. We predict that antigen detection can achieve sensitivities of >90% in patients with fever of less than 3 days duration. This assay is the first candidate dengue diagnostic test with potential to guide medical management of febrile patients at risk for hemorrhagic fever.

196 COMPARATIVE SEQUENCE ANALYSIS OF THE S SEGMENT RNA FROM SEVEN STRAINS OF CCHF VIRUS AND DEVELOPMENT OF A PCR-BASED DIAGNOSTIC SYSTEM. Lofts RS*, Hodgson LA, Ksiazek TG, and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious diseases, Ft. Detrick, MD; and Disease Assesment Division, U.S Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Crimean-Congo Hemmorhagic Fever (CCHF ) virus, a tick-borne member of the *Nairovirus* genus of the Bunyaviridae family, has a wide geographic distribution encompassing most of sub-Saharan Africa and southern Eurasia. Human disease is often associated with severe hemorrhagic manifestations with mortality rates as high as 40%. Rapid diagnosis is desirable to identify patients who might benefit from antiviral chemotherapy and to minimize exposure to attending medical staff. We have developed a rapid, sensitive, and specific diagnostic system for early detection of viral RNA using PCR technology. The S segments of six geographically distinct strains from Africa and Asia were compared after direct dideoxy sequencing of PCR-amplified products. Sequence heterogeneities of 5-15% were observed upon alignment of these sequences with the 10200 strain of CCHF virus. Simultaneous alignment of all seven sequences allowed for the selection of primers capable of detecting all CCHF strains in the study. In a direct comparison with an ELISA-based, antigen-capture detection system, ethidium bromide staining of PCR amplification products in agarose gels was 10-100 times more sensitive for the detection of virus in cell culture. The PCR technique also detected virus-specific sequences in blood and liver tissues of experimentally infected infant mice. These data will be used to develop a comprehensive, clinical diagnostic system for the early detection of CCHF virus infections.

197 ENZYME IMMUNOSORBENT ASSAYS FOR EBOLA VIRUS IgG AND IgM ANTIBODIES. Ksiazek TG*, Rollin PE, Jahrling PB, and Peters CJ. Disease Assessment Division, U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Institute Pasteur, Paris, France.

We have developed enzyme immunosorbent assays for Ebola virus IgG and IgM antibodies and evaluated them using sera collected from infected primates and humans. The anti-Ebola (Reston) IgM response of experimentally infected monkeys and a single infected human were measured using an IgM capture format. Experimental monkeys responded with detectable IgM antibody as early as day 6 post-

infection and it persisted for < 90 days. IgM antibody was also detectable in 5 of 5 animals at the time they died of Ebola virus infection. The IgG response was reliably detectable only at 10-13 days post-infection. However, anti-Ebola IgG persisted in 3 surviving animals for greater than 400 days; titers and optical densities remained high in these sera.A single human, reported infected with Ebola (Zaire) virus, remained IgG positive when tested approximately 10 years after infection. Although limited by the available sera, these data suggest that the IgG test has good sensitivity for detection of anti-Ebola virus antibodies. Long-term persistence of anti-Ebola IgG antibodies suggests that the IgG assay will be useful in field investigations of Ebola virus ecology. The IgM assay holds promise both as a diagnostic tool and as an epidemiological aid for determining incidence.

198 IMPROVEMENT OF AN ALKALINE PHOSPHATASE BASED NON-RADIOACTIVE DETECTION SYSTEM TO DETECT RIFT VALLEY FEVER VIRUS RNA BY FILTER HYBRIDIZATION. Knauert FK* and Parrish BA. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD

Although intrinsically more sensitive then horseradish peroxidase-based detection systems, we have found alkaline phosphatase-dependent systems to be more erratic in terms of background and nonspecific staining patterns. To take advantage of this intrinsic sensitivity, we examined the various components of this detection system to determine those conditions that would result in consistent background and nonspecific reactivity characteristics. We have been able to establish conditions that result in consistent, minimal background and nonspecific reactivity by eliminating carrier nucleic acid from the sample diluent; altering the constituents and conditions of blocking; carefully selecting the conjugate concentration; incorporating treatment with levamisole, an inhibitor of non-intestinal alkaline phosphatase, into the procedure; and meticulously avoiding mechanical damage to the nitrocellulose sheets. The improvement in background and nonspecific reactivity resulted in a concomitant, approximate twofold improvement in sensitivity. The availability of a sensitive and specific non-radioactive diagnostic filter hybridization assay should significantly improve the utility of such a procedure.

199 DIRECT AMPLIFICATION OF HANTAVIRUSES FROM HUMAN SERUM BY POLYMERASE CHAIN REACTION. Xiao SY* and LeDuc JW. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Short and low viremia in hantavirus infection has long been an obstacle in the detection of this virus from clinical samples. Serological tests are not able to detect viral antigen from blood and urine specimens, and virus isolation from patient materials using cell culture or laboratory animals requires several weeks and needs containment facility. To circumvent these problems, we established an RNA polymerase chain reaction method for hantavirus genomic amplification, and it was successfully used to detect hantaviruses from experimentally infected cell culture and animals. In addition, a universal primer pair was prepared which recognized isolates of all five established viruses of the Hantavirus genus. To apply this technique to patient material such as blood or urine, an acid guanidine thiocyanate-phenol-chloroform extraction method was adopted to recover viral RNA from serum specimens for PCR amplification. Using purified virions of Hantaan virus serially diluted in human serum, a preliminary study found a positive amplification from 150 µl sample containing 90 pfu of virus. This suggests that this method may be suitable for use on clinical samples, but further optimization might be needed to increase the sensitivity. Serum specimens from patients of hemorrhagic fever with renal syndrome are being tested with this technique.

200 COMPARATIVE AGGLUTINABILITY OF ERYTHROCYTES FROM DIFFERENT SPECIES OF ANIMALS BY HEPATITIS A VIRUS. Silor DL*, Dubois DR, Eckels KH, and Summers PL. Walter Reed Army Institute of Research, Washington DC.

Recently it has been demonstrated that Hepatitis A virus (HAV), a member of the Picornaviridae family, can hemagglutinate (HA) erythrocytes. The ability of HAV to bind to red blood cells has been used in the development of a hemagglutination-inhibition (HAI) test to detect antibodies to HAV. The HAI test, using goose red blood cells (GRBC), has been shown to be as sensitive as commercial ELISA and RIA tests. The present study was undertaken to determine the ability of HAV to hemagglutinate erythrocytes from other species of animals and to compare their hemagglutinability with that of GRBC. Concentrated cell lysates from HM175 strain of HAV infected BS-C-1 cells were used as hemagglutinin. Goose, chicken, horse, cow, goat, sheep, pig, dog, rhesus monkey, and African green monkey erythrocytes were evaluated.When blood was collected using the anticoagulants EDTA or heparin, HAVHA was inhibited, whereas the anticoagulant Alsever's did not interfere with the HA pattern. Using 128-256 HA units no significant differences were noted with erythrocytes from chicken, horse, dog, rhesus monkey,and African green monkey. Sheep erythrocytes exhibited poor HAV agglutinability and erythrocytes from cow and goat were found not to agglutinate even at the highest concentration of HAV antigen used. The experiments demonstrated a marked difference in the comparative agglutinability of different species of red blood cells. Data suggests that there may be receptor differences between different species of erythrocytes which effect their ability to bind to HAV. The data also identifies a suitable alternate source of erythrocytes in the event that GRBC are not readily available.

201 LACK OF CROSS-REACTIVITY BETWEEN ANTIBODIES TO MALARIAL ANTIGENS AND HUMAN T-LYMPHOTROPIC VIRUS TYPE-I/II. Lal RB*, Sulzer A, Shi YP, Sinha S, Alpers M, Povoa M, Roberts C, and Lal AA. Retrovirus Diseases Branch, DVRD, Centers for Disease Control, Atlanta, GA; Malaria Branch, DPD, Centers for Disease Control, Atlanta, GA.; Papua New Guinea Institute of Medical Sciences, Goroka, New Guinea; Instituto Evandro Chagas, Belem, Brazil; and Department of Diagnostic Retrovirology, Walter Reed Army Institute of Research, Washington, DC.

Recent studies have shown that malaria infections can give rise to antibodies that cross-react with HTLV-I/II antigens. To further investigate this cross-reactivity, we have determined the cross-reactivity of HTLV-I/II antigens with antibodies against malaria antigens in serum/plasma specimens from malaria-endemic (n=346) and nonendemic regions (n=137). Using Western blot analysis of HTLV antigens, we found that 49 of 178 (28%) specimens from the Lindu valley,Indonesia; 14 of 118 (12%) from Madang, PNG; and 3 0f 50 (6%) from Paragaminos, Brazil reacted with HTLV gag proteins (HTLVind), whereas only 2 (1.12%) of Lindu valley, 4 (3.4%) of PNG, and 1 (2.0%) of Brazilian specimens reacted to both gag and env gene products (HTLVpos). The serum/plasma samples from malaria endemic-regions of Indonesia, PNG, and Brazil contained high to moderate levels of antibodies to *Plasmodium falciparum* and/or *P. vivax* malarial sporozoite and blood-stage antigens. There was no correlation between the antimalarial antibody level in serum/plasma samples and the cross-reactivity with HTLV-I/II antigens. None of the specimens from the non-malaria endemic regions demonstrated any seroreactivity to HTLV antigens. Furthermore, HTLVpos serum samples from non-malarious regions (n=13) did not react with blood-stage malaria parasites, as determined by IFA. Contrary to earlier findings this lack of correlation between HTLV and malaria antigens suggests that antibodies to malaria antigens do not cross-react with HTLV sero-testing.

202 DENGUE INFECTION OF HUMAN STROMAL CELLS IN CULTURES OF HUMAN BONE MARROW. LaRussa V, Putnak R*, and Knight R. Department of Hematology and Department of Viral Diseases, Walter Reed Army Institute of Research, Washington, DC.

Infections with dengue (DEN) virus and other arboviruses are known to be associated with bone marrow failure. Recent studies have suggested that hematopoietic progenitors may be targets. In our study, adherent stromal cells developed in long-term cultures of human bone marrow (LTBMC) were assayed for ability to support DEN 2 virus replication. Marrow stromal cells were established using a modified version of LTBMC described previously. Fully-developed 3 to 4 week old adherent stromal cell monolayers were incubated with DEN 2 virus for 2 hrs, cultures were then washed to remove virus inoculum, and incubated at 37 C and 5% CO2. Virus titers increased from <20 plaque-forming-units (PFU) per plate to greater than 10,000 PFU per plate at 48 hrs post-infection and remained at this level for at least 5 days. No gross cytopathology of stromal cells was observed. Virus antigen was detected by immunofluorescent assay. A minor population of brightly fluorescent cells was observed; these included monocytes. From these experiments we conclude that human adherent stromal cells support DEN virus replication. Implications for possible bone marrow failure due to DEN infection and immunological enhancement of virus replication will be discussed.

203 INHIBITION OF EBOLA VIRUS *IN VITRO* AND IN A SCID MOUSE MODEL BY S-ADENOSYLHOMOCYSTEINE HYDROLASE INHIBITORS 3-DEAZAADENOSINE AND 3-DEAZANEPLANOCIN A. Huggins JW*, Zhang ZX, and Monath TP. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

Primary human outbreaks of the filoviruses Ebola and Marburg cause the most severe viral hemorrhagic fever known, with mortality of 40-90% in sporadic outbreaks. S-adenosylhomocysteine hydrolase inhibitors, carbocyclic 3-deazaadenosine (AVS-303) and 3-deazaneplanocin A (AVS-4275), which mimic a transition state intermediate, inhibited Ebola viral replication, assayed by reduction of viral antigen detected by monoclonal ELISA (effective concentration50 ($EC_{50}$)=4 μg/ml, toxic concentration50 ($TC_{50}$)≥250 μg/ml; and $EC_{50}$=0.3 μg/ml, $TC_{50}$=125 μg/ml, respectively). Evaluation of antiviral compounds has been hampered by lack of an adequate small animal model. Infection of multiple adult mouse strains (C3H/HeJ, A/J, Beige, CBA/N, SCID-c.d.-17) with Mayinga strain of Ebola produced disease only in the adult SCID mouse, where ≥0.1 PFU produced a uniformly lethal infection in animals 4 to 8 weeks old. After infection, virus was recovered from all major organs (heart, lung, liver, spleen, kidney, brain) by day 5 post [illegible], with a peak titer by day 19-25. Clinical and pathological features were consistent with a wasting syndrome. Prophylaxis, with carbocyclic 3-deazaadenosine (minimum effective dose i.p = 2.5 mg/Kg bid) or 3-deazaneplanocin A (minimum effective dose i.p = 0.05 mg/Kg bid), begun at time of infection extended mean time to death (MTD) by 15 and 10 days over controls (MTD 42 days). Viremia and viral replication were reduced in major organs by 3 or 1 logs, respectively, in heart, lung, liver, spleen, kidney, brain. The significant inhibition of viral replication in an immuno-compromised host strongly recommends continued evaluation in primates.

204 BACULOVIRUS EXPRESSION OF TRUNCATED HANTAAN VIRUS SEGMENT GENES: EPITOPE MAPPING USING MONOCLONAL ANTIBODIES TO THE G1 AND G2 PROTEINS. Pennock DG* and Schmaljohn C. Virology Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

Hantaan virus, a member of the Hantavirus genus of the Bunyaviridae family, is the etiologic agent of Korean hemorrhagic fever. The viral genome consists of three segments of single-stranded RNA designated as large (L), medium (M) and small (S). The M genome segment encodes two envelope glycoproteins, G1 and G2, in a single open reading frame with a gene order of 5'-G1-G2-3' with respect to the virus-complementary sense RNA. Both proteins are involved in eliciting a protective response to viral infectivity in animal models, and antibodies to either G1 or G2 have been demonstrated to neutralize viral infectivity. In order to localize potentially important antigenic and immunogenic sites on G1 and G2, we prepared a series of baculovirus recombinants that express truncated Hantaan M genes.

To construct the recombinants, cDNA representing the M segment was linearized near the 3' terminus and deletion mutants made by digestion with exonuclease III. The truncated genes were inserted into the polyhedrin gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus by homologous recombination. The exact termini of the genes were located by nucleotide sequence analyses of the transfer vectors. Nine recombinants containing the truncated G1 gene and nine recombinants containing the entire G1 gene and truncated G2 gene were examined. Six G1 and four G2 expression products were identified by immunoprecipitation with polyclonal or monoclonal antibodies to authentic Hantaan virus proteins. Immunoprecipitation and indirect immunofluorescence techniques were used to generate a spatial map of the Hantaan epitopes defined by these antibodies.

205 SEQUENCE AND GENETIC ORGANIZATION OF THE S RNA SEGMENT OF CRIMEAN-CONGO HEMORRHAGIC FEVER (CCHF) VIRUS. Smith JF*, Hodgson LA, and Lofts RS. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

CCHF virus is endemic throughout most of Sub-Saharan Africa and areas of Southeastern Europe and Central Asia where human disease is associated with exposure to ticks or with nosocomial outbreaks. We have carried out a molecular and antigenic analysis of this virus to define the genes of particular relevance to diagnosis and immunoprophylaxis. Studies with monoclonal antibodies have shown that the CCHF NC protein is the most type-specific polypeptide, and our diagnostic studies have focused on a sequence analysis of the S RNA segment to enable the development of PCR diagnostic assays and eventual expression of this protein. RNA was extracted from viral nucleocapsids purified from infected cells by equilibrium centrifugation, and L, M, and S RNA segments were separated by gel electrophoresis. Based on results from 3' end sequencing, a single S RNA-specific primer was selected to initiate first strand CDNA synthesis and subsequent full-length amplification by PCR procedures. Both strands of the DNA product were sequenced using T4 DNA polymerase and dideoxy sequencing reactions. The S genome segment of CCHF virus is 1671 nucleotides with a single ORF encoding a protein with a predicted molecular weight of 53,891 daltons. The genetic organization is similar to that reported for Dugbe virus, however, the nucleotide sequences are only 61% homologous. Northern blot analyses using a labelled full-length CCHF S segment PCR product, detected no hybridization with RNA from other Nairoviruses (Qalyub, Bandia, Ganjam, or Dugbe).

206 THE NON-STRUCTURAL GENOME OF DENGUE-1 VIRUS CV1636/77: COMPARISON OF THE NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES WITH THE OTHER DENGUE SEROTYPES. Chu MC*, Putvatana R, and Trent DW. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

The determination of the entire nucleotide and deduced amino acid sequences of the DEN-1 Jamaica CV1636/77 virus together with the known sequences of DEN-2, DEN-3 and DEN-4 viruses provide a complete sequence database of the DEN serotype complex. We have previously presented analyses of the nucleotide sequences encoding the structural and the non-structural NS3-NS4-NS5 regions. Sequence analyses of the entire genomic RNA and cloned cDNA of DEN- 1 reveal that the genomic RNA contains 10,650 nucleotides and encodes an open reading frame of 10,224 nucleotides that translate into 3408 amino acid residues. A comparison of the DEN-1 sequence with DEN-2 (Jamaica), and DEN-3 (H87) reveal that the genomic sequences encoding the structural (C-prM-M-E) and some of the non- structural (NS1, NS3, and NS5) regions are conserved (>70% homology). The 3'non-coding regions of DEN-1, DEN-2, DEN-3, and DEN-4 (Dominica) are of different nucleotide lengths comprising 325, 454, 432, and 385 base pairs respectively. The entire non-structural sequences for DEN-1 and DEN-4 share >95% identity. These results reveal a close evolutionary relationship between Caribbean DEN-1 and DEN-4 viruses which cannot be detected by conventional serological methods. Thus the sequence database of all the DEN virus serocomplex will be a useful tool in studying the genetic variation and evolution of these viruses.

207 RNA POLYMERASE, TYPE II, ACTIVITY IN BRAIN NUCLEI OF RATS INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS. de Lopez ET*, Rangel P, Belloso J, Benitez Y, and Martinez M. Instituto de Investigaciones Clinicas, Universidad del Zulia, Maracaibo, Venezuela.

Previous studies have demonstrated that in rat brain, several enzymes from different metabolic pathways were modified several days after Venezuelan Equine Encephalomyelitis (VEE) virus infection. Knowledge of enzyme alterations in early stages of VEE virus disease are very important to ascertain viral morphogenesis. We inoculated Sprague-Dawley male rats (150-200g) with a 100 $LD_{50}$ of the Goajira strain of VEE virus and sacrificed them by decapitation after 15, 20, 24, and 48 hours of post infection-(pi). Cerebral hemispheres were homogenized and tested for enzyme activity and viral titer by Reed-Muench method. Viremia was detected as soon as 5 hours pi while on brain viral replication started only after 20 hours pi with a very low titer ($<10^{-2}LD_{50}$). Titer values increased very slowly at 24 hours pi ($10^{-3.5}LD_{50}$) and continued rising to $10^{-4.2}LD_{50}$ at 48 hours pi. RNA polymerase was already altered at 15 hours pi showing a 30.8% reduction ($p<0.01$) on its activity when the virus was not detected on brain and this inhibition increased constantly reaching 36.1% and 41.2% at 24 and 48 hours pi respectively. These findings suggest that enzyme alterations are probably not due to direct viral effects.

208 ANTIBODY ENHANCEMENT OF DENGUE-3 VIRUSES. Kuno G*. Centers for Disease Control, Division of Vector-Borne Infectious Diseases, Dengue Branch, San Juan, PR.

Although strains of dengue viruses have been used to demonstrate *in vitro* antibody-dependent enhancement (ADE), variation in enhanceability among strains of a given serotype has not been extensively investigated. In this study, the ADE of DEN-3 strains with both type-specific and non-specific polyclonal human antibodies from 3 geographic areas was studied. An ADE profile, which was unique for each strain, was obtained, when enhancement values with all antibodies were plotted. In general, geographic strains were divided into highly enhanceable and less enhanceable groups. Nevertheless, several strains (e.g., India, Indonesia, Niue, Puerto Rico, Samoa, Tahiti, and Thailand) were notable for the lack of ADE with most antibodies. All type-specific antibodies were enhancing, although levels of virus growth varied considerably among strains with a given antibody. For the strains from Indonesia and Thailand, temporal change in enhanceability was also studied and no significant change between the isolates from the 1970's and 1980's were observed.

## POSTER II: KINETOPLASTIDA

209 THE FIRST CONFIRMED ISOLATION OF *LEISHMANIA MAJOR* FROM SOUTHERN SINAI, EGYPT. Kamal H*, Shehata M, Osman A, Doha A, El Hoosany S, and Schnur LF. Ain Shams University Research and Training Centre on Vectors of Diseases, Cairo, Egypt; and Kuvin Centre of Infectious and Tropical Diseases, Hebrew University Medical School, Jerusalem, Israel.

Proven cases of cutaneous leishmaniasis caused by *Leishmania major* have been reported from Northern Sinai near El Arish and along the northern Sinai border with Israel. *Gerbillus pyramidum* and *Phlebotomus papatasi* also harbor infections of *L. major* and serve as a reservoir and the vector respectively. Two leishmanial stocks were recently isolated from children living at Wadi Feiran, southern Sinai. The first (RTC-22) came from a six-year old girl living at Sabah, presenting a single wet ulcer on her right arm; the second (RTC-26) from a five-year old boy living at El Haswa, presenting multiple wet lesions on both ankles. The isolates were grown and typed by enzyme electrophoresis on cellulose acetate, examining 12 enzymes: ME, PGI, G6PD, PGM, 6GPD, ICD, LDH, ALAT, HK, GOT, FUM and 6APD. Both isolates typed identically to a Moroccan *L. major* reference strain MHOM/MA/81/LEM 265, representing

zymodeme MON 25. The excreted factor subserotype of RTC-22 was A4 and that of RTC-26 is being determined. Potential vectors in this focus are *Phlebotomus papatasi, P. bergeroti,* and *P. sergenti.* The range of *G. pyramidum* is restricted to northern Sinai, suggesting other rodent species are the reservoir.

210 SPECIES-SPECIFIC DETECTION OF *LEISHMANIA MEXICANA* PROMASTIGOTES WITHIN SAND FLY HOMOGENATES ON NYLON BLOTS USING A RECOMBINANT KINETOPLAST DNA PROBE. Stiteler JM*, Bruckner PR, and Perkins PV. Walter Reed Army Institute of Research, Department of Entomology, Washington, DC

A characterization technique for the identification of *Leishmania mexicana* (*Lm*) promastigotes in sand fly blots using a recombinant-kinetoplast DNA (rec-kDNA) probe was developed for use in studying the epidemiological relationship(s) of leishmaniasis with respect to *Lm* and vector sand fly species. The rec-kDNA probe, pT15b-12, which was constructed by the cloning of kDNA minicircle class-specific *Eco* R sequence from WR#669 (*Lm. amazonensis*) into a pUC8 plasmid vector, was used for the species-specific detection of *Lm* promastigotes within sand fly homogenates on nylon (Nytran, Schleicher, and Schuell) blots. Detection was achieved by autoradiographic visualization of $^{32}P$ labeled pT15b-12 rec-kDNA probe following post-hybridization stringency washes of the DNA:DNA hybridizations. Although the mincircle class-specificity of the rec-kDNA probe results in a decreased sensitivity for kDNA, detection of the promastigotes with the pT15b-12 probe is sufficient to allow for the positive identification of *Lm* promastigotes within sand fly mid-gut homogenates. A relative quantification of promastigote number can be made by comparison of signal generated in serial dilutions of spot blotted kDNA and/or promastigote dot blots.

211 LEISHMANIASIS IN DESERT SHIELD/STORM. Grogl M*, Mendez J, Milhous WK, Nuzum EO, Martin RK, Berman JD, Schuster BG, and Oster CN. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC and Department of Infectious Diseases, Walter Reed Army Medical Center, Washington, DC.

Historically, infectious diseases have always had a significant impact on military operations. By 18 February 1991, a total of 285,000 U.S. Army personnel was operating in the Kuwaiti Theater of Operations. The total number of leishmaniasis cases during Desert Storm will never be known due to self-healing, treatment with ketoconazole before evacuation, and reporting differences between the Department of Defense branches. A partial after-action assessment indicates that leishmaniasis was the most reported and clinically recognized vector-borne disease. At Walter Reed, a total of 12 cases of leishmaniasis was confirmed by visualization of the parasite. Ten patients had cutaneous disease and 2 visceral. The incubation period ranged from 15-165 days with a mean incubation time of 57.1 days. Seven of the 10 cutaneous cases had multiple lesions with the mean number per patient being 12 (range from 3-34 lesions).Parasites isolated from cutaneous lesions were identified by isoenzymes as *L. major* (3) and *L. tropica* (2). Two additional *L. tropica* isolates were isolated from bone marrow aspirates in two patients with visceral leishmaniaisis. This finding of visceralizing *L. tropica* in U.S.soldiers is unusual and indicates how little we know about the disease in the Middle East and how poorly leishmaniasis is understood in a naive host.

212 VENEZUELAN KALA AZAR AND *LEISHMANIA COLOMBIENSIS N. SP.* Delgado O, Castes M, White AC*, and Kreutzer RD. Instituto de Medicina Tropical,Universidad Central de Venezuela, Caracas,Venezuela; Instituto de Biomedicina, Caracas, Venezuela; Department of Medicine, Baylor College of Medicine, Houston, TX; and Biology Department, Youngstown State University, Youngstown, OH.

Recently a new peripylarian species, *Leishmania colombiensis*, was described. It causes cutaneous leishmaniasis in Colombian human hosts and both cutaneous and visceral disease in Panama animal hosts (*Choloepus hoffmanni*). The isozyme (allomorph-allele observed by electrophoresis) analysis indicated the two populations are genetically very similar, identity=I=0.923 (1.0=identical) and distance=D= 0.080 (0.0=identical), but they differ for allomorphs of the enzymes ACP and PFK. Two strains (MHOM/VE/ /Chuao from bone marrow and MCAN/ VE/ /Talisman from the spleen) isolated by Pifano in an endemic focus of kala azar in eastern Venezuela were examined by enzyme analysis, and they were determined to be very similar, possibly identical to *L. colombiensis*. The enzyme profiles (20 enzymes) of the three populations were compared using the statistics of I and D. The Venezuela sample differed from the other two for the enzyme PK and I with the Colombia sample was 0.937 and with the Panama sample 0.872. Enzyme and genetic analysis data will be presented. The data suggest that the distribution of *L. colombiensis* extends into Venezeula and that the parasite can be involved with visceral as well as cutaneous leishmaniasis.

213 THE EFFECT OF *LUTZOMYIA LONGIPALPIS* SALIVARY GLAND MATERIAL ON THE ABILITY OF MACROPHAGES TO PRESENT ANTIGEN TO *LEISHMANIA MAJOR*-SPECIFIC T CELLS. Theodos CM* and Titus RG. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Previous studies from our laboratory demonstrated that salivary gland material from the sand fly *Lutzomyia longipalpis* enhanced the infectivity of *Leishmania major* when both were injected subcutaneously into the hind footpad of resistant or susceptible strains of mice. Further studies demonstrated that the enhancement of infectivity was due to an effect of the lysate on the host and not the parasite. In an attempt to discern the mechanism(s) responsible for the enhanced infectivity, the present study was designed to determine whether sand fly salivary gland material alters the ability of macrophages to present antigen. This hypothesis was supported by the observation that sand fly salivary gland material was able to enhance the infectivity of *L. major* even when the parasites were injected four days after the injection of the salivary gland material. Sand fly salivary gland material was found to markedly inhibit the ability of *Leishmania*-infected macrophages to present antigen to *L. major*-specific T cells. Preincubation of the macrophages with sand fly salivary gland material was not required in order to inhibit antigen presentation. We are currently attempting to isolate and characterize the immunosupressive factor present in sand fly salivary gland material. Once identified, this immunosuppressive factor could be used as a candidate vaccine antigen to protect against infection by *Leishmania*.

214 THE EFFECT OF SAND FLY SALIVA ON MACROPHAGE FUNCTION IN *LEISHMANIA MAJOR* INFECTION. Hall LR* and Titus RG. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Recent work in our laboratory has demonstrated that a factor(s) present in the saliva of the phlebotomine sand fly enhances disease progression, and may be required for establishment of infection with the protozoan parasite *Leishmania major*. The mechanism by which saliva enhances infection is not known. Since Leishmania is an obligate intracellular parasite of the macrophage, our work has been primarily focused on elucidating the effect of saliva on macrophage function. We have investigated the effect of saliva on the ability of various cytokines to stimulate the macrophage to destroy the intracellular parasites. In addition, we have determined the ability of macrophages to express various markers of activation at the protein level when incubated in the presence of saliva. Finally, preliminary studies have indicated that the induction of a specific immune response to the parasite is affected by saliva. These results suggest an altered cytokine profile of parasite-specific T cells when induced in the presence of saliva. The apparent role of saliva in the establishment of *Leishmania* infection could have important implications for vaccine development strategies. It may be possible to control disease development by

immunizing against the saliva of the vector, thus avoiding the difficulties inherent in using the parasite as a vaccine.

215 INTERACTIONS OF MURINE AIDS AND CUTANEOUS LEISHMANIASIS IN C57BL/6 MICE. Barral-Netto M*, Barral A, Silva JS, and Reed SG. Seattle Biomedical Research Institute; Seattle, WA; Faculdade de Medicina-Universidade Federal da Bahia; Salvador-Bahia Brazil; and Faculdade de Medicina, USP, Ribeirao Preto-SP, Brazil.

The ocurrence of AIDS and leishmaniasis has been reported in humans but a detailed understanding of their interaction is lacking. We took advantage of the murine model of AIDS (infection with LP-BM5 murine leukemia virus), to investigate its effect on the course of *Leishmania amazonensis* (*La*)infection on C57BL/6 mice. Infection with the virus was performed either 2 months after or 1 month before *La* infection ($5x10^6$ amastigotes, s.c. into the foot-pad) lesions and lymphocyte proliferative responses were evaluated periodically. MAIDS infection initiated after leishmania infection led to an increase in lesion size (3.2±.7 vs. 0.8+0.1 mm [mean±SEM] in *La* +MAIDS and *La* alone, respectively, after 12 weeks of leishmania infection). On the other hand, *La* infection 1 month after MAIDS did not lead to faster disease progression (0.49±0.13 vs. 0.58± 0.13 mm after 8 weeks of leishmania infection). In both cases MAIDS infection led to immunosuppression, as evidenced by lack of spleen cell proliferation to ConA (2311±844 CPM in MAIDS+*La* vs 154732±18471 in *La* alone) or leishmania antigen (1131±856 vs 15276±2847, respectively). This model shows the importance of timing on the influence of infection-induced immunosuppression in leishmaniasis and suggests its utility for further investigation of MAIDS-leishmaniasis interactions.

216 ANALYSIS OF *LEISHMANIA MAJOR* SPECIFIC T CELLS GENERATED *IN VITRO* FROM NONSENSITIZED MICE. Shankar AH* and Titus RG. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The outcome of experimental murine *Leishmania major* infection is associated with preferential expansion of either Th1 cells in resistant mice or Th2 cells in susceptible mice. We have developed a technique whereby *L. major*-specific T cells can be generated *in vitro*. This allows analysis of factors which may affect preferential activation of Th1 or Th2 cells. *L. major*-specific T cells were generated *in vitro* from nonsensitized mice by coincubation of normal spleen cells with live *L. major* promastigotes. These T cells generated by primary *in vitro* immunization (PIV T cells) were obtained from all mouse strains tested: BALB/c, BALB/b, C57BL/6, B10.D2, B10.A(5R), CBA/Ca, CBA/J, and DBA/2. Flow cytometry revealed that PIV T cells were predominantly Thy $1.2^+$ and $CD4^+$. Upon restimulation, the cells were found to respond to live *L. major* promastigotes and showed some cross-reactivity to *L. donovani*. No reactivity was found with live *L. mexicana* promastigotes, freeze-thawed *L. major* promastigote antigen, promastigote conditioned culture medium, or *L. major* metacyclic LPG. Restimulation of PIV T cells required APC with syngeneic IA. PIV T cells from all mouse strains tested produced IL-2, IL-4, and IFN-γ after 7 days exposure to *L. major*. Stimulation of Balb/c PIV T cells an additional 10 days significantly enhanced IL-4 production. The affects of cytokines and antigen presenting cells on preferential induction of Th1 and Th2 cells in this system are being investigated.

217 *TRYPANOSOMA CRUZI* INFECTION IN β-2-MICROGLOBULIN DEFICIENT MICE. Tarleton RL*, Postan M, Koller BH, Latour A, and Smithies O. Department of Zoology, University of Georgia, Athens, GA; and Department of Pathology, University of North Carolina, Chapel Hill, NC.

cells. In this study, we examined the ability of mice in which the β-2-microglobulin (β-2-M) gene has been disrupted by homologous recombination, to resist infection with *T. cruzi*. β-2-M deficient mice fail to express functional class I MHC molecules and consequently lack mature $CD8^+$ T cells but otherwise appear normal. The absence of class I MHC and $CD8^+$ T cells was confirmed in both *T. cruzi*-infected and non-infected homozygous β-2-M deficient mice by flow cytometric analysis. When infected with the Brazil strain of *T. cruzi*, β-2-M deficient mice uniformly generate high parasitemias and die during the acute phase of infection. In comparison, β-2-m +/+ or +/- litter mates are highly resistant: all have very low parasitemias and most survive the acute phase of infection. Spleen cells from β-2-M deficient and +/- or +/+ mice have comparable *in vitro* proliferative responses to parasite antigens. Preliminary experiments suggest that spleen cells from infected -/- mice, are significantly less suppressed for IL-2 production than are infected +/+or +/- mice. These studies confirm the requirement for functional $CD8^+$ T cells in immune control of *T. cruzi* infection in mice and add additional support for the role of $CD8^+$ T cells in immunosuppression in *T. cruzi* infection.

218 CELLULAR IMMUNITY AS A DETERMINANT OF CHRONIC PATHOLOGY IN HUMAN *TRYPANOSOMA CRUZI* INFECTION IN NORTHEAST BRAZIL. Cetron MS*, Basilio FP, Moraes AP, Sousa AQ, Paes JN, and Van Voorhis WC. Department of Medicine, Infectious Disease, University of Washington, Seattle, Washington; HEMOCE Division of Serology; Department of Infectious Disease, Hospital Sao Jose, Brazil; and Department of Cardiology, Federal University of Ceara, Fortaleza-Ceara, Brazil.

*Trypanosoma cruzi*, the etiologic agent of Chagas' disease, causes both acute and chronic (indeterminate, cardiac, gastrointestinal) manifestations in humans. Although the pathogenesis of these syndromes is poorly understood, cell-mediated immune (CMI) responses are felt to be intricately important to the pathogenesis of Chagas' disease. We investigated the CMI responses in *T. cruzi*-infected persons and normal controls from an endemic area in Northeastern Brazil. Thus far, 61 patients with suspected chronic *T. cruzi* infection were studied. An epidemiological survey, physical exam, and Chagas serology was performed on each subject. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured *in vitro* in the presence of *T. cruzi* antigens, PHA, and Ag-free controls. PBMC proliferation was assayed by $^3H$ thymidine incorporation. Proliferation index (PI) was defined as: mean $^3H$ cpm of stimulated PBMCs + mean $^3H$ cpm of unstimulated PBMCs. Our data demonstrate that extract of *T. cruzi* trypomastigote antigens stimulates PBMCs from infected persons, but not uninfected controls. The mean PI of infected individuals in indeterminate, gastrointestinal, and cardiac subgroups was 12.5, 1.5, and 5.9 respectively; compared to 1.8 for controls. Mean serum titers of antibodies to *T. cruzi* were equivalently elevated in all clinical subgroups, but negative in controls. Overall, patients with clinically symptomatic Chagas' disease had diminished CMI responses but equivalent serological responses to *T. cruzi* antigens compared to those with indeterminate stage. Thus, cellular (rather than serologic) immunity may be a critical determinant in the various Chagas syndromes.

## POSTER II: *FILARIA*

219 DIAGNOSTIC POTENTIAL OF RECOMBINANT EXCRETORY/SECRETORY PRODUCTS OF MALE ADULT WORMS OF *BRUGIA MALAYI*. Philipp M*, Bakeer M, Lillibridge CD, and Jayaraman K. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA; and Centre for Biotechnology, Anna University, Madras, India.

A specific and sensitive diagnostic method to confirm diagnosis of clinical filariasis in the absence of microfilaraemia is still greatly needed. Antigen detection methods, conceptually ideal for this purpose, have been most successful in the detection of patent infections, but have proven so far to be insensitive in occult conditions. Amicrofilaraemic active infections could be detected by quantification of circulating antibody specific for non-microfilarial antigens expressed and released by living adult worms, such as

occult conditions. Amicrofilaraemic active infections could be detected by quantification of circulating antibody specific for non-microfilarial antigens expressed and released by living adult worms, such as E/S molecules of male adult worms. A cDNA library was constructed using *B. malayi* male adult worm mRNA. The library was made in the expression vector lambda gt11 and screened with a rabbit antiserum raised against E/S products collected *in vitro* from 100% viable *B. malayi* male and female adult worms. Of an initial number of 13 (out of 60) recombinants selected on the basis of their reactivity with rabbit anti-E/S antiserum, six reacted strongly with a pool of three sera from southern Indian patients infected with *Wuchereria bancrofti*. These six recombinants (clones BmES 12, 14, 55, 62, 86 and 102) were further analysed for reactivity with sera from three patients with tropical pulmonary eosinophilia, two with chronic pathology, and three with asymptomatic microfilaraemia. Only clone12 (600 bp), reacted with all serum samples. The remaining recombinants reacted with some but not all of the test sera, in a manner that suggests the need for a polyvalent diagnostic reagent. Such a reagent could be generated by simple mixture of purified recombinant antigens or by engineering a fusion protein encompassing the appropriate antigenic domains.

220 A MODEL FOR THE PROTEOLYTIC PROCESSING OF THE *DIROFILARIA IMMITIS* ANTIGEN, DI5. Poole CB*, Benner J, Grandea A, and McReynolds LA. New England Biolabs, Inc., Beverly, MA.

Di5 was identified as a potential vaccine candidate for canine heart worm through screening a *D. immitis* cDNA library with serum from dogs immunized with irradiated *D. immitis* L3's. The gene coding for Di5 is composed of 25-50 copies of a 399 base pair tandem repeat. Antiserum raised against purified recombinant Di5 protein detects a protein ladder on immuno-blots of a *D. immitis* PBS extract. This ladder ranges in size from approximately 14 to > 200 kd with steps approximately 14-15 kd apart. Pulse-chase studies with metabolically labelled adult parasites showed that Di5 is synthesized as a large molecular weight precursor which is processed into smaller molecular weight units. N-terminal sequencing of the 14 kd monomer showed a cluster of lysine and arginine residues amino terminal to the cleavage site. To better understand the specificity of the parasite protease which cleaves Di5, a series of *in vitro* digestions were performed with varying concentrations of Endoproteinase Lys-C, Endoproteinase Arg-C, Trypsin, Papain and Proteinase K. Immuno-blots of the digested extracts demonstrated that all the proteases digested the highest molecular weight bands in the ladder into lower molecular weight forms with little or no smearing of the protein ladder. Eventually, the ladder was digested into a single band the size of the monomer which was resistant to digestion by all the Trypsin-like proteases tested despite the occurance of 23 lysine and 6 arginine residues per monomer. The monomer was also resistant to digestion by Proteinase K. These data suggest that the high molecular weight Di5 precursor consists of protease resistant core regions linked together by short protease-sensitive regions. This structure would enable a parasite protease with little or no specificity to digest Di5 and generate a specific product. Experiments directed toward identifying the protease(s) responsible for cleaving Di5 are currently in progress.

221 *CARDIOFILARIA KALIMANTANI* N. SP. (FILARIOIDEA: ONCHOCERCIDAE) FROM NATURAL AND EXPERIMENTAL HOSTS, LIFE CYCLE AND EPIDEMIOLOGIC SIGNIFICANCE. Purnomo, Atmosoedjono S, and Bangs MJ*. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.

Lymphatic filariasis remains a significant problem in many areas of Indonesia. Although only three species of filariae are known to commonly infect humans in the archipelago, a diverse range and number (38 species, 18 genera) of zoophilic filarial worms co-exist with anthropophilic *Brugia* and *Wuchereria* species. As part of a longitudinal filariasis study in South Kalimantan (Borneo) nearly 100,000 mosquitoes (49 species, 9 genera) were dissected for filarial larvae. Of 27,883 *Coquillettidia crassipes* examined, 0.7% had L-3 larvae. Mongolian jirds experimentally exposed to these larvae produced adult

worms in the peritoneal cavity after a minimum of 27 days. All were identified as an undescribed species of *Cardiofilaria*. The microfilariae were morphologically identical to those found in a naturally infected silvered leaf monkey, *Presbytis cristata*. Previously described only from birds, this represents the first report of a *Cardiofilaria* species present in a natural mammalian host. All stages of the life cycle, including larval development in *Cq. crassipes*, have been described. Further studies have shown complete larval development in laboratory-bred *Aedes togoi*, and adult development in quails and chickens. Because *C. kalimantani* develops in natural and experimental avian and mammalian hosts, and given *Cq. crassipes*, often considered ornithophilic, will readily feed on humans, the possibility of this parasite infecting humans or domesticated animals is intriguing. No evidence to date suggests this actually occurs. The taxonomic analysis of animal filariae such as *C. kalimantani*, often found sympatrically with human filarial pathogens, will help to alleviate epidemiologic confounders associated with misidentification and subsequent overestimation of local transmission indices. This, coupled with recent advances in species-specific DNA hybridization assays and stage-specific monoclonal antibody techniques, should help to avoid the erroneous incrimination of non-vectors of human filariids and further assist national research surveillance and control programs.

222 $CD8^+$ T LYMPHOCYTES ARE NOT REQUIRED FOR MURINE RESISTANCE TO THE HUMAN FILARIAL PARASITE, *BRUGIA MALAYI*. Rajan TV*, Nelson FK, Koller BH, Shultz LD, and Greiner DL. Department of Pathology, University of Connecticut Health Center, Farmington CT; Department of Pathology, University of North Carolina, Chapel Hill, NC; Department of Medicine, University of Massachusetts Medical Center, Worcester, MA; and The Jackson Laboratory, Bar Harbor, ME.

Mice are non-permissive for infection with the nematode parasite *Brugia malayi*, an etiologic agent of human lymphatic filariasis. We have recently shown that the T and B lymphocyte deficient SCID mice are permissive for infection with this parasite, while co-isogenic, immunocompetent C.B-17 mice are resistant. This observation suggests that the antigen-specific immune system mediates murine resistance to *B. malayi*. In order to define the component of the antigen-specific immune response that is responsible for this phenomenon, we examined mutant mice that are deficient in beta2-microglobulin (B2m-) for susceptibility to infection. These mice do not express antigens of the major histocompatibility complex (MHC Class 1-) and lack CD8 antigen expressing cytotoxic T lymphocytes. Twelve such mice were injected with 50 infective stage larvae each. One died early due to unknown causes. The other 11 were necropsied 3 months after injection. We were unable to a find a single adult worm or microfilarial larvae in any of these mice. In contrast, 29 of 33 SCID mice that we have thus far injected with *B. malayi* larvae have been found to contain an average of $6.6 \pm 6.4$ worms each. The difference between the yield of worms in these two groups is statistically significant at the $p<0.01$ level. These data suggest that the presence of CD8+ T lymphocyte subset is not an obligate requirement for murine resistance to human filarial parasites.

223 CHARACTERIZATION OF *BRUGIA MALAYI* PARASITE ANTIGENS USING BIOTINYLATED LECTIN PROBES. Rao UR, Vickery AC*, Kwa BH, and Nayar JK. College of Public Health, University of South Florida, Tampa, FL; and Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL.

The antigenic determinants of *Brugia malayi* were characterized with lectins using an enzyme linked avidin-biotin assay. All lectins used reacted with soluble adult worm antigen suggesting the presence of numerous carbohydrate molecules. Microfilarial antigens reacted with only wheat germ agglutinin (WGA), concanavalin-A (Con-A) and pokeweed mitogen. Adult worm antigens were resistant to heat treatment and some proteases but sensitive to trypsin, N-acetyl-D-glucosamine, N-acetylgalactosamine, L-fucose and sodium periodate indicating that they contained carbohydrate moieties. Adult worm

soluble antigens were evaluated by SDS-PAGE, electroblotted and were identified using biotinylated lectins. Numerous lectin reactive carbohydrate epitopes were recognized by this method. Under reducing conditions, adult worm antigens conjugated with fluoresceinated lectins showed reactivity with low molecular weight precipitin bands. Of all the lectins, Con-A predominantly bound to the adult worm antigen fraction. When used in a dot-blot assay, biotinylated WGA could detect 25 ng/ml adult worm antigen. The data suggest that *B. malayi* adult worm or microfilaria antigens are complex and display an intriguing mixture of biochemical components. Carbohydrate moieties alone or in association with glycoproteins may be important in the immune response to the parasite.

224 NUMBER AND DISTRIBUTION OF *HHA I* REPEAT CLUSTERS IN THE GENOME OF THE FILARIAL WORM *BRUGIA MALAYI*. Sachar R*, Li Z, Spiegelman M, and Williams SA. Department of Biological Sciences, Smith College, Northampton, MA.

A DNA sequence of 322 base pairs, called the *Hha I* repeat, occurs 30,000 times as tandem repeats in the genome of *Brugia malayi*. We have found that this amount constitutes 12% of the 85 Mb genome. As previously determined, the haploid chromosome number is five, consisting of one chromosome about 1.0 μm in length and four others, each about 0.5 μm long. In order to determine the genomic organization of the *Hha I* repeat, we are using both pulsed-field gel electrophoresis (PFGE) and *in situ* hybridization. Results are consistent with distribution of the repeat in about two large clusters per chromosome. Agarose plugs of *B. malayi* DNA were subjected to PFGE following digestion of *Hha I* flanking sequences by restriction endonucleases. The number of clusters is about 10 and the size of the clusters ranges from 85 kb to 3400 kb. *In situ* hybridization at the light microscopic level shows that all five chromosomes, prepared by squashes of *B. malayi* spermatozoa, strongly bind a DNA probe specific for *Hha I*. The probe is a biotinylated 45-mer oligonucleotide homologous to a sequence embedded in the *Hha I* family. Detection of hybridization is by an avidin-biotin-horseradish-peroxidase detection system. In contrast, *Dirofilaria immitis* controls exhibit distinctly weaker nonspecific binding of the probe. The extremely small size of *B. malayi* chromosomes precludes precise localization of the hybridization signal by light microscopy. SEM samples are being prepared to enable the precise localization of the repeat clusters.

225 DEVELOPMENT OF *DIROFILARIA IMMITIS* IN UNITED STATES STRAINS OF *AEDES ALBOPICTUS*. Scoles GA* and Craig GB. Vector Biology Labs, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

While Asian strains of *Aedes albopictus* have been shown to be susceptible to infection with *Dirofilaria immitis* (dog heartworm), United States strains have not been shown to be suitable hosts. Three U.S. strains of *Ae. albopictus* were tested for susceptibility to *D. immitis*. *Ae. aegypti* and *Ae. triseriatus* were used as controls. Mosquitoes were raised under standard conditions and allowed to feed on an infected dog. On day 15 post feeding the mosquitoes were decapitated in individual wells of a tissue culture plate. Nematodes were allowed to migrate into medium. Bodies were examined for nematodes remaining in the Malpighian tubules, thorax and head. Nematodes that migrated from the body were cultured for 3 days; >75% molted to the 4th stage in all 3 species. Parasite development varied considerably among the 3 strains of *Ae. albopictus*. One strain did not support development beyond the sausage stage, a second strain had sausage and $L_2$ stages. In the third strain 56% of the individuals contained $L_3$'s (5.2 per infective mosquito). However, microfilarial to $L_3$ developmental efficiency was low (37%) with nematodes arrested at all stages in the Malpighian tubules. In comparison *Ae. triseriatus* produced 14.4 $L_3$'s per individual with 97% efficiency and *Ae. aegypti* produced few $L_3$'s (efficiency <1%). Mortality was lowest in the strains most susceptible to infection. *Ae. albopictus*, recently introduced into the U.S. has strong potential to become a vector of dog heartworm.

226 CLONING MF1, A TRANSMISSION-BLOCKING ANTIGEN FROM *BRUGIA MALAYI*. Southworth MW*, Fuhrman JA, and Perler FB. New England Biolabs, Inc., Beverly, MA; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The MF1 antigen is a developmentally regulated protein of mature microfilariae (mf), previously defined by a monoclonal antibody which caused clearance of peripheral microfilaremia by passive transfer into infected gerbils. The purified proteins, p70 and p75, which bind the monoclonal, were partially sequenced. Degenerate oligonucleotide primers derived from the N-terminal amino acid sequence and from a random MF1 tryptic peptide sequence, were used to PCR amplify MF1 sequences from the *B. malayi* genome. λgt11 cDNA libraries were screened with this probe. No full-length clones were obtained, so the gene was sequenced and cloned directly from mf mRNA by PCR techniques. The PCR product was cloned into pmal-c; 30/32 white colonies had the right size insert; 18/32 gave a fusion protein of ~100 kDa. So far, 9/18 have been tested on a Western blot for reactivity with the MF1 monoclonal, all 9 positive. Most of the soluble fusion protein appears to bind well to amylose; however, around 90-95% is insoluble. Factor X cutting of the fusion protein gives a product of ~60 kDa, which reacts with MF1 monoclonal, as well as another monoclonal that recognizes a different MF1 epitope (2D5). The recombinant MF1 antigen will provide a valuable tool in immunodiagnostic and biochemical studies, and protective immunization trials.

227 CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF MONOCLONAL ANTIBODIES SPECIFIC TO NEMATODE TUBULIN. Bughio NI*, Faubert GM, and Prichard RK. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

Anti-*Brugia pahangi* tubulin monoclonal antibodies (MAbs) P3D and 1B6,were used: (1) to investigate the heterogeneity of tubulins from nematodes and mammals; (2) to determine their effect on the viability of adult *B. pahangi*; and (3) to examine the distribution of β-tubulin in *B. pahangi*. Western blot analysis of one-dimensional SDS-PAGE showed that MAbs P3D and 1B6 react with an epitope(s) specific to filarial and intestinal nematode β-tubulins, but not to mammalian tubulins. In two-dimensional SDS-PAGE, MAb P3D recognized one β-tubulin isoform and MAb 1B6 reacted with two β-tubulin isoforms in adult *B. pahangi*. Results of limited proteolysis showed that MAb P3D reacted with 42 and 34 kDa amino-terminal fragments, however MAb 1B6 recognized the 21 kDa carboxy-terminal fragment of β-tubulin. MAbsP3D and 1B6 caused 80% and 60% reduction in worm viability respectively, 48 h post treatment. Immunogold labeling of *B. pahangi* demonstrated the presence of tubulin in the cuticle, hypodermal layers and somatic muscles of *B. pahangi*. The reduction in the viability of adult worms may be due to the disruption of microtubules in the body wall muscle.

228 THE DEVELOPMENTALLY REGULATED EPITOPE RECOGNIZED BY MAB MF2 IS LOCATED IN A FILARIAL ISOACTIN. Sritharan M* and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The monoclonal antibody designated MF2 reacts with a developmentally regulated epitope on a 44 kDa antigen of *Brugia malayi* microfilariae, which is also present in adult male worms. A partial amino acid sequence of the purified 44 kDa antigen indicates that the MF2 epitope is located on an isoform of actin. Mab MF2 crossreacts with actin from other invertebrate sources, but not with vertebrate isoforms of the protein. The 44 kDa filarial antigen is also recognized by an antiserum to chicken gizzard actin, which broadly crossreacts with actins from many invertebrate species. The MF2 epitope is located on 31 and 33 kDa peptide digestion fragments resulting from the action of SV8 protease on native 44 kDa filarial antigen. Mab MF2 reacts with fragments of the same size from purified clam adductor muscle digested with SV8 protease. Sequence analysis of a PCR product generated by amplification of genomic DNA of *B. malayi* with primers based on the amino acid sequence of the putative filarial actin is in progress. This should permit us to determine the precise nature and location of the MF2 epitope.

229 IMMUNITY IN ONCHOCERCIASIS: ANTIGEN INDUCED IL-2 PRODUCTION BY PUTATIVELY IMMUNE INDIVIDUALS IS REFLECTED BY INCREASED IL-5 SYNTHESIS. Steel C*, Abrams JS, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and DNAX Research Institute, Palo Alto, CA.

Although antigen-specific T cell responses have been implicated in the induction of a protective immune response to onchocerciasis, the nature of the signals generated by these T cells remains unclear. To elucidate the role of both TH1- and TH2 -type cytokines in this process, supernatants from *Onchocerca volvulus* (Ov) antigen- and mitogen-stimulated peripheral blood mononuclear cells (PBMC) previously collected from putatively immune (PI; n = 7) and Ov-infected (INF; n = 10) individuals were assessed for the production of IL-2, IL-4, IL-5 and interferon-(γ) (IFN-γ). As shown previously, the PI individuals made significantly more IL-2 ($p < 0.01$) than the INF patients. Interestingly, the IL-5 response to Ov antigen paralleled that of IL-2 with signficantly more IL-5 being produced by the PIs than by the INF patients (p=.018). There was an extremely strong positive correlation ($R^2$=.91, p=.001) between the ability to produce IL-2 and the IL-5 response that followed. There was neither IL-4 nor IFN-γ production to Ov antigen in either group of patients; however, mitogen stimulated IL-4 production was increased in the PIs compared to those with active infection. To examine more closely the relationship between IL-2 production and the IL-5 response it engenders, IL-5 mRNA induction was assessed using PCR. In response to even small amounts of IL-2 (0.1 units/ml) IL-5 mRNA could easily be noted. In contrast, there was no IL-4 mRNA induced by IL-2, and there was no IL-2 induced induction of IFN-γ mRNA. The mechanisms underlying the regulation of IL-5 in onchocerciasis along with the role of this cytokine in mediating protective immunity to Ov infection await clarification.

230 AN EXAMINATION OF THE IMMUNE RESPONSE TO CLONED *ONCHOCERCA VOLVULUS* ANTIGENS OI3 AND OI5. Strang G*, Southworth MW, and Perler FB. New England Biolabs, Inc. Beverly, MA

We are interested in studying the immune response to *Onchocerca volvulus* using recombinant proteins. OI3 and OI5 were selected using sera from putatively immune individuals; both encode portions of a 200 kDa OV antigen. Fewer than 20% of chronically infected individuals had detectable levels of specific antibody to OI3. Further characterization of the antibody response was carried out to examine cross-reactivity of the antigens to sera from individuals with different parasitic infections. A series of ELISAs showed that there was considerable cross-reactivity to sera from individuals infected with *Brugia malayi* and *Wuchereria bancrofti*, but none to sera from individuals with Schistosomiasis, Trichinellosis and Toxocariasis. ELISAs with OI5 and OI3 were also run on sera taken from experimentally infected chimpanzees that had differed in their response to OV infection. Next, methods of immunization were characterized as a preliminary step before starting murine protection trials with the L3 chamber model. Different routes of immunization and adjuvants were compared for their ability to stimulate OI5 and OI3 specific antibody and T cell responses in mice. Results suggest that subcutaneous (SC) and intraperitoneal (IP) injections stimulate equivalent antibody responses, but that IP priming is more effective than SC priming at stimulating a T cell response. Further experiments are underway to define the OI3 immune response in vaccinated mice.

231 USING THE POLYMERASE CHAIN REACTION TO AMPLIFY AND SEQUENCE DNA FROM MUSEUM SPECIMENS OF *BRUGIA MALAYI* AND *BRUGIA PAHANGI*. Xie H*, Bain O, and Williams SA. Molecular and Cellular Biology Program, University of Massachusetts at Amherst, MA; Laboratoire de Zoologie-Vers, associe au CNRS, Museum National d'Histoire Naturelle, Paris; and Department of Biological Sciences, Smith College, Northampton, MA.

The *Hha I* family of highly repeated DNA elements are 322 bp long, are tandemly repeated and have been cloned from *Brugia malayi, Brugia timori, Brugia pahangi,* and *Brugia patei.* In order to expand our database and to maximize the utility of these probes as diagnostic and taxonomic tools, we wished to develop methods for collecting *Hha I* repeat sequence data as efficiently as possible. This was accomplished by amplifying the *Hha I* repeats from *Brugia* genomic DNA by symmetric polymerase chain reaction (PCR). Briefly, genomic DNA was released from adult worm(s), L3 larvae or microfilariae by boiling. Two primers that hybridize within the repeat are used in symmetric PCR reactions. After simple purification procedures, the amplified double-stranded DNA is sequenced directly without cloning. Consensus *Hha I* repeat sequence data can be obtained from a single worm in two days. Consensus sequences from different *Brugia* species, different biotypes within species, and different geographic isolates are being determined. Data were obtained from a single Indian *Brugia malayi* adult male worm preserved for several years in ethanol. These data indicate that the geographic isolate from India has a DNA sequence that differs greatly from those obtained from Indonesia and China. This method is also being used to obtain data from periodic, sub-periodic and anthropophilic isolates of *Brugia* species. This will aid in the design of species- or biotype-specific DNA probes, which will aid in the diagnosis of filariasis and in epidemiological studies. Such data will also prove invaluable in a detailed phylogenetic analysis of the lymphatic filariae.

232 GTP-BINDING *RAS* P21 PROTEINS OF FILARIAL PARASITES HAVE A UNIQUE C-TERMINAL SEQUENCE. Xu M*, Dissanayake S, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Because GTP-binding *ras* proteins are increasingly implicated in various aspects of the metabolism of a wide range of organisms, we cloned and characterized *ras* P21 cDNAs of the filarial parasites, *Brugia malayi, Onchocerca volvulus* from Mali and *O. volvulus* from Toubaro. The *B. malayi* gene codes for a protein of 215 amino acids; the *Onchocerca* equivalents encode 218 residues. All three cDNAs contain the GTP-binding domains as well as other amino acid motifs that are highly conserved among all *ras* proteins. However, in contrast to all previously described *ras* P21 proteins, the three filarial homologs have no C-terminal cysteins and lack the common C-terminal CAAX sequence, which are believed to be essential for the biological function of these molecules. Instead, all three filarial P21 proteins have the unique C-terminus: ala-ala-ala-ala-asn-ala-glu-leu-pro-asp-asp-asp-glu-as p-leu. Because the overall deduced amino acid sequence of the filarial *ras* P21 proteins has the greatest homology to that of the rab/SEC/YPT subfamily of P21 proteins, we speculate that these molecules may function in vesicular transport in the parasites. The unique C terminus of filarial P21 proteins might serve as a suitable target for antifilarial drug development.

## POSTER II: MALARIA CHEMOTHERAPY

233 INTRINSIC ANTIMALARIAL ACTIVITY OF CIPROFLOXACIN ALONE OR IN COMBINATION WITH CHLOROQUINE OR MEFLOQUINE. Coyne PE*, Gerena L, and Milhous WK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Fluroquinolone antibiotics represent a major breakthrough in the management of serious bacterial infections especially gram-negative pathogens. Their antimalarial activity has also been demonstrated *in vitro* and clinical trials have been successfully conducted in India. Select groups of American soldiers recently deployed in Operation Desert Storm required antimalarial prophylaxis with chloroquine (CQ), but also used ciprofloxacin (CIPRO) for prophylaxis against anthrax or for treatment of bacterial diarrheas. The current study was undertaken to evaluate the intrinsic antimalarial *in vitro* activity of CIPRO alone and in combination with CQ or mefloquine (MFQ) against falciparum malaria clones from different geography regions. Although CIPRO exhibited an intrinsic activity equivalent to doxycycline, it was less effective against multidrug resistant strains from Indochina. Clinical trials recently conducted

in Thailand have also demonstrated limited utility against multidrug resistant strains. When combined *in vitro* with either CQ or MFQ, a marked degree of antagonism of intrinsic antimalarial activity was observed. Although advanced studies in rodent and primate models will be required to establish potential clinical relevance, these findings suggest that use of these drug combinations could result in prophylactic or treatment failures.

234 THE EFFECTS OF ARTEMISININ (QINGHAOSU) ON THE RED CELL CYTOSKELETON. Yang Y* and Meshnick SR. Department of Microbiology, City University of New York Medical School, New York, NY.

Artemisinin (qinghaosu), derived from a Chinese herbal remedy, is a promising new antimalarial drug. We have previously shown that the drug appears to react with intraparasitic hemin to generate toxic free radicals. Since red cell membranes are known to be exquisitely sensitive to oxidant damage, we have now characterized the effects of artemisinin on red blood cell membrane preparations. When red cell membranes are incubated with artemisinin, a high-molecular weight membrane polypeptide aggregate forms which is unable to penetrate 4% polyacrylamide/SDS gels. At the same time, the spectrin band and Band 3 diminish in intensity. More of this aggregate forms when the membranes are incubated in the presence of both artemisinin and hemin. When the membranes are incubated in the presence of [$^{14}$C]-artemisinin, some of the recovered radioactivity co-migrates with the polypeptide aggregate. When the aggregate is eluted and dissociable by treatment with mercaptoethanol, it is found to contain spectrin, Band 3 and hemoglobin. Thus, artemisinin, particularly in the presence of hemin, has potentially toxic effects on the red cell cytoskeleton. Similar effects may play a role in both the antimalarial activity and potential host toxicity of the drug.

235 CHINESE HERBAL ANTIOXIDANTS IN MALARIA CHEMOTHERAPY. Meshnick SR*, Hong YL, Scott MD, Yang YZ, Ranz A, and Pan HZ. Department of Microbiology, City University of New York Medical School, New York, NY; Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China; and Childrens' Hospital Oakland Research Institute, Oakland, CA.

Primaquine is an important antimalarial drug which can cause hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency. The anemia is probably due to the generation of activated oxygen by primaquine metabolites. Oxidant generation by one metabolite (5,6-dihydroxy-8-aminoquinoline (AQD)), was measured by chemiluminescence (CL). AQD-dependent CL was inhibited by catalase, unaffected by mannitol, and stimulated by superoxide dismutase, suggesting that it is mediated by $H_2O_2$, not superoxide or hydroxyl radicals. The CL was also partially inhibited by deferoxamine, an iron chelator, implying that it is dependent on iron. Three antioxidants (daphnetin, ferulate and maltol), derived from Chinese herbal remedies, inhibited AQD- and $H_2O_2$-mediated CL, whereas a fourth, anisodamine, had no effect. Daphnetin also proved to be a potent antagonist of lipid peroxidation, as measured by the $H_2O_2$-mediated production of thiobarbituric acid-reacting substances. In addition, daphnetin was found to have antimalarial activity both *in vitro* and *in vivo*. Daphnetin has been used clinically in China for the treatment of coagulation disorders with no apparent toxicity. Thus, daphnetin, or an agent like it, might prove useful when administered in conjunction with primaquine.

236 ABSOLUTE STEREOCHEMISTRY OF THE ENANTIOMERS OF MEFLOQUINE HYDROCHLORIDE IN RELATION TO THEIR ANTIMALARIAL ACTIVITY. Karle JM*, Karle IL, Gerena L, and Milhous WK. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC.; Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC.; and Department of Parasitology, Walter Reed Army Institute of Research, Washington, DC.

The more active enantiomer of mefloquine hydrochloride was found to share the same absolute configuration as quinidine, the more active of the two cinchona alkaloids quinine and quinidine. (+)-Mefloquine hydrochloride was 1.81 and 1.69 (± 0.17 S.D., n=5) times more active than (-)-mefloquine hydrochloride in inhibiting radiolabeled hypoxanthine uptake into chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*, respectively. In the same assay system, quinidine was 2.3 to 2.6 (n=5 to 6) times more active than quinine. The absolute configuration of (-)-mefloquine hydrochloride was determined by x-ray crystallography and was found to have the opposite configuration of quinidine as demonstrated by the O-C-C-N torsion angles of -56 and +65 degrees in (-)-mefloquine and quinidine, respectively. Mefloquine, quinine, and quinidine all share an erythro conformation. The enantiomers of the threo diastereomer of mefloquine also display differential activity in the hypoxanthine uptake assay with the (+)-enantiomer 1.85 to 1.95 (n=5) times more active than the (-)-enantiomer. These results show that amino alcohol antimalarial agents whose structures are based upon the structures of the cinchona alkaloids can demonstrate stereospecific antimalarial activity.

237 QUANTITATIVE ISOBOLOGRAPHIC ANALYSIS OF ANTIMALARIAL DRUG INTERACTIONS.
Brueckner RP*, Milhous WK, and Canfield CJ. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Pharmaceutical Systems, Gaithersburg, MD.

The effects of drug combinations are becoming increasingly more important in the therapy of infectious and neoplastic diseases. Drug synergy, antagonism and reversal of drug resistance are of particular interest in the therapy of malaria. Isobolograms have been used to pictorially represent these interactions. However, ideal methods to mathematically quantify the degree of drug interaction do not exist. For example, Fractional Inhibitory Concentrations (F.I.C.) values vary widely, depending on the relative proportion of the 2 drugs in any given experiment. A mathematical equation describing the isobole was developed. The equation incorporates one drug interaction parameter that numerically quantifies the degree of synergy or antagonism. A computer program was written which finds the best value of this parameter by minimizing the sum of the squared perpendicular distances from each data point to the isobole. This method was then applied to several antimalarial combinations. It is more useful than other methods used to quantify drug interactions, and can been used to select optimal drug combinations.

238 IDENTIFICATION OF NEW REVERSAL MODULATORS FOR *PLASMODIUM FALCIPARUM*.
Bass GT*, Sr., Gerena L, Milhous WK, Andersen SL, Oduola AM, Kyle DK, and Martin RK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; University of Ibadan, Ibadan, Nigeria; and Department of Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

*Plasmodium falciparum*, a significant source of morbidity and mortality in man, has developed resistance to chloroquine, the drug of choice for chemoprophylaxis and chemotherapy. Mefloquine, a recent alternative antimalarial drug for malaria, has shown evidence of drug resistance in Southeast Asia and in specific areas of Africa. In order to preserve the clinical utility of these drugs, new chemotherapeutic strategies must be continually developed and implemented to circumvent the resistance phenomenon. One promising approach has been the description of reversal modulators that upon simultaneous exposure with chloroquine and other antimalarial drugs act to potentiate their efficacy. We have identified additional drugs (amitriptyline and its derivitives) capable of reversing chloroquine and mefloquine resistance. Results from studies of falciparum clones from Southeast Asia and West Africa indicated that these agents reverse chloroquine and quinine resistant isolates equally well. These reversal agents were not effective modulators of mefloquine resistance in West Africa; however, amitriptyline did reverse mefloquine resistance in Southeast Asian isolates. With reduced toxicity and improved

pharmacokinetics, these compounds may provide new insight for a lead-directed synthesis of reversal modulators.

239 REVERSAL OF CHLOROQUINE RESISTANCE IN THE ISOLATES OF *PLASMODIUM FALCIPARUM* FROM AFRICA. Basco LK and Le Bras J*. Laboratoire de Parasitologie, Hopital Bichat-Claude Bernard, Paris, France.

The emergence of chloroquine-resistant *P. falciparum* has been reported from most of the malaria endemic regions. Recent studies show that a combination therapy of chloroquine (CQ) and calcium antagonists, tricyclic antidepressants, tricyclic antihistaminics, or phenothiazines can reverse CQ resistance in the experimental malaria models. Since most studies are based on culture-adapted *P. falciparum* strains, we examined the ability of desipramine, cyproheptadine, chlorpromazine, and prochlorperazine to potentiate CQ action *in vitro* against the fresh isolates obtained from 52 non-immune travelers and 7 semi-immune indigenous patients. Fifty percent inhibitory concentration ($IC_{50}$) of CQ and the "reversing agents," alone and in combination, was determined by the semi-micro drug susceptibility test. With the CQ resistance level set at $IC_{50}$ > 100 nM, resistance was reversed at the concentration of 625 nM of "reversing agents" in 28/34 CQ-resistant parasites. At 1250 nM, resistance was reversed in all isolates, except for the most resistant isolate from Rwanda ($IC_{50}$ 823 nM). In contrast, the $IC_{50}$ values were not modified in 21/25 CQ-susceptible isolates. The $IC_{50}$ values were lowered in 4/25 susceptible isolates which were relatively more susceptible to the "reversing agents" alone. The results suggest that CQ resistance can be reversed in most African isolates at the *in vitro* concentration between 625 and 1250 nM, independently of the immune status of the patient, and that higher concentration may be required to restore the efficacy of CQ against the highly resistant isolates.

240 TRANSPORT AND INCORPORATION OF [$^3$H-] P-AMINOBENZOIC ACID BY *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES. Zhang Y*, Merali S, and Meshnick SR. Department of Microbiology, City University of NY Medical School, New York, NY.

*De novo* folate biosynthesis is the target of several important antimalarial agents. We have characterized transport and incorporation of the folate precursor p-aminobenzoic acid (PAB) into normal and *Plasmodium falciparum*-infected red blood cells. Normal red cells transport PAB in a saturable and energy-dependent manner, with a dissociation constant of 83 nM. Parasite-infected red cells may use the same mechanism, as demonstrated by similarities in time course, concentration-response, and dissociation constant (101 nM). The transport capacity of red cells is closely correlated with the intracellular ATP level, but is insensitive to sodium ionophores nigericin and monensin. p-Aminosalicylic acid (PAS) inhibits PAB transport competitively, with a inhibition constant of 378 nM. Phloritin, flufanamic acid, and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DITS), which are inhibitors of the anion transporter (Band 3), and oxalic acid, a substrate of this transporter, partially inhibit PAB transport into both normal and infected red cells.These data suggest that the PAB is partially transported via Band 3. Furthermore, in both normal and infected red cells, the inhibitory effects of PAS and the anion transport inhibitors are additive. In contrast to the transport of PAB, the incorporation of PAB into folate and its derivatives takes place in parasite-infected red cells but not in normal red cells. Infected red cells (10% parasitemia) synthesize 0.46 nmol folate per day per mg protein from [$^3$H-] PAB. These findings have important implications for developing new therapies.

241 THE RELATIONSHIP OF *PF-MDR1* TO MEFLOQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM*. Wilson CM*, Thaithong S, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA and WHO Collaborating Centre, Chulalongkorn University, Bangkok, Thailand.

The purpose of these studies was to examine the role of *Pf-mdr1* in mefloquine (MFQ) resistance in clinical isolates from northern Thailand and in *in vitro* derived MFQ resistant mutants. The isolates were all (11/11) resistant to MFQ, chloroquine (CLQ), quinine (QNE), pyrimethamine, halofantrine (HAL) and qinghaosu (QHS). Southern analysis of genomic DNA with *Pf-mdr1* shows clear evidence of increased gene copy number in 8/11 isolates. Sequence analysis of *Pf-mdr1* in these isolates shows only a single nucleotide change in the entire coding region along with variability in the poly-asparaginated region of the linker domain. In addition, MFQ resistant mutants were selected from a mutagenized HB3 clone (MFQ $IC_{50}$ 4 ng/ml) with either continuous (HB3-mef10) or discontinuous (HB3-mef20) pressure. The $IC_{50}$ by hypoxanthine uptake assay was 8-11 ng/ml for HB3-mef10 and 17-22 ng/ml for HB3-mef20. Both mutants are also resistant to QNE but remain sensitive to CLQ, HAL, and QHS. Southern analysis with *Pf-mdr1* shows a gene duplication in HB3-mef20 while HB3-mef10 remains the same as the parent. Sequence analysis of *Pf-mdr1* in these mutants reveals no change from the parent. These studies indicate a potential role for*Pf-mdr1* in MFQ resistance.

242 ACCESSIBILITY AND COMPLIANCE IN THE USAGE OF MEFLOQUINE IN ITS FIRST YEAR OF AVAILABILITY AT A US TRAVEL CLINIC. Eaton M* and Kozarsky P. Emory University School of Medicine, Atlanta, GA.

In 3/90 mefloquine was recommended by the Centers for Disease Control for the chemoprophylaxis of malaria in travelers to areas with chloroquine-resistant *P. falciparum* and became available in the US in 5/90. Shortages and potential neuropsychiatric and cardiac side effects caused hesitation in prescribing/taking the drug. To determine availability and usage of mefloquine by patients seen at the Travel Clinic of Emory University, 643 charts (patients seen 5/90-4/91) were reviewed. Mefloquine was recommended in 269 but was contraindicated in 40; 9 refused the drug. Phone survey of 154/269 revealed 10 had not travelled as planned, 145 (94%) obtained mefloquine but with difficulty, 3 were noncompliant, 9 could not complete the course because of adverse side effects. 73 (47%) did not take the drug due to unavailability, contra-indications, adverse reactions, or refusal. In the first year of mefloquine usage, counseling travelers about malaria chemoprophylaxis has been complicated by mefloquine's novelty and periodic shortages. Mefloquine was available to most, however, and compliance was good. We conclude that increased availability, better education, and further study and monitoring of contraindications to mefloquine should improve usage.

243 RECRUDESCENCE OF FALCIPARUM MALARIA OCCURRING FIVE YEARS AFTER EXPOSURE IN WEST AFRICA. Furlong WB*, Gerena L, Waltersdorff RL, Oduola AM, and Milhous WK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Washington Adventist Hospital, Takoma Park, MD.

Although recrudescence following several years after initial exposure is frequently encountered in vivax malaria, it is rare for cases of falciparum malaria. A 48 year old woman originally from Sierra Leone had been living in the United States for five years. She was hospitalized with a history of fever, chills, intermittent dyspnea, and headache. A rare number of ring stage parasites could be demonstrated on thick smears.The patient denied having a blood transfusion and her most recent visit to Africa was five years prior to her admission. She was treated initially with quinine and tetracycline with a presumptive diagnosis of falciparum malaria, but quinine therapy was stopped after two days because of severe nausea and vomiting and a chloroquine treatment regimen was initiated. The patient was afebrile 24 hours later and was eventually discharged and treated with primaquine. Falciparum malaria parasites were successfully recovered in culture and susceptibility testing performed. Parasites were resistant to pyrimethamine, exhibited decreased sensitivity to mefloquine, but were markedly susceptible to

chloroquine, quinine, sulfadoxine, halofantrine, artemisinin and the desethylchloroquine. This *in vitro* susceptibility pattern resembles those reported during the previous 5-10 years in West Africa.

244 EPIDEMIOLOGIC AND ENTOMOLOGIC CONDITIONS RESPONSIBLE FOR STABLE HYPERENDEMIC TRANSMISSION OF MALARIA IN THE CENTRAL HIGHLANDS OF IRIAN JAYA. Bangs MJ*, Hamzah N, Purnomo, Basri H, and Anthony RL. US Naval Medical Research Unit, No.2, Jakarta, Indonesia; and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

In early 1990, NAMRU was requested by the local Health Department to investigate an outbreak of alarmingly high malaria associated deaths in the Oksibil area. Quarterly malariometric surveys from May, 1990 to April, 1991 established that stable malaria transmission was present in Oksibil, a remote highland community, 4100-4300 feet elevation, in the eastern Jayawijaya Mountains of Irian Jaya, Indonesia. Despite some marked differences in prevalence rates among the four villages surveyed, the overall combined average was 53% for children <5 years, 40% for the 5-9 year olds, 35% for the 10-14 year olds and 20% for adults >15 years. *Plasmodium falciparum* accounted for slightly more than 50% of all infections but *P. vivax* appeared to be the predominant species in the younger age groups. Over the year, four cases were diagnosed as *P. ovale*. Although spleen rates were only taken intermittently, palpable enlargement was obvious, regardless of age groups, in >50% of the valley's population. While currently hyperendemic, elevated titers of anti-malarial antibodies and the apparent higher clinical tolerance of most adults implied that malaria had been endemic in Oksibil for a number of years. Entomologic and epidemiologic data indicate that a recent upsurge in disease occurred, almost simultaneously, with the replacement of traditional housing by the more modern social housing. This replacement involved extensive landscaping activities and environmental modifications which inadvertently created numerous, ideal larval habitats for the principle vector species, *Anopheles punctulatus*. Clustering of disease with respect to household was apparent in several locations and indicative of intense focal transmission. This Oksibil experience serves to emphasize how quickly malaria can overwhelm a community and, in the absence of effective means of control and treatment, significantly reduce the chances of survival of the younger population.

245 MALARIA SURVEILLANCE IN 15 SOUTHERN PROVINCES OF VIETNAM, 1976-1989. Nguyen Long G*, Phan Dinh L, Nguyen Van K, and Nguyen-Dinh P. Sub-Institute of Malariology, Parasitology and Entomology (Sub-IMPE) and Cho Ray Hospital, Ho Chi Minh City, Vietnam; and Malaria Branch, Centers for Disease Control, Atlanta, GA.

Surveillance of malaria, a major public health problem in Vietnam, is essential for the cost-effective use of scarce resources available for control. Since 1975, the Sub-Institute of Malariology, Parasitology and Entomology (Sub-IMPE) of Ho Chi Minh City has conducted malaria surveillance in the 15 southernmost of the country's 40 provinces, through a network of peripheral health posts, hospitals, and malaria stations. The trend of greatest concern was the continuous increase, during the final 6 years (1984-1989), of reported positive slides (160% increase), clinically diagnosed cases (63%), severe cases (207%) and deaths (129%). In 1989, 3 provinces (Lam Dong, Dong Nai, and Song Be), totalling 15.8% of the southern population, reported 47.6% of the slide-confirmed cases of *Plasmodium falciparum*, 79.3% of the severe malaria cases and 72.2% of the malaria deaths. These 3 provinces share characteristics previously shown to contribute to malaria transmission and disease: presence of hilly forested areas, agricultural activities, rubber plantations, new economic zones, and ethnic minorities. Areas and population groups identified as being at greatest risk should be the focus of efforts to control the increasing trends of malaria in Vietnam.

246 USE OF BED NETS IMPREGNATED WITH PERMETHRIN FOR MALARIA CONTROL IN EASTERN GUATEMALA. Richards FO*, Zea Flores RM, Klein RE, Sexton JD, and Gatica Palacios MR. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; Centro para Investigaciones en Enfermedades Tropicales, Universidad del Valle de Guatemala, Guatemala; and Division de Malaria, Ministerio de Salud Publica, Guatemala.

We evaluated the impact of bed nets impregnated with 500 mg/$m^2$ permethrin (PERIPEL®, Wellcome) on anopheline behavior and malaria transmission during an ongoing longitudinal study. Twelve hundred persons in 251 households among five communities participated or served as controls. Mosquito densities and domiciliary exit and mortality rates were studied. The residual effect of the permethrin in the nets was evaluated using WHO "cone" bioassays. Malaria cases were identified by local voluntary collaborators. Questionnaire surveys and domiciliary visits allowed evaluation of the community acceptance of the bed nets. Preliminary results show a trend for increased mosquito exit and mortality rates in houses having impregnated bed nets. There were no significant differences in mosquito densities or malaria cases. Bioassays showed mosquito mortality rates of 90% after 6 months. The participants were enthusiastic about the nets, which they saw as a means for avoiding nuisance insects more than preventing malaria. They expressed a desired to wash the bed nets every 4-8 weeks, a habit that could limit the effectiveness of residual insecticide action. Impregnated bednets should be considered a major vector control component in community-based malaria programs in Central America.

247 FORMALDEHYDE/DETERGENT SOLUTION PREVENTS BLOOD BORNE TRANSMISSION OF *PLASMODIUM* INFECTION IN A MOUSE MODEL. Ager AL*, Andersen SL, Louderback AL, May R, and Milhous WK. Center for Tropical Parasitic Diseases, University of Miami, Miami, FL; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Clinical Chemistry Consultants, Inc., Arcadia, CA.

Transfusion transmitted *P. falciparum* infections as well as other blood borne infections are a major public health problem worldwide. A method of sterilizing blood from a donor before transfusion would have significant clinical advantages, especially in developing countries where current strategies for protection of the blood supply are impractical. We report the successful prevention of blood borne *P. berghei* infection by treating infected blood with a solution of formaldehyde, and Brij detergent known as Louderback Sterilizing Medium (LSM), originally developed for treatment of virus contaminated blood.Ten out of ten mice given an inoculum of $5x10^6$ parasites treated with LSM for 30 minutes injected intraperitoneally were entirely free of infection after 21 days. Control mice given the same (untreated) inoculum all died on day six. No hemolysis was noted, and no adverse effects to the mouse were observed. Toxicological studies and evaluation of this solution against other infectious agents are in progress. This study raises the possibility that pretreatment of blood with a sterilizing agent prior to transfusion may help prevent transfusion malaria.

248 THE RELATIVE FITNESS OF PYRIMETHAMINE-RESISTANT AND SUSCEPTIBLE LINES OF *PLASMODIUM BERGHEI* IN THE SPOROGONIC CYCLE IN *ANOPHELES STEPHENSI*. Shinondo CJ*, Lanners HN, Lowrie, Jr. RC, and Wiser MF. Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane Univiversity, New Orleans, LA; and Parasitology Department, Tulane Regional Primate Research Center, Covington, LA.

An *Anopheles stephensi-Plasmodium berghei* mouse model was used to study if pyrimethamine resistance (PR) confers any biological advantage during mosquito transmission. A drug-resistant line (PR) was derived from the parental, pyrimethamine sensitive line (PS) by a five-step relapsing technique with 200 mg/kg pyrimethamine. The PR and the PS lines were compared according to parameters of the sporogonic cycle, gametocytemia, oocyst size, ookinete, oocyst and sporozoite rates, gut infection index,

salivary gland index and sporozoite infectivity *in vivo* and *in vitro*. The PR line did not produce sporozoites 21 days p.i. at 19.5°C, but non-infective sporozoites were produced 28-30 days p.i. The other parameters were similar bycomparison in the two lines. Twenty-four-hour treatment of mice with subcurative doses of 10-20 mg/kg pyrimethamine enhanced infectivity of the PR line to the mosquito but abolished that of the PS line. The PR line had no biological advantage in the absence of drug pressure.

249 LACK OF CAUSAL PROPHYLACTIC ACTIVITY OF PROGUANIL PLUS SULFAMETHOXAZOLE AGAINST *PLASMODIUM CYNOMOLGI BASTIANELLII* IN RHESUS MONKEYS. Edstein MD*, Shanks GD, Smith CD, Corcoran KD, Chedester AL, Sattabongkot J, Ngampochjana M, Hansukjariya P, and Webster HK. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Recent clinical studies on the Thai-Cambodian border have shown proguanil (PROG) plus sulfamethoxazole (SMX) and PROG plus dapsone to be associated with a lower prevalence of vivax malaria compared to Maloprim (pyrimethamine plus dapsone). Based on these observations we assessed whether PROG plus SMX possessed causal prophylactic activity against *P. cynomolgi bastianellii* in the rhesus monkey (*Macaca mulatta*). This simian model has been routinely used to evaluate tissue and blood schizontocidal activity of candidate antimalarial compounds. A pilot study in 2 healthy monkeys revealed plasma elimination half-lives of 8.4 and 10.0 h for SMX and 7.2 and 11.4 h for cycloguanil, the active metabolite of the pro-drug PROG. Based on these half-lives a 5 day treatment schedule (-2, -1, 0, +1, +2) was selected. PROG (40 mg)/SMX (100mg)/kg were administered via stomach tube to 6 monkeys at 24 h intervals and 2 monkeys were used as controls. On day 0 all monkeys were inoculated intravenously with about $10^6$ sporozoites. The control monkeys developed parasitemia on days 9 and 23 while the PROG/SMX treated monkeys showed infection from days 28 to 44 (mean 38). These findings suggest that PROG plus SMX delays but does not prevent a primary parasitemia in the *P. cynomolgi*-rhesus monkey test system.

250 FIELD USE OF THE MICRO *IN VITRO* TEST FOR PREDICTING CHLOROQUINE SENSITIVITY IN *PLASMODIUM VIVAX*. Andersen EM*, Purnomo, Masbar S, Murphy GS, and Bangs MJ. U.S. Navy Medical Research Unit No. 2, Jakarta, Indonesia.

A micro *in vitro* test for predicting chloroquine sensitivity which had been previously described was evaluated in a field trial of chloroquine tolerance in *P. vivax* in Arso PIR II, Irian Jaya, Indonesian New Guinea, from March through May 1991. Forty-five volunteers with single *P. vivax* infections were enrolled and 500 µl of blood was taken for an initial *in vitro* test. Only parasitemias with young trophozoite stages were suitable for culture. The volunteer was then treated with 25 mg/kg chloroquine base over a 3-day period. If a volunteer became smear-positive for *P. vivax* during the ensuing 2-week period more blood was taken for a second *in vitro* test. For the *in vitro* test, resistance was defined as inhibition of parasite growth, by thick smear evaluation, in the presence of 1 to 64 picomoles of chloroquine base (compared to control wells with no chloroquine) over a 48 hour period. Fifteen isolates from the 45 volunteers enrolled grew in culture at the higher drug concentrations. *Plasmodium vivax* from 9 volunteers from whom isolates were cultured developed resistance clinically as confirmed by thick smear. The isolates from 5 of these volunteers grew very well in culture with a progression of parasite maturity and schizont development over a 48 hour period. Growth occurred in all chloroquine concentrations, however an arithmetic decrease in growth was seen as the concentration increased. Although these results are preliminary, it appears that the micro *in vitro* test has predictive value in assessing chloroquine sensitivity in *P. vivax*.

## POSTER II: ENTAMOEBA AND GIARDIA

251 *GIARDIA LAMBLIA* ENCYSTMENT IN THE MONGOLIAN GERBIL. Campbell JD*, Mohammed SR, Faubert GM. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

We studied *G. lamblia* (WB strain) encystment in the Mongolian gerbil model in order to determine if encystation specific vesicles (ESV), which appear in trophozoites encysting *in vitro*, also appear during encystment *in vivo*. Forty gerbils were infected with 1 x $10^6$ trophozoites by oral gavage and 4 were killed every 3 days over a 30 day period. The gerbils' small intestines were removed and cut in 3 equal sections, which were incubated in cold PBS baths, and the cells were counted. Trophozoites with ESV were present in all 3 sections, but the trophozoite to encysting trophozoite ratio was most consistant in the jejunum, suggesting that this is the major site of encystation in the gerbil. Trophozoites with ESV appeared in the small intestine (day 6) before cysts (day 9). In encysting culture medium, trophozoites with ESV appear as early as 6 hours and there are greater numbers of trophozoites with than without ESV. *In vivo*, however, there is a consistant predominance of cells without ESV (95-99%). The higher percentage of cells with ESV and the high numbers of cysts seen *in vitro* may be due to the continuous optimal conditions required for encystation. The appearance of trophozoites with ESV *in vivo* confirms that these forms are not *in vitro* artifacts, but a natural occurrence during encystment.

254 THE EFFECTS OF AXENISATION ON THE ZYMODEME STATUS OF *ENTAMOEBA HISTOLYTICA*. Gathiram V*, Jackson TG, Suparsad S, and Anderson CB. Research Institute for Diseases in a Tropical Environment, Congella, Durban, Republic of South Africa; and Medical School, University of Natal, Congella, Durban, Republic of South Africa.

The aims were to investigate the effects of axenisation of xenic cultures of pathogenic and non-pathogenic zymodemes of *Entamoeba histolytica*: particularly, (i) the growth rate and, (ii) stability of the characteristic isoenzyme patterns of these cultures were observed. The initial isolations were made in either Robinson's or Diamond's TYS-GM medium. Cultures were then transferred into Diamond's TYI-S33 medium containing various combinations of antibiotics to limit and eventually eradicate concomitant bacteria. Cultures were supplemented with trypanosomes or crithidia until monaxenic culture with either of these flagellates had been established. At the stage where prolific monaxenic growths had been established, the amoebae were gradually weaned off the concomitant organisms into axenic culture. Successful axenic culture was only achieved with pathogenic zymodemes while only monaxenic growths were obtained with non-pathogenic zymodemes. Conversions from pathogenic to non-pathogenic zymodemes and vice versa were not observed in any case.

253 ASSOCIATION OF SALIVARY IgA SPECIFIC FOR THE 260KD ADHERENCE PROTEIN OF *E. HISTOLYTICA* WITH THE PRESENCE OF AMEBIC LIVER ABSCESS. Kelsall BL*, Jackson TG, Pearson RD, and Ravdin JI. University of Virginia, Charlottesville, Virginia; RIDTE, Durban, Republic of South Africa; Case Western Reserve University, Cleveland, OH; and the Cleveland VA Medical Center, Cleveland, OH.

Antibodies to the 260kd galactose specific adherence protein (AP) of *E. histolytica* (EH) block the *in vitro* attachment of amebic trophozoites to purified colonic mucins, suggesting a role for anti-AP secretory IgA (sIgA) in host defense. We measured anti-AP salivary IgA by ELISA in throat washings from South African (S. Afr.) patients with and without amebic liver abscess (ALA) and health U.S.A. controls. We compared these results to serum anti-AP IgG antibody responses, as measured by ELISA. Anti-AP sIgA was present in the saliva of 5/5 patients with ALA (mean OD=0.447±0.244), in 0/5 patients with non-amebic illness (mean OD=0.049±0/038, p=0.007), and in 0/7 health U.S.A. controls (mean OD=0.036±0.023, p=0.001; criteria for positivity OD>0.100 in samples balanced for 10 μg/ml total IgA).

There was no significant difference in ELISA ODs between the S. Afr. and U.S.A. controls (p=0.454). The level of serum anti-AP IgG antibodies was concordant with salivary anti-AP sIgA in patients with ALA (5/5 positive), serum antibodies were absent in matched hospitalized S. Afr. patients with other disorders (0/5 positive)(mean OD=0.493±0.279 vs 0.049±0.035, p=.017; criteria for positivity OD>0.100 at 1:1000 dilution). To date, we studied one S. Afr. subject having intestinal infection with Eh. The patient had a clinical diagnosis of schistosomiasis, no serum anti-AP antibodies (OD>0.001) but had a positive salivary anti-AP ELISA (*OD=0.220*). As previously reported, the zymodeme of that Eh culture is unknown; however, the negative serum anti-AP ELISA indicates infection by a nonpathogenic strain with anti-AP sIgA being produced at mucosal surfaces. In summary, assay of salivary anti-AP IgA may have a role in diagnosis of amebiasis and the study of mucosal immunity to Eh.

254 KILLING OF *ENTAMOEBA HISTOLYTICA* TROPHOZOITES BY MACROPHAGES IS MEDIATED BY NITRIC OXIDE FROM L-ARGININE. Lin JY* and Chadee K. Institute of Parasitology of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

The killing of *Entamoeba histolytica* (Eh) trophozoites by phagocytes involves oxidative and nonoxidative mediators. In this study, we determine whether L-arginine-derived nitric oxide is involved in the killing of Eh trophozoites by activated murine macrophages *in vitro*. Elicited peritoneal or bone marrow-derived macrophages activated with rIFN-γ alone or with LPS induced 50-60% amoebicidal activity, concomitant with increased levels of $NO_2^-$. L-$N^G$-monomethylarginine (L-NMMA), a specific inhibitor of the L-arginine:nitric oxide(NO) pathway, inhibited amoebicidal activity and $NO_2^-$ release in adose-dependent fashion, but without affecting $H_2O_2$ production. Addition of excess L-arginine reversed the inhibitory effect of L-NMMA. The addition of catalase, a scavenger of $H_2O_2$, also inhibited $NO_2^-$ release and amoebicidal activity. Excess iron ($FeSO_4$) in the medium prevented macrophage cytotoxicity without affecting $NO_2^-$ production, *indicating that metabolic inhibition by iron-nitrosyl complexes may play a* role in macrophage-mediated effects. These results demonstrate that amoebicidal activity of activated macrophages is dependent on L-arginine-derived nitrogen oxidation products and is independent from $H_2O_2$ release.

255 *ENTAMOEBA HISTOLYTICA* ELICITS SECRETION OF NEUTRAL AND ACIDIC MUCINS IN RAT COLON AND COLONIC ADENOCARCINOMA CELLS *IN VITRO*. Tse S*, Keller K, and Chadee K. Institute of Parasitology of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

*Entamoeba histolytica* (Eh) invasion is preceded by luminal and colonic goblet cell mucin depletion. In rat colon, pathogenic Eh was shown to exhibit mucin and nonmucin secretagogue activity. In this study we determine the secretion of neutral and acidic mucins in response to Eh(HM1-IMSS) in rat colonic loops and *in vitro*, in the mucus-producing colonic adenocarcinoma cell LS174. $^3$H-glucosamine- labelled glycoproteins were obtained from loops or LS174 cells incubated with Eh trophozoites or known mucus secretagogues cholera toxin (loop studies) and $Ca^{2+}$ ionophore (LS174 cells). High $M_r$ (>$10^6$) $^3$H-labelled mucins from Sepharose 4B column chromatography ($V_o$ fractions) was loaded onto ECTEOLA ion-exchange columns and eluted with increasing concentrations of NaCl in 4 mM phosphate buffer (pH 6.4). A minor neutral mucin specie was eluted with 0.05 M NaCl, while the majority of the acidic mucins (<98 %) was eluted with 0.5 M and 2.0 M NaCl, respectively. Histochemical mucin stains (alcian blue and PAS) of 1 μm sections of rat colon exposed to Eh showed equal secretion of neutral and acidic mucins from goblet cells in the interglandular epithelium and crypts. Unlike other infectious agents or disease conditions that affect different mucin species, our results demonstrate a generalized secretion of mucins in response to Eh that may facilitate Eh invasion.

256 RIBOSOMAL PROTEINS OF *GIARDIA LAMBLIA*: ISOLATION AND NOMENCLATURE. Montanez C*, Depardon F, Sinker S, and Ortega-Pierres MG. Department of Genetics and Molecular Biology, Center for Research and Advanced Studies of IPN, Mexico, DF, Mexico.

The characteristics of the components involved in the translation mechanisms of *Giardia lamblia* are poorly understood. An initial step in the study of these mechanisms is the identification and characterization of ribosomal components. We have characterized the ribosomal subunits of this parasite. Polysomes of G. *lamblia* were isolated from trophozoites. Subunits derived from these polysomes were fractionated by treatment with $10^{-5}$ M $MgCl_2$ and ultracentrifugation in sucrose gradients. Ribosomal proteins from each subunit were obtained by extraction with acetic acid-$MgCl_2$. Ribosomal RNAs were recovered by phenol-chloroform extraction in the presence of LiCl. Approximately 78 proteins were identified by two-dimensional gels pH 5-SDS. Of these, 36 were associated with the small ribosomal subunit (SS) and 42 with the large subunit (LS) and had molecular weights of 11 to 60 kDa. Based on this, a proposal is presented for the nomenclature of ribosomal proteins in *G. lamblia*. The localization of ribosomal RNAs was analyzed by denaturing gels following deaggregation of purified subunits. The 15S and 21S ribosomal RNAs were associated with the SS and LS respectively as occurs with other organisms. Association of small ribosomal RNAs with the two subunits is currently in progress. This study helps to elucidate structure and function of ribosomes in this parasite.

## POSTER II: SCHISTOSOMA IMMUNOLOGY

257 HISTOPATHOLOGIC STUDY OF SCHISTOSOMAL PERIOVULAR GRANULOMAS IN CHALLENGED MICE PREVIOUSLY EXPOSED TO EITHER MODERATELY OR HIGHLY IRRADIATED CERCARIAE. Ramos EA*, Reynolds SR, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, FIOCRUZ, Bahia, Brazil.

The formation of periovular granulomas was studied in the *Schistosoma mansoni* irradiated cercariae vaccine model. C57Bl/6J mice were multiply exposed to either 15 Krad (moderate dose) or 50 Krad (highdose) irradiated cercariae and challenged with normal cercariae. The granulomas were examined 7 weeks after challenge. Previously, we had found greater protection in the moderate dose exposed mice which correlated with a differential lymphokine production profile. Here we report that granulomas were not only fewer in number but had significantly smaller area in square microns in the moderate dose irradiated cercariae exposed mice than either the high dose exposed or infected control groups. An *in vitro* granuloma assay of the spleen cells showed that mice multiply exposed to moderate dose cercariae also had a significantly smaller granuloma index than the other groups. Although upon histopathologic exam, there were no striking differences in granuloma cell composition between the two groups, fibrosis was discrete, starting at the granuloma borders in the high dose group whereas it was nearly absent in the mice exposed to moderately irradiated cercariae. We concluded that multiple exposure to moderately irradiated cercariae led to protection from granuloma formation.

258 LARVICIDAL PROPERTIES OF MACROPHAGES INDUCED BY CLONED MURINE SCHISTOSOMAL EGG ANTIGEN-SPECIFIC CD4 POSITIVE T HELPER LYMPHOCYTES. Kanazawa T* and Stadecker MJ. Tufts University School of Medicine, Boston, MA.

The role of T helper (TH) lymphocytes in activating peritoneal macrophages (PM) to kill larvae of the helminth *Schistosoma mansoni* (schistosomula), was investigated with the use of egg antigen-specific CD4 positive TH clones of both the TH-1 (IL-2, IFN-$\gamma$ secreting) and TH-2 (IL-4, IL-5 secreting) types. Results showed that stimulated TH-1 clones, in exceedingly small numbers, or supernatants thereof, conferred on PM the ability to kill schistosomula. The molecule responsible for this activity was found to be IFN-$\gamma$.

IFN-γ-induced PM larvicidal activity was dependent on live cells, energy, as well as protein synthesis, and appeared to be mediated by toxic nitrogen metabolites. In contrast, egg antigen-specific TH-2 clones, or their supernatants, failed to induce PM larval killing, as they did not secrete IFN-γ, or any other equivalent macrophage activating factor. Immune effector mechanisms accounting for the resistance to schistosomal reinfection are currently thought to depend on the helminthotoxicity of activated macrophages. Inasmuch as macrophage activation appears to be primarily induced by IFN-γ, the existence of specific TH-1 clones is of critical importance for the maintenance of the state of concomitant immunity.

259 IMMOBILIZATION OF *SCHISTOSOMA MANSONI* MIRACIDIA BY ACTIVATION OF THE ALTERNATE PATHWAY OF COMPLEMENT AT EXTREMELY HIGH DILUTIONS OF NORMAL SERA. Knopf PM* and McLaren DJ. Division of Biology and Medicine, Brown University, Providence, RI; and National Institute for Medical Research, Mill Hill, London, UK.

Free-swimming miracidia were immobilized by the addition of normal mammalian serum to the water. This miracidial immobilizing activity (MIA) was demonstrated to be the result of activating the alternate pathway of complement (APC). MIA was heat sensitive; neutralized by EDTA but not EGTA; and greatly reduced in C6-deficient serum. Half-maximal MIA in normal serum was detected at final dilutions exceeding 1/200 and normal rat serum was particularly potent, with MIA at dilutions exceeding 1/2000. The detection of APC activity at such high dilutions is quite extraordinary and was attributed to the hypotonic conditions of the reaction. In addition to demonstrating APC-dependent MIA, we also confirmed and extended previous findings that heat-inactivated infection sera also displayed MIA, sera from chronically infected animals having higher titers. MIA in vaccine rat serum co-fractionated with rat IgG and anti-SWAP antibody activity. Antibody-dependent MIA titers were much lower than for APC-dependent MIA. Based upon light and electron microscope studies, immobilization of miracidia by APC activation does not appear to be equivalent to miracidial transformation into sporocysts, but is the result of tregumental damage. Moreover, miracidia within eggshells are insensitive to MIA.

260 THE ROLE OF MACROPHAGES IN MURINE *SCHISTOSOMA JAPONICUM* INFECTION. Laxer MJ* and Tuazon CU. Division of Infectious Diseases, Department of Medicine, George Washington University, Washington, DC.

Macrophages, eosinophils, and platelets have been implicated as effector cells of the immune response in the *Schistosoma mansoni* mouse model. Immunization studies using various antigens demonstrated the major role of macrophages as a protective mechanism against challenge infection. The role of macrophages in immunity to *S. japonicum* infection is less well studied. *In vitro* cytotoxicity assays against L929 cells and mechanically transformed schistosomules were utilized to study the role of macrophages in *S. japonicum* infection in the mouse model. Peritoneal macrophages from control and infected C57Bl/6 mice harvested at weeks 1,4,6,8 and 12 after infection were used in this experiment. $4 \times 10^4$ $^3H$ labeled L929 cells were added to $8 \times 10^5$ macrophages. Percent cytotoxicity was determined after 48 hours incubation. Schistosomulacidal activity was determined by incubating macrophages with transformed somules at effector:target ratio of $10^4$:1. Larvicidal activity was expressed as mean % of nonviable larvae after 48 hours. A progressive increase of tumoricidal and larvicidal activity was observed with peaks at 6 weeks (19%) and 8 weeks (47%) respectively. Thereafter, macrophage cytotoxicity diminished until 12 weeks after infection. Results of this experiment will be correlated with IFN-γ and TNF-α concentrations from supernates of soluble adult worm stimulated spleen cells.

261 FURTHER CHARACTERIZATION OF THE MAJOR LYMPHO-STIMULATORY ACIDIC COMPONENTS OF SCHISTOSOME EGGS RECOGNIZED BY SPECIFIC MURINE TH-1 TYPE

CLONES. Chikunguwo SM*, Quinn JJ, Ham DA, and Stadecker MJ. Tufts University School of Medicine, Boston, MA; and Harvard School of Public Health, Boston, MA

Granulomatous inflammation in schistosomiasis mansoni is a result of CD4 positive T helper lymphocyte (TH) sensitization to soluble schistosomal egg antigens (SEA). In previous studies we have shown that responses of polyclonal murine lymphocytes, as well as of cloned specific TH, are predominantly directed against acidic molecules within SEA. In this study, four SEA-specific, CD4 positive, IL-2-secreting, TH type 1 (TH-1) clones were subjected to stimulation by components of SI A, fractionated, on the basis of charge, with the technique of rotofor isoelectric focusing. SEA were focused on a pH gradient of 3-10 and subsequently eluted from 20 fractions, which were normalized for protein content. All clones responded to acidic molecules present in two closely juxtaposed sets of fractions between pH 4 and 6. However, in the case of one clone, which by Southern blot analysis was shown to bear a distinct TCR β chain rearrangement, stimulatory components extended into the neutral range, suggesting recognition of additional antigenic material. When SEA components with pH 4-6 were pooled and re-fractionated, all four clones responded to at least four discernible constituents in this range. It is not known whether they recognized an epitope(s) present on different molecules, or on split products of the same molecule. Two of theseclones also exhibited strong responses to hatching fluid. This finding is of considerable biological significance and suggests that the determinant (s) that they recognize in SEA are also present in the hatching fluid. Additional immunological and chromatographic techniques are currently used in pursuit of the identification and isolation of the major lympho-stimulatory egg components.

262 CROSS-REACTIVE IDIOTYPES ON RABBIT ANTI-SEA ANTIBODIES STIMULATE ANTI-IDIOTYPIC LYMPHOCYTE RESPONSES OF MICE INFECTED WITH *SCHISTOSOMA MANSONI*. Amano T*, Nakazawa M, Oshima T, Bosshardt S, and Colley D. Department of Parasitology, Yokohama City University School of Medicine, Yokohama, Japan; Veterans Administration Medical Center, Nashville, TN; and Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN.

Some antibodies (Abs) to soluble egg antigens (SEA) of *Schistosoma mansoni* have been shown previously to express idiotypes (Ids) that stimulate proliferation of regulatory anti-Id T lymphocytes from infected patients or mice. We now find cross-reactive Ids (CRI) on rabbit anti-SEA Abs. Anti-SEA Ab preparations were immunoaffinity-purified from sera from 5 rabbits immunized (3 in Japan/2 in USA) with *S. mansoni* SEA. They were mainly immunoglobulin (Ig) on SDS-PAGE, with some heterogeneity of anti-SEA Abs by Western blots. In 3 day lymphocyte cultures anti-SEA Abs had no proliferative effect on cells of uninfected mice or mice infected for 6 weeks. With spleen cells from mice infected for 8 weeks, 4 of 5 anti-SEA preparations stimulated moderate-to-strong proliferation (mean E-C values = 21,340 cpm). Responses by cells from mice infected for 10 weeks or >16 weeks were somewhat lower. Normal rabbit Ig did not stimulate cells from infected or uninfected mice. Infected mice have lymphocytes that react with rabbit (as well as mouse and human) CRI on some anti-SEA Abs. The presence of CRI systems adds to the hypothesis that Id/anti-Id interactions contribute to immunoregulation in schistosomiasis.

263 CLONAL HAEMATOPOIETIC RESPONSE IN MURINE SCHISTOSOMAL INFECTION Lenzi HL*, Lenzi JA, and Mota EM. Departmento Pathologia, IOC-Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

The striking association between eosinophilia and schistosomiasis is well known. We studied the overall haematopoietic response in the bone marrow and other tissues during murine schistosomiasis from 15 to 160 days after infection. The percentage of medullary eosinophils varied from 4.0 to 80% day 70 p.i. eosinophils in bone marrow and blood were synchronized. The following observation were made: neutrophils predominate in the bone marrow except on day 70 p.i. when they were surpassed by

eosinophils; the lowest levels of neutrophils coincided with the highest levels of immature eosinophils (from 50 to 90 days after infection); lymphocytic lineage was more or less constant and increased only from day 90 to 160 p.i.; plasma cells only appeared 90 days p.i.; erythroid and megakaryocytic lineages showed little variation; eosinopoiesis in the spleen of infected animals was significantly higher than in controls; hepatic and lymph node eosinophilic metaplasia began on day 40; and hepatic neutrophilic metaplasia began on day 50. These results suggest that murine *S. mansoni* infection has three distinct haematopoietic phases: a) non- or low-productive phase (before day 35-40 p.i.); b) acute productive phase (from day 35-40 to day 70-90 p.i.); and c) chronic productive phase (after day 70-90 p.i.). The acute productive phase is characterized by an increase in eosinophils and monocyte/macrophages and a decrease in neutrophils. The C precursor cell (myeloid committed cell) appears to be the principal target of CSFs.

264 OMENTUM MILKY SPOTS BEHAVE AS LYMPHOHEMATOPOIETIC ORGANS IN MURINE SCHISTOSOMIASIS CAUSED BY *SCHISTOSOMA MANSONI*. Lenzi JA*, Borojevic R, Oliveira DN, and Lenzi HL. Departmento Pathologia, IOC-Fiocruz, Rio de Janeiro, Brazil; and Departmento Bioquimica, Instituto Bioquimica, UFRJ, Rio de Janeiro, Brazil.

Adult schistosomes live in the mesenteric venous system, where they secrete highly antigenic and toxic materials. We studied the kinetics of the cellular response and the role of milky spots in the peritoneum of mice infected percutaneously with 70 cercariae of *S. mansoni*. The number of eosinophils increased significantly in a cyclical pattern from 0.08 to 16% of the cellular infiltrate. Mast cell also exhibited a cyclic pattern with an inverse or direct relationship to that of eosinphils depending on the duration of infection. There was an inverse relationship between the decrease of endothelial cells and the increase in lymphoblastoid mononuclear cells. The number of resident macrophages remained essentially unchanged. Mature lymphocytes and neutrophils also exhibited a cyclic pattern, the former peaking on day 60 and the latter on day 50 of infection. No plasma cells were detected. Milky spots were very active during infection. From day 45 p.i., they exhibited a "bone marrow-like" transformation characterized by intense eosinophilc metaplasia, lymphocytosis with immunoblastoid and plasma cells, Russel bodies, monocytes/macrophages, neutrophils and mesothelial cells. Thus, the increase in the number of peritoneal cells can be explained in part by the intense reactivity of the milky spots, with local production and release of cells into the peritoneal fluid.

265 *IN VIVO* TREATMENT WITH ANTI-IL-2 ANTIBODIES INHIBITS GRANULOMA FORMATION, FIBROSIS AND IL-5 PRODUCTION IN MURINE SCHISTOSOMIASIS MANSONI. Hieny S*, Cheever AW, Caspar P, Macedonia J, Finkelman F, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, Bethesda, MD; and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.

Mice (C3H/HeN strain) infected with *S. mansoni* were injected with mAbs against IL-2 and/or IL-2 receptor (IL-2R) on the 6th and 7th wk of infection and sacrificed on the 8th wk. This treatment resulted in a 17 to 31% reduction in liver egg granuloma volumes and a more striking 51 to 63% inhibition in fibrosis (tissue hydroxyproline) when compared to animals treated with an irrelevant control mAb. While treatment with anti-IL-2R alone failed to induce a significant reduction in granuloma size or fibrosis, combined treatment with anti-IL-2R plus anti-IL-2 appeared to cause a greater inhibition than anti-IL-2 mAb alone. Spleen cells from the anti-IL-2 treated animals were stimulated with schistosome egg antigen (SEA) or concanavalin-A (Con-A) and the supernatants assayed for IL-2, IL-4, IL-5 and IFN-$\gamma$. These mice produced significantly less IL-5 in response to SEA and Con-A but higher levels of IL-4 than did control animals. The latter difference was reflected in significant decreases in peripheral blood and tissue (granuloma) eosinophils and increases in total serum IgE in the anti-IL-2 treated mice. In contrast, no significant changes were evident in the low level IL-2 and IFN-$\gamma$ responses typically seen

during patent infection. These results confirm the involvement of IL-2 in the granulomatous response and, in addition, suggest that IL-2 manipulation could be used to alleviate fibrosis induced pathology. Nevertheless, it is not clear whether IL-2 plays a direct role in granuloma formation or is indirectly required for the growth and differentiation of effector CD4+ T cells producing other cytokines such as IL-5.

266 ROLE OF PARAMYOSIN IN THE HOST-PARASITE RELATIONSHIP. Laclette JP*, Nicholson-Weller A, Richter D, Pante N, Cohen C, Bing D, and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.; Department of Immunology, Instituto de Investigaciones Biomedicas, UNAM, Mexico D.F., Mexico; Infectious Diseases Division, Beth Israel Hospital, Boston, MA; Center for Blood Research, Boston, MA; and Rosensteil Center, Waltham, MA.

Antigen B of the cestode parasite *Taenia solium* is a collagen-binding protein that interferes, *in vitro*, with the classical pathway function of the complement system. Recent data, including sequencing of full-length cDNA clones, indicate that antigen B is the paramyosin of *T. solium*. We now report the results of studies showing that collagen-binding and inhibition of complement are properties of several invertebrate paramyosins. Paramyosins from *T. solium*, *Schistosoma mansoni*, and the mussel *Mytilus edulis* can be isolated from crude extracts by collagen affinity. These paramyosins inhibit C1 function whether the C1 is isolated or contained in C2-deficient serum. Since *T. solium* paramyosin (TPmy) was the best inhibitor, we concentrated further studies on this molecule. TPmy binds purified C1q in solution with a kinetics similar to C1r/C1s. Further studies of the C1-antigen B interaction indicate that: 1) C4 is not activated, 2) C42 decay is not affected; and 3) there is no effect on the efficiency of C3-9 in lysing sheep erythrocytes. Thus, TPmy inhibition is directed at the initiation of the classical pathway. The results suggest that paramyosins of helminth parasites may have a role in modulation of the host immune response through complement inhibition at C1.

267 NITROGEN OXIDES: DIFFERENTIAL EFFECTS ON *SCHISTOSOMA MANSONI* AND DOWN-REGULATION OF MURINE MACROPHAGES. Smith JM* and Prichard RK. Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

Hydrogen peroxide, tumor necrosis factor-α (TNF-α) and nitrogen oxides are products released when activated mouse macrophages (Mϕ) are stimulated *In vitro* and are cytotoxic to schistosomula of *Schistosoma mansoni*. Adult schistosomes are normally refractory to attack by immune mechanisms, but when pretreated with the drug oltipraz (OPZ) are susceptible to schistosomulacidal concentrations of $H_2O_2$. $H_2O_2$ and TNF-α productions are down-regulated in Mϕ from mice with patent *S. mansoni* infections. The potency of $NO_2$ for killing adult schistosomes, in the absence and presence of oltipraz, and levels of $NO_2$ produced by stimulated Mϕ from infected mice was investigated. High levels of toxicity were observed when schistosomula were exposed to $NO_2$, but no adult mortality was obtained at concentrations up to 120 μM $NO_2$. OPZ decreased the viability of adult worms, but this effect was not enhanced in the presence of $NO_2$. The ability of OPZ-treated worms to metabolize $NO_2$ *in vitro* was significantly impaired compared to untreated worms. Mϕ from infected mice produced lower levels of $NO_2$ compared with those from BCG elicited mice, with a corresponding decrease in schistosomula killing. *S. mansoni* murine infections result in the down-regulation of Mϕ cytotoxicity, the underlying mechanisms of which are being investigated.

268 PROGRESS AND REFINEMENT OF A MURINE MODEL FOR KATAYAMA FEVER. Weina PJ*. Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Katayama Fever Syndrome (Acute Toxemic Schistosomiasis) is a debilitating early manifestation of infection with one of the three human species of schistosomes. Until recently there has been no useable small animal model to study this syndrome. We report the progress and expansion of a biotelemetric murine model that enables us to study the syndrome and eventually evaluate new lead therapeutic interventions. Early success with the model led us to the discovery of the unique fever response exhibited by mice and the behavioral response (activity changes) taken by mice to compensate for increasing core temperature. Recent monitoring of the elevation of leukocytes and eosinophilia of up to 25% have shown the concurrent nature of these index increases with the fever event. Application of a severity index that takes into account our radiotelemetry data (fever and activity) along with changes in leukocytosis, eosinophilia, and weight have allowed us to refine our model and grade the level of response in infected animals.

269 *SCHISTOSOMA MANSONI*: ANTIGENS RELEVANT TO DIAGNOSIS OF PREPATENT INFECTION. Mikhail MM*, Mansour MM, Farid Z, and Harrison RA. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Prepatent or acute schistosomiasis is difficult to differentiate clinically from febrile infections and diagnosis depends on recovery of eggs from feces or urine. This study was designed to identify antigens which could diagnose prepatent schistosomiasis. Pooled sera of inbred and individual sera of outbred mice were collected at sequential times after infection with *S. mansoni* cercariae. Sera were immunoblotted with worm antigens of an Egyptian strain of *S. mansoni* and IgG and IgM reactivities determined. A 31/32 kD doublet reacted with IgG from 6 of 12 outbred mice after 8 days of infection and with all inbred mice after 3 weeks. A 34/35 kD doublet after 3 weeks' infection. Sera of 25 Egyptians with acute *S. mansoni* and 2 with *S. haematobium* were evaluated and showed that IgG and IgM antibodies of all patients recognized the 31/32 doublet while IgG antibodies of 22 patients recognized the 34/35 and 70kD antigens. The potential of these *S. mansoni* antigens in diagnosis of prepatent and acute schistosomiasis is discussed as is cross reactivity with other parasites.

270 MOLECULAR BASIS OF GRANULOMA FORMATION IN SCHISTOSOMIASIS. Phillips SM*, Perrin PJ, Gaafar T, and Wahba SN. Allergy and Immunology Section, University of Pennsylvania, Philadelphia, PA; Navy Medical Research Institute, Bethesda, MD; University of Cairo, Cairo, Egypt; and Dr. Osman Hospital, Maadi, Cairo, Egypt.

We have studied the regulation of the granulomatous reaction and its dependency upon a cascade of interacting cells and the soluble suppressor substances which they induce. TseF, a direct suppressor of granuloma formation, is produced by regulatory Lyt-2+ T-suppressor cells and shares the basic molecular structure of the T cell receptor. The alpha chain is responsible for antigenic specificity. The beta chain bears V-beta determinants and imparts both genetic specificity and functional characteristics. The two chains are disulfide linked and weigh a total of 72Kd. The factor induces a Ti dependent transmembrane signal and leads to decreased IL-2 receptor expression, IL-2 production, and interferon-gamma production in target L3T4+ T cells. TseF has no apparent effects on IL-4 and IL-5 production. As a consequence of interaction with TseF, increases in glutathione and ornithine decarboxylase (indicators of intercellular differentiation) are noted in L3T4+ TH 1 target cells. Conversely, antigen mediated blast transformation is decreased. Consonant changes are also found in cells which are exposed to TseF *in vivo* as a consequence of chronic infection. The effects of TseF are mediated by direct membrane interactions in the absence of accessory cells and are potentiated by IL-2. Exogenous IL-2 can prevent TseF production but does not inhibit its action. Effects are independent of antigen recognition per se. The induced differentiation is associated with increased Protein kinase- C dependent phosphorylation. Thus

TseF reduces granulomatous hypersensitivity through an alternation of terminal differentiation of effector T-cells without effecting their initial response to antigen.

271 COMPARISON OF SEA FRACTIONS-ELICITED LYMPHOKINE PRODUCTION BY SPLENIC AND GRANULOMA LYMPHOCYTES OF *SCHISTOSOMA MANSONI*-INFECTED MICE. Lukacs NW and Boros DL*. Department of Immunology/Microbiology, Wayne State University School of Medicine, Detroit, MI.

In schistosomiasis mansoni circumoval granulomatous inflammation is regulated by soluble egg antigen (SEA)-induced cytokine responses. The present study examines intragranulomatous vs splenic IL-2 and IL-4 production in acutely and chronically infected mice. Crude SEA separated by SDS-PAGE yielded 9 fractions, <21, 25-30, 32-38, 40-46, 50-56, 60-66,70-90, 93-125, and >200 kD. Acute infection spleen cells produced IL-2 (>50 pM) to 6 of 9 fractions (25-30, 32-38, 40-46, 50-56, 60-66, 93-125, and >200) and significant IL-4 (20-50 pM) to the same 6 fractions. In contrast, with acute infection granuloma T cells only 2 fractions (50-56 and 93-125) elicited IL-2 (>50 pM) but a similar set of 6 elicited higher IL-4 (50-100 pM). The chronic infection spleen cells produced substantial IL-2 (20-40 pM) to 4 fractions (<21, 25-30, 32-38, and 93-125) but produced minimal amounts of IL-4 (<10 pM) to all 9 fractions. Chronic infection granuloma T cells produced significant IL-2 (20-40 pM) to 3 fractions (60-66, 70-90, and >200) and significant IL-4 (30-60 pM) to 3 different fractions (40-46, 50-56, and 93-125). The results demonstrate higher IL-4 production in acute, chronic granuloma T cells, whereas splenic T cells demonstrate higher IL-2 production. Identification of SEA fractions that elicit lymphokines will provide information on their role in granuloma formation.

## L: LYME DISEASE

272 NATIONAL LYME DISEASE UPDATE. Dennis DT*, Paul WS, Campbell GL, and Craven RB. Centers for Disease Control, Division of Vector-Borne Infectious Diseases, Fort Collins, CO.

Lyme disease is the leading vector-borne infectious disease in the United States. Nearly 8,000 cases were reported by 46 states to the Centers for Disease Control in 1990; this represents a 16-fold increase in annual reported cases since 1982. Approximately 80% of cases continue to be reported from just 8 states in the northeastern, north-central, and Pacific coastal areas. Regional estimates of disease incidence are positively correlated with the geographic distribution of ticks in the *Ixodes ricinus* complex and their rates of infection with *Borrelia burdorferi*. The geographic range of *Borrelia burdorferi* and its principal vector, *Ixodes dammini*, appears to be expanding, as does the range of Lyme disease endemicity. Most human infections appear to be related to exposure in residential settings, but recreational and occupational exposures are also important. Surveillance of Lyme disease is hampered by the absence of reliable and accurate laboratory tests and by ill-defined clinical case criteria. A national study of commercial Lyme Disease serologic test kits revealed unacceptable levels of chance-adjusted agreement between test methods and between laboratories performing the tests. A new Lyme disease surveillance case definition was adopted in 1990 for uniform national case reporting by state health departments to the Centers for Disease control. The impact of the new case definition on surveillance statistics is being evaluated.

273 SPATIAL VARIATION IN THE PROPORTION OF TICKS INFECTED WITH THE LYME DISEASE SPIROCHETE IN NORTHERN CALIFORNIA. Kimsey RB*. Department of Entomology, University of California, Davis, CA.

The Lyme disease spirochete is reported to infrequently infect *Ixodes* tick vectors in Northern California, particularly when compared to infection rates in New England tick populations. However, in three diverse regions of Northern California where *Ixodes pacificus* ticks occur, local distribution of infectious ticks appears focal and coincidentally, independent of the numbers of ticks. Although deer uniformly

inhabit these locations, potential reservoir rodents do not. As numbers of spirochete infected ticks relate directly to rodent populations, deer may thus maintain tick vector populations over relatively broad regions lacking rodent reservoirs of the Lyme disease spirochete.

274 CRITICAL ABUNDANCE OF HOSTS PERPETUATING THE TICK THAT TRANSMITS THE AGENT OF LYME DISEASE. Awerbuch T*, Sandberg S, and Spielman A. Department of Biostatistics, Harvard School of Public Health, Boston, MA; Department of Mathematics, Framingham State College, Framingham, MA; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Although the deer ticks, *Ixodes dammini*, that transmit the agent of Lyme disease, seem to perpetuate solely where deer are abundant, their subadult stages feed abundantly on mice, the main reservoir of infection. These hosts appear to contribute to the development of the tick. To explore this possibility, we devised a conceptual basis for estimating the importance of these rodents in determining the abundance of these ticks. Using a spread sheet, we constructed, a computer model that links the ecological components involved in the life cycle of the tick taking into account particular seasonal interactions with its various hosts. Patterns of tick population growth were explored as a function of host density. Data representing the ecological parameters of an island location in coastal Massachusetts were used to implement the model. We conclude that the density of mice critically affects the tick population. Such tick populations may fail to perpetuate unless the abundance of mice rises above a certain threshhold.

275 MODEL SYSTEM FOR TESTING THE INFECTIVITY OF *BORRELIA BURGDORFERI* TO TICKS. Piesman J*. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Ft. Collins, CO.

Studies measuring the vector competence of ticks to acquire and transmit *Borrelia burgdorferi* have used various animal hosts, routes of inoculation, and sources of spirochetes. In an attempt to standardize vector competence studies, we infected laboratory hamsters and mice with *B. burgdorferi* from low passage cultures in BSK media, tick homogenates, and by tick feeding. Needle-injected spirochetes were introduced by the i.p. or i.d. route. Animals exposed to the JD1 strain of spirochete served as hosts for xenodiagnostic *Ixodes dammini* larvae at 1 month post infection. Animals infected by tick feeding or injection with tick homogenates were highly infectious to ticks, infecting >70% of xenodiagnostic ticks. Animals inoculated i.d. were more infectious to ticks than were animals inoculated i.p., but this difference was not significant. Hamsters inoculated with low passage cultured spirochetes infected <30% of xenodiagnostic ticks, but mice similarly inoculated with cultured spirochetes infected >70% of xenodiagnostic ticks. We have used this model system to establish the type strain (B31) of *B. burgdorferi* in ticks and an isolate from Wisconsin. A standardized system for infecting ticks with *B. burgdorferi* will prove useful in conducting vector competence studies with various tick species.

276 NEST-ASSOCIATED FEEDING OF RUBIDIUM-MARKED DEER TICKS (*IXODES DAMMINI*). Ratanatham S, Kimsey RB, Hamlin D*, Pollack RJ, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Entomology, University of California, Davis, CA.

The striking spatial aggregation of deer ticks (*Ixodes dammini*), infected by the Lyme disease spirochete (*B. burgdorferi*), suggests that questing ticks might concentrate in the rodent burrow in which the previous developmental stage had detached. To explore this hypothesis, we adapted a rubidium-marking technique to identify ticks that had fed on particular rodent hosts. In laboratory studies, ticks were placed on animals injected with RbCl. Rb was detectable for 9 days after injection by AA spectrophotometry of blood from each animal as well as the ticks that fed upon them. Rb was present in

developmental stage and the eggs of Rb-fed females. Some was lost in exuvia and feces. In field sites, mice (*Peromyscus leucopus*) were live-trapped, injected with Rb and released. During the following 2 years, nymphal ticks were removed from wild mice, and adults were swept from vegetation. Rb was detected in about half of the nymphs subsequently found on mice. Certain mice consistently harbored Rb-marked nymphs, particularly when retrapped at specific sites. Other mice trapped nearby consistently harbored unmarked nymphs. This suggests that the burrows of mice may be the main venue of transmission of the agent of Lyme disease.

277 CLEARANCE OF *BORRELIA BURGDORFERI* FROM GUTS OF TICKS ENGORGING UPON RECOMBINANT OSP-A VACCINATED MICE. Telford SR*, Fikrig E, Barthold SW, Flavell RA, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Section of Immunobiology, Yale University School of Medicine, New Haven, CT; and Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT.

Vaccination with recombinant *B. burgdorferi* OSP-A/glutathione transferase fusion protein elicits protective antibody titers in the C3H/He mouse model. Interestingly, antibody and complement appear to concentrate within the gut of ticks during the course of their bloodmeal. Spirochetes may be particularly vulnerable to destruction within the gut of infected ticks if vaccinated animals serve as hosts. Accordingly, we allowed nymphal *Ixodes dammini*, infected by the N40 strain of *B. burgdorferi*, to engorge upon vaccinated mice, and determined whether spirochetes persisted within these ticks at varying times after repletion. Identical groups of ticks were fed upon control mice vaccinated with glutathione transferase. Of nymphs engorging upon immune mice, examined 5 days after their repletion, 14% were sparsely infected according to a direct fluorescent antibody procedure. In contrast, 80% of nymphs fed upon control mice were infected ($p<0.001$). Similar results were observed for ticks examined 14 days post-repletion (0% vs 73%, $p<0.001$). Thus, spirochetes appear to be eliminated in ticks feeding upon OSP-A vaccinated mice. Because oral immunization may also produce a protective antibody response in mouse reservoirs, the distribution of bait containing the vaccine recombinant may be a promising method to reduce the abundance of infected ticks in nature.

278 PROTECTION OF MICE AGAINST LYME DISEASE INFECTION BY EAR PUNCH BIOPSY. Shih CM*, Pollack RJ, Telford SR III, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The mechanism of initial dissemination of the Lyme disease spirochete in animal hosts is poorly understood. Experiments were designed to determine whether mice can be protected from infection by excising the site where infected vector ticks had attached and fed. Nymphal *Ixodes dammini* ticks, infected with the JD1 strain of *Borrelia burgdorferi*, were placed on the ear of CD1 mice and allowed to feed to repletion. The sites of infecting bites were excised by punch-biopsy at several time intervals before and after tick drop-off. Xenodiagnosis results indicate that none of the 34 mice exposed to infected nymphal ticks became infected when the site of the infecting bite was removed within 3 days after tick drop-off. Only 20% of the mice in which the biting site was excised on days 4 or 7 became infected. In contrast, all mice in the non-excision group became infected. Thus, excision of the site of inoculation appears to abort systemic infection. This suggests that the spirochete may reside in the skin near the feeding site for a surprisingly long period of time following detachment of the infecting tick.

279 EFFECT OF ORAL OR SUBCUTANEOUS ADMINISTRATION OF IVERMECTIN IN HAMSTERS ON SUBADULT DEER TICK *IXODES DAMMINI* FEEDING AND SURVIVAL. Korch, GW, Jr.* and Miller JA. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD; and U.S. Livestock Insects Research Laboratory, U.S. Department of Agriculture, Kerrville, TX.

Ivermectin is an anthelminthic drug used in veterinary practice that has been shown to control a wide variety of agricultural and veterinary arthropod pests. We evaluated this compound for its effect on subadult deer ticks, *Ixodes dammini*. Female hamsters were given ivermectin either in a daily oral dose of a peanut butter and oatmeal bait formulated to deliver 200 mcg/kg of ivermectin per 1.0 g of bait, or by a single subcutaneous dose (1.0 mg/kg, Ivomec). Oral dose groups received either 200 μg/kg or 400 μg/kg of ivermectin. Seventy-five larvae were infested on each of 5 hamsters per treatment, and 20 nymphs were seeded on each of two hamsters per treatment. Recovery of molted larvae ranged from 18% (60/340 larvae) for the 400 mcg oral treatment group to 60% (167/278) for untreated hamsters. Recovery rates of engorged nymphs ranged from 5% (2/39) for the subcutaneous treatment group to 100% (40/40) for untreated controls. The mean weight of both fed larvae and nymphs from ivermectin-treated animals was significantly lower than that from untreated animals. Subadult ticks also took longer to feed on ivermectin-treated animals. These results suggest that ivermectin-treated baits may be effective in controlling deer ticks under field conditions. Bait mixtures could be formulated and delivered to treat a wide range of natural host species.

## M: KINETOPLASTIDA CHEMOTHERAPY AND EPIDEMIOLOGY

280 S-ADENOSYLMETHIONINE SYNTHETASE IN *TRYPANOSOMA BRUCEI*. Bacchi CJ*, Yarlett N, Garofalo J, Ciminelli M, and Goldberg B. Haskins Laboratories and Department of Biology, Pace University, New York, NY.

The licensed trypanocide Ornidyl (difluoromethylornithine; DFMO) has profound effects on the metabolism of African trypanosomes. Although the most notable effects are reduction of polyamine content and inhibition of ornithine decarboxlase, significant changes in S-adenosylmethionine (AdoMet) metabolism also occur. AdoMet is the aminopropyl donor in spermidine synthesis and the methyl group donor in transmethylase reactions. AdoMet synthetase catalyzes AdoMet formation from methionine and ATP. The partially purified enzyme from *T. b. brucei* blood forms is a single isoform with a native molecular weight (gel filtration) of 145 KD, and a subunit weight of 78 KD (SDS-PAGE). $K_m$ values were 77 μM for methionine and 55 μM for ATP. One kinetic property which distinguishes the trypanosome enzyme from most mammalian enzymes is the high $K_i$ value for product inhibition by AdoMet (240 μM). Most mammalian isoforms have $K_i$ values <60 μM, resulting in close control of AdoMet production. The decreased product regulation exhibited by the parasite enzyme is responsible for the ~50-fold increase (5 mM intracellular levels) in AdoMet pools found in DFMO treated parasites. DFMO treated mammalian cells only double their intracellular AdoMet pools. The increase in AdoMet seen in DFMO-treated trypanosomes may well be the key factor in the biochemical action of this agent.

281 ANTIMONTAL TREATMENT IN HAMSTERS AS A MODEL FOR ASSESSING PARASITOLOGICAL CURE IN TEGUMENTARY LEISHMANIASIS. Travi BL*, Martinez JE, and Zea A. Fundacion CIDEIM, Cali, Colombia and Departmento de Microbiologia, Universidad del Valle, Cali, Colombia

The objective of the present study was to determine the curative systemic dose of Glucantime for hamsters infected with *Leishmania braziliensis* s.l. as a standard for testing candidate drugs *in vivo*. Hamsters were inoculated with $5x10^6$ stationary phase *L. panamensis* promastigotes in the right hind foot. Six weeks PI animals were randomly assigned to groups of 8-10 animals each according to the following antimonial treament schedules; Group A untreated control; Group B, C, and D, 20, 40, and 60 mg/kg/day for 20 days respectively. In another set of assays the efficacy of local vs. systematic antimonial treatment was evaluated. Clinical evolution as measured by the decrease in the diameter of the inoculated foot having the contralateral foot control, showed that the 20 mg/kg/day regime was inadequate. The 40 mg/kg/day regimin resulted in marked improvement of the inoculated foot, and

parasite clearance of the lesion site, but viable amastigotes were detected in the draining lymph nodes (DLN). The 60 mg/kg/day regime showed clinical improvement, and parasite clearance at the lesion site. Seven hamsters were negative at the DLN's while 3 had a low number of viable amastigotes. Local treatment consisting of 4 intralesional injections of 4.2 mg meglumine antimoniate, at 5 day intervals showed clinical improvement, and parasite clearance at the lesion site and DLN in all hamsters. This study suggested that: 1) clinical resolution may require larger antimonial doses in hamsters than humans. 2) clinical improvement is not strictly correlated with parasitological cure and 3) a combined systemic-local treatment schedule should be tested in human clinical trials.

282 DIFFERENTIAL SENSITIVITY TO THE PENTAVALENT ANTIMONIALS, PENTOSTAM AND GLUCANTIME, OF SOME LEISHMANIAL ISOLATES. Jackson JE* and Tally JD. Experimental Therapeutics Division, Walter Reed Army Institute of Research, Washington, DC.

Pentavalent antimonials (SbV), Pentostam (P) and Glucantime (G), are not consistently effective against all leishmanial diseases. Also, reports of treatment failure are increasing. We found marked differences in leishmanial sensitivity to P and G even among isolates having similar disease form, clinical outcome, geographic region, and isolation date. Testing included isolates from major disease types (cutaneous, CL;diffuse cutaneous, DCL; mucocutaneous, MCL; visceral, VL; and recidivans, LR), with clinical histories reflecting clinical cure to unresponsive to chemotherapy, from various endemic areas. Drug sensitivity was done using an *in vitro* method, RAM. Results were based on Sb concentration. A few examples are: Brazilian VL sensitivity to P varied from 3->33 μg/ml Sb and between 6->70 μg/ml Sb G among isolates. (One Brazilian VL was sensitive to 6 μg/ml Sb P and insensitive to >70 μg/ml Sb G.) A Panamanian CL was sensitive to 3 μg/ml P and insensitive to >20 μg/ml G; and an Iraqi LR was sensitive to 6 μg/ml G and insensitive to 200 μg/ml P. Some isolates were resistant to 200-600 μg/ml Sb Gor P: some VL and CL of Kenya, DCL of the Dominican Republic, CL of the U.S.A. In many endemic regions, there seems to be a positive correlation of most common SbV and of isolate resistance to P or to G.

283 ANTILEISHMANIAL ACTIVITY OF MEDICINAL PLANTS USED IN NIGERIAN TRADITIONAL MEDICINE. Iwu MM*, Jackson JE, Tally JD, and Klayman DL. Experimental Therapeutics Division, Walter Reed Army Institute of Research, Washington, DC.

Pentavalent antimonials (SbV) remain the drugs of choice for the chemotherapy of human leishmaniases despite their cardiac and renal toxicity. Because these antimonials and alternative drugs are not consistently effective against certain forms of the disease, there is a need for more effective and less toxic drugs. Using a radiorespirometry microtest based on *in vitro* inhibition of catabolism to $^{14}CO_2$ of a battery of $^{14}C$-substrates by promastigotes, we have examined 11 plants used in Nigerian traditional medicine for possible antileishmanial activity. Methanol extracts from 6 of the plants, *Gongronemalatifolia, Dorstenia multiradiata, Picralima nitida, Colaattiensis, Desmodium gangeticum,* and *Rothmania withfieldii,* were found to be active at concentrations of 5-50 μg/ml against visceral *Leishmania (Leishmania) chagasi* and cutaneous *Leishmania (Leishmania) mexicana.* The extracts differentially inhibited (10-90%) the catabolism of certain sugars, amino acids, or fatty acid precursors by promastigotes. Differential metabolic inhibition suggests diversity of antiparasite mode of action for the various plant extracts.

284 SCHIZODEME AND ZYMODEME CHARACTERIZATION OF *LEISHMANIA* IN THE INVESTIGATION OF A FOCUS OF INFANTILE VISCERAL LEISHMANIASIS IN ALEXANDRIA, EGYPT. Karim AM, Osman AM*, Al Gauhari AI, and Shehata MG. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

A number of *Leishmania* isolates from a focus of infantile visceral leishmaniasis near Alexandria, Egypt have been previously characterized by starch gel enzyme electrophoresis. They were shown to be variants of the common mediterranean *L. infantum*: strain MON-1 and were termed MON-98. Restriction endonuclease fingerprinting patterns for MON-98 kinetoplast DNA (kDNa) are examined here and compared with results using cellulose acetate enzyme electrophoresis. We show that human and dog isolates that are similar by cellulose acetate electrophoresis using a 12 enzyme system are indeed identical at the organelle genotype level as demonstrated by 3 restriction enzyme fingerprints. Comparison with MON-1 zymodeme and schizodeme profiles shows similarity between MON-1 and MON-98 in 11 out of 12 enzyme migration patterns studied. No similarity was observed in any of two restriction fingerprints compared. Those results confirm indications that dogs are reservoirs for the strain causing visceral leishmaniasis at El Agamy, Alexandria. They demonstrate the high resolving power of schizodeme analysis.

285 *LEISHMANIA INFANTUM* MON-98 ISOLATED FROM NATURALLY INFECTED *PHLEBOTOMUS LANGERONI* (DIPTERA: PSYCHODIDAE) IN EL AGAMY, EGYPT. Doha S* and Shehata M. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

A total of 21 (1.3%) leishmania-like flagellates in the anterior mid gut and in the head regions were detected in 1634 *P. langeroni*. None of1847 *P. papatasi* were found infected. Eight (38%) of the 21 cultures were successfully grown in NNN medium cultures and were characterized by CAE technique using 12 enzyme systems. The eight positive cultures gave zymogram patterns indistinguishable from that of the MON-98 reference strain of *L. infantum*. The present results together with anthropophilic behaviour of *P. langeroni* its distribution and abundance in relation to VL cases and stray dogs and its documented feeding on humans and dogs meet with the essential criteria required to incriminate this species as a vector of visceral leishmaniasis in the area. *P. langeroni* can be now added to the list of sand fly species of the subgenus *Larroussius* found naturally infected with typed isolates of *L. infantum*.

286 CUTANEOUS (CL) AND VISCERAL (VL) LEISHMANIASES INDIGENOUS TO ISRAEL AND EGYPT : REAPPRAISAL, CURRENT STATUS AND ANOMALIES. Schnur LF*, Youssef M, Wahba MM, and Shehata M. Department of Parasitology, Hebrew University - Hadassah Medical School, Jerusalem, Israel; and Ain Shams University Research and Training Centre on Vectors of Diseases, Cairo, Egypt.

*Leishmania major* causes zoonotic CL in northern Egypt, Sinai, the Negev, Arava and Jordan Valley. *P. papatasi* is the vector, rodents the reservoir: *Gerbillus pyramidum* in Sinai; *Psammomys obesus* and *Meriones crassus* in Israel, yet to be implicated in Egypt. *L. major* was also isolated from dogs in Egypt. *L. tropica* causes anthroponotic CL and rare cases of leishmaniasis recidivans and VL. It is widely spread in Israel where cases are few, but has not not been recorded in Egypt. A recent Israeli case came from Eilat, bordering Sinai. The putative vector is *P. sergenti*. Egyptian VL has been rare: 32 cases in 90 years; 20 infantile from ElAgamy since 1982. The other cases were adults and children. Many more cases were reported from Israel: all, but two, infants and children. *L. infantum* has been isolated in both countries from dogs and recent VL cases but not CL cases. The putative vector in Egypt is *P. langeroni*, found in abundance near El Agamy. The vector in Israel is unknown. Israeli and Egyptian *L. major* and *L. infantum* strains display serotypic and enzymic heterogeneity with regional distribution of variants: *L. major* expressing 7 different excreted factor (EF) serotypes and variation in 6PGD and NH; *L. infantum* two EF serotypes and variation in ME. Israeli *L. tropica* strains show serotypic homogeneity but marked enzymic variation in MPI, 6PGD, ALAT, ASAT, SOD, PK, MDH and NH.

287 A QUANTITATIVE STUDY OF LEISHMANIASIS TRANSMISSION AT A SITE IN THE NORTHEASTERN SINAI DESERT OF EGYPT DURING 1990. Fryauff DJ*, Modi GB, Mansour NS, Mikhail EM, Youssef FG, and Wassif KM. Medical Zoology and Basic Sciences Divisions, US Naval Medical Research Unit Number Three, Cairo, Egypt; and Zoology Department, Faculty of Science, Ain Shame University, Cairo, Egypt.

A longitudinal epidemiological study of cuaneous leishmaniasis (CL) was initiated in a 3000 $km^2$ sector of the northeastern Sinai desert monitored by the Multinational Force and Observers (MFO). Data obtained from over two years of study (August 1989-October 1991) confirmed only one of four suspected sites as a significant focus of CL transmission. During 1990, *Phlebotomus papatasi*, the only man-biting species encountered, comprised more than 96% of the sand fly population, and frequently attained landing desities exceeding 100 sand flies/man/hr. This vector was present from April to November, with highest densities occurring during the period of May-September. A peak promastigote infection rate of 2.4% (13/534) was determined for *P. papatasi* during July, 1990. Among 80 persons at risk of acquiring CL during 8 months of vector activity at this site, 9 of 11 suspected CL cases were confirmed by culture. *Leishmania* infection was also acquired by 2 of 22 sentinel hamsters used during the same time period. *L. major* has been the only species identified from human, vector, and sentinel hamster innoculations. Gerbil species accounted for more than 97% of the 731 animals trapped at the principal study site. *Gerbillus pyramidum* is the only species from which *Leishmania* has thus far been isolated.

## N: INTESTINAL PROTOZOA

288 PREVALENCE OF *BLASTOCYTIS HOMINIS* AMONG ASYMPTOMATIC AND SYMPTOMATIC INDIVIDUALS. Markell EK and Udkow MP*. Department of Internal Medicine, Kaiser Permanente Medical Center, Oakland, CA.

The classification of *Blastocytis hominis* remains unclear, and its pathogenicity has been the subject of considerable debate in the last few years. Previous studies of human blastocystosis have been done with symptomatic patients and have thus been inherently biased. In an ongoing study, we exclude this bias by determining the rate of infection in two groups of patients, one symptomatic and one asymptomatic. The asymptomatic study group (ASG), numbering 182 volunteers, was recruited from well persons, further selected by eliminating all who had any but short-term gastrointestinal complaints during the preceding year. The symptomatic control group (SCG), with 170 subjects of comparable age and sex distribution, was composed of persons from the same geographic area whose physicians ordered stool examinations for typical clinical reasons. The health records of both groups were checked to verify the distinct nature of the two groups. Single stool samples from both groups were examined by an observer (EKM) blinded as to their origin. *Blastocystis* was found in 11.5% (21/182) and the ASG and 12.9% (22/170) of the SCG. Analysis of these results demonstrated no significant difference ($p = .405$) in prevalence of this organisms in the two groups. We consider our results to date persuasive evidence of the nonpathogenicity of this organism, treatment of which currently includes administration of potentially toxic drugs.

289 INDUCTION OF STRESS RESPONSES IN *TRICHOMONAS VAGINALIS*. Davis SR* and Lushbaugh WB. Parasitology Division, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS.

Stress responses may be important in host-parasite relationships. The heat shock response (HSR) induced in *Trichomonas vaginalis* (TV) by incubation at various temperatures was traced by metabolic labeling with $^{35}S$-methionine. Increased temperature (43°C) depressed normal protein production and enhanced synthesis of heat shock proteins(Hsp's). The Hsp's included molecules of about 85, 78, 66, 60, 39-32,20-15 and 12 kDa. TV switched from normal protein synthesis to Hsp production over a 20 to 60 minute

period. A comparison of clinical isolates revealed that major Hsp's of 85, 77, and 66 kDa were present in fresh and culture adapted forms of all isolates examined to date; however, variation in the presence or intensity of other Hsp's occurred among both fresh isolates and culture adapted forms. Culture adaptation altered the HSR in some strains but not in others. The effects of oxidative stress on TV protein synthesis were studied in similar experiments utilizing $H_2O_2$ as the stressor. Micromolar concentrations (450-550) induced a stress response resembling HSR in that the 85, 77, 66 kDa proteins were induced. Oxidative stress was distinctive because it induced synthesis of other molecules (167, 120, 43 kDa). Also some constituitively expressed molecules remained evident.

290 CHARACTERIZATION OF A *GIARDIA LAMBLIA* VARIANT-SPECIFIC SURFACE PROTEIN (VSP) GENE FROM ISOLATE GS/M AND ESTIMATION OF THE VSP GENE REPERTOIRE SIZE. Nash TE* and Mowatt MR. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, Md.

*Giardia lamblia* undergoes surface antigenic variation. Studies to date have shown that the VSPs of isolate WB are cysteine-rich, can vary dramatically in size, contain Cys-X-X-Cys motifs, and are differentially expressed. Expression of some VSP epitopes is restricted among certain isolates. GS/M(H7) is a *Giardia* clone which expresses an epitope not found in WB. The VSP gene encoding this epitope was selected by differential hybridization using radiolabelled cDNA from H7 and variant siblings that express other VSPs. The VSPH7 probe gene detects a 1800 nt transcript abundant in H7 but undetectable in variant siblings. Primer extension directly from RNA was used to complete the gene sequence which predicted a protein with a molecular weight of 56,832 . The protein showed many of the characteristics of 2 previously sequenced WB VSPs including many Cys-X-X-Cys tetrapeptides and a conserved 3' terminal sequence. Genomic Southern analysis indicated the presence of two distinct VSPH7 genes in H7 which were undetectable in WB. An oligonucleotide from the conserved region was used in combination with one specific for the *VSPH7* gene to estimate the *VSP* repertoire size at between 133 and 151. VSPs, even from isolates expressing unique epitopes, constitute a family of related proteins.

291 EXPRESSION OF ANTIGENS RECOGNIZED BY ANTIBODIES TO CONSERVED HEAT SHOCK PROTEINS DURING ENCYSTATION OF *GIARDIA LAMBLIA*. Reiner DS*, Aley SB, and Gillin FD. Department of Pathology, University of California, San Diego, San Diego, CA.

The alkaline pH and high bile concentration of the gut are cellular stress factors which induce cultured *G. lamblia* trophozoites to encyst and to express a large number of new antigens, some of which are in the molecular weight range (~60 to 102 kD) of heat shock or stress proteins (HSP). In these studies, we have used the following specific antibodies against HSP to characterize these antigens: Antiserum against (1) the conserved GroEL (HSP 60):GroES complex purified from *Legionella*, (2) recombinant *Mycobacterium* BCG GroEL (HSP 60) alone (from T. Shinnick), (3) *Drosophila* HSP 70 (from S. Lindquist) and (4) a cloned fragment of a *P. falciparum* 75 kD surface HSP (from F. Ardeshir and R. Reese). Antiserum (1) reacted with a 27 kD protein in encysting and with 65 and 100 kD antigens in HS *Giardia* . The other sera reacted with proteins of ~60 to 70 kD which were either unique to encysting cells or shared with HS cells, but not controls. The complexity of these patterns is not surprising since HSP 60 proteins have conserved and non- conserved epitopes and the HSP 70 family has constitutive and induced proteins. We now propose that the *Giardia* HSP 60 and 70 analogs function as molecular chaperonins during assembly and import of cyst antigens into the encystation specific vesicles we have described.

292 EXPERIMENTAL GIARDIASIS IN THE PROTEIN MALNOURISHED HOST. Leitch GJ*, Udezulu IA, and Visvesvara GS. Department of Physiology, Morehouse School of Medicine, Atlanta, GA; and Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

Adult male gerbils were infected with 100,000 *Giardia lamblia* cysts and the course of the resulting enteritis was studied. Animals were fed defined low fiber isocaloric diets containing 25% casein (control; C) or 6% casein (low protein; LP). In a preliminary study, animals were fed these two diets ad libitum for 10 weeks prior to being infected with *Giardia* cysts. Fourteen days later, the animals were sacrificed. There was a significant reduction in both the number of cysts excreted and the number of trophozoites recovered from the intestine of the LP diet group animals. These animals also had significantly greater enteropooling than the C diet group animals, exemplified as both an increase in small intestine and cecum weights. In a second experiment, animals were pair fed the C and LP diets for 3 weeks prior to and during the infection. Body weights of LP group animals were always significantly lower than for paired C group animals. The LP diet group animals exhibited an additional statistically significant weight loss 15 days after infection while the C group animals did not. This weight loss was reversed following clearing of the parasite. On days 14 and 15, postinfection there was significant steatorrhea in the LP diet group animals but not in the C group animals. Brush border enzyme activities were reduced by infection with *Giardia* in both C and LP diet group animals, and selected brush border enzyme activities were reduced by the LP diet alone. Control group animals stopped excreting cysts 2-3 days prior to LP group animals. These data suggest that giardiasis has a greater nutritional and physiological impact on the malnourished host than on the adequately nourished host.

293 PULSED FIELD GEL ELECTROPHORESIS OF *GIARDIA DUODENALIS* ISOLATES FROM A WATERBORNE OUTBREAK. Sarafis K*, Shahriari H, Isaac-Renton JL. Division of Medical Microbiology, University of British Columbia, Vancouver, British Columbia, Canada.

The recent advent of pulsed field gel electrophoresis (PFGE) has allowed karyotypic characterization of protozoa at the species and subspecies level. To determine if the chromosome complement of *Giardia duodenalis* isolates associated with an epidemic was homogeneous, we examined clinical, animal and environmental isolates using CHEF-PFGE. The molecular weight of the *Giardia* chromosomes was estimated to be between 1 and 4 Mb. Significant differences were observed between banding patterns of reference and epidemic associated isolates. The reference strain WB (ATCC #30957) and reference strain P-1 (ATCC # 30888) all displayed 4-5 major chromosome bands with migration pattern differences exhibited between them. The epidemic associated isolates all displayed 5 major higher molecular weight bands. As well, some isolates displayed a minor lower molecular weight band. The degree of polymorphism exhibited within the epidemic associated isolates was minor when compared to each of the reference strains. We observed the greatest degree of polymorphism within an estimated size range of 3.6 and 2.2 Mb.

294 PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF SEVERAL CLONES OF *ENTAMOEBA HISTOLYTICA*. Orozzco E*, Lazard D, Gamboa Y, Sanchez T, Valdes J, and Hernandez F. Department of Experimental Pathology, CINVESTAV I.P.N. Mexico D.F. Mexico.

We have studied the virulence phenotype of three closely related clones and determined their molecular karyotype by transverse alternate electrophoresis (TAFE). Their "chromosomal" patterns were very similar and actin and ribosomal genes were located in a 1.2 megabases (Mb) molecule, although, other chromosomes were also lighted with the ribosomal probe. We also cloned and localized in the amebic chromosomes a sequence encoding for a transcript specific for virulent trophozoites. By differential plaque hybridization of virulent and non virulent trophozoites we isolated a 3.5 kbp cDNA clone (pMD). Some subfragments of the clone recognized at least four transcripts in both virulent and non-virulent trophozoites. Interestingly, another pMD subfragment hybridized with a transcript of 0.63 kb present only in the highly virulent trophozoites. Southern blot analysis of both amebic chromosomes and digested total DNA showed that all these transcripts are encoded by a linked piece of DNA localized in two amebic "chromosomes" that comigrate with yeast chromosomes of 1.4 and 1.3 Mb. PCR experiments

demonstrated that the 0.63 kb sequence is absent or highly modified in the nonvirulent trophozoites. As far as we know this is the first report on chromosomal localization of a DNA fragment that encodes a transcript specific for virulent trophozoites.

295 DIFFERENTIATION OF *ENTAMOEBA HISTOLYTICA* STRAINS BY OLIGONUCLEOTIDE PROBES. Burch DJ, Li E, Kuntz-Jenkins CR, and Stanley SL, Jr. Department of Medicine, Washington University School of Medicine, St. Louis, MO; and Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO.

We have previously described the isolation and characterization of a 0.7kb *Entamoeba histolytica* HM1:IMSS cDNA clone, C2, that exhibits strain specificity. This cDNA hybridizes with DNA isolated from xenically cultured *E. histolytica* clinical isolates that express pathogenic zymodemes but not DNA isolated from strains expressing nonpathogenic zymodemes. This clone distinguishes among axenic strains possessing the same pathogenic zymodeme in that it hybridizes with RNA and DNA isolated from strains HM1-IMSS and 200:NIH but not from strains HK-9 and Rahman. To further study this gene and its strain specificity, we have isolated and sequenced the corresponding genomic 1.6kb Sau3a fragment. The genomic clone contains 0.54kb of sequence 5' to the C2 sequence, 0.7kb corresponding to the C2 cDNA clone sequence, and 0.31kb of sequence 3' to the C2 gene. Antisense oligonucleotides corresponding to the 5' and 3' flanking sequences were used to probe RNA and DNA isolated from a number of axenic strains. While the oligonucleotides derived from the 5' sequences exhibited the same strain specificity as the C2 cDNA clone, an oligonucleotide derived from the 3' sequences hybridized with RNA from all axenic strains tested. This approach of mapping with oligonucleotide probes may allow us to define the strain specific portion of this genomic clone.

## O: FILARIASIS PATHOLOGY & DIAGNOSIS

296 *ONCHOCERCA VOLVULUS* DNA PROBE CLASSIFICATION CORRELATES WITH EPIDEMIOLOGICAL PATTERNS OF BLINDNESS. Zimmerman PA, Dadzie KY, De Sole G, Remme J, Soumbey Alley E, and Unnasch TR*. Onchocerciasis Control Programme in West Africa, Ouagadougou, Burkina Faso; and Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

The causative agent of onchocerciasis, *Onchocerca volvulus*, appears to exist in two different strains in the rainforest and savanna zones. Furthermore, epidemiological evidence suggests that distinct patterns of *O. volvulus* induced ocular disease exist in the rainforest and savanna zones. The most favored hypothesis to explain this finding is that the two strains of the parasite differ in their pathogenic potential. To test this hypothesis, parasites were collected from 17 villages in five West African countries. Detailed ophthalmological studies were undertaken to establish the epidemiological disease pattern present in each village. A total of 152 parasite samples collected from these villages were then classified with *O. volvulus* strain specific DNA probes. The results shows a strong correlation of epidemiological disease pattern with DNA probe classification, supporting the hypothesis of a strain association with ocular pathogenicity. The DNA probes maybe used to predict the pathogenic potential of a given parasite population throughout large parts of West Africa with a sensitivity of 95% and a specificity of 93%. The ability to use the strain specific DNA probes in this fashion should be useful in the strategic planning of ongoing control efforts, as well as in monitoring new outbreaks of transmission within regions under control.

297 PATHOGENIC ANTIGENS OF *ONCHOCERCA VOLVULUS* CAUSING SCLEROSING KERATITIS IN A GUINEA PIG MODEL. Chakravarti B*, Lass J, Diaconu E, Herring TA, Chakravarti DN, and Greene BM. Division of Geographic Medicine, Department of Medicine, University of Alabama

at Birmingham, Birmingham, AL; and Division of Ophthalmology, Department of Surgery, Case Western Reserve University, Cleveland, OH.

A guinea pig model of experimental sclerosing keratitis, clinically similar to that found in human onchocerciasis involving intrastromal injection of soluble antigens into corneas of preimmunized Hartley guinea pigs was used to identify and characterize *O. volvulus* antigens causing sclerosing keratitis. Initial fractionation of soluble antigens in adult worm extract was carried out by molecular sieve chromatography (Bio-Gel A-5m) followed by anion-exchange HPLC (Mono Q). The Mono Q fraction eluting between 180 to 350 mM NaCl was further fractionated by anion-exchange chromatography on DEAE-Sepharose CL-6B into four different pools. Pool C (eluting between 130 to 330 mM NaCl) contained the most pathogenic antigen(s) capable of inducing experimental sclerosing keratitis in guinea pigs. Pool C was further fractionated by cation exchange (Mono S) HPLC, into six different pools. Two pools (a and e) were found to be most active. SDS-PAGE (7.5%, w/v acrylamide) analysis showed that, pool a (eluted unbound at 10 mM NaCl) contained eight major proteins (Mr 31 to 200 K) while pool e (eluted between 130 to 420 mM NaCl) contained sixteen major proteins (Mr 31 to 200 K). Characterization of these pathogenic antigen(s) at the molecular level and their mechanism(s) of action will be helpful in further understanding the molecular mechanism of blinding sclerosing keratitis.

298 MONOCLONAL ANTIBODIES TO CIRCULATING *ONCHOCERCA VOLVULUS* ANTIGENS. Chandrashekar R*, Ogunrinade AF, and Weil GJ. Washington University School of Medicine, St. Louis, MO; and University of Ibadan, Ibadan, Nigeria.

We have previously identified and characterized circulating *O. volvulus* antigens in sera from onchocerciasis patients. We now report production of 2 murine monoclonal antibodies (MAB) that bind to these antigens. MAB OV-1 and OV-5 are $IgG_2$ antibodies that bind to parasite antigens (23, 62-70kDa) in Western blots of PEG-precipitated immune complexes from human onchocerciasis sera. The target epitopes of the MABs are heat stable and resistant to trypsin, but they are destroyed by Pronase and metaperiodate treatments. Western blot analysis performed with adult worm extract and ELISA inhibition studies demonstrate that OV-1 and OV-5 bind to different epitopes, neither of which is phosphorylcholine. Preliminary studies have shown that these antibodies can be used to detect *O. volvulus* antigens in human sera by Western blot and by ELISA. In addition, OV-5 binds to a 28 kDa antigen that is present in urine from onchocerciasis patients. We hope that these studies will lead to improved methods for diagnosis and quantitation of *O. volvulus* infection based on antigen detection.

299 EFFICIENT ASSESSMENT OF FILARIASIS ENDEMICITY BY SCREENING FOR FILARIAL ANTIGENEMIA IN A SENTINEL POPULATION. Ramzy RM*, Hafez ON, Gad AM, Faris R, Buck AA, and Weil GJ. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

We have previously reported that a monoclonal antibody-based antigen detection assay (AD12) is sensitive and specific for *Wuchereria bancrofti* infection in Egypt. The purpose of the present study was to demonstrate the use of this assay in a sentinel population as a means of efficiently screening for filariasis endemicity. Antigen testing was performed with finger prick blood collected during the day from 745 middle school children (ages 12-16). The school draws students from 5 villages situated within 5 km of Tahoria village in Qalubia Governate. The prevalence of filarial antigenemia in the school was 17.4%. Antigenemia rates in children from the 5 villages were 28, 20, 17, 17, and 9% (nonuniformity significant by Chi square, $P = .02$). These data agree with Ministry of Health rankings of relative endemicity for these villages based on night blood surveys. The village with 28% antigen prevalence in the school study was surveyed in 1990, one year before the present study. The prevalence rates of antigenemia and microfilaremia at that time for a different sample of children now aged 12-16 were 34% and 23%,

respectively. We conclude that antigen detection in middle school children is an efficient means of assessing filariasis endemicity in Egypt.

300 COMMUNITY DIAGNOSIS OF LYMPHATIC FILARIASIS IN EGYPT: A COMPREHENSIVE APPROACH. Faris R*, Ramzy RM, Gad AM, Weil GJ, and Buck AA. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

The objective of this study was to determine the best tests for efficiently estimating the true prevalence of bancroftian filariasis in endemic areas. 424 persons (ages 10-75) in an endemic village were studied. The evaluation included a clinical examination, night blood exams for microfilariae (50 µl thick smears and 1 ml filtration), and a test for circulating filarial antigen (AD12). 190 subjects (44.8%) had at least one positive test and were considered filarious (FIL). The sensitivities of clinical examination, thick smears, filtration and antigen testing for FIL were 22%, 50%, 64% and 88%, respectively. Compared to membrane filtration, antigen detection had a sensitivity of 98%, a positive predictive power of 71%, and a negative predictive power of 99%. The high diagnostic capacity of antigen detection and the fact that finger-prick day blood can be used for the test make it the most suitable single test for field studies. However, none of the blood tests was a sensitive indicator of clinical filariasis; 70% of clinical cases were negative in all 3 blood tests and would have been missed if clinical examinations had not been performed. Therefore, we recommend a combination of clinical examination and antigen testing for community diagnosis of filariasis in endemic areas.

301 DO ELEPHANTIASIS AND HYDROCELE REPRESENT DISTINCT PARASITOLOGIC AND IMMUNOLOGIC OUTCOMES OF FILARIAL INFECTION? Lammie PJ*, Addiss DG, and Eberhard ML. Parasitic Diseases Branch, Centers for Disease Control, Atlanta GA.

Chronic obstructive disease in lymphatic filariasis has been associated with increased antifilarial immune responsiveness. The present study investigated this relationship further in the Haitian community of Leogane where *Wuchereria bancrofti* is endemic. Pronounced differences in microfilarial prevalence were observed between patients with hydrocele and those with lymphedema or elephantiasis. Only 2/76 patients (66 females/10 males) with leg involvement (E+) were MF+; in contrast, 23/38 (60.5%) of men with hydrocele (H+) were MF+. The immunological basis of these differences was examined by ELISA and invitro blastogenesis assays. Antifilarial IgG levels were comparable for E+ and H+ individuals (geometric means of 128.7 vs. 140.3 units); however, amicrofilaremic H+ men had higher IgG levels than MF+/H+ men(194.9 vs 112.1). Peripheral blood lymphocytes from MF- patients, regardless of type of clinical manifestation, were more responsive to filarial antigens (adult and MF) than cells from MF+/H+ individuals. The H+ group is more heterogeneous, immunologically and parasitologically, than the E+ patients. These results may be difficult to reconcile with infection models which predict that the occurrence of microfilaremia in symptomatic individuals is proportional to the incidence of infection.

302 PATHOPHYSIOLOGICAL AND IMMUNOLOGICAL CHANGES IN INGUINAL LYMPH NODES FROM RHESUS MONKEYS WITH EXPERIMENTAL *BRUGIA MALAYI* INFECTION. Dennis VA*, Lasater BL, Blanchard JL, Lowrie, Jr. RC, and Campeau RJ. Parasitology Department, Tulane Regional Primate Research Center, Covington, LA; Veterinary Sciences Department, Tulane Regional Primate Research Center, Covington, LA; and Radiology Department, Tulane University School of Medicine, New Orleans, LA.

Rhesus monkeys can be experimentally infected with *Brugia malayi* yet the host-parasite relationship is poorly defined. We examined the pathophysiological and immunological changes that occur in the lymphatic system of 5 *B. malayi*-infected rhesus monkeys. Third stage larvae were inoculated

subcutaneously in the right calf of these animals, and 5 weeks later the inguinal nodes on the right side began to show signs of enlargement, the maximum being observed 10-16 weeks postinoculation (WPI). Radioisotopic lymphangioscintigraphy showed the lymph flow rate in the right leg was significantly more retarded than in the contralateral leg in 4/5 and 3/5 monkeys at 7 and at 15 WPI, respectively. Right and left nodes from the 5 infected and 5 control monkeys were biopsied at 24 WPI and lymph node cells (LNC) analyzed for *in vitro* blastogenic reactivity to soluble *B. malayi* adult (Ad) and microfilaria (mf) antigens, production of parasite-specific antibody (Ab) *in vitro*, and quantification of T and B lymphocytes populations by flow cytometry. Right LNC from 3/5 and 4/5 infected monkeys were more responsive to Ad and mf antigens, respectively in blastogenesis assays than the left LNC. Parasite-specific Ab was only produced in response to mf stimulated right LNC from 1/5 monkeys. LNC from the other monkeys either spontaneously produced Ab or did not produced Ab at all. Flow cytometric analysis of right and left LNC lymphocyte subsets indicated no differences in CD2 (total T), CD8 (suppressor T), CD4 (helper T), CD45R (suppressor inducer T), CDW29 (helper-inducer T) and CD20 (total B) between right and left LNC. An increase was only seen in HLA-DR positive cells of right LNC from infected monkeys. These results suggest acute lymphatic blockage in filarial-infected rhesus monkeys and that different immune mechanisms regulate T and B cell functions in this filarial model system.

303 AFFECTS OF PROTECTIVE RESISTANCE ON THE DEVELOPMENT OF LYMPHATIC LESIONS AND GRANULOMATIOUS HYPERSENSITY IN *BRUGIA*-INFECTED JIRDS. Petit TA, Klei TR*, Enright FM, Coleman SU, and Jones K. Louisiana State University, Baton Rouge, LA.

Protective immune responses have been hypothesized to play a role in the induction of filariae associated lymphatic lesions. This hypothesis was tested in the jird-*B. pahangi* model. Jirds were immunized with irradiation attenuated $L_3$. Lymphatic lesions were compared in vaccinated (V) and nonvaccinated (NV) jirds following challenge (C) with100 $L_3$. Challenge worm burdens were reduced in V jirds at both acute (67%) and chronic (41%) periods of infection. The ratio of lymph thrombi/lymphatic worms was significantly greater in V-C jirds than in NV-C jirds during acute infections ($P<0.01$), but not chronic infections($P$ 0.07). Granulomatous hypersensitivity to *B. pahangi* adult extracts (SAWA) was assessed by measurements of granuloma areas around SAWA-coated sepharose beads embolized in lungs prior to necropsy. Vaccinated-nonchallenged jirds showed a marked response at both acute and chronic time periods. Similar marked responses seen during acute periods of C infections in V and NV jirds were significantly ($P<0.01$) reduced during chronic periods in both groups. Lymphatic lesion severity was greater in partially resistant individuals during acute infections. However, this inflammatory response was subsequently modulated potentially by the parasites which survive the protective immune response.

## P: VIRAL VACCINES

304 PRECLINICAL TESTING OF A RECOMBINANT VACCINIA VIRUS VACCINE CANDIDATE FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME. Hasty SE*, Dalrymple JM, Malinoski FJ, Schmaljohn AL, and Schmaljohn CS. U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

A candidate vaccine for hemorrhagic fever with renal syndrome (HFRS) is being considered for safety tests in humans. The vaccine candidate is a recombinant vaccinia virus expressing Hantaan virus structural proteins (G1, G2, NC). Parent vaccinia virus was a plaque-purified derivative of the Connaught smallpox vaccine virus and cDNAs representing Hantaan virus M and S RNA genome segments were inserted into the vaccinia thymidine kinase gene. Master seed and production seed as well as an initial lot of vaccine were prepared in MRC-5 cells under conditions suitable for the production of human vaccines. The complete neutralization of the vaccine virus as a prerequisite to testing for adventitious agents was problematic. Sequential neutralizations by monoclonal antibodies

were required to significantly reduce the titer of the recombinant vaccinia virus. The candidate vaccine was evaluated for virulence by comparing it with a battery of standard or reference vaccinia viral strains. Virulence tests included the examination of lesions produced in rabbits after intradermal inoculations, hemorrhagic manifestations of pocks formed on chorioallantoic membranes of fertile hens eggs, and infection and mortality of both adult and suckling outbred mice. These data indicated that the recombinant vaccine virus was less virulent than any of the reference strain controls including the Bureau of Biologics standard. Infection of experimental animals with the experimental HFRS vaccine candidate resulted in detectable antibodies to each of the three Hantaan viral proteins in immunized hamsters that resisted infection when challenged with virulent Hantaan virus.

305 STUDIES ON A MUTAGEN-ATTENUATED RIFT VALLEY FEVER VACCINE CANDIDATE (MP12) IN NON-HUMAN PRIMATES. Morrill JC* and Peters CJ. Disease Assessment Division, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

The mutagen-attenuated vaccine strain of Rift Valley fever virus (RVF MP12), when tested in sheep and cattle, is immunogenic, avirulent, nonabortigenic, and protects both dam and fetus against virulent virus challenge. Neurovirulence tests done in juvenile rhesus monkeys showed that this vaccine was highly attenuated and minimally neuroinvasive. We have subsequently investigated the safety and efficacy of parenteral immunization in the rhesus macaque. Rhesus monkeys inoculated intramuscularly with 3000 PFU of RVF MP12 developed 80% plaque-reduction neutralization antibody titers of >1:320 within 10 days of inoculation and were protected against intravenous or small-particle aerosol challenge with $10^5$ PFU of the virulent ZH-501 strain of RVF virus. After intravenous or aerosol challenge, no evidence of disease was detected in any of the vaccines, whereas unvaccinated challenge-control monkeys developed viremia titers of 5.4 to 6.6 log 10 PFU/ml of serum and had three- to four fold increases in serum ALT values. These data enhance our confidence in MP12 as a potential vaccine for human use. The MP12 vaccine is produced in cells certified safe for human vaccine production and the current vaccine lot is undergoing review prior to human testing at USAMRIID.

306 THE MUTAGEN-ATTENUATED RIFT VALLEY FEVER VACCINE (MP12): POTENTIAL FOR A MUCOSAL VACCINE. Pitt ML* and Morrill JC. Pathophysiology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

The mutagen-attenuated Rift Valley fever vaccine strain (RVF MP12), which is nonpathogenic in rhesus monkeys, neonatal lambs, pregnant ewes and cows, has been developed only as a parenteral vaccine. However, a vaccine that could be administered noninvasively, would have numerous advantages. This is especially true in field situations where the ability to maintain sterility of hypodermic equipment is limited and successful immunizations may be done by lay people. Preliminary studies have shown that MP12 administered orally to mice protects them against aerosol/parenteral challenge with the virulent ZH-501 strain of RVF virus. We investigated the potential for MP12 when administered mucosally to produce protective immunity in rhesus monkeys. Three groups of monkeys were treated with $3 \times 10^4$ PFU of MP12 via aerosol, oral and intranasal routes. The development of the immune response was monitored. All the aerosol and intranasally immunized monkeys and 2/4 of the orally immunized monkeys developed good neutralizing antibody titers to RVFV. The immunized monkeys and a control group were challenged by aerosol, 56 days after immunization, with $10^5$ PFU of the virulent ZH-501 RVF viral strain. The results of both the immunization and challenge will be presented. This study strongly suggests that there is great potential for development of MP12 as a mucosal vaccine.

307 INTERFERENCE BETWEEN ALPHAVIRUS VACCINES. Malinoski FJ*, Ksiazek T, Schmaljohn A, Ramsburg HH, and Monath TP. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Live, attenuated vaccines are now available for Venezuelan equine encephalitis (VEE) and chikungunya (CHIK) viruses, two distantly related mosquito-borne alphaviruses of the enveloped RNA Togaviridae family. As these vaccines may be required for the protection of workers using both viruses, we examined the potential complications of sequential immunization. Human volunteers received either: (1) live, attenuated CHIK vaccine (CHIK Vax) or placebo followed by live, attenuated VEE vaccine (VEE Vax) (2) CHIK Vax or placebo then formalin inactivated VEE vaccine (VEE Inact-Vax), or (3) VEE Vax with or without VEE Inact-Vax boosting followed by CHIK Vax. Although neither live, attenuated vaccine induced cross-neutralizing antibody, each significantly interfered with seroconversion to the other. In contrast, the VEE Inact-Vax induced neutralizing antibody when administered one month after CHIK Vax. To address the question of protection in the absence of cross reactive neutralizing antibody, adult hamsters were immunized in groups similar to the volunteer groups and then challenged with virulent VEE. Animals that received VEE Vax or VEE Inact-Vax alone or one month after CHIK Vax were fully protected. Interestingly, animals given only CHIK Vax exhibited a significant increase in survival time. Taken together these data indicate that the absence of cross-neutralizing antibody is not sufficient to predict the susceptibility to lethal challenge after immunization with alphavirus vaccines and suggests further that it may be possible to develop a generic vaccine for protection against multiple alphaviruses if cross protective epitopes can be identified.

308 EXPERIENCE WITH AN INACTIVATED HEPATITIS A VACCINE. Nalin DR*, Calandra G, Ryan J, Lewis J, Miller W, Clements M, and Shouval D. Merck, Sharp and Dohme Research Labs, West Point, PA; Johns Hopkins Medical College, Baltimore, MD; and Hadassah Hospital, Jerusalem, Israel.

To date, 870 adults and 154 children have received one or more doses of a highly purified Merck formalin-inactivated, alum-adjuvanted hepatitis A vaccine. Dose regimens tested include 100, 200, 400 and 800 ng in 3 dose (0, 1 or 2, and 6 mo.) or 2 dose (0,6 mos) regimens. All doses tested have been highly immunogenic and well tolerated; no serious adverse experiences have occurred. Seroconversion rates after a single dose of 200 ng. or above have been 100% in children and adults <30, and >90% in older adults. Neutralizing antibody was detectable by weeks 2 to 4 post one dose. Antibody titers after a second 400 ng dose at 6 mos. were similar to those after three 100 ng or 400 ng doses at 0, 1 and 6 mos. Neutralizing antibody titers reached levels above those seen 3 mos. post immune serum globulin (IG); GMT's (mIU/ ml, RIA) after 2 doses of 400 ng (0,6 mos.) were >200 fold higher than during the first week after IG. The Merck inactivated hepatitis A- vaccine induces higher and much longer lasting titers of antibody ag- against hepatitis A virus than afforded by IG, and is very well tolerated.

309 SAFETY OF HEPATITIS A VACCINE (HM175, INACTIVATED-SMITHKLINE, BEECHAM) IN U.S. SOLDIERS: COMPARISON OF IMMUNIZATION SCHEDULES Kanjarpane DD*, DeFraites RF, Hoke CH, Sanchez JL, Malis DJ, Gelnett JM, Fleming JL, Krauss MR, Egan JE, Sjogren M, Moonsammy G, and Krause D. Walter Reed Army Institute of Research, Washington, DC; Madigan Army Medical Center, Tacoma, WA; and SmithKline Beecham Pharmaceuticals, Inc., Philadelphia, PA.

Hepatitis A is an important source of morbidity for military personnel and other travelers to the developing world. Safe and immunogenic vaccines are needed to replace passive immunoglobulin prophylaxis, which is effective for only several months. We studied the safety of an inactivated hepatitis A (HM175 strain) vaccine produced by SmithKline, Beecham (720 ELISA units/dose) in U. S. soldiers at

Fort Lewis, WA. Volunteers were randomly allocated to receive two intramuscular injections of vaccine in the following schedules: Ninety soldiers received an injection in each arm on the same day (GRP 1), 83 received the second dose 14 days after the first (GRP 2), and 84 received the second injection 30 days after the first (GRP 3). Recipients were directly observed for immediate adverse reactions, and later questioned about any side effects which may have occurred in the 72 hours after the injection. Average age of the sample was 27 years; there were no substantial differences in composition of the vaccine groups with respect to age, sex (95% male), race (80.5% white, 10.5% black/Afro-american) or military rank. The most commonly reported side effect was local soreness (20.6% after the first injection, 19.4% after the second). Soreness was also more commonly noted by participants in GRP 1 (29%). Systemic symptoms were rare; headache was reported by 2.3% of the entire group after the first dose and 2.7% after the second, while 'fever' was noticed by 2.3% and 1.6%, respectively. Most symptoms did not interfere with daily activities. No serious adverse vaccine reactions occurred. This vaccine was well tolerated by this group of young American adults.

310 LIMITED REPLICATIVE CAPACITY OF A LIVE-ATTENUATED HEPATITIS A VACCINE. Sjogren MH*, Purcell RH, McKee K, Binn LN, Macarthy P, Lackovic M, Ticehurst JR, Hoke CH, Feinstone SM, Bancroft WH, and D'Hondt E. Walter Reed Army Institute of Research, Washington, DC; National Institutes of Health, Bethesda, MD; US Army Medical Research Institute of Infectious Diseases, Frederick, MD; Food and Drug Administration, Bethesda, MD; and SmithKline Beecham, Belgium.

Formalin-inactivated vaccines are being studied for the prevention of hepatitis A virus (HAV) infection in humans. However, more than one injection is required to obtain adequate levels of anti-HAV. An oral vaccine would be desirable because of ease of administration and likely long-lasting antibody response. A live-attenuated HAV vaccine candidate was prepared by multiple passages of HAV, strain HM175 in MRC-5 cell culture. The vaccine was immunogenic in 3/4 chimpanzees and 1/4 marmosets inoculated intravenously with $10^4$ $TCID_{50}$. Hepatitis was not detected in any animal. Two volunteers each received $10^4$, $10^5$,$10^6$ or $10^7$ tissue culture infectious doses ($TCID_{50}$) of the candidate vaccine by the oral route. The presence of infectious virus in the vaccine was confirmed following inoculations. All volunteers were clinically well for 3 months following inoculation and had no evidence of hepatitis or shedding of virus in stool. Anti-HAV (tested by standard HAVAB) was not detected during follow-up (range 8 weeks to 1 year). These data suggest that humans are resistant to infection by oral inoculation with this live attenuated hepatitis A vaccine. Immunization by the parenteral route is in progress. If parenteral vaccine is immunogenic in humans as it is in animals, it is unlikely to be transmitted to contacts by the fecal-oral route.

## Q: MALARIA IMMUNOLOGY I

311 HYPNOZOITES OF *PLASMODIUM SIMIOVALE*. Cogswell FB*, Collins WE, Krotoski WA, and Lowrie RC. Parasitology Department, Tulane Regional Primate Research Center, Covington, LA ; Malaria Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Gillis W. Long Hansen's Disease Center, Carville, LA.

Latent tissue stages (hypnozoites) of *P. simiovale* were found by immunofluorescence in a rhesus monkey inoculated with $5 \times 10^6$ sporozoites from 198 heavily infected *Anopheles dirus* mosquitoes.The pre-erythrocytic forms of 6 μm in diameter are described from liver biopsies taken 8 days after sporozoite inoculation. Hypnozoites persisted in hepatic parenchymal cells for over 180 days until eliminated by a curative course of primaquine phosphate. Hypnozoites were shown to decline in absolute numbers after a series of relapses, thus supporting one of the testable tenets of the latent stage theory of relapse. This is the first report of hypnozoites from an ovale-type relapsing malaria.

312 IMMUNOGENICITY OF MULTIPLE ANTIGEN PEPTIDE (MAPS) CONTAINING T AND B CELL EPITOPES OF THE REPEAT REGION OF THE *PLASMODIUM FALCIPARUM* CS PROTEIN. Munesinghe DY, Clavijo PJ, Calvo Calle JM, Nussenzweig RS, and Nardin E*. Department of Zoology, University of Colombo, Sri Lanka; and Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

The immunogenicity of Multiple Antigen Peptide (MAPs) constructs containing T and B cell epitopes located in the repeat region of the *P. falciparum* circumsporozoite (CS) was examined. All the MAPs constructs that contained the T cell epitope (DPNANPNVDPNANPNV), located in the 5' end of the CS protein repeat region, stimulated proliferation and γ-interferon production by a human T cell clone which recognizes this epitope. These human T cells did not recognize MAPs that contained only the B cell epitope, (NANP)3, which is located in the 3' repeat region of the CS protein. Optimal antibody responses were obtained in mice immunized with MAPs containing four copies of tandemly arranged T and B cell epitopes, (TB)4. The murine immune response to the MAPs constructs was genetically restricted. Mice of a high responder strain, C57BL, recognized both the 5' and 3' repeat sequences in the MAPs as T, as well as B, cell epitopes and developed very high anti-MAPs and anti-sporozoite antibody titers. A/J and C3H mice, which were intermediate responders, developed lower antibody titers which varied according to the orientation of the T versus the B cell epitope within the MAPs constructs. Balb/c mice were nonresponders and did not develop antibodies following immunization with any of the MAPs constructs.

313 CYTOTOXIC CD4+ T CELLS FROM A SPOROZOITE-IMMUNIZED VOLUNTEER RECOGNIZE THE *PLASMODIUM FALCIPARUM* CS PROTEIN. Moreno A*, Clavijo P, Edelman R, Davis J, Sztein M, Herrington D, Nardin E, Nussenzweig RS. Department of Medical and Molecular Parasitology. New York University School of Medicine, New York, NY; Center for Vaccine Development, University of Maryland, Baltimore, MD; and Bowman-Gray School of Medicine, Wake-Forest University, Winston-Salem, NC.

Protective immunity against malaria sporozoites is mediated, in part, by neutralizing antibodies to circumsporozoite (CS) repeat sequence. Recently, studies in the rodent malaria model have shown that cytotoxic T cells play a role in sporozoite-induced immunity directed at liver stages of the parasite. In order to investigate the cytotoxic T lymphocyte (CTL) in man, we assayed a series of T cell lines derived from peripheral blood lymphocytes obtained from a volunteer who had been immunized against *P. falciparum* malaria by multiple exposure to the bites of irradiated infected mosquitoes. Cytotoxic CD4+ cells were generated by *in vitro* expansion with a pool of synthetic peptides spanning the entire CS protein. A CD4+ class II restricted CTL was obtained which specifically lysed autologous Epstein-Barr virus transformed B cells pulsed with a synthetic peptide representing a carboxy terminal sequence of the *P. falciparum* CS protein. The same peptide, as well as recombinant and native CS protein, also stimulated proliferation and γ-interferon production by the CD4+ CTL. The CTL epitope overlaps a highly conserved, as well as a polymorphic region of the CS protein. Our data provide the first evidence that the malaria parasite can induce CD4+ CTL in man.

314 FURTHER CHARACTERIZATION OF THE *PLASMODIUM FALCIPARUM* SPOROZOITE GENE CSP-2. Sina BJ*, Sakhuja K, Anders J, and Hollingdale MR. Biomedical Research Institute, Rockville, MD; and Walter Reed Army Institute of Research, Washington, DC.

CSP-2 is a 42/54kd cross-reactive antigen found on the surface of *P. falciparum* and *P. berghei* sporozoites. We have previously shown that passive transfer of a CSP-2 specific monoclonal antibody protected mice to *P. berghei* sporozoite infection, and neutralized *P. falciparum* and *P. berghei* sporozoite infectivity *in*

*vitro*. Current efforts are focussed on cloning and sequencing the CSP-2 gene and determining the protective capacity of the recombinant protein as a candidate malarial vaccine. Fragments of CSP-2 were initially isolated by immunoscreening from a *P. falciparum* genomic library. The 3' end of the CSP-2 gene was obtained from *P. falciparum* sporozoite amplified by PCR. The sequence of the CSP-2 coding strand obtained from cDNA was confirmed by metabolic labeling and immunoprecipitation of CSP-2 protein from *P. falciparum* sporozoites. Recombinant CSP-2 fusion protein was purified and used to vaccinate mice to determine the immunogenicity and protective capacity of the antigen against *P. berghei* sporozoite challenge. Studies are also underway to express the CSP-2 gene in various vaccine carrier organisms such as Salmonella, Vaccinia virus and Hepatitis B virus antigen particles produced in yeast.

315 INDUCTION OF CYTOTOTOXIC T LYMPHOCYTES AGAINST THE *PLASMODIUM FALCIPARUM* CS PROTEIN BY IMMUNIZATION WITH SOLUBLE PROTEIN WITHOUT ADJUVANT. Malik A*, Gross M, Ulrich T, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Ribi Immunochemcial Research Inc., Hamilton, MT; and Smith Kline Beecham, King of Prussia, PA.

Cytotoxic T lymphocytes (CTL) against the circumsporozoite (CS) protein can mediate protection against malaria. Accordingly there are efforts to construct vaccines that induce CTL against the CS protein. Induction of CTL is generally thought to require cytosolic expression of proteins, and exportation of target peptides to the cell surface in the class I MHC pathway. This has been achieved by immunization with recombinant live vectors including vaccinia, pseudorabies virus and Salmonella sp., and recently by immunization with peptides emulsified in Freund's Complete adjuvant. To determine if we could induce CTL against the CS protein by immunization with soluble protein, we immunized B10.BR mice with a recombinant fusion protein including only the flanking regions of the *P. falciparum* CS protein fused to 81 amino acids from the non-structural protein of influenza A (RLF). Immunization was carried out by a number of routes, and with and without an adjuvant containing monophosphoryl lipid A, cell wall skeleton of mycobacteria, and squalane (DETOX). Mice immunized i.p. or i.m. did not produce CTL, but mice immunized with or without adjuvant by the iv route produced CTL against RLF, and against peptide 368-390, which we have shown to be a human CTL epitope. This first demonstration of induction of CTL by immunization of soluble protein without adjuvant opens exciting possibilities for the development of human vaccines designed to produce protective cytotoxic T lymphocyte responses.

316 SYNERGY BETWEEN ANTIBODIES TO *PLASMODIUM FALCIPARUM* SPOROZOITE ANTIGENS ENHANCE NEUTRALIZING ACTIVITY. Appiah A* and Hollingdale MR. Biomedical Research Institute, Rockville, MD.

Antibodies from human volunteers immunized with *P. falciparum* CS protein vaccines inhibited sporozoite invasion (ISI) *in vitro*, and the $ISI_{50}$ (that concentration that reduced invasion by 50%) was significantly greater in protected (2-10 μg/ml) than non-protected volunteers (>100 μg/ml). However, we have developed MAb 36 against CS protein that has an $ISI_{50}$ of 0.1 μg/ml, suggesting that the ISI activity achieved in these volunteers was not optimal. One approach to increase neutralizing activity is to elicit antibodies to more than one sporozoite antigen. We have tested the ISI activity of antibodies to three distinct *P. falciparum* sporozoite surface antigens; CS protein(57/65/67 kd), CSP-2 (42/54kd) and CSP-3 (55/60kd). The $ISI_{50}$ of each was calculated, and then each was tested at that concentration in combination with increasing concentrations of the other antibodies. A distinct synergistic effect between anti-CS, anti-CSP-2 and anti-CSP-3 antibodies was observed. This was confirmed by demonstrating ISI activity in combination at concentrations that alone have no activity. These results suggest that a successful pre-erythrocytic vaccine should contain more than one sporozoite antigenic component.

317 PROTECTION AGAINST *PLASMODIUM YOELII* BY CD8+ CYTOTOXIC T LYMPHOCYTE CLONE THAT RECOGNIZES AN EPITOPE ON SPOROZOITE SURFACE PROTEIN 2. Khusmith S*, Sedegah M, Mellouk S, Houghten RA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; and Torrey Pines Institute for Molecular Studies, San Diego, CA.

BALB/c mice immunized with irradiated *Plasmodium yoelii* sporozoites produce antibodies and cytotoxic T cells (CTL) against a 140 kD protein, sporozoite surface protein 2 (SSP2), and against the CS protein. Mice immunized with P815 cells transfected with either SSP2 or CS genes are partially protected, and those immunized with a mixture of SSP2 and CS genes transfectants are completely protected, against malaria. The partial immunity induced by SSP2 or the CS protein, and the complete protection induced by the combination are reversed by depletion of CD8+ T cells. To determine if CD8+ T cells against SSP2 are adequate to protect against malaria, we produced a CD8+ T cell clone (TSL B7) by stimulating spleen cells from mice immunized with irradiated sporozoites under limiting dilution conditions, with an irradiated clone of P815 cells transfected with the SSP2 gene (SSP2 3.9). The TSL B7 cell clone lyses P815 cells transfected with the SSP2 gene. Epitope mapping with multiple peptides has demonstrated that this CTL clone recognizes an epitope included within a 20 amino acid region amino-terminal to the repeat region of SSP2, and that cytolytic activity is antigen specific and MHC restricted. Adoptive transfer of this CTL clone protects naive mice, demonstrating that CTL against SSP2 alone are adequate to protect against sporozoite challenge.

318 A MURINE T CELL CLONE WHICH PROTECTS AGAINST INFECTION BY BOTH *PLASMODIUM YOELII* AND *P. BERGHEI* SPOROZOITES. Weiss WR*, Houghten R, Sedegah M, Berzofsky JA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Torrey Pines Institute for Molecular Studies, San Diego, CA; and Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

We have characterized a murine T cell clone which protects mice against challenge with *P. yoelii* or *P. berghei* sporozoites. This clone was grown from the spleen of a BALB/c mouse immunized with irradiated *P. yoelii* sporozoites. The cells are CD8+ and CD4- and carry Vb8.1 TCR. They recognize a 10 amino acid peptide from position 281-290 of the *P. yoelii* CS protein, and they cross-react with peptides from the homologous region of the *P. berghei* CS protein. This clone lyses tumor cell targets sensitized with these peptides if they express the Kd molecule. The cloned cells produce large amounts of INF-γ in culture. When transferred to naive mice, this clone protects mice against moderate but not high doses of either *P. yoelii* or *P. berghei* sporozoites. This data reinforces previous work implicating T cells in the immunity to the pre-erythrocytic stages of malaria. It also shows that a single cell, directed at one cross-reactive epitope, can protect against two distinct malaria parasites.

319 PFS40: A CALCIUM BINDING SEXUAL STAGE PROTEIN PREDICTED TO BE A MALARIA TRANSMISSION BLOCKING TARGET ANTIGEN BY AN IMMUNOGENETIC APPROACH. Rawlings DJ*, Keister DB, and Kaslow DC. Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

Pfs40, a 40 kDa sexual stage specific surface protein of *Plasmodium falciparum* parasites, was first identified as a potential target antigen of transmission blocking (TB) immunity by an immunogenetic approach. Limited host MHC Class II interaction with Pfs40 in a series of congenic mouse strains suggested that this molecule had a paucity of T helper epitopes as a consequence of immune selection. Pfs40 was purified by two dimensional gel electrophoresis (2DGE), digested with trypsin, and three of the resulting HPLC-purified tryptic peptides microsequenced. Oligonucleotides were used to clone and sequence the full length cDNA and genomic DNA encoding Pfs40. The deduced amino acid sequence

predicted an integral membrane polypeptide of 43 kDa, containing all three tryptic peptides microsequenced, and five EF hand calcium binding domains. The biological activity of one or more of these domains was confirmed by binding of 45Ca to both native and recombinant Ffs40. Antisera to the latter specifically immunoprecipitated a radio-iodinated 40 kDa protein present in gametes, recognized the native 40 kDa calcium binding protein by 2DGE immunoblot, and reacted specifically with sexual stage parasites by indirect immunofluorescence. Ultrastructural location of Pfs40, TB activity of anti-Pfs40 sera, and the role of Pfs40 in calcium dependent events of exflagellation and fertilization will be discussed.

320 EXPRESSION-PCR STRUCTURE-FUNCTION ANALYSIS OF AN ERYTHROCYTE BINDING ANTIGEN (EBA-175) AND A CYTOADHERENCE RECEPTOR (CD36) FOR *PLASMODIUM FALCIPARUM*. Kain KC*, Ockenhouse CF, Orlandi PA, and Lanar DE. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

We recently described a rapid system called expression-PCR (E- PCR) for *in vitro* synthesis of functional protein from genomic, plasmid, or reversed transcribed DNA. In this system a universal promoter (UP) is used containing an untranslated leader sequence from alfalfa mosaic virus directly downstream from the T7 bacteriophage promoter. When this UP is spliced to a DNA segment it produces a suitable template for *in vitro* transcription and translation. In this study, E-PCR was used to characterize structure-function relationships of proteins that are vaccine candidate antigens for *Plasmodium falciparum* including EBA-175 and the cytoadherent receptor for infected RBCs (CD36). The functional domains of EBA-175 and CD36 were mapped by binding analysis of a series of overlapping polypeptides of EBA-175 and CD36 produced by E-PCR. In addition E-PCR synthesized proteins were used as immunogens to produce antisera. Unlike standard methods for *in vitro* transcription and translation, E-PCR is not dependent upon specialized transcription vectors, cloning, plasmid purification, or restriction enzyme sites. E-PCR represents a significant improvement over current *in vitro* expression systems, and is a rapid and simple method to define biologically significant domains of vaccine candidate antigens and to generate antisera.

321 THE INFECTED ERYTHROCYTE RECEPTOR(S) FOR CD36 AND THROMBOSPONDIN ARE RESTRICTED TO KNOBS. Nakamura K*, Howard R, Hasler T, and Aikawa M. Case Western Reserve University, Institute of Pathology, Cleveland, OH; and DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA.

Cytoadherence of parasite-infected red blood cells (PRBC) to the cerebral microvasculature is considered to be a major contribution to the pathogenesis of cerebral malaria. Both CD36 and thrombospondin (TSP) are expressed on the surface of endothelial cells and are believed to be receptors for PRBC. There has been no clear demonstration, however, of the distribution of receptor molecules for CD36 and TSP on the surface of PRBC. In this study, CD36 and TSP were purified from human platelets and mixed with PRBC to allow affinity binding to their respective receptors. After one hour incubation, receptor-bound glycoproteins were visualized immunocytochemically using specific antibodies to CD36 and TSP and gold-conjugated secondary antibodies. Samples were then fixed with 2.5% glutaraldehyde, embedded in epon and thin sections cut and observed with a Zeiss CEM 902 electron microscope. Affinity labeling of CD36 and TSP to the PRBC surface showed gold particles associated specifically with knobs. Our study demonstrated for the first time that the distribution of receptors is restricted to the knobs.

322 MACROPHAGE PRODUCTION OF $NO_2^-$ DURING *PLASMODIUM CHABAUDI* AS INFECTION IN C57BL/6 MICE. Nowotarski ME* and Stevenson MM. Centre for the Study of Host

Resistance, McGill University, Montreal, Quebec, Canada; and The Montreal General Hospital Research Institute, Montreal, Quebec.

Intraperitoneal infection of C57BL/6 (B6) mice with $10^6$ *Plasmodium chabaudi* AS parasitized red blood cells results in a transient course of infection characterized by a peak parasitemia of 35% on days 9-10 and clearance of the parasite by day 28. The development of acquired immunity to blood-stage infection with this murine *Plasmodium* species is dependent upon an antibody-independent, cell-mediated mechanism and requires T cells and macrophages. We have previously demonstrated that Ia antigen expression by peritoneal and splenic macrophages increases significantly during infection in B6 mice and that these cells produce significant levels of TNF as well as $H_2O_2$ and $O_2^-$ *in vitro*. The peak of these three responses, which are indices of macrophage activation, occurs just prior to the peak parasitemia. As another marker of macrophage activation, we examined the production of $NO_2^-$ *in vitro* in response to LPS by macrophages recovered from B6 mice during infection. Both peritoneal and splenic macrophages produced significantly higher levels of $NO_2^-$ than cells recovered from normal, uninfected animals; similar to the other markers of macrophage activation examined, the peak production occurred just prior to the peak parasitemia. Maximum $NO_2^-$ production by peritoneal macrophages from *P. chabaudi* AS infected B6 mice was approximately 2-fold that of the normal response while splenic macrophages produced greater than 10-fold as much $NO_2^-$. These results, thus, demonstrate that macrophages recovered from *P. chabaudi* AS infected B6 mice produce significant levels of $NO_2^-$.

## R: HELMINTH IMMUNOLOGY

323 COMPARISON OF ANTIGENS RECOGNIZED BY MICE VACCINATED WITH MODERATE OR HIGH DOSE IRRADIATED CERCARIAE OF *SCHISTOSOMA MANSONI*. Richter D* and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA

Cercariae exposed to a moderate dose of irradiation (15 kRad) have been shown in our laboratory to confer a higher degree of protection to mice than do cercariae exposed to 50 kRad irradiation. To define antigens that might cause this increased level of resistance, mice of two strains (C57BL/6 and CBA/J) were repeatedly vaccinated with either 15 or 50 kRad irradiated cercariae. Using soluble preparations of cercariae as well as male and female adult worms fractionated by isoelectric focussing in the Rotofor cell, the antibody response and T-cell proliferation of the four groups of mice were compared. C57BL/6 mice generated antibodies to the same antigens, no matter whether they were vaccinated with moderate or high dose irradiated cercariae. In the other strain, however, a differential humoral response dependent on irradiation dose was observed. Overall, antigens of approximately 22-23, 28, 70, and 97 kD, present in all three preparations, were detected by the antisera. Peak proliferation of T cells was stimulated by the same pI fractions that contained antigens recognized by the antisera. Using these antigens in purified or recombinant form, at least one (22-23 kD) has been shown to stimulate T-cell proliferation. This is the first study characterizing antigens recognized by both the humoral and cellular arms of the immune response of mice vaccinated with irradiated cercariae.

324 PROTECTIVE ACTIVITY OF T CELL LINES AND CLONES FROM MICE VACCINATED AGAINST *SCHISTOSOMA MANSONI*. Williams M*, Caspar P, Hieny S, Sher A, and James S. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

T cell responses are important for the development of protective immunity to schistosomiasis in several experimental models. One postulated mechanism is the secretion by T cells of cytokines, such as IFN-γ, that activate macrophage effector cells. We have isolated several T cell clones from C57BL/6 mice immunized intradermally with soluble *S. mansoni* worm antigen plus BCG. These clones are CD4+ Th1 cells as judged by IFN-γ and IL-2 secretion and lack of IL-4 and IL-5 production. One of these clones has

been found to transfer protection in an intraperitoneal transfer and challenge model. This clone does not appear to recognize *S. mansoni* paramyosin, an antigen that was previously shown to protect using this vaccination protocol, but responds to a limited number of worm antigen fractions separated by anion exchange FPLC. We have also produced an IL-5 secreting spleen cell line from BALB/c mice vaccinated once with 500 radiation attenuated cercaria, a model in which antibody production is believed to play a role in protection. Preliminary evidence suggests that this line is also protective in the i.p. transfer system. Attempts to further characterize the antigens recognized by these clones and by T cells obtained directly from animals are currently underway in an effort to identify potential immunogens for vaccine production.

325 *IN VIVO* ROLE OF EOSINOPHILS IN *SCHISTOSOMA MANSONI* IMMUNITY IN MURINE MODEL OF IRRADIATED VACCINE. Othman MI* and Higashi GI. University of Michigan, Department of Epidemiology, Ann Arbor, MI.

Schistosomiasis immunity is a complex phenomenon where cells, cytokines and antibodies play a role. This work was initiated to study the *in vivo* role of eosinophils in this immunity engendered by the irradiated vaccine model using 2CD11 monoclonal antibody (MAb) specific for eosinophils. Six experiments were performed using CBA or DBA female mice divided into 4 groups (10 mice/group). Two groups were immunized with 500 irradiated *Schistosoma mansoni* cercariae and the other 2 served as controls. All groups were challenged with 200 normal cercariae 6 weeks later. Mice were perfused 6 weeks post challenge. Blood samples were collected bi-weekly for eosinophil count and serum. One of the immunized and the control groups received i.p injections of 2CD11 mAb on day -2,-1,0,+1 and +2 of challenge at 16-20 µg /mouse /injection. The results of this study show partial abrogation of protection due to 2CD11 treatment of the immunized mice with average of 21.3% (range 9-37%) compared to 35.5% (range 22-60) of the immunized untreated groups. Eosinophilia levels which were lower in the 2CD11 groups after the first injection, continued for 10 days post challenge and then rose steadily. There were no significant differences in antibody isotype responses to different *S. mansoni* antigens due to 2CD11 mAb as measured by ELISA or western blotting.

326 LYMPHOPROLIFERATIVE RESPONSE OF MURINE SPLENIC LYMPHOCYTES TO THE MAJOR LYMPHOSTIMULATORY PEAK OF SOLUBLE EGG ANTIGEN. Quinn JJ*, Secor WE, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Splenic lymphocytes from mice 8-10 weeks post-infection with *Schistosoma mansoni* were examined for their proliferative response to the major lymphostimulatory fraction (F5), which was obtained by isoelectric-focusing of soluble egg antigen (SEA). Preliminary lymphokine analysis suggests a TH-2 response to this antigen. As previously reported, proteins within this fraction exhibited marked proliferative effects and are recognized by a partially-protective monoclonal antibody E.1. Moieties of this fraction eluted from a E.1 immunoaffinity column were further fractionated by FPLC gel filtration. The resultant fractions were tested for their ability to induce proliferation of bulk splenic lymphocytes. These fractions consistently elicited a proliferative response as high or greater than that of unfractionated SEA or F5 and was contained within two peaks (gel filtration) corresponding to E.1 binding by Elisa. Further analysis of the FPLC peaks is needed to determine the antigen(s) responsible for this proliferation as well as their ability to induce granuloma formation both *in vitro* and *in vivo*.

327 SCHISTOSOMAL EGG ANTIGEN-SPECIFIC CLONED MURINE CD4 POSITIVE TH-1 TYPE LYMPHOCYTES MEDIATE LOCAL DTH REACTIONS AS WELL AS GRANULOMA FORMATION *IN VIVO*. Stadecker MJ and Chikunguwo SM*. Tufts University School of Medicine, Boston, MA.

It is now well extablished that the granulomatous inflammation surrounding the eggs of *Schistosoma mansoni* is mediated by T helper (TH) lymphocytes. Our laboratory has recently cloned murine CD4 positive TH cells specific for schistosomal egg antigens (SEA). In the current study SEA-specific IL-2-producing TH-1-type clones were tested for their ability to mediate local delayed-type hypersensitivity (DTH) reactions as well as granuloma formation *in vivo*. Marked, delayed-onset erythema and induration developed in footpads of normal syngeneic hosts injected with SEA together with SEA-specific TH-1 clones. Histological examination of these lesions revealed typical predominantly mononuclear cell infiltrates characteristic of DTH reactions. Conversely, no reactions were observed in allogeneic hosts, or in the absence of SEA, or with the use of a control TH-1 clone. Moreover, adoptive transfer of cloned SEA-specific TH-1 cells to normal syngeneic mice, mediated, in four days, the formation of vigorous granulomas around schistosomal eggs embolized in the lungs. Such granulomas, which were quantitated by computer-assisted morphometric analysis, were comparable in size to those elicited by lung-embolized eggs in SEA/CFA immunized mice. In contrast, significantly smaller granulomas were observed in normal recipients of eggs plus a control TH-1 clone, or of eggs alone. Our data indicate that local DTH reactions, as well as egg granuloma formation *in vivo*, can be mediated by monoclonal SEA-specific TH-1 cells. They suggest that T cell sensitization to only small numbers of SEA determinants may be sufficient to elicit the hepato-intestinal granulomatous inflammation associated with schistosomiasis.

328 CHARACTERIZATION OF IMMUNE RESPONSES OF BRAZILIAN SCHISTOSOMIASIS MANSONI PATIENTS TO PARTIALLY PURIFIED EGG ANTIGENS. Secor WE*, Reis MG, Reis E, Quinn JJ, David RA, David JR, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, FIOCRUZ, Bahia, Brazil.

Peripheral blood mononuclear cells (PBMC) from intestinal (INT), hepatosplenic (HS), and former (FRM) patients and spleen mononuclearcells (SMC) from HS patients were tested for their ability to proliferate in response to schistosome soluble egg antigens (SEA) which had been partially purified by isoelectric focussing. Cells from all groups responded most strongly to antigens in a pool of fractions (R.3) with a pI of 3.5 - 4.3. As noted in other studies, the percentage and magnitude of former patients' responses to SEA or R.3 was higher than that of intestinal and hepatosplenic patients. In addition, intestinal patients with low or moderate numbers of eggs excreted in their feces had stronger proliferative responses to SEA and R.3 than patients with greater than 800 eggs per gram feces. In contrast, the vaccine candidate triose phosphate isomerase (TPI) stimulated patients' cells at similar percentages and magnitudes (although at lower levels than to SEA or R.3) regardless of clinical form or egg excretion levels. All patients were also typed for MHC class II and selected patients' cells were used to produce lymphokine production profiles for the various antigens. Continuing work includes further fractionation and testing of the stimulatory antigens.

329 SEA REACTIVE HUMAN T CELL CLONES: ANALYSIS OF GRANULOMA FORMATION AND LYMPHOKINE PRODUCTION. Moyes RB*, Kennedy DA, Cao M, and Doughty BL. Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

We have developed *Schistosoma mansoni* soluble egg antigen (SEA) reactive human T cell clones in order to further elucidate the complex immunological events which are associated with granulomatous hypersensitivity. Using the *in vitro* granuloma model to screen for granuloma producing T cells, we have characterized several SEA reactive T cell clones. Some clones are associated with the formation of granulomas while others are not. All of the SEA reactive clones are CD3+, CD4+, CD8- but possess different defined antigenic specificities as well as different lymphokine profiles. Recombinant egg proteins were cloned from an egg cDNA library, sequenced, expressed, and purified. The recombinant

proteins were used to further define the antigen specificity of SEA reactive T cell clones and to analyze granuloma formation. These T cell clones will provide us with the reagents to analyze the antigenic and cellular components of granulomatous hypersensitivity in human schistosomiasis at the molecular level. Information from the analysis of these effector functions will provide insight into the immunopathology associated with this disease.

330 CONSEQUENCES OF NEONATAL IDIOTYPIC MANIPULATIONS ON SUBSEQUENT SCHISTOSOME INFECTIONS IN MALE AND FEMALE CBA/J MICE. Eloi-Santos S*, Harrell H, Bosshardt S, Nix N, Correa-Oliveira R, and Colley D. Department of Microbiology and Immunology, Vanderbilt University School of Medicine, and Veterans Administration Medical Center, Nashville, TN; Centro de Pesquisas, "Rene Rachou", FIOCRUZ, and Dept. Bioquimica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; and Meharry Medical College, Nashville, TN.

During asymptomatic schistosomiasis mansoni patients express dominant, cross-reactive idiotypes (CRI) on anti-SEA Abs that stimulate anti-idiotypic (anti-Id) T lymphocytes and regulatory responses. These CRI are present throughout chronic infection, and are expressed on some human anti-SEA mAbs (such as E5). Anti-CRI T cells develop in utero in children of infected mothers. Some of these CRI occur in sera of acutely infected mice, but decrease from 8-16 weeks after infection. Mice born of mothers with or without schistosomiasis were infected as adults. No differences were seen in the survival of these 2 groups. Most were born of multiparous, chronically-infected mothers, not expected to have high levels of CRI. To test possible effects of CRI more directly, at birth, normal mice were injected with 50 μg of mouse CRI preparations (8WkId), >20WkId, normal Ig, mAb E5, or mouse anti-E5 mAbs. They were infected 8 weeks later. Survival curve data show that mice injected once neo-natally with 50 μg of 8WkId or E5 live, on average, 3-5 weeks longer than if injected at birth with anti-E5 mAbs or >20WkId. We are currently trying to establish if there is a definable immunological basis for these survival curve differences which appear to be due to differential neonatal exposure to CRI+/CRI- anti-SEA Abs or anti-CRI.

331 IMMUNOLOGICAL PROFILE OF INDIVIDUALS RESIDING IN AN ENDEMIC AREA FOR SCHISTOSOMIASIS MANSONI. Alves-Oliveira LF, Silveira AM, Souza A, Filho J, Parra JC, Doughty BL, Colley DG, Correa-Oliveira R, and Gazzinelli G. Faculdade de Odontologia de Governador Valdares, Brazil; Fundacao Servico Hospitalar de Governador Valadares, Brazil; College of Veterinary Medicine, Texas A & M University, College Station, TX; Veterans Administration Medical Center; Vanderbilt University, Nashville, TN; and Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, Brazil.

An epidemiological survey of endemic areas for schistosomiasis mansoni revealed a large variation of the parasitemia of the infected population. This variability could be reflecting the frequency of contact with infected water, and susceptibility to infection. Recent studies on the incidence of reinfection and access to transmission sites, suggest that partial resistance to superinfection occurs in relationship to age. Our goal was to investigate cellular and humoral immunological parameters related to resistance to reinfection. Preliminary studies in the area of Corrego do Bernardo, city of Governador Valdares, Brazil, showed a prevalence of 80% between the ages 5 to 39 years, and maximal parasitic load of 428 egg/g of feces in male patients between 20 and 24 years old. The lymphocyte blastogenesis response to *S. mansoni* antigens from intestinal schistosomiasis patients confirmed previous results, showing immunoregulation of the proliferative response of peripheral blood mononuclear cells (PBMC) to soluble egg antigen (SEA). "*In vitro*" granuloma studies were performed by incubating PBMC in the presence of SEA conjugated polyacrylamide beads. Granuloma reactivity, measured as a granuloma index (GI), did not reveal any differences with regard to age or sex.

332 HUMAN IDIOTYPE AND ANTI-IDIOTYPE MOLECULES IN SCHISTOSOMIASIS JAPONICA. Wisnewski A*, Cheever LW, Chireau M, Olds GR, and Kresina TF. Brown University, Providence, RI.

Idiotypic antibodies binding soluble egg and worm antigens of *Schistosoma japonicum* and anti-idiotype antibodies binding specific egg antigen associated idiotypes were generated by EBV transformation of peripheral blood lymphocytes. One polyclonal idiotypic EBV transformant, LO2C2 derived from an acutely infected 30 yr old male, expresses a major cross-reactive idiotype with virtually identical Western Blot antigen binding patterns as polyclonal pooled serum antibodies derived from acutely infected patients. Three human monoclonal antibodies derived from LO2C2 serologically define the human major cross-reactive idiotype. Anti-idiotype EBV transformants free of antigen binding activity were generated which recognize 30-72% of the LO2C2 idiotype. Two such anti-ids LO16AC2 and LO12BD4 suppressed antigen specific blastogenesis of human lymphocytes derived from *S. japonicum* infected patients (44 and 66%, $p<0.05$). Alternatively anti-id LO11BB4 stimulated lymphocyte blastogenesis (greater than 200 fold) in the absence of antigen, thus exhibiting internal image of antigen activity. The data show the presence of a major cross reactive idiotype associated with human *S. japonicum* egg and worm antigen binding antibodies and immunoregulatory anti-idiotypic antibodies associated with this idiotype.

333 ANALYSIS OF CELLULAR IMMUNITY TO FRACTIONATED *FASCIOLA HEPATICA* ANTIGENS IN A HYPERIMMUNE BOVINE MODEL. Nodland KI, Hasan S, Moyes RB, Suderman MT, and Doughty BL. Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

*Fasciola hepatica* is a parasitic trematode that infects cattle, sheep and man. Fascioliasis is characterized by fever and eosinophilia during the acute migratory phase and by biliary obstruction, biliary ductal hyperplasia, and cholangitis during the chronic phase. Significant production losses occur in herds infected with *F. hepatica*. Drug treatment is the only method of cure and does not provide continued protection. To understand the host protective immune response, we have studied T cell responses to *F. hepatica* soluble adult worm antigen proteins (SWAP) in cattle made immune to challenge infection. Our results indicate that these hyperimmune cattle have a heterogeneous T cell response to a number of fractionated *F. hepatica* antigens. These observations were noted in four separate experiments where longitudinal studies were conducted by antigen specific lymphocyte transformation assays with peripheral blood mononuclear cells (PBMC's). Anti-*F. hepatica* antibody titers and IL-2 production in PBMC's stimulated by SWAP gave increased values in hyperimmune cattle. The cellular immune responses of hyperimmune cattle in a T cell immunoblot correlated with increased resistance as measured by decreased worm burden. The immunodominant T cell antigens are approximately 125, 85,65, 50, 44, 41, 36, 30, 24, 18, 16, and 14 kD.

334 PRESENCE OF IgE OR IgE-LIKE RECEPTOR IN THE NEMATODE PARASITE OF MICE *HELIGMOSOMOIDES POLYGYRUS*. Enriquez FJ*, Bradley-Dunlop D, and Boggavarapu J. Hybridoma Technology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ.

Serum IgE levels increase during helminth infections, but this increase has not been adequately studied following infections with *Heligmosomoides polygyrus*, an intestinal nematode parasite of mice and an excellent laboratory model. Unsuccessful attempts to identify parasite-specific serum IgE responses by ELISA following infections, prompted us to study the possibility that an IgE or an IgE-like receptor present in the parasite and its homogenates would consistently cause false positive results. For this purpose, an *in vivo* worm modified ELISA was developed. Live adult parasites, previously incubated in Tris buffer pH 3.0 (to dissociate bound Ig) or pH 7.2, were incubated in suspension with either immune serum, normal serum, or an irrelevant murine IgE monoclonal antibody (mAb) to DNP, followed by goat

anti-mouse IgE peroxidase labelled antiserum. IgE was present in eluate washes from worms incubated with Tris buffer pH 3.0. In addition, irrelevant IgE mAb to DNP, IgE present in normal mouse serum, as well as high amounts of IgE present in immune serum, were independently identified on the surface of live adult worms and all of these IgE Abs increased when worms were previously dissociated of bound Igs by low pH treatment. It was concluded that luminal *H. polygyrus* adult worms may attract parasite-specific and non-parasite-specific IgE via an IgE or an IgE-like receptor in a strategy to promote their own survival in the intestinal lumen.

## S: OPPORTUNISTIC INFECTIONS

335 EVALUATION OF AN IMMUNOASSAY FOR THE DETECTION OF *CRYPTOSPORIDIUM* IN STOOL SPECIMENS. Sloan LM* and Rosenblatt JE. Mayo Clinic and Foundation, Rochester, MN.

We evaluated a commercially produced ELISA (LMD Laboratories, Inc.) for the detection of *Cryptosporidium* in 231 stool specimens submitted to the Mayo Clinic parasitology laboratory for routine examination. The specimens examined were either fresh (4), or were stored frozen at -65C (46), or were formalin preserved (181). Results were compared to those obtained by indirect fluorescent antibody (IFA) detection (Merifluor-TM, Meridian Diagnostics, Inc.). Sixty-two of the specimens were positive by IFA and ELISA while 159 were negative by all methods; 88 of these negative stools contained 116 other parasites belonging to 16 different species. Nine ELISA "false negatives" were observed. These were all formalin preserved samples positive for *Cryptosporidium* by IFA. One "false positive" was noted on a frozen stool. The ELISA sensitivity was 87%, specificity was 99%, and positive predictive value was 98%. Storage of specimens preserved in formalin or fresh frozen at -65C for up to 18 months did not appear to affect the results. There was no cross reactivity with *Giardia lamblia* (52 negative specimens) nor with 15 other parasites present in the ELISA negative stools. The ELISA is a fast, easy-to-read, and accurate method for the detection of *Cryptosporidium* in stool specimens.

336 DETECTION OF *CRYPTOSPORIDIUM PARVUM* DNA IN FIXED, PARAFFIN-EMBEDDED TISSUE BY THE POLYMERASE CHAIN REACTION. Laxer MA*, D'Nicuola ME, and Patel RJ. Department of Infectious and Parasitic Disease Pathology, Armed Forces Institute of Pathology, Washington, DC; and American Registry of Pathology, Armed Forces Institute of Pathology, Washington, DC.

Previously constructed primers (26 bp) and probe (20 bp) specific for a 452 base sequence of *Cryptosporidium parvum* DNA were used to detect organisms in fixed, paraffin-embedded tissue by polymerase chain reaction (PCR). Six paraffin-embedded specimens positively diagnosed as cryptosporidiosis by light microscopy were obtained from the archives of the Armed Forces Institute of Pathology (AFIP). From each sample, six 6 μM-thick sections were cut from the blocks and placed in 1.5 ml microcentrifuge tubes. The sections were deparaffinized in xylene, rehydrated through a series of alcohols, vacuum dried, resuspended in digestion buffer with Proteinase K and sodium dodecyl sulfate (SDS), and incubated for 2 hours at 65°C. The DNA was purified by routine methods, added to the PCR mix, and run for 35 cycles. Positive control was 10 ng of purified 452 base target sequence DNA. Negative controls were known *C. parvum*-negative fixed, embedded tissue, *C. parvum* positive tissue run with human primers, and one each reaction tube minus *Taq* polymerase, primers, and template DNA. PCR products were electrophoresed in agarose gels, vacuum blotted to nylon membranes, hybridized with digoxigenin-11-dUTP labeled probe, and detected by chemiluminescent substrate exposing Kodak XAR film. All six histologically positive samples showed amplification in ethidium bromide-stained agarose gels; specificity was confirmed by lumigrams. No amplification was detected in the negative controls. We believe this system offers a specific and sensitive method of detecting *C. parvum* in fixed, paraffin-embedded tissue that will be useful in retrospective studies.

337 DETECTION OF *CRYPTOSPORIDIUM* BY PCR AMPLIFICATION OF SMALL SUBUNIT RIBOSOMAL RNA. Pieniazek NJ*, Arrowood MJ, Mathews HM, and Slemenda SB. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

*Cryptosporidium parvum*, a coccidian parasite, is a frequent cause of diarrhea in humans. Water-borne epidemic outbreaks have been described. Currently, epidemiologic investigations are limited as no sensitive and selective method exists for detecting *C. parvum* oocysts in environmental samples (e.g. water sources). The objective of this study was to develop molecular probes that would both facilitate such detection and help to exclude other coccidians not of epidemiologic importance. DNA was isolated from *C. parvum* oocysts, and a region of small subunit ribosomal RNA (srRNA) was amplified using primers binding to conserved srRNA regions designed after universal rRNA sequencing primers and the known srRNA sequences of other coccidians. A 1008 bp fragment was amplified, cloned and, sequenced. Sequence comparison to other coccidian srRNA sequences showed that *C. parvum* srRNA contains an unusual structure of the V4 srRNA variable region and a unique site for the restriction nuclease SspI. PCR primers designed to amplify this unique segment of srRNA were found to be a good tool for sensitive and specific detection of *C. parvum* in samples seeded with oocysts.

338 EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS IN THE NORTHEAST OF BRAZIL. Newman RD*, Lima AM, Castro MX, Guerrant RL, and Weikel CS. Johns Hopkins University School of Medicine, Baltimore, MD; University of Virginia School of Medicine, Charlottesville, Va; and Universidade Federal do Ceara, Fortaleza, Brazil.

*Cryptosporidium sp.* is an important cause of diarrheal disease in children worldwide. To better understand the epidemiology of cryptosporidiosis, we have begun a prospective, 5 year study of this disease in a newborn cohort in an impoverished urban community of 405 families in Fortaleza, Brazil. We report here our observations on 84 children enrolled during the first 15 months of 3 time weekly surveillance for diarrheal disease and for whom at least 3 months of data were available. Of 159 diarrheal episodes sampled, 23 stool smears (14.5%) were positive by acidfast and auramine stains for oocysts of *Cryptosporidium.*; only 2 of 98 (2%) formed control stools were *Cryptosporidium* positive. Thus, 25 cohort children (29.8%) were diagnosed with cryptosporidiosis in the first 2 years of life. Eighty-seven percent of cases occurred during the rainy months. The median age of children with *Cryptosporidium* was 10 months with no cases documented before 3 months of age despite the introduction of nonbreast milk foods at a median of 1 month of age. Diarrhea lasted a mean of 12 days (range 3-36 days) and was associated with vomiting (70%), and fever (59%). Dehydration was mild to moderate; one child was hospitalized. No stools were grossly bloody but 3 had ≥5 leukocytes/hpf. Only 2 children were documented to have concomitant infections (*Trichuris, Giardia*) by routine stool examination. In summary, cryptosporidiosis is the leading recognized cause of diarrheal disease documented by routine stool examination in children less than 2 years of age in the northeast of Brazil. Illnesses are lengthy and strikingly seasonal. Further studies are necessary to identify the sources of the parasite and the long term impact on growth and development.

339 CRYPTOSPORIDIOSIS AMONG PATIENTS WITH THE ACQUIRED IMMUNODEFICIENCY SYNDROME IN VENEZUELA. Bonilla LC*, Guanipa N, Raleigh X, Cano G, and Quijada L. Instituto de Investigaciones Clinicas. Universidad del Zulia, Maracaibo, Venezuela.

In patients with the acquired immunodeficiency syndrome (AIDS), the coccidian protozoan *Cryptosporidium* has been commonly implicated as a cause of chronic diarrhea. In the United States, as many as 11%-28% of these patients have the infection; in Brazil, 12%-17%; in Africa, 30%-50%; and in Haiti, 46%. In Venezuela, there is scarce information about cryptosporidiosis. Infection rate of 4% to 10.8% in hospital children with gastroenteritis and 15.5% of seropositivity, for both specific IgG and IgM antibodies, determined with an enzyme-linked immunosorbent assay, have been reported in children.

Here we present a study designed to evaluate the prevalence of *Cryptosporidium* in patients with AIDS from Maracaibo, the capital city of Zulia State. Twenty one out of the twenty five outpatients that attend the Regional Immunology Unit were studied. They were aged 7 to 46 years and had mild to severe diarrhea. Three stool specimens were collected from each patient. For the recovery and identification of *Cryptosporidium* the modified Ziehl-Neelsen carbolfuchsin stain on 10% formalin preserved stool was used. For the diagnosis of other parasites, direct wet mounts, iron-hematoxylin stained smears and formol-ether concentrates were examined. Eight (38%) of the patients had *Cryptosporidium* oocysts, most of them revealed an inflammatory exudate and two had Charcot-Leyden crystals. Other pathogenic parasites observed were: *G. lamblia* (23.8%), *B. hominis* (19%), *E. histolytica* (9.5%), *S. stercoralis* (9.5%), *T. trichiura* (4.7%) and *A. lumbricoides* (4.7%). *Cryptosporidium* appears to be very frequent in patients with AIDS in Venezuela. The infection rate observed is higher than the observed in the United States and Brazil and similar to the frequencies reported from Haiti and Africa.

340 PROLIFERATIVE RESPONSIVENESS OF LYMPHOCYTES FROM *CRYPTOSPORIDIUM PARVUM* EXPOSED MICE TO TWO SEPARATE ANTIGEN FRACTIONS FROM OOCYSTS. Moss DM* and Lammie PJ. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA

Neonatal SWR-$H^q$ mice inoculated with $3 \times 10^4$ *Cryptosporidium parvum* oocysts develop a self-limited infection. Parasites were demonstrable in the intestines through day 13 post inoculation (PI). Proliferative responsiveness of lymphocytes from spleen and lymph nodes of *C. parvum* exposed mice was determined by culturing the lymphocytes with two different antigen preparations, a water-soluble (WS) and a urea-soluble (US) antigen fraction from *C. parvum* oocysts. Lymphoproliferation was assessed at day 10 PI and then every third day through day 28 PI. Lymphocytes from lymph nodes of exposed mice cultured with WS antigen exhibited a significant and antigen-specific response compared to age-matched unexposed mice at days 10, 19, 22, and 28 PI. US antigen cultured with lymphocytes from lymph nodes demonstrated a significant and antigen-specific response at days 10 and 19 PI. No significant response occurred when splenic lymphocytes were cultured with WS or US antigen. Experiments with intestinal epithelial lymphocytes are planned. This model may be useful for defining the nature of the lymphocyte populations elicited by *Cryptosporidium* infection and their relationship to elimination of the parasite.

341 GROWTH CHARACTERISTICS, ELECTRON MICROSCOPY, AND ANTIGENIC ANALYSIS OF THE MICROSPORIDIAN ISOLATED FROM THE URINE OF A PATIENT WITH AIDS. Visvesvara GS*, Leitch GJ, Moura HM, Wallace S, Weber R, and Bryan RT. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Morehouse School of Medicine, Atlanta, GA.

Microsporidian spores isolated from the urine of a dysuric patient with AIDS were inoculated onto several different cell cultures. The parasite successfully infected only 2 cell lines (HLF & Vero). Infected host cells were filled with developing stages of the parasite, including mature spores. The proliferative forms were monokaryotic and attached to the parasitophorous vacuole. In culture flasks with established infections, spores measuring 2.25 to 2.28 µm long and 1.25 to 1.85 µm wide were released into the culture medium every 3 days. Proteins extracted from the spores and separated on SDS-PAGE revealed more than 30 bands. In the immunoblot two of the patient's serum samples reacted with the spore proteins and produced several bands ranging in molecular weight from 10,000 to 200,000. Major bands, however, occurred in the area between 67 to 94 kDa. The proteins from our isolate also reacted with the sera of 2 patients with ocular microsporidiosis (OM). In the IIF test, our patient's sera as well as those of the 2 patients with OM reacted with our isolate and gave a titer of 64. None of 6 sera from patients with

intestinal microsporidiosis reacted with our isolate in either the IIF or the immunoblot assays. Based on these observations, we conclude that the parasite isolated belongs to the genus *Encephalitozoon*.

342 PRESENTATION OF *TOXOPLASMA GONDII* ANTIGENS TO CD8+ T LYMPHOCYTES INVOLVES A RESTRICTED SET OF ANTIGENIC POLYPEPTIDES Denkers EY*, Gazzinelli RT, Hieny S, and Sher A. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

Murine bone marrow macrophages were sensitized for CTL-mediated lysis and were able to stimulate CD8+ interferon-γ production when incubated with soluble *T. gondii* extract. Sonicated parasites were centrifuged at 10,000 x g and the resulting supernatant was centrifuged at 100,000 x g. When these supernatants and the associated pellets were tested in the cytotoxic assay, the supernatant remaining after high speed centrifugation was found to contain most of the antigenic activity. This active fraction was subject to anion exchange chromatography, and virtually all of the antigenic activity was associated with two discrete fractions. Size separation of the soluble antigens demonstrated that proteins of $M_r$>5000, rather than peptide fragments, induced the CD8+ T cell responses. However, protease treatment of these macromolecules enhanced the ability to drive CD8+ responses. Several additional cell types were tested for ability to present *T. gondii* antigens, and although all could present antigen when infected with live tachyzoites, none could present soluble antigen. In contrast, these other cell types were able to present soluble *T. gondii* antigens that had been pre-incubated with bone marrow macrophages. Together, the results suggest that the CD8+ response may be induced by relatively few immunodominant *T. gondii* antigens, and that bone marrow macrophages extracellularly process the antigens for CD8+ recognition. We are currently using this system to identify the antigenic polypeptides serving as targets for CD8+ mediated immunity.

343 ASSOCIATION OF CD8+ LYMPHOCYTE SUBSETS WITH IgM RESPONSES IN *TOXOPLASMA* INFECTED MACAQUES. Warren JJ*, Morton WR, and Fritsche TR. Department of Laboratory Medicine, University of Washington, Seattle,WA; and Regional Primate Research Center, University of Washington, Seattle, WA.

Recent studies have demonstrated that CD4+ and CD8+ lymphocytes play important roles in the immunologic containment of *Toxoplasma*. The evidence in murine models suggests a more important role for CD8+ than for CD4+ cells. To determine the relative roles for both CD4+ and CD8+ lymphocytes in acute *Toxoplasma* infection in a macaque model, lymphocyte marker studies have been performed during the first six weeks post infection (pi). Lymphocytes were marked with Lue 3a for CD4 and Lue 2a for CD8. CD4 activation was determined with the antibody 3AC5 to identify CD45RA. In addition, serologic monitoring for IgM (antibody capture assay) and IgG (indirect immunofluorescence assay) has been performed. All 5 animals displayed typical IgG seroconversion and peak IgM responses at 2 weeks pi. In 3 animals the IgM was undetectable at 6 weeks, but remained positive in 2 others. Those animals with undetectable IgM at 6 weeks had an average increase in CD8 cells of 17% at 2 weeks pi. In those with persisting IgM the average increase was only 9%, and was not apparent until 5 weeks pi. All animals showed activation of CD4 lymphocytes with an average increase in CD4+/CD45RA+ cells of 20%, 21%, and 20% at 1, 3, and 6 weeks pi, respectively. Lymphocyte marker studies will also be performed after 4 of these animals are infected with simian immunodeficiency virus. Our goal is to establish a primate model of toxoplasmic encephalitis, and evaluate the roles of CD4+ and CD8+ lymphocytes in immunologic containment not only in acute infection but also during reactivation. These results suggest CD8+ lymphocytes function in containment of *Toxoplasma* in macaques.

344 A MUTANT STRAIN OF *TOXOPLASMA GONDII* RESISTANT TO BENZIMIDAZOLES. Schwartzman JD*, Roberts K, and Tirrell R. Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA.

In order to study the relationship between parasite cytoskeleton, parasite motility and invasiveness we have selected strains of *Toxoplasma gondii* which are resistant to mebendazole and albendazole, two drugs which are known to interact with tubulin and cause microtubule depolymerization in other systems. Although coccidia have been thought to be resistant to the effects of benzimidazoles, we found that high concentrations of these drugs effect the morphology and invasiveness of *T. gondii*. The parental strain of *T. gondii* (RH) undergoes a morphological change to a rounded configuration and invasion of host cells is decreased by more than 50% at 100 micromolar mebendazole. A cloned mutant strain selected from an ethyl nitrosourea mutagenized population of RH exposed to 200 micromolar mebendazole exhibits no morphological change at 100 micromolar mebendazole and invasiveness is not inhibited 50% until 150 micromolar mebendazole. This strain has sensitivity equal to the RH parental when exposed to the antimetabolite 5'fluorouracil, suggesting that the mutant is not resistant to the drug on the basis of a change in permeability to uncharged molecules. The mutant strain is also resistant to similar concentrations of albendzole. The effect of benzimidazoles on *T. gondii* microtubule organization is under study. Mutant strains with defined defects of cytoskeletal organization will be useful in studying the process of host cell invasion.

345 CLOSE ASSOCIATION OF *PNEUMOCYSTIS CARINII* TROPHIC FORMS WITH CULTURE CELLS AS SHOWN BY IMMUNE SPECIFIC STAINING AND ELECTRON MICROSCOPY. Bartlett MS*, Goheen MP, Queener SF, Durkin MM, Shaw MM, and Smith JW. Indiana University School of Medicine, Indianapolis, IN.

*Pneumocyctis carinii* from infected rat lung has been used in culture systems employing feeder cells to produce large numbers of organisms and to study the effects of drugs on organisms. The role of feeder cells has been questioned. Using antibody from rats convalescing from *P. carinii* pneumonia and rabbit anti-rat immune globulin labeled with peroxidase to stain *P. carinii* infected and control cell monolayers, a close association of *P. carinii* with cell surfaces of human embryonic lung cells (HEL) was demonstrated. Electron micrographs of HEL, human embryonic lung fibroblasts (WI-38) and embryonic feline lung fibroblasts (AKD) demonstrated *P. carinii* trophic forms invaginating into cell surfaces. With WI-38, HEL and AKD cells the *P. carinii* cell association is prominent and frequent; however, other cell lines such as Chinese hamster ovary (CHO) with which there is no organism proliferation have no attachment of *P. carinii* to cells. In culture of rat *P. carinii* with cells, attachment appears to be essential for proliferation.

346 AGE-RELATED DISTRIBUTION AND SEVERITY OF HUMAN BABESIAL INFECTION Pollack RJ*, Telford SR III , Krause PJ, Ryan R, Zemel L, Brassard P, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; University of Connecticut Health Center, Hartford, CT; and Block Island Clinic, Block Island, RI.

Human illness, due to infection by *Babesia microti*, has been reported in hundreds of adults, but relatively few children. This suggests either that children are infected less frequently than are adults or that infection in children in less readily diagnosed. To explore these possibilities, we conducted a prospective serological survey for *B. microti* infection in residents of an endemic region (Block Island, RI). Blood was obtained from 71% of the 800 residents, including 51 children (range 5-16, mean=11 years) and 516 adults (range 17-83, mean=49 years). A standard IFA test was used to detect antibody against *B. microti*. IgG against *B. microti* was detected in 12% of children and 9% of adults; none had measurable IgM. Although babesiosis had not previously been diagnosed in any of our *Babesia*-seropositive subjects, 25% of children and 20% of adults reported that they had experienced illness compatible with babesiosis during the

previous year. Infection appears to be underreported in all age groups, but especially so in children. Diagnosis is difficult, and any clinical findings may be attributed to other more prevalent pediatric infections. These results demonstrate that babesiasis is as prevalent in children as in adults in an endemic area.

## T: FILARIAL IMMUNOREGULATION AND PROTECTIVE IMMUNITY

347 PREFERENTIAL INDUCTION BY PARASITE ANTIGEN OF IL-4 AND IL-5 SECRETING LYMPHOCYTES IN HUMAN HELMINTH INFECTION: A PRECURSOR FREQUENCY ANALYSIS. Mahanty S*, King CL, Abrams JS, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and DNAX Research Institute, Palo Alto, CA.

Interleukin (IL)-4 and IL-5 have been shown to regulate, in part, the elevated serum IgE and eosinophilia associated with helminth infections. To determine if parasite antigen (Ag)-reactive populations of T cells exist that preferentially secrete IL-4 and IL-5, CD4+ -enriched peripheral blood mononuclear cells (PBMC) obtained from helminth infected and normal individuals were assayed for the frequency of cytokine producing cells responding to parasite Ag or the non-parasite Ag tetanus toxoid (TT). The number of cells producing IL-4, IL-5, interferon-γ (IFN-γ) and granulocyte-macrophage colony stimulating factor (GM-CSF) was enumerated using a filter immunoplaque assay. In infected individuals, parasite Ag (compared to spontaneous production) induced a 100-fold increase in IL-4-secreting lymphocytes (mean = 0.12%, range: 0.06-0.21%) while TT generated only a 3.5-fold increase (mean = 0.0042%, range: 0.001-0.01%). Parasite Ag generated parallel increases in the frequency of IL-5 secreting lymphocytes (mean = 0.053% range: 0.009-0.091%). There were no detectable spontaneous or TT-induced IL-5-secreting lymphocytes. In marked contrast, the parasite Ag-induced increase in IFN-γ and GMCSF did not differ from that induced by TT. Parasite Ag failed to induce any cytokine secreting lymphocytes in normal individuals. These data indicate that parasite Ag preferentially induces IL-4 and IL-5 secretion from parasite sensitized CD4+ cells and suggest that a Th2-like subpopulation of T cells may exist in humans. This subpopulation of lymphocytes may play an important role in the development of IgE and eosinophilia.

348 *IgE PRODUCTION IN HUMAN HELMINTH INFECTION: RECIPROCAL INTERRELATIONSHIP* BETWEEN INTERLEUKIN-4 AND INTERFERON-γ. King CL*, Low CC, Mahanty S, Abrams JS, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and DNAX Research Institute, Palo Alto, CA.

To determine if the serum IgE levels that accompany human helminth infection are directly related to an increased capacity to produce IL-4 and inversely related to interferon-γ (IFN-γ) synthesis, PBMC from infected and normal individuals with excessive (EX; n=9, range 4,129 - 18,400 μg/ml), elevated (EL; n=6, range 403-1,018 μg/ml) and normal (NL; n=7, range 4-159 μg/ml) serum IgE levels were stimulated *in vitro* mitogen, parasite Ag, or tetanus toxoid (TT). Supernatants were assessed for IL-4 and IFN-γ by ELISA. Precursor frequency analysis for IL-4 producing lymphocytes was assessed using a filter immunoplaque assay. While the mitogen-induced IFN-γ synthesis was equivalent among the groups, PBMC from EX produced ~10-fold higher levels of IL-4 (geometric mean [GM] = 997pg/ml for EX and 107pg/ml for EL and 115pg/ml for NL; $p<0.01$) and was significantly correlated with serum IgE levels ($r2=0.8$, $p<0.01$). Parasite Ag (but not TT) also stimulated IL-4 production by PBMC from 8 of 9 EX (range 49-150 pg/ml), but was undetectable in EL or NL. In contrast, PBMC from EL produced significantly higher levels of IFN-γ to parasite Ag (GM=591pg/ml for EL, 98pg/ml for EX and 4 pg/ml for NL; $p=0.02$). Parasite Ag induced significant expansion of CD4+ lymphocyte populations (GM = 0.12%; range 0.05 - 0.21%) whose numbers were significantly correlated with serum IgE levels ($p=0.03$). These data indicate that helminth induced serum IgE levels are directly related to an increased capacity

of PBMC to expand and produce IL-4 and inversely related to IFN-γ production. It further supports the concept that IL-4 and IFN-γ reciprocally regulate IgE *in vivo*.

349 THE REGULATION OF EOSINOPHILIA IN ONCHOCERCIASIS: THE ROLE OF EOSINOPHILOPOIETIC CYTOKINES AND CHANGES WITH IVERMECTIN TREATMENT. Nutman TB*, Steel C, Lujan-Trangay A, and Abrams JS. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; SNEM, Ministry of Public Health, Guatemala City, Guatemala; and DNAX Research Institute, Palo Alto, CA.

IL-3, IL-5, and GM-CSF have each been implicated in the regulation of the eosinophil response. As eosinophilia accompanies infection with *Onchocerca volvulus* (Ov), the present study sought to examine 1) the role of these 3 cytokines in the regulation of the eosinophil response in onchocerciasis, and 2) the effect of ivermectin therapy on the regulation of Ov-induced eosinophilia. Eosinophil levels along with peripheral blood mononuclear cells (PBMC) were obtained from Ov– infected Guatemalans (n=27) before and at 6 monthly intervals during 2 years of semi-annual treatment with ivermectin; the PBMC were stimulated with either Ov antigen (Ag) or the non-parasite Ag streptolysin-O (SLO) and supernatants collected for measurement of IL-3, IL-5, and GM-CSF. As expected, pretreatment eosinophil levels were elevated (geometric mean [GM] = 1144/μl). This elevation was accompanied by Ov-induced IL-5 production (range 14- 150 pg/ml) in 22/27 patients (81%). This IL-5 induction exceeded the response to SLO in all individuals ($p < 0.0001$). In contrast, there was no Ag-specific induction of IL-3; for GM-CSF parasite antigen actually suppressed production relative to media controls. After 4 courses of ivermectin therapy, the eosinophil levels fell significantly (GM= 175/μl, $p < 0.01$) reaching values of 25% of those pretreatment. There was a concurrent drop in the ability to make IL-5 to parasite antigen; in all but one individual IL-5 production diminished (range 35%-100% reduction). For the other cytokines, there was either no change or an increase (GM-CSF) at the end of the study. These findings suggest that IL-5 is the critical eosinophilopoeitic cytokine in onchocerciasis.

350 SPECIFICITY OF EOSINOPHIL AND IL5 RESPONSES TO *BRUGIA* ANTIGENS IN MICE. Pearlman E*, Kroeze WK, Hazlett, Jr. FE, Mawhorter SJ, Boom WH, and Kazura JW. Case Western Reserve University, Cleveland, OH.

BALB/c mice immunized with *Brugia malayi* extract (BM Ag) and challenged intraperitoneally (ip) with microfilariae develop local eosinophilia and a predominant TH2-response (i.e., IL-4 and IL-5 production). To examine the Ag specificity of this response, mice were immunized with either BM Ag, mycobacterial proteins (PPD), or both, and cellular and Ag-driven IL-5 responses determined following ip injection of either Ag. Peritoneal cavities of mice immunized with BM Ag alone or BM Ag plus PPD developed eosinophilia following injection of either Ag. In contrast, mice immunized with PPD alone failed to develop eosinophilia after ip injection of either Ag. BM Ag-driven IL-5production by peritoneal, spleen, or lymph node cells paralleled the number of eosinophils (e.g., 825 ± 225 pg/ $5 \times 10^6$ splenocytes for BMAg- or BM Ag plus PPD-immunized mice vs. 25 ± 5 pg for PPD-immunized mice). IL-5 production by lymphoid cells of BM Ag plus PPD-immunized mice was 800 ± 350 pg following *in vitro* stimulation with BM Ag vs. 97 ± 40 pg following coculture with PPD. These data imply that Brugia Ags per se and not the mode or frequency of sensitization account for the TH2-like response that characterize this helminthic infection.

351 CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN EXPRESSION BY *IN VIVO* ACTIVATED MURINE EOSINOPHILS. Mawhorter S*, Kazura J, Pearlman E, and Boom H. Case Western Reserve University, Cleveland, OH.

To understand the possible accessory or antigen presenting function of eosinophils in helminthiases, we are investigating the expression and regulation of Class II MHC antigen (Ia) by murine eosinophils. Eosinophils were obtained from the peritoneal cavities of BALB/c mice sensitized to *B. malayi* antigens and purified to >95% by discontinuous Percoll gradient centrifugation. FACS analysis of eosinophils showed constitutive expression of class II MHC antigen which increased in parallel with the degree of hypodensity (the percentage eosinophils staining Ia positive were 35.4, 8.6 and 7.9 at the 54, 59,and 66% Percoll interfaces, respectively). Exposure of eosinophils to rIL-4 for 18 hours in the presence of conditioned medium containing IL-3 and GM-CSF increased Ia expression from 57.3% to 65.6% positive-staining cells, whereas IFN-$\gamma$ decreased Ia expression to 9.0%. rIL-5 had no effect on Ia expression. These data indicate that activated murine eosinophils constitutively express Ia and that the pattern of cytokineregulation of these molecules is similar to that documented for B cells.

352 IL-2 RESTORATION OF LYMPH NODE CELL PROLIFERATIVE RESPONSES TO BRUGIA ANTIGENS INHIBITED DURING ONSET OF PATENCY IN DOGS. Schreuer D*, Orton S, and Hammerberg B. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

We have shown that during chronic infections of dogs with *Brugia pahangi*, cells from the lymph node that drains the site of infection often fail to proliferate when stimulated with adult parastie antigen. In dogs that responded, however, unresponsiveness could be induced bysubcutaneous injection of *B. pahangi* excretory/secretory products into the infected limb. We now report that in 13 dogs infected with 10 or 20 third stage larvae, cell proliferative response to *Brugia* antigen (BpA) was detected by infected lymph node cells from all dogs in at least 1 of 3 node biopsies taken at 5, 8 and 12 weeks p.i. Moreover, 12 of the 13 dogs showed a proliferative response before the onset of patency, around 5 weeks p.i. In 7 of the 13 dogs a loss ofproliferative response to BpA by the infected node cells was detected most frequently about the time of patency onset. The response to BpA could be restored by addition of substimulatory amounts of human recombinant IL-2. In contrast, there was no loss of response to mitogens at any time by any of the dogs. Finally, the proliferative responses of peripheral blood leucocytes (PBL) did not parallel that of infected node cells, suggesting that PBL do not reflect the immunomodulatory events in the infected limb. Thus, *Brugia* infected dogs permit the study of site-of-infection immune modulation.

353 ANTI-FILARIAL IMMUNE RESPONSIVENESS OF CORD LYMPHOCYTES. Hitch WL*, Eberhard ML, Hightower AW, and Lammie PJ. Emory University, Atlanta; GA; and Parasitic Diseases Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

House to house surveys in an area endemic for Bancroftian filariasis have demonstrated that maternal infection is a risk factor for the acquisition of filarial infection and is associated with altered parasite-specific immune reactivity. The effect of in utero exposure to filarial antigens on filaria-specific immune reactivity was examined with paired cord and maternal lymphocytes. Preliminary analysis of responses in blastogenesis assays using a crude extract of the adult or mf stage of *Brugia pahangi* demonstrated limited T cell sensitization as evidenced by responsiveness (SI>3) to adult worm antigens by cells from several infants, not all of whom had microfilaremic mothers. Approximately 40% of maternal blood samples were responsive. A small proportion of cord and maternal samples were responsive to crude mf antigen. Exogenous rIl-2 was unable to reconstitute the response of nonresponsive cord lymphocytes. No significant anti-filarial IgM titers were detected in cord sera by ELISA. The presence of circulating antigen in sera was detected by ELISA using polyclonal rabbit antibody to crude adult Bp. There was no obvious relationship between the presence of Ag and cellular responsiveness in the cord samples. Examination of additional parameters of T cell activation may indicate the presence of nonresponsive or tolerant cord PBLs.

354 EVIDENCE FOR HUMORAL MECHANISMS CONTROLLING *LOA LOA* MICROFILAREMIA IN ENDEMIC RESIDENTS. Pinder M*, Martin-Prevel Y, Everaere S, and Egwang TG. International Medical Research Center of Franceville, Franceville, Gabon.

The level of microfilaremia is a critical determinant of the intensity of transmission of filarial infections. In loiasis several lines of evidence will be summarized indicating that the majority of infected subjects are amicrofilaremic even in highly endemic areas. In such areas both transmission and seropositivity are high and yet microfilaremia prevalence rarely exceeds 30% and the intensity of infection does not increase with age . Data on the relative stability of microfilaremia with time will be presented. In addition in 68 subjects with a verified ocular passage 45 were amicrofilaremic (AML). Antibody reactive with living microfilariae (mf) was present in many of these AML sera (22/37) and not in heavily microfilaremic nor control sera. Symptoms did not differ between reactive and non-reactive AML subjects. Many (11/22) IFA positive AML sera agglutinated mf and no other sera were active. Many AML sera were able to cause neutrophils and eosinophils from uninfected donors to adhere to mf in the presence of complement.

355 ANTIBODY RESPONSES TO *BRUGIA MALAYI* $L_3$ ANTIGENS IN JIRDS PROTECTED BY IMMUNIZATION WITH IRRADIATED $L_3$. Li BW*, Chandrashekar R, Liftis F, and Weil GJ. Washington University School of Medicine, St. Louis, MO.

Vaccination with irradiated third stage *B. malayi* larvae ($L_3$) has been reported to induce partial protective immunity to $L_3$ challenge in jirds. The purpose of this study was to identify antigens that may be targets of protective immunity in this model. Jirds were immunized by s.c. injection of irradiated $L_3$ and challenged either s.c. or i.p. Necropsy was performed 3 mo after challenge. Partial protection was achieved in s.c. challenged animals; worm recovery was only 30% of that observed in controls, and worms recovered from immunized animals were stunted. No effect of vaccination was observed in animals challenged i.p. Parasite antigen levels in sera collected 1 and 2 mo after larval challenge were consistent with parasitological findings obtained at necropsy. Antibody studies compared prechallenge sera from immunized animals to sera from infected (unimmunized) controls. Antibody responses to $L_3$ surface antigens (assessed by IFA) were much stronger after immunization than after infection. Immunoblot studies showed preferential recognition of several $L_3$ antigens (97, 55-60 kDa) by antibodies in sera from immunized animals. Additional studies are needed to determine whether immunization with antigens preferentially recognized by irradiated vaccine recipients can induce protection to larval challenge.

356 PREFERENTIAL RECOGNITION OF ANTIGENS OF *ONCHOCERCA VOLVULUS* BY PUTATIVELY IMMUNE INDIVIDUALS. Gallin M*, Adams AZ, Schumacher M, and Erttmann KD. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Federal Republic of Germany.

In order to identify antigens of *Onchocerca volvulus* which may be related to protective immunity we examined the antibody response to *O. volvulus* in 120 Liberian individuals exposed to infection with the parasite. Sera were obtained from forty individuals with the generalised form of onchocerciasis (GEN), thirty with the sowda form (SOW) and fifty individuals who had no clinical or parasitological signs of onchocerciasis (END). Immunoblot analysis of adult worm antigens with human sera showed preferential recognition of an antigen of 90 kD by serum from 55% of END, 20% of SOW and none of GEN. Immunoblots using antigens from *O. volvulus* infective larvae (L3) showed that this antigen is also present at this stage of the parasite development. Serum from END reacting only with the 90 kD antigen showed no reactivity to purified or recombinant *O. volvulus* paramyosin. These sera were used to screen an *O. volvulus* cDNA library and several clones were identified. Immunoblot analysis of O. volvulus L3

with sera from END revealed preferential recognition of antigens of 220 kD, 55 kD and 16 kD, of which the 16 kD antigen is not detected in adult worms or microfilariae.These results suggest that *O. volvulus* larval antigens may be involved in protective immune responses in human onchocerciasis.

357 DIFFERENTIAL ANTIBODY RESPONSES TO *ONCHOCERCA VOLVULUS* IN ECUADOR. Guderian R, Chico M*, Cordova X, Kron MA, Mackenzie CD, and Sisley B. Division of Clinical Investigation, Community Development Services, Hospital Vozandes, Quito, Ecuador; Department of Pathology, Michigan State University, East Lansing, MI; and Department of Immunology, London School of Hygiene and Tropical Medicine, London, England.

Genetic factors have been implicated in the modulation of immune responses to infection with filarial parasites. In the coastal province of Esmeraldas in Ecuador, onchocerciasis has spread rapidly throughout the villages where two distinct racial groups coexist. Previous epidemiological studies of onchocerciasis in this population, consisting of blacks of African origin and Amerindians of the Chachi tribe, have demonstrated different rates of clinical disease manifestations in each racial group. To explore the possibility of differential antibody responses to *O. volvulus* antigens, sera were collected from 100 persons with onchocerciasis from a hyperendemic hyperendemic region in northwestern Ecuador. Comparative Western blot analysis of these sera was performed using crude nodule derived *O. volvulus* antigen. IgG 1-4, IgM, IgA and IgD levels were determined by ELISA assay in 50 persons: 20 Chachi (11 male 9 female) and 19 blacks (9 male, 10 female). Antibody responses in the Chachi group were more intense in Western blot analysis using Protein A-horseradish peroxidase IgG1, IgG2 and IgG4 levels in the Chachi were significantly greater than comparable age matched male and female blacks. These data suggest that the intensity of IgG responses of humans with onchocerciasis in Ecuador may be influenced by genetic factors.

358 MECHANISM OF IMMUNE-MEDIATED KILLING OF LARVAL *ONCHOCERCA VOLVULUS* IN A MOUSE MODEL. Lange A*, Yutanawiboonchai W, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

Vaccination of BALB/c mice with irradiated infective larvae (L3) of *Onchocerca volvulus* results in a 51 to 72 percent reduction in survival of challenge infections contained in diffusion chambers. If cells were prevented from entering the diffusion chambers implanted in immune mice, the larvae were not killed. The goal of the present study was to determine the specific cell types involved in the immune elimination and to ascertain when the elimination occured. Worm survival was assessed at time points ranging from 24 hours to 8 weeks. Diffusion chamber contents were collected and analyzed using standard histologic methods to determine the cell types present when parasites were being killed. By day 7 post challenge, there was a 52% reduction in parasite survival in immune mice. This level of reduction remained constant over the next 7 weeks. Coincident with worm killing in immune animals, there was a 98% increase in the numbers of eosinophils found in the diffusion chambers of these mice. The numbers of macrophages, neutrophils and lymphocytes found in immune and nonimmune animals were not significantly different at any time point examined. These data suggest a role for eosinophils in the immune mediated killing of *O. volvulus* larvae.

359 IMMUNIZATION OF CHIMPANZEES WITH X-IRRADIATED *ONCHOCERCA VOLVULUS* THIRD STAGE (L3) LARVAE. Prince AM*, Brotman B, Johnson EH Jr., Smith AB, Pascual D, and Lustigman S. Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia; and Department of Virology and Parasitology, The Linsley F. Kimball Research Institute of the New York Blood Center, New York, NY.

To provide a theoretical basis for the potential development of vaccines against *Onchocerca volvulus* (Ov) a trial has been conducted to assess the protective efficacy of immunization of chimpanzees with X-irradiated L3 larvae. Approximately 1000 larvae were injected at 0,1, and 7 months. The immunized animals, and unimmunized controls, were then challenged with 250 live L3. In order to provide possibly protective exposure to the immunologically distinct L4 epicuticle, a radiation dose (45 Krads) was chosen which preserved about 50% of the molting ability of unirradiated larvae. Despite the presence of a strong immune response to crude adult worm extracts, and to clone Ovantigens, at the time of challenge no protection against patent infection was observed: 3 of 4 immunized animals developed patent infection as compared to 2 of 4 controls. One immunized animal, however, failed to become patent or to manifest the late antibody response to adult worm antigens seen in both sub-patent infections in this model, and may have been protected from infection. The implications of these studies for future attempts to immunize against *O. volvulus* are discussed.

360 ANALYSIS OF ISOTYPE-SPECIFIC ANTIBODY RESPONSES TO A SOLUBLE *BRUGIA PAHANGI* L3 ANTIGEN IN A HAITIAN POPULATION. Bailey JW, II*, Lammie PJ, Hightower AW, Hitch, WL, Walker EM, and Eberhard ML. Parasitic Disease Branch, Centers for Disease Control, Atlanta, GA; and Statistics Division, Centers for Disease Control, Atlanta, GA.

Recent studies suggest that adults living in an area endemic for lymphatic filariasis may be resistant to infection with the infective stage larvae (L3). Sera obtained by finger prick from 179 individuals (age = 1 to 75 yrs) from Leogane, an area endemic for *Wuchereria bancrofti*, were assayed using mouse monoclonal antibodies directed against human IgG subclasses and IgM to determine isotype-specific anti-L3 antibody levels. Thirty of 179 individuals from randomly selected households were microfilaremic (Mf+); amicrofilaremic (Mf-)individuals were younger than Mf+ ($p = 0.0012$). There was no association between microfilaremic status and IgG1, IgG2 or IgG3. Anti-L3 IgG4 andIgM responses were significantly higher in Mf+ and Mf- individuals, respectively. Anti-L3 IgG1-specific antibodies did not correlate with age. However, anti-L3 antibodies of the IgG2, IgG3 or IgG4 subclass increased with age while anti-L3 IgM-specific antibodies decreased with age. A more detailed analysis of associations among age, microfilaremic status, familial clustering and anti-L3 isotype-specific antibody response may permit the development of models to predict exposure to filariasis and to describe the evolution of the host response to larval antigens.

## U: MALARIA CHEMOTHERAPY I

361 INVOLVEMENT OF REACTIVE OXYGEN SPECIES IN THE DESTRUCTION OF *PLASMODIUM FALCIPARUM* Golenser J*, Marva E, and Chevion M. Department of Parasitology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; and Department of Cellular Biochemistry, the Hebrew University-Hadassah Medical School, Jerusalem, Israel.

A delicate balance exists between oxidative stress and protective mechanisms in erythrocytes parasitized with plasmodia. Derangement of this balance expressed by increased fluxes of free radicals often leads to the death of the parasite. Among these, hydroxyl radicals (HR) are highly reactive and capable of causing damage to proteins, lipids and nucleic acids.Increased levels of HR were found in erythrocytes containing advanced stages of *P. falciparum* (PE). Addition of polymorphonuclear cells (PMN) to PE yielded HR levels higher than the amount produced by PMN and normal erythrocytes (NE), PE, NE or PMN alone. Similar results were obtained when PE were treated with ascorbic acid. Erythrocytes containing young parasites reacted to ascorbate like NE, indicating stage specific sensitivity that increases with parasite development. The uptake of ascorbate into PE increased by 150%. This change provides a partial explanation for the elevated levels of HR in PE treated with ascorbate. A complementary explanation stems from the increase in the level of endogenous redox-active iron-containing structures during the intracellular development of the parasite. These structures promote

oxidative stress by virtue of catalyzing the production of HR. This is further substantiated by the effect of diethylenetriamine penta-acetic acid, a chelator of transition metals, which markedly reduced the generation of HR in both untreated and treated PE. It is demonstrated that the interaction of PE with physiological factors, such as PMNs or vitamin C, lead to excessive production of HR which derange the host-parasite delicate balance and results in plasmodial death.

362 1,2,4,5-TETRAOXANES; A NEW GROUP OF PEROXIDE ANTIMALARIAL DRUGS WITH POTENTIAL FOR CLINICAL UTILITY. Andersen SL*, Vennerstrom JL, Hong-Ning F, Ellis WY, Ager AL, Gerena L, and Milhous WK. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; College of Pharmacy, University of Nebraska Medical Center, Omaha, NE; and Center for Tropical Parasitic Diseases, University of Miami, Miami, FL.

Several 1,2,4,5-tetraoxanes are active antimalarial agents. These compounds are the only peroxides reported to date that are curative in a single dose *in vivo* model against *Plasmodium berghei* in the "Rane test." We resynthesized three of the most active 1,2,4,5-tetraoxanes and isolated two as mixtures of meso and d,l stereoisomers and the third (WR 1448999) as a single meso stereoisomer.The curative activity of these 1,2,4,5-tetraoxanes against *P. berghei* was confirmed at a single doses of 320 and 640 mg/kg. By contrast, artemisinin, a structurally-related trioxane, was not curative in a single dose experiment. The drug was then tested in a multiple dose experiment using the same mouse/*P. berghei* model, designed to estimate the therapeutic index by evaluating efficacy and toxicity in the same experiment. Results revealed similar efficacy of WR 148999 compared to artemisinin and equivalent toxicity. A relatively simple, one-step chemical synthesis as recently described makes this class of compounds more economically feasible for developing countries. Further preclinical evaluation of WR 148999 and lead directed synthesis of more compounds in this class are indicated.

363 SYNTHETIC 1,2,4-TRIOXANES, A NEW CLASS OF ANTIMALARIALS. Jefford CW*, Kohmoto S, Rossier JC, Peters W, and Milhous W. Department of Organic Chemistry, University of Geneva, Geneva, Switzerland; Department of Medicinal Protozoology, London School of Hygiene and Tropical Medicine, London, UK; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

The discovery of the naturally occurring trioxane, artemisinine, has marked the beginning of a new era in malaria chemotherapy since it is unlike the usual drugs in being active against multi-resistant organisms and also being much safer. Taking artemisinine as a lead we have devised and prepared several third generation trioxanes. Over 200 new synthetic 1,2,4-trioxanes have been tested against *P. falciparum in vitro* and *P. berghei in vivo* using the methods of Desjardins and Peters. As a result we have identified a new trioxane pharmacophore in a molecular environment different from that in artemisinine. Certain bicyclic trioxanes, termed fenozans, display significant *in vivo* and *in vitro* activities when compared to those of conventional nitrogen-containing anti-malarial drugs and artemisinine. The fenozans show promise as future anti-malarial agents on account of their high activity, availability and ease of derivatization.

364 AN EFFECTIVE TOPICAL TREATMENT OF *PLASMODIUM BERGHEI*-INFECTED MICE WITH ARTELINIC ACID. Klayman DL*, Ager, Jr. AL, Fleckenstein LL, and Lin AJ. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Center for Tropical Parasitic Diseases, University of Miami, Miami, FL.

Artelinic acid (dihydroartemisinin p-carboxybenzyl ether), a derivative of the naturally-occurring antimalarial qinghaosu, has been incorporated into a gel suitable for topical application and evaluated in *Plasmodium berghei*-infected mice for both curative and prophylactic properties. A dose of 0.9 mg of

artelinic acid was applied 2X daily to the partially denuded backs of the test animals beginning on days 3 and 0, respectively, of injection of parasitized erythrocytes. In the curative experiments, rapid elimination of the parasitemia and 60 day survival of 5/5 mice resulted when the gel was applied for 3 days beginning on day 3. In the prophylactic trials, the establishment of parasitemia was prevented and 60 day survival was achieved in 5/5 mice by administration of the same dose for 2 days beginning on day 0.

365 BISQUINOLINES. 1. ANTIMALARIALS WITH POTENTIAL AGAINST CHLOROQUINE-RESISTANT MALARIA. Vennerstrom JL*, Ellis WY, Ager AL, Andersen SL, Gerena L, and Milhous WK. College of Pharmacy, University of Nebraska Medical Center, Omaha, NE; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Center for Tropical Parasitic Diseases, University of Miami School of Medicine, Miami.

Based on observations that several bisquinolines such as piperaquine possess notable activity against chloroquine-resistant malaria, thirteen new N,N-bis-(7-chloroquinolin-4-yl)alkane diamines were synthesized and screened against *Plasmodium falciparum in vitro* and *P. berghei in vivo*. Twelve of the thirteen bisquinolines had a significantly lower resistance index (0.2-2.3) than did chloroquine (11.2); the resistance index was apparently unrelated either to *in vitro* or *in vivo* activity. Except for two compounds, there was a reasonable correlation between *in vitro* and *in vivo* activities. Seven of the thirteen bisquinolines had $IC_{50}$ values of less than 6 nM against both chloroquine-sensitive (D-6) and -resistant (W-2) clones of *P. falciparum* and were curative against *P. berghei* at a dose of 640 mg/kg. In contrast to chloroquine, these bisquinolines did not show any toxic deaths at curative dose levels. Four bisquinolines, however, caused skin lesions at the site of injection. Maximum activity was seen in bisquinolines with a connecting bridge of two carbon atoms where decreased conformational mobility seemed to increase activity. (±-trans-$N^1$,$N^2$-Bis- (7-chloroquinolin-4-yl)cyclohexane-1,2-diamine was not only the most potent bisquinoline *in vitro*, but was clearly unique in its *in vivo* activity - 80% and 100% cure rates were achieved at single doses of 160 and 320 mg/kg, respectively. In summary, these preliminary results support the premise that bisquinolines may be useful agents against chloroquine-resistant malaria.

366 NEW ALTERNATIVES TO CYCLOGUANIL AND PYRIMETHAMINE. Milhous WK*, Peterson DS, Wellems TE, Lehnert EK, Gerena L, Andersen SL, and Schuster BG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

Resistance to the antifolate antimalarial drugs cycloguanil (CYC) and pyrimethamine (PYR) is mediated by specific point mutations in the enzyme target dihydrofolate reductase (DHFR) that apparently alter binding affinity of the drugs to the parasite enzyme. Resistance to the antifolate drugs is not significantly modulated by drugs that reverse resistance to chloroquine or quinine (such as verapamil) or by penfluridol (a drug which reverses resistance to mefloquine). These findings suggest tha. low molecular weight antifolate drugs may not be subject to efflux by the putative transport mechanisms that mediate resistance to chloroquine. A structural analogue of CYC, WR99210 (2',4',5'-trichlorophenoxy substituted triazine) was found to be remarkably active ($IC_{50}$ of less than 0.003 ng/ml) against ten parasite clones which had previously been characterized with regard to specific point mutations on their DHFR and levels of resistance to CYC or PYR. WR99210 was 15-200 fold more active than either CYC or PYR against the respective susceptible clones and 7000-40000 fold more active than either CYC or PYR against the resistant clones. This broad spectrum of enhanced activity of WR99210 and lack of cross resistance to other antifolates provides valuable insight for lead directed synthesis of new drugs targeted at the parasites' DHFR enzyme.

367 EFFLUX OF $^{3}$H-DIHYDROQUININE FROM CHLOROQUINE-RESISTANT *PLASMODIUM FALCIPARUM*. Krogstad DJ*, Gluzman IY, Wellems TE, and Schlesinger PH. Washington University School of Medicine, St. Louis, MO; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

Previous studies have shown that rapid efflux is the basis of chloroquine resistance, and that chloroquine resistance is linked to quinine resistance. These studies were performed to determine whether efflux is responsible for the ability of verapamil to enhance the activity of quinine against chloroquine-resistant (but not susceptible)*P. falciparum*. $^{3}$H-Dihyhdroquinine was used because $^{3}$H labeling by currently available methods reduces the double bond in the vinyl group of quinine. Preliminary studies demonstrated similar pKs for quinine and dihydroquinine, and similar $IC_{50}$s with chloroquine-susceptible and resistant clones of *P. falciparum*.Verapamil increased the $^{3}$H-dihydroquinine accumulation of chloroquine-resistant parasites from 111 to 180 fmol per $10^{6}$ parasitized red blood cells, but had no effect on accumulation by susceptible parasites. The initial efflux half-time of $^{3}$H-dihydroquinine from chloroquine-resistant *P. falciparum* was shorter than from susceptible *P. falciparum* (2 vs > 60 minutes), and was prolonged by verapamil (to > 60 minutes). These results indicate that the chloroquine-resistant parasite excretes $^{3}$H-dihydroquinine by a process similar to chloroquine efflux; they suggest that quinine efflux occurs in the chloroquine-resistant parasite.

368 PRELIMINARY LOCALIZATION OF THE EFFLUX PROCESS IN *PLASMODIUM FALCIPARUM*: PERSISTENCE OF EFFLUX AFTER LYSIS OF THE HOST RED CELL. Gluzman IY* and Krogstad DJ. Washington University School of Medicine, St. Louis, MO; and Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

The subcellular site of the efflux process responsible for chloroquine resistance has not been defined. The purpose of these studies was to develop a subcellular preparation to study efflux after removal of the host red cell and its cytosol. Because verapamil enhances chloroquine accumulation by resistant parasites, candidate preparations were tested for this effect after red cell lysis. Three preparations (saponin, hypertonic [$NH_4Cl$], and hypotonic lysis) no longer demonstrated the verapamil effect with resistant parasites, and had marked reductions in chloroquine accumulation by susceptible parasites. Conversely, digitonin and sorbitol treated preparations retained the verapamil effect. Digitonin preparations resuspended in high potassium RPMI have $^{3}$H-chloroquine accumulations similar to those of intact parasitized red blood cells (1260 vs 1135 or 116 vs 131 fmol chloroquine per $10^{6}$ susceptible or resistant parasites); similar increments with verapamil in resistant parasites (to 315 and 342 fmol chloroquine per $10^{6}$ parasites); and similar initial efflux half-times (1.5-2.0 vs 2.0 and 50 vs > 50-75 minutes). These preparations are suitable for the study of efflux by parasites freed from their host red cell. These results suggest that the molecule(s) responsible for efflux is (are) in the parasite, the parasite membrane or the parasitophorous vacuole.

## V: SCHISTOSOMIASIS MOLECULAR BIOLOGY AND BIOCHEMISTRY

369 UTILIZATION OF FLUORESCENT FATTY ACID AND PHOSPHOLIPID ANALOGS BY SCHISTOSOMULA OF *SCHISTOSOMA MANSONI*. Furlong ST* and Thibault KS. Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA; and Department of Medicine, Harvard Medical School, Boston, MA.

To gain insight into how schistosomula process lipids we have followed the incorporation into these organisms of 1-pyrene- decanoic acid (PDA),a fatty acid analog, and 1-palmitoyl-2[6-[(7-nitro-2-1-3-benzoxadiazol-4-yl) amino]caproyl]phosphatidylcholine(NBD-PC), a fluorescent phospholipid analog.

The incorporation of the lipid analogs was followed both morphologically and biochemically. In 50 μM PDA, label can be seen in worms within 15 minutes by fluorescence microscopy. The majority of the label localized to areas of the worm that also stained with Nile Red and which are most likely intracellular lipid droplets. Biochemical analysis showed that the principal lipid classes labeled with PDA were triglycerides and phosphatidylcholine. By contrast to the studies with PDA, when schistosomula were incubated with NBD-PC significant staining of the worm surface was evident. Furthermore, NBD-PC was preferentially incorporated into specific cell types within the worm. These studies demonstrate that fluorescent lipid analogs are incorporated into distinct compartments of schistosomula and that the incorporation of these precursors can be followed biochemically. Such studies may provide more data on the mechanisms by which the parasite processes lipids than those using radiolabeled precursors alone.

370 INHIBITION OF THE PHOSPHOINOSITIDE RESPONSE WITH PRAZIQUANTEL IN *SCHISTOSOMA MANSONI*. Wiest PM*, Li Y, Burnham D, Olds GR, and Bowen WD. Department of Medicine, The Miriam Hospital, Brown University, Providence, RI.

The phosphoinositide response is a major signal transduction mechanism in regulation of intracellular events. The presence of this pathway was examined in *Schistosoma mansoni* by radiolabeling intact schistosomula and adult worms with 25 μCi of [3H]-myoinositol for 24 hr and stimulating with 25 mM NaF/10 μM $AlCl_3$ in the presence of 10 mM LiCl. An increase in total inositol phosphates was seen within 2 min and maximal accumulation occurred after 30 min. NaF-stimulated inositol phosphate production was significantly greater in females (8,925 ± 3,396 cpm/mg protein, N=3) than males (2,484 ± 353). Adult worms stimulated with 100 μM of the GTP analogue GppNHp in 0.001% saponin demonstrated a 110% increase in inositol phosphates. Neomycin (25mM) inhibited NaF-induced inositol phosphate production by 86% and induced surface bleb formation similar to Praziquantel. Praziquantel (200 μg/ml) blocked NaF-induced inositol phosphate turnover by 56% with maximal inhibition achieved within 15 min. These data suggest that a GTP-binding protein activates phospholipase C resulting in inositol phosphate turnover in *S. mansoni*. Furthermore, inhibition of the phosphoinositide response by Praziquantel may be a new mechanism of action of this anthelminthic drug.

371 THE NOT-SO-APPARENT ACTIVE SITE OF *SCHISTOSOMA MANSONI* CYSTEINE PROTEINASE, CP1. Chappell CL* and Rege AA. Department of Family Medicine, Baylor College of Medicine, Houston, TX; Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, TX.

"Hemoglobinase" has been used to describe the proteolytic activity found in the digestive tract of adult *S. mansoni*. Present evidence suggests that at least two hemoglobin-degrading enzymes are associated with the parasite gut, while another is located in the parenchymal tissues. We term these enzymes as CP1, CP2 and CP3, respectively. All were thought to be cysteine proteinases until recently, when the amino acid sequence of CP1 was noted to be quite different from CP2 and other cathepsin-like proteinases. Difficulties in the purification of CP1 from CP2 raised the possibility that CP1 may not be an enzyme at all. We have used specific monoclonal antibodies to address this question. Three lines of evidence have been explored: 1) interaction of CP1 with the proteinase inhibitor, alpha-2 macroglobulin, 2) irreversible binding of CP1 to a cysteine proteinase-specific, radiolabelled inhibitor, and 3) immuno- precipitation of cysteine proteinase activity with a CP1-specific monoclonal antibody. These experiments suggest that CP1 does, in fact, display cysteine proteinase activity. Sequence analysis of the putative active site suggests that CP1 is similar to a family of cysteine proteinases characterized by streptococcal CP.

372 MAMMALIAN AND BACTERIAL EXPRESSION OF RECOMBINANT SM23 - IMMUNE RECOGNITION AND EPITOPE MAPPING. Reynolds SR*, Shoemaker CB, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

SM23 is a highly immunogenic integral membrane protein found in *Schistosoma mansoni* larval and adult stages. It is a member of a newly recognized family of proteins having identical tertiary structure but unknown function that includes melanoma protein ME491 and human leukocyte surface markers CD37, CD53 and R2. The availability of properly folded recombinant SM23 would be useful for studies of function and structure and vaccine investigation. The full-length coding DNA forSM23 was transfected into two mammalian cell lines - COS and CHO. Extracts of transfected cells showed a wide band of approximately 23 kDa recognized on Western blots when probed by rabbit polyclonal anti-SM23 and all immune mouse sera tested to date including C57Bl/6, BALB/c, CBA, and outbred CD-1. A large hydrophilic region (AA's 108-204) was also expressed in bacteria as a maltose binding fusion protein. The purified recombinant protein contained a conformational B cell epitope when probed with all of the above antisera and a T cell epitope recognized by C57Bl/6 and BALB/c mice vaccinated with irradiated cercariae. Further epitope mapping is in progress.

373 ALTERNATE SPLICING GENERATES mRNA ENCODING TRUNCATED PRODUCTS OF THE *SCHISTOSOMA MANSONI* EGF RECEPTOR HOMOLOGUE Ramachandran H*, Landa A, Stein L, and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Instituto de Investigaciones Biomedicas, Universidad National Autonoma de Mexico, Mexico City, Mexico.

A full-length cDNA encoding the *Schistosoma mansoni* homologue of epidermal growth factor receptor (SER) has been cloned and is predicted to encode a protein of 200 kD. In addition to the full-length SER cDNA, three variant cDNA classes of SER have been identified. The predicted protein products encoded by the cDNAs share identity with SER at the amino terminus but diverge into different short C-termini. Subsequent genomic cloning and sequencing studies have demonstrated that the variant transcripts are generated from a single gene by the process of alternate splicing. Northern blot and quantitative PCR analyses have indicated that SER and one of the variant transcripts are about equally represented in different stages of the parasite's life-cycle. We are currently performing in-situ hybridization studies in order to localize the full-length and variant SER transcripts within *S. mansoni*. Antisera generated against several domains of the full-length SER product are also being characterized and will be used in immuno-localization and functional studies. Although no function can yet be assigned to the variant classes of SER, one hypothesis is that they may act to interfere with the host's immune response against the full-length protein.

374 LOCALIZATION STUDIES ON THE *SCHISTOSOMA MANSONI* HOMOLOGUES OF P-GLYCOPROTEIN. Bosch I* and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The cDNAs encoding two different *Schistosoma mansoni* homologues (SMDR1 and SMDR2) of the multidrug resistance gene known as P-glycoprotein have been isolated and characterized. Based on the amino acid translation, one homologue (SMDR1) contains only a single copy of the characteristic P-glycoprotein heterodimeric repeat and is, thus, similar to the bacterial export proteins in stucture. SMDR2 has a heterodimeric structure and is very homologous to P-glycoprotein in hydropathy profile and sequence. Transcription analysis indicates that both mRNAs are expressed in cercarial and adult stages. Several hydrophilic domains from both proteins have been chosen as immunogens and the corresponding regions of the genes have been amplified by PCR and inserted into bacterial expression vectors. The resulting recombinant products havebeen purified and used to immunize mice and rabbits.

The immune and nucleic acid reagents are being used to study expression of SMDR1 andSMDR2 during all stages of the life cycle and to localize the site ofexpression within the parasite by in situ techniques.

375 CHARACTERIZATION OF THE TRIOSE PHOSPHATE ISOMERASE GENE FROM *SCHISTOSOMA MANSONI*. Reis MG*, Gross A, Harn D, and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz FIOCRUZ/UFBa, Salvador, Bahia, Brazil.

A 28 kDa antigen of *Schistosoma mansoni*, that induces partial protection as an immunogen, has previously been identified as triosephosphate isomerase (TPI). Using an *S. mansoni* TPI cDNA clone as a probe, we report here the isolation and characterization of genomic clones containing the complete *S. mansoni* TPI gene and substantial additional 5' and 3' sequences. DNA sequencing reveals that the TPI gene contains six exons. The location of all five introns is precisely conserved relative to those identified in mammalian TPI genes although one mammalian intron is missing in the *S. mansoni* TPI gene. Introns contain typical splice donor and acceptor sites. In most cases the introns are substantially larger than the corresponding mammalian introns. The first intron, though, is only 42 base pairs in lengthmaking it one of the smallest ever characterized. A putative promoter element has also been identified.

376 CHARACTERIZATION OF CDNA CLONES ENCODING GLUCOSE TRANSPORTERS FROM *SCHISTOSOMA MANSONI*. Skelly PJ* and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The cDNA encoding two distinct *S. mansoni* glucose transporter homologues have been isolated from an adult cDNA library. The predicted amino acid sequences of the two proteins share nearly identical hydropathy profiles with mammalian and plant glucose transporters. Using database homology search software, one *S. mansoni* glucose transporter protein (SGTP1) has its greatest sequence similarity (54%)to the human insulin responsive GTP (GLUT4) while the second glucose transporter (SGTP2) has its highest homology (55%) to the human liver GTP (GLUT2). Each are more homologous to mammalian glucose transporters than to one another. The genes encoding these proteins are expressed in both cercarial and adult life stages. A third cloned *S. mansoni* transporter protein gene (SGTP3) was isolated by low stringency hybridization of the cDNA library with an SGTP1 probe. The predicted amino acid sequence of this protein has a hydropathy profile very different from glucose transporters and exhibits its greatest similarity (25%) to a yeast histidine transporter (HIP). As schistosomes rely on their hosts for glucose and other nutrients, we expect that transporters for these nutrients will be serum exposed and represent potential vaccine or chemotherapeutic targets.

377 IDENTIFICATION AND CHARACTERIZATION OF GENES ENCODING *SCHISTOSOMA HAEMATOBIUM*-SPECIFIC ANTIGENS. Blanton RE*, and Aman R. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH and Institute of Primate Research, National Museums of Kenya, Nairobi, Kenya

*Schistosoma haematobium* (SH) is similar genetically and antigenically to *S. mansoni* (SM). There is, however, a species-specific component to protective immune responses. SH-specific antigens 1) may be vaccine candidates, 2) may be key to the differing biology of these two species, and 3) may be diagnostic or epidemiologic tools. To screen, we used an SM adsorbed pool of human sera from individuals infected only with SH.We identified 11 cDNA clones from an SH adult worm library that express SH-specific antigens. From Northern blots probed with cDNA inserts, we find that the 11 clones code for 6-7 different antigens. There is a corresponding SM gene expressed for most of the antigens, but the gene in most cases is expressed at 1/10th the level or shares only weak sequence similarity with the SH gene. Three distinct genes code for human immunodominant antigens corresponding to clones 1-1, 4-1 and 4-2.

Interestingly, the protein corresponding to another antigen, 3-1, was immunodominant for a pool of baboon sera, while clone 4-2 was not. Southern blots using these clones revealed a complex hybridization pattern for SH DNA and a simple "single gene" pattern for SM genomic DNA. These results suggest that there may be significant differences in antibody responses between baboons and humans and that all of the genes we have so far examined may have arisen by amplification of SM genes.

## W: MALARIA EPIDEMIOLOGY AND FIELD STUDIES

378 LOW FREQUENCY OF ANTI-*PLASMODIUM FALCIPARUM* CS REPEAT ANTIBODIES AND HIGH MALARIA TRANSMISSION RATE IN ENDEMIC AREAS OF RONDONIA STATE - NORTHWESTERN BRAZIL . Oliveira-Ferreira J*, Teva A, and Daniel-Ribeiro C. Department of Immunology, WHO Collaborating Center for Research and Training in Immunology of Parasitic Diseases, IOC - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

To evaluate the anti-plasmodial immunity status of populations exposed to malaria in 4 endemic areas of Rondonia State we studied the prevalence of anti-plasmodial antibodies. This was done by an immunoradiometric assay (IRMA) using $(NANP)_4$ synthetic peptide corresponding to the repetitive epitope of the circumsporozoite protein of *Plasmodium falciparum* and sera from 1617 individuals. Antibodies to blood stages were measured by immunofluourescence using *P. falciparum* parasitized red blood cells. Our results showed that the prevalence of anti-sporozoite antibodies in the human population, mostly migrants living in this state for 2 to 4 years, ranged from 3.3% to 7.8%, was higher in individuals with more than 30 years of age and related both to the number of past attacks of malaria and to antibodies to blood stage antigens. Anti-blood stage antibodies were present in 50% of the population and was related to the number of past attacks of malaria. Taken together the short time of residence of the migrants in the studied localities and the relatively high malaria transmission rate by local anopheline (7.5 infective bites per man per month) these data suggest that the studied population were not yet exposed enough to malaria to develop a high degree of anti-*P. falciparum* immunity and constitute, therefore, a priority group for a immunoprophylactic campaign in the future.

379 POLYMORPHISM IN THE CS PROTEIN OF FIELD ISOLATES OF *PLASMODIUM FALCIPARUM* FROM MADANG, PAPUA NEW GUINEA. Shi YP*, Alpers M, and Lal AA. Division of Parasitic Disease, Center for Disease Control, Atlanta, GA and Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea.

The polymorphism of B- and T-cell epitopes in biologically representative field isolates of malaria parasite may be a problem in malaria vaccine development. We have found polymorphism in the CS protein gene from *P. falciparum* from Madang, Papua New Guinea (PNG). The nucleotide and the deduced amino acid sequences from 39 recombinant CS clones representing 18 field isolates contained both previously identified and new amino acid substitutions in the 3 immunodominant regions of the CS protein, *Th1R-N1, Th2R, and Th3R. In the Th1R-N1 region, 33 of the 39 clones had sequences similar to* the 7G8/Wel CS protein, 4 clones had a sequence similar to NF54/LE5 CS protein, and the remaining 2 clones were new variants. In the Th2R region, 34 of the 39 clones had a sequence similar to the WEL CS protein sequence, three were like 7G8, and the remaining 2 clones exhibited previously unknown mutations. In the most polymorphic region, Th3R, 21 clones were WEL type, 11 were 7G8 type, and the remaining 7 exhibited a previously unknown sequence. Comparison of the CS protein sequences of parasites from PNG with the CS protein sequences from the Gambia and Brazil revealed that the 3 immunodominant epitopes of parasites from PNG and the Gambia were more polymorphic than those of parasites from Brazil. Whether the extent and nature of epitope variability is associated with the degree of endemicity of malaria in these regions needs to be further explored. We have also found that, in the 3 immunodominant targets, substitution leads to change in hydrophobic hydrophilic balance only in the CTL-containing Th3R, suggesting that the parasite may be evolving both in an epitopic as well as an agrotopic fashion to evade host immune(CTL) functions.

380 MALARIA ENDEMIC SERA IDENTIFY B CELL EPITOPES WITHIN NON REPEAT REGIONS OF THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM FALCIPARUM*. Calvo Calle JM*, Cochrane A, Clavijo PJ, Collins W, Herrington DA, Boudin C, Stuber D, Tam JP, Nussenzweig RS, and Nardin E. New York University School of Medicine, New York, NY; Centers for Disease Control, Atlanta, GA; Center for Vaccine Development, University of Maryland, Baltimore, MD; Department of Parasitology, University of Grenoble, Grenoble, France; F. Hoffman-La Roche, Basel, Switzerland; and The Rockefeller University, New York, NY.

The fine specificities of antibodies reacting with the circumsporozoite (CS) protein of *P. falciparum* were investigated using sera of volunteers immunized with irradiated *P. falciparum* sporozoites or individuals living in a malaria endemic area (Burkina Faso, West Africa). The sera were assayed by ELISA using either recombinant proteins, or a series of multiple antigen peptide (MAPs) constructs, which contained nonrepeat sequences from the N- and C- terminal regions of the *P. falciparum* CS protein. Antibodies in sera of the sporozoite immunized volunteers reacted predominantly with the repeat region of the CS protein which contains the immunodominant B cell epitope. In contrast, endemic sera contained antibodies which reacted with both the repeat epitope and with epitopes contained in the N- and C-terminal regions of the *P. falciparum* CS protein. The sera of children living in endemic areas, which did not have detectable anti-repeat antibodies, as well as the sera of the individuals given a *P. falciparum* blood induced infection as treatment for neurosyphilis, also contained antibodies which reacted with these non-repeat regions. The epitopes recognized by some of the endemic sera were defined by reaction with MAPs containing C- terminal sequences of *P. falciparum* CS protein.

381 POLYMORPHISM OF THE ALLELES OF THE MEROZOITE SURFACE ANTIGENS MSA1 AND MSA2 IN *PLASMODIUM FALCIPARUM* WILD ISOLATES FROM COLOMBIA. Snewin VA, Herrera M, Sanchez G, Scherf A, Langsley, and Herrera S*. Unite de parasitologie Experimentale, Departement d' Immunologie Institut, Paris, France; and Department of Microbiology, School of Health, Universidad del Valle, Cali, Colombia.

The degree of polymorphism and the allelic distribution of two major *Plasmodium falciparum* merozoite surface antigens (MSA1 and MSA2) has been analyzed in clinical isolates from Colombia. DNA was prepared directly from patients' blood and used in PCR reactions to amplify block 2 of MSA1 and the central region from MSA2. Thirty one samples were analyzed and a marked degree of length polymorphism was detected especially for MSA2. A high proportion of multiple bands was also observed, most probably resulting from mixed infections. Allele specific oligonucleotides were used to type both alleles. For MSA1, 26 out of 31 clinical isolates were of the RO33 type, 15 were MAD20 and three were typed as KI. When the MSA2 allele was analyzed, nine isolates hybridized with a CAMP specific probe and four with an FC27 derived oligonucleotide. Two samples, which showed multiple bands, hybridized with both probes. Interestingly, in 16 out of 27 isolates the MSA2 allele remained unassigned by the specific probes. Five of these were cloned and their DNA sequenced. These sequences are discussed.

382 HUMAN CYTOTOXIC T LYMPHOCYTES AGAINST THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN AFTER NATURAL EXPOSURE TO MALARIA. Sedegah M*, Sim KL, Malik A, Mason CA, Sherwood J, Koech D, Ware B, Roberts C, and Hoffman SL. Naval Medical Research Institute, Bethesda, MD; Pan American Health Orangization, Washington, DC; Walter Reed Army Institute of Research, Washington, DC; US Army Medical Research Unit, Nairobi, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

Humans immunized with irradiated *Plasmodium falciparum* sporozoites produce cytotoxic T lymphocytes (CTL) against a peptide reflecting amino acids 368-390 of the *P. falciparum* CS protein, as previously reported. As reported earlier Kenyans whose lymphocytes proliferate after stimulation with CS protein peptides 361-380 and 371-390 are less likely to become infected with malaria than their neighbors whose lymphocytes do not proliferate after stimulation with these peptides suggesting that CTL against an epitope in this region contribute to their resistance to infection. We selected 11 adult male Kenyans whose lymphocytes proliferated after stimulation with peptides 361-380, 371-390, or 368-390 and who were relatively resistant to reinfection with malaria, and at the end of the dry season, just prior to an increase in malaria transmission, determined at a single time point, if they had circulating CTL against peptide 368-390. Three of the eleven had antigen specific, genetically restricted, CD8+ T cell-dependent CTL against peptide 368-390. This first demonstration of circulating CTL against a malaria protein among villagers exposed to endemic malaria, provides an impetus and methodology for further studies to examine the relationship between circulating CTL and resistance to malaria infection.

383 IDENTIFICATION OF *PLASMODIUM FALCIPARUM* PARASITES USING THE POLYMERASE CHAIN REACTION (PCR). Courval JM*, Barker, Jr. RH, Banchongaksorn T, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Malaria Center, Region 2, Chieng Mai, Thailand.

In previous studies we described protocols for detecting *Plasmodium falciparum* in clinical samples using PCR. We developed simple methods utilizing lysed blood samples directly for PCR amplification, without organic extraction of DNA. In the present studies we have examined parameters associated with sample processing to assess their effects on the efficiency of PCR amplification. Blood samples were collected by digital puncture from 790 patients presenting at malaria clinics in Mae Sod, Thailand. Thick smears were also prepared for microscopic examination. Samples for PCR were treated with saponin lysis mix (0.2%NaCl, 1 mM EDTA, 0.015% saponin), incubated at room temperature for 10 min, 4 hr, or overnight, then filtered onto 903 paper. Once dry, a 3 mm piece of paper was added directly to PCR reaction mix and amplified for 30 cycles. PCR product was detected by hybridization with the *P. falciparum*-specific probe pPF14. Preliminary analysis showed intensity of hybridization was greatest from samples incubated in lysis mix overnight, and was lowest for samples incubated 4 hrs ($p < 0.001$). Results of PCR amplification using lysed samples and using organically extracted samples are being compared with microscopy. Simple modifications in blood sample processing methods can result in improved sensitivity, and may overcome previously described PCR amplification problems associated with inhibition.

384 DETECTION OF *PLASMODIUM FALCIPARUM* PARASITEMIAS USING AN ANTIGEN-TARGETED ELISA AND A DNA PROBE IN THE GAMBIA. Schaeffler BA*, Taylor DW, Parra ME, Voller A, Bidwell D, Tam MR, Hassan-King B, Greenwood B, Subramanian S, and McLaughlin GL. Program for Appropriate Technology in Health, Seattle, WA; Georgetown University, Washington, DC; Institute of Zoology, London, UK; Medical Research Council Laboratories, The Gambia; and University of Illinois, Champaign, IL.

Two assays for the detection of malaria blood parasitemias were tested side-by-side and compared with thick blood films at the Medical Research Council Laboratories in The Gambia. The ELISA assay is a two-sited antigen-capture assay which uses two monoclonal antibodies directed against different epitopes of histidine-rich protein-2. The DNA assay uses an alkaline phosphatase-conjugated synthetic DNA probe that hybridizes to the 21-base repetitive DNA family of *P. falciparum*. Both assay protocols were adapted for completion within 2.5 hours. After finger pricks, 35-$\mu$l blood was collected into separate capillary tubes and dispensed into microtiter plate wells containing lysis buffers for ELISA or DNA assays. For the ELISA assay, signal intensities were enhanced using lysed whole blood, relative to serum. For the DNA probe assay, signal intensities were enhanced using chemiluminescence, but results were similar

for dye or light. The ELISA and DNA assays were well-correlated (ca 95%) and each assay correlated with early microscopic scores using Giemsa-stained thick smears (ca 90%). Each of these rapid biotechnology assays appears to reliably detect parasitemias above 0.05% infected erythrocytes. The current assays may be useful for large-scale population monitoring, and alternative configurations are feasible.

385 DEVELOPMENT OF A TWO-SITE ELISA USING MONOCLONAL ANTIBODY AGAINST A 50-KDA CATABOLITE FROM *PLASMODIUM FALCIPARUM* MEROZOITES FOR THE DIAGNOSIS OF MALARIA INFECTION. Ferreira-da-Cruz MF*, Machado-Paso R, Fortier B, and Daniel-Ribeiro CT. Department of Immunology, IOC - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; and INSERM U-42, Villeneuve d'Asc, France.

Since the serodiagnosis of malaria infection based on the presence of antibodies is of limited value in endemic areas, we have developed an ELISA for the detection of *P. falciparum* and *P. vivax* antigens in serum specimens. We used a monoclonal antibody in the solid phase and a pool of IgG from hyperimmune patients as the second antibody. Plasmodial antigens can be detected in 100% of the individuals with *P. falciparum* malaria and in 91.7% of those with *P. vivax* malaria at levels of 0.0003% and 0.01% of parasitemia respectively. The data obtained by testing symptomatic individuals with a past history of malaria[after ≤30, >30 <180, and ≥180 ≤360 days elapsed after last positive thick blood smears (TBS)], showed that this assay can be more sensitive than microscopic examination since we found positive results in 33.3%, 68.8%, and 20% of these individuals, respectively. This assay was also capable of detecting circulating plasmodial antigens among 40.6% of symptomless individuals with negative TBS studied up to 30 days after the last parasitologically confirmed attack of malaria. These findings suggest that the research of *Plasmodium* soluble antigens by ELISA may be a valuable alternative approach for the detection of malaria infection in endemic areas which can be used for blood donor screening in these regions.

386 VILLAGE-WIDE TRIAL OF INSECTICIDE-IMPREGNATED BED NETS AND CURTAINS: EPIDEMIOLOGICAL AND ENTOMOLOGICAL RESULTS FOR YEAR ONE. Beach RF*, Ruebush TK, Sexton JD, and Oloo AJ. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Vector Biology and Control Research Centre, Kenya Medical Research Institute, Kisumu, Kenya.

The effectiveness of permethrin-impregnated ($0.5g/m^2$) bed nets and eave, window and door curtains for malaria control is being evaluated in western Kenya. Six villages with a total population of 5200 were assigned to 1 of 3 study groups: bed net, curtain or control. Baseline *Plasmodium falciparum* prevalence was similar in all 3 groups (range: 54-62%). Twelve months after the trial was initiated significant differences ($\chi^2$, $P<0.05$) were observed between the control and the bed net and curtain vilages for the following parameters: 1)*P. falciparum* prevalence (control: 45%, bed net: 31%, curtain: 25%); 2) children with a positive blood smear and an axillary temperature >37.5°C (Control: 56%, bed net: 24%, curtain: 37%). 3) *P. falciparum* sporozoite antigen rate for *An. gambiae* and *An. funestus* (Control: 13%, bed net 8%, curtain 7%). A significant difference in *P. falciparum* parasitemia>$2500/mm^3$ was observed between the control and curtain groups (control: 48%, curtain: 34%). Incidence was not significantly different in the 3 groups. These results suggest that village-wide use of insecticide-treated bed nets and curtains may reduce both malaria prevalence and sporozoite infection rates as well as the severity of illness. The study will continue for a second year to see if the downward trend observed during year one continues.

## X: AMEBIASIS

387 P-GLYCOPROTEIN GENES OF *ENTAMOEBA HISTOLYTICA*. Samuelson J*, Descoteaux S, Ayala P, and Orozco E. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Experimental Pathology, CINESTAV-IPN, Mexico D.F., Mexico.

Previously we have shown that emetine resistant mutants of *E. histolytica* overexpress mRNAs for amoeba homologues of the P-glycoprotein gene, which pumps therapeutic drugs out of some multidrug resistant tumor cells and parasites. Now we have identified at least 4 different amoeba P-glycoprotein genes from a genomic library made from the emetine resistant amoebae. Three of these P-glycoprotein genes were chosen for sequencing, because they contain DNA sequences overexpressed by the mutant as shown by Northern blotting and by analysis of cDNAs. These amoeba P-glycoprotein genes appear similar to each other, contain no introns, and have two homologous halves each with an ATP binding site as has been shown for other P-glycoprotein genes. A fourth P-glycoprotein gene identified appears to be a pseudogene, because a stop codon is present in the middle of the 5' ATP binding site.

388 PCR METHOD FOR THE IDENTIFICATION OF *E. HISTOLYTICA* AND OTHER ENTERIC PATHOGENS IN STOOL SAMPLES Acuna-Soto R*, Samuelson J, De Girolami P, Schoolnick G, and Wirth D. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Clinical Laboratory, Deaconess Hospital, Boston, MA; and Division of Geographic Medicine, Stanford University, Stanford, CA.

A method has been developed for a simple, field based extraction of DNA from stool samples and subsequent PCR amplification of *E. histolytica* specific sequences. The stool samples were washed, subjected to freeze-thaw, dissolved in a detergent-buffer solution and digested with proteinase K. The sample is then boiled and desalted. This DNA is the substrate for PCR-based amplification using primer sequences derived from *E. histolytica* specific probes. This method has been used with clinical samples as well as with field samples collected in Chiapas, Mexico. A comparison of this method with standard microscopy and direct DNA probe analysis on 300 field samples is currently in progress. In addition, this method of stool sample treatment has been used for PCR detection of other enteric pathogens such as *Ascaris lumbricoides, Escherichia coli,* and enterovirus. It may have use as a general diagnostic procedure for stool samples. This method can be used with fresh stool, formalin-fixed samples and samples treated with the ethyl acetate.

389 PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF SEVERAL CLONES OF *ENTAMOEBA HISTOLYTICA*. Lazard D, Gamboa Y, Valdes J, Hernandez F, Sanchez T, and Orozco E*. Department of Experimental Pathology, CINVESTAV I.P.N., Mexico D.F., Mexico.

We have studied the virulence phenotype of three closely related clones and determined their molecular karyotype by transverse alternative electrophoresis. Their "chromosomal" patterns were very similar and actin and ribosomal genes were located in a 1.2 Mb molecule, although other chromosomes were also lighted with the ribosomal probe. We cloned and localized a sequence encoding for a transcript specific for virulent trophozoites. By differential screening we isolated a 3.5 kbp cDNA clone (pMD). Some pMD subfragments recognized several transcripts in both virulent and non-virulent trophozoites. Interestingly, another pMD subfragment hybridized with a 0.63 kb transcript exclusively in virulent trophozoites. Southern blot analysis of both chromosomes and DNA showed that all the transcripts are encoded by a linked piece of DNA localized in the 1.3 and 1.4 chromosomes. FCR experiments demonstrated that the 0.63 kb sequence is absent or modified in the non-virulent trophozoites. As far as we know, this is the first report on chromosomal localization for a DNA fragment that encodes a transcript specific for virulent trophozoites.

390 REGULATION OF CHITIN SYNTHETASE ACTIVITY IN ENCYSTING *ENTAMOEBA INVADENS*. Das S, Aley SB*, and Gillin FD. Department of Pathology, University of California, San Diego, CA.

Although the cyst wall of *Entamoeba invadens* contains chitin, synthesis of this structural polymer during encystation has not been described before. Here we report that conditions which stimulate encystation of the parasite lead to increased chitin synthetase (ChS) activity, measured by incorporation of $^{3}$H-GlcNAc from UDP-GlcNAc. The radiolabeled product was precipitable by TCA or alcohol and identified as chitin because it was digested by purified chitinase to radioactive chitobiose and GlcNAc. Cell fractionation indicated that approximately 60% of the enzyme is in the high speed supernatant. pH activity profiles showed that soluble ChS has an optimum at 6.0, while particulate ChS has a peak at 7.0-7.5. Both the activities were dependent on divalent metal ions, especially $Mn^{2+}$ and $Mn^{2+}$ plus $Co^{2+}$. In contrast to the ChS of other organisms, neither the particulate nor the soluble ChS of *E. invadens* enzyme was activated by trypsin treatment. Soluble and particulate ChS were also stimulated by digitonin and phosphatidylserine, while phosphatidylethanolamine stimulated only the soluble ChS. The enzyme activities were inhibited by UDP, UDP-glucose and UDP-GalNAc, but not by the analogs Polyoxin-D or Nikkomycin. This is the first report of an enzyme which is developmentally regulated during encystation.

391 ISOLATION AND PARTIAL CHARACTERIZATION OF A PORE-FORMING PROTEIN OF PATHOGENIC *ENTAMOEBA HISTOLYTICA*. Leippe M*, Ebel S, Horstmann RD, and Muller-Eberhard HJ. Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Federal Republic of Germany.

A protein that forms pores in target cell membranes is implicated in the potent cytolytic activity of pathogenic *Entamoeba histolytica*. Confirming previous reports pore-forming activity was detected in the 150,000 x g supernatant of an amoeba lysate using liposomes as targets. The pore-forming activity of the amoeba extract was found to be optimally expressed at pH 5.2, and active material preferrentially inserted into negatively charged lipid vesicles. Upon molecular sieve chromatography of the amoeba extract, the pore-forming activity was associated with material which eluted at a position corresponding to approximately 11 kDa. The pore-forming material was further purified to apparent homogeneity by a multi-step procedure. SDS-polyacrylamide gel electrophoresis of the purified material revealed one polypeptide of 4-5kDa under non-reducing as well as under reducing conditions. Treatment of the purified polypeptide with glutaraldehyde in solution or bound to liposomes revealed oligomers upon SDS-polyacrylamide gel electrophoresis, suggesting functionally relevant peptide-peptide interactions. The primary structure of the polypeptide was solved by protein sequencing. The polypeptide constitutes a potential candidate for vaccine development.

392 A MONOCLONAL ANTIBODY TO THE 170 KDA SUBUNIT OF THE GAL-ADHESIN ABROGATES THE RESISTANCE OF *E. HISTOLYTICA* TO LYSIS BY HUMAN COMPLEMENT C5B-9. Braga L*, Ninomiya H, Wiedmer T, McCoy J, Sims P, and Petri W, Jr. Department of Medicine, University of Virginia, Charlottesville, VA; and Oklahoma Medical Research Foundation, Oklahoma City, OK.

Pathogenic strains of *E. histolytica* (Eh) activate the alternative complement pathway, deplete serum C3, C5, C7, and C5b-9 hemolytic activity, bind C9, but are not lysed. Our hypothesis was that Eh has a cell surface protein that blocks the action of late complement components in a manner similar to the homologous restriction factors (HRF). Our aim was to identify molecules involved in complement resistance by producing anti-amebic monoclonal antibodies (mAb) that inhibit their activity. A mAb (3D12, IgG1 isotype) was isolated that increased susceptibility to complement lysis. Lysis of amebae in

50% normal human sera (NHS) was increased from 53 ± 3.4% to 85 ± 4.6% by 50 μg/ml of purified mAb 3D12. This effect of mAb 3D12 did not require C1 activation: In the presence of 3D12, amebic lysis in 30% NHS containing EGTA increased to 40 ± 3% versus 11 ± 12% (no mAb). In order to determine whether mAb 3D12 directly increased the lytic sensitivity of Eh to the human C5b-9 complex, C5b67 was first deposited (by incubation of ameba with either purified C5b6 ± C7, or in C8-def. human serum) before serum-free incubation with mAb 3D12 and C8 (2 μg/ml) and C9 (5 μg/ml). Under these conditions, C5b-9 mediated lysis increased from 2 ± 1% (no mAb) to 48 ± 2% in the presence of mAb 3D12. 3D12 immunoprecipitated a protein that was previously identified as the 170kDa subunit of the galactose adhesin. These results indicate that Eh resistance to the human C5b-9 complex can be overcome with a mAb against the galactose adhesin. The mechanism of the mAb neutralization of complement resistance and the role of the galactose adhesin in this process are being pursued.

393 ISOLATION OF AN UNIQUE *ENTAMOEBA HISTOLYTICA* CDNA CLONE ENCODING A PROTEIN WITH A ZINC FINGER DOMAIN. Stanley SL, Jr., Li E. Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO.

Little is known regarding the regulation of gene expression in the protozoan pathogen *Entamoeba histolytica*. Recently we used differential screening of a cDNA library derived from the pathogenic *E. histolytica* strain HM1:IMSS to isolate clones expressed in HM1:IMSS, but not in the *E. histolytica*-like Laredo strain. One of the clones contained an insert of 251 bp, and was designated c3. RNA hybridization analysis under high stringency conditions demonstrated that the c3 clone hybridized with a transcript of approximately 330 bp in all 5 axenic strains of *E. histolytica* examined, but not to other *Entamoeba ssp.* Similar specificity was seen with Southern blotting. The complete c3cDNA is 290 bp, and encodes a protein of 84 amino acids. Inspection of the c3 derived amino acid sequence revealed a region of 23 amino acids with the characteristics of a Cys-$X_2$-Cys-$X_n$-Cys-$X_2$-Cys "zincfinger". This region was identical in 9 of 23 amino acids (40%) with the putative zinc binding domain of the human *c-erb*-A gene. Studies are now underway to express this protein in prokaryotes to determine whether the c3 protein product can bind zinc, and whether, like other proteins with zinc fingers, it binds nucleic acids. The study of a zinc-finger protein from *E. histolytica* may provide insights into the regulation of gene expression in this pathogen.

394 APPLICATION OF TWO-DIMENSIONAL ELECTROPHORESIS TO CHARACTERIZE PROTEINS OF *ENTAMOEBA HISTOLYTICA*. Shen P, Nokkaew C, McCoomer NE, Pohl J, and Bailey GB*. Department of Biochemistry, Morehouse School of Medicine and Microchemical Facility, Emory University, Atlanta, GA.

We are utilizing two-dimensional gel electrophoresis (2-DE) to characterize *E. histolytica* proteins. More than 400 proteins were detected between Mr 100 kD - 14 kD and pI 3 - 9.5 with silver stain or by autoradiography after labeling cells in culture for 48 hr with $^{35}$S-methionine. Over 60 phosphorylated and 58 glycosylated proteins were detected after labeling of cells for 1 hr with $^{32}PO_4$ and for 48 hr in culture with $^{3}$H-mannose, respectively. More than 100 proteins were labeled after 48 hr in culture with $^{3}$H-palmitate. Eight isoforms of actin, the most abundant cellular protein, were detected by immunoblotting, three at Mr 43 kD and five at Mr 45 kD. Sufficient quantities of the more abundant proteins for amino acid analysis and microsequencing were obtained by transfer to PVDF membranes from as few as four 2-DE gels. All of four proteins analyzed to date were blocked at the N-terminus. A 15 amino acid internal sequence was obtained for one of these proteins (Mr 37 kD) by *in situ* cleavage with V-8 Protease followed by isolation on a 16% SDS-Tricine gel, blotting to PVDF and microsequencing of a Mr 21 kD fragment. These technologies, in combination with other tools of molecular biology, provide a means for identification and characterization of numerous *E. histolytica* proteins.

395 MODULATION OF ARACHIDONIC ACID (AA) METABOLISM IN MACROPHAGES BY *ENTAMOEBA HISTOLYTICA*. Wang W*, Chadee K. Institute of Parasitology of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

*Entamoeba histolytica* (Eh) infections cause transient suppression of cell-mediated immunity. We investigated whether macrophage (mϕ) dysfunction in amoebiasis is associated with altered mϕ AA metabolism. Resident peritoneal mϕ (PMO) from naive gerbils produced enhanced levels of prostaglandin $E_2$ ($PGE_2$) and leukotriene $C_4$ ($LTC_4$) in response to live Eh, diffusible excretory/secretory products from Eh and soluble Eh proteins that were inhibitable by indomethacin and nordihydroquiaretic acid, respectively. PMO from animals with amoebic liver abscesses released high basal levels of $PGE_2$ and $LTC_4$. In response to zymosan stimulation, PMO produced 2-and 4-fold less $PGE_2$ and $LTC_4$ respectively, as compared to uninfected controls. Liver abscess-derived mϕ (AMO) showed high constitutive basal release of $PGE_2$ and $LTC_4$. In response to Eh and zymosan stimulation, AMO at 10 days p.i. produced significantly higher levels of $PGE_2$ than AMO at 20 days, while AMO at 30 days p.i. were unresponsive. AMO were refractory to Eh and zymosan stimulation for enhanced $LTC_4$ release. Pretreatment of AMO with AA substrate restored $PGE_2$ and $LTC_4$ biosynthesis. These results demonstrate that Eh can induce profound alteration in AA metabolism in mϕ which can modulate immunoregulatory mechanisms in amoebiasis.

396 METRONIDAZOLE THERAPY IN AMEBIC LIVER ABCESS (ALA) AND RECOGNITION OF A CARRIER STATE. Jackson TG*, Irusen EM, and Simjee AE. Research Institute for Diseases in a Tropical Environment, Congella, Durban, Republic of South Africa.

The aims of the study were to (i) evaluate the efficacy of metronidazole 2.4g stat versus a conventional regimen in the therapy of amoebic liver abscess (ALA), and (ii) to identify carriers of pathogenic strains of *Entamoeba histolytica* (Eh) post treatment. Patients with uncomplicated ALA were randomly allocated to receive metronidazole 2.4g immediately or 800mg tds for 5 days. Their responses were assessed clinically, ultrasonically (U/S) and with stool cultures. The results can be summarized as follows:

| | 2,4 stat | | 800 mg tds x 5 days | |
|---|---|---|---|---|
| No. of patients | 25 | | 25 | |
| Resolution of temperature (mean days) | 3 | | 3 | |
| U/DS at discharge - decrease in size | 16 | | 17 | |
| U/DS at discharge - no significant change | 9 | | 8 | |
| Evidence of Eh in stool pre-treatment | 20 | | 16 | |
| Evidence of Eh in stool post-treatment | 11 | (55%) | 9 | (56%) |
| Zymodemes | ALL PATHOGENIC | | | |

Metronidazole 2.4g stat is equally effective in curing ALA as a conventional regimen. However, asymptomatic intestinal colonization by pathogenic zymodemes of Eh was present in 72% of patients and persisted post-treatment in over half of them, irrespective of the drug regimen. This carrier rate predisposed patients to recurrent bouts of invasion. Moreover, carriers are an important source of spread of disease to the community.

## Y: ARBOVIRUS EPIDEMIOLOGY

397 EPIDEMIOLOGY OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN AN AREA OF RURAL PEOPLE'S REPUBLIC OF CHINA (PRC) WITH MIXED APODEMUS AND RATTUS DERIVED INFECTIONS. Fisher-Hoch SP*, Ruo S, Li YL, Tang YW, Xu ZY, Liu ZL, Tong Z, Ma QR, and McCormick JB. Special Pathogens Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA; Department of Epidemiology, Shanghai Medical University, Shanghai, PRC; and Jiande County Antiepidemic Station, Jiande, Zhejiang Province, People's Republic of China.

Hemorrhagic fever with renal syndrome (HFRS) is a major public health problem in the People's Republic of China. The main reservoirs are *Apodemus agrarius* and *Rattus norvegicus* which carry viruses causing the severe and moderate disease respectively. Our study location was in a mountainous region in Zhejiang Province. In 1987, 219/1811 individuals (12%) had antibody to hantaviruses, with prevalence increasing with age. Illness to infection ratio was 1:5.5, with the earliest recalled case in 1973. In 1988, 30/1325 (2.3%) individuals, negative in 1987, had developed antibody to hantavirus, but only two had recorded HFRS illness. Illness to infection ratio was 1:14. Highest infection rates were in teenage males. Risk factors included location of the house, and straw stacks outside the house, but the strongest association was with keeping cats ($p<0.01$, OR 5.0,95% CI 1.56-15.92). *Rattus norvegicus* were trapped within houses and *Apodemus agrarius* in the fields. In a local survey of 457 hospitalized patients, the hemagglutination inhibition test which distinguishes patients with infections from the two rodent species, established that there was a single peak due to Hantaan virus infection in the late fall, just after the rice harvest and another peak in May/June around the wheat harvest. In contrast, most of the Seoul virus infections were found during the colder, winter months.

398 FILOVIRUS OUTBREAK AMONG PHILIPPINE NONHUMAN PRIMATES IN SOUTH TEXAS. Hendricks KA*, Taylor JP, Pearson SL, Simpson DM, Jahrling PB, and Fisher-Hoch SP. Bureau of Disease Control & Epidemiology, Texas Department of Health, Austin, TX; Infectious Diseases Program, Epidemiology Division, Texas Department of Health, Austin, TX; Texas Primate Center, Hazleton Research Products, Inc., Alice, TX; Associateship for Disease Prevention, Texas Department of Health, Austin, TX; Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; and Division of Viral and Rickettsial Diseases, Special Pathogens Branch, Centers for Disease Control, Atlanta, GA.

From February 1 through March 27, 1990 an outbreak of a newly-described filovirus, antigenically related to Ebola and Marburg viruses, occurred among 100 Philippine cynomolgus, *Macaca fasicularis*, monkeys in two rooms of a quarantine building in south Texas. Multiple specimens were available from 96 monkeys. Sixty-four of 96 monkeys tested were positive by either antigen capture test for filovirus (14), a single high titer or a four-fold rise in antibody (39), or both (11). Signs for 19 laboratory-confirmed cases with a single clearly defined clinical episode included: anorexia (100%), loose stools (55%), lethargy (48%),and epistaxis (17%). Necropsy observations in 15 of these included:splenomegaly (100%), myocardial hemorrhage (47%), enlarged kidneys (40%), hepatomegaly (20%), and petechia (7%). From February 2 through March 15, 46 of 52 animals in one of the rooms died; the six surviving animals were positive for filovirus. Cages were numbered horizontally and stacked in two tiers. Monkeys were more likely to die within two days of a horizontal neighbor (40%) than within two days of a vertical neighbor (26%). Because animals were consistently fed, handled, and treated in ascending order by cage number, these data suggest that handling procedures may be implicated in transmission.

399 A CASE-CONTROL STUDY OF RISK FACTORS FOR KOREAN HEMORRHAGIC FEVER AMONG SOLDIERS IN THE REPUBLIC OF KOREA. Dixon KE, Nang RN*, Kim DH, Huh J, Park J, and Hwang Y. United States Army Medical Research Unit-Republic of Korea, APO San Francisco, CA.

Each year, approximately 500 or more cases of Korean Hemorrhagic Fever (KHF) occur in the Republic of Korea (ROK). Over 100,000 cases of KHF are reported in the People's Republic of China with case fatality rates ranging from 3 to 15%. Despite the discovery of the etiologic agent for KHF, Hantaan virus, in 1976 by Lee et al., little is known about the specific risk factors or activities that predispose to KHF. We conducted a case control study on the risk factors for KHF on 369 Republic of Korea soldiers. The study was conducted from13 October 1989 to 3 January 1991 at the Capital Armed Forces General Hospital located in Seoul, Republic of Korea. We interviewed 146 KHF patients and 223 controls on specific activities and exposures that they experienced, 2 months prior to the onset date of their disease. Major risk factors identified include advanced tactical training, sleeping in tents, living in dwellings with dirt floors, digging trenches, exposure to both indoor and outdoor dust, sightings of the field mice *Apodemus*, the use of camouflage, and having a combat military specialty. Protective factors identified include basic training, sightings of rats, and living in modern quarters. It is hoped that the knowledge gained from this study will help in the development of preventive measures to combat risk factors identified for KHF.

400 CORRELATES OF HANTAVIRAL INFECTION IN PATIENTS FROM BALTIMORE, MD, USA. Gurri Glass GE*, Childs JE, Watson AJ, LeDuc JW. Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD; Division of Nephrology, Johns Hopkins University, School of Medicine, Baltimore, MD; and Disease Assessment Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick MD.

Sera from 1341 patients with proteinuria at Johns Hopkins Hospital were screened for serological evidence of hantaviral infection. Testing was by ELISA, and infection and incrimination of viral source were confirmed by plaque reduction neutralization assays. Twenty-two individuals (1.64%) were confirmed positive; all to Baltimore rat virus, a local Seoul-like hantavirus. Four of the individuals showed at least a four-fold rise in neutralizing antibody titer. Review of clinical records during seroconversion showed that all were acutely ill with nausea, vomiting, fever, epigastric pain, proteinuria, liver involvement, and oliguric renal failure. The remaining seropositive patients were age and sex-matched with seronegative patients. Seropositive patients had increased risk of hypertension, and hypertensive renal disease. The data indicate acute HFRS occurs in the United States, and may be associated with significant sequelae.

401 SEVERE DENGUE INFECTIONS AMONG CHILDREN IN METROPOLITAN BANGKOK 1973-90. Nisalak A*, Nimmannitya S, and Innis BL. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Children's Hospital, Bangkok, Thailand.

Bangkok has the most protracted problem of epidemic dengue hemorrhagic fever (DHF) in the world. To determine what dengue (DEN) types caused severe illness, an observational study of children admitted to Bangkok Children's Hospital with suspected DHF has been conducted since 1973. 2-month hospitalization rates for lab confirmed cases were computed. DEN epidemics (rates>150 admission/2 months) occurred in 1977, 1980, 1984,1987 and 1989. Each of the last 3 epidemics resulted in peak hospitalization rates greater than before. For 17 of 18 years, peak rates occurred Jul-Oct; >2 DEN types were isolated. In 1980 and in 7 of 10 subsequent years, 4 DEN types were isolated during rate peaks. DEN-1 and -4 emerged and disappeared several times; DEN-3 was a major type only over 4 years in the 1980's; DEN-2 was the type most consistently isolated. We drew the following inferences: (1) DEN is increasing in Bangkok, (2) all types may cause epidemic DHF, (3) epidemic DEN in Bangkok involves

simultaneous circulation of several types, (4) high rates of DEN-1 and -4 transmission can not be sustained in the face of simultaneous DEN-2 and/or DEN-3 transmission. Additional descriptive data about case severity and the virulence of DEN types will be presented.

402 FOCALITY OF DENGUE TRANSMISSION IN RURAL THAI VILLAGES AND PROPOSALS FOR COMMUNITY-BASED VECTOR CONTROL. Strickman D*, Kittayapong P, Innis BL, and Wannapong R. Department of Entomology, Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand; Walter Reed Army Institute of Research, Washington, DC; Department of Virology, Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand; and Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand.

A 1 yr study of dengue transmission and vector (*Aedes aegypti* and *albopictus*) populations was performed in a district of 9000 residents,100 km east of Bangkok. Transmission was evaluated by measuring IgG and IgM levels against dengue and Japanese encephalitis in 750 primary students each 6 weeks. Two hundred pre-schoolers (<6 yrs old) were sampled once. Vector populations in a 3-village area (appox. 15% of pop.) were measured by biting and ovitrap collections in 39 homes (3x/wk), biting collections in 2 schools (1/wk), and larval surveys of the 3-village area (3x/yr). Female mosquitoes were evaluated for dengue virus, wing length, and ovarian status. There was an increase in transmission of dengue from 1989 to 1990 (overall positive in September 6.8% to 25.3%). During part of the study, transmission in the school was concentrated in certain buildings. Cases in September 1990 were concentrated within certain areas of the village. Analysis is not complete at this date, but concentration in at least one village was correlated to concentration of children <6 yrs old. Most important sources of vectors are water jars, bathroom basins, and ant traps. A dengue suppression trial is in progress, using techniques based on locally available, cheap materials applied by local residents and targetted according to transmission risk.

403 A MODEL OF DENGUE FEVER TRANSMISSION, WITH AN EVALUATION OF THE PROBABLE IMPACT OF ULTRA LOW VOLUME (ULV) ADULTICIDING MEASURES ON DENGUE EPIDEMICS. Newton EA* and Reiter P. Caribbean Epidemiology Center, Port-of-Spain, Trinidad, West Indies; and Dengue Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, San Juan, PR.

We have developed a mathematical model of dengue fever transmission which enables us to explore conditions for epidemic and endemic transmission, and to experiment with vector control practices. It is a deterministic SEIR model: populations of each species are divided into compartments representing disease status (Susceptible, Exposed, Infectious, Resistant), and the flow between compartments is described by differential equations. Examination of the equilibrium points of the model leads to a formulation of the basic reproduction rate ($R$) of the disease. With a base set of parameters, $R = 2.076$, and the model realistically reproduces epidemic transmission in an immunologically naive population. Endemic transmission occurs in a partially immune population. Control of adult mosquitoes by ultra-low volume (ULV) aerosols is simulated by an abrupt decrease in vector densities, followed by gradual recovery of the vector population. The model indicates that, although the peak of the epidemic may be delayed, ULV has little impact on disease incidence, even when multiple applications are made. Source reduction or other means of decreasing the carrying capacity of the environment for mosquitoes, and thus the basic reproduction rate of the disease, is more effective in reducing transmission.

404 RIFT VALLEY FEVER VIRUS ACTIVITY IN WEST AFRICA, 1989-1991. Zeller HG*, Wilson ML, Schmidt EA, Thiongane Y, Cornet JP, Camicas JL, Gonzalez JP, Bessin R, Teou K, Kpomassi M, Formenty P, Digoutte JP, and Akakpo JA. Institut Pasteur, Dakar, Senegal; Yale University School of Medicine, New Haven, CT; Institut Senegalais de Recherches Agricoles, Dakar, Senegal; ORSTOM, Dakar, Seneal; Laboratoire National d'Elevage, Ouagadougou, Burkina Faso; Ecole inter-

Etats des Sciences et Medecine Veterinaires, Dakar, Senegal; and Laboratoire Central de Pathologie Animale, Bingerville, Cote d'Ivoire.

Rift Valley Fever (RVF) virus in East Africa produces epizootics that persist for 1-3 years and then disappear for 5-15 year intervals. In West Africa, however, little is known of RVF virus transmission. The recent epizootic period there (1987-88) also produced human infection in some sites as well as epidemic RVF in southern Mauritania and northern Senegal. Since then, we have been monitoring RVF virus transmission by studying domestic ungulates from this region and from Burkina Faso, Togo, Cote d'Ivoire and Cameroon. Sera were tested by ELISA for anti-RVF virus specific IgG and IgM. From hundreds of sheep and cattle sampled in the river and savannah regions of northern Senegal, only 3 seroconversions were recorded in 1989 and none in 1990. Similarly, few new RVF virus infections were found in the Sahelian and Sudanian habitats of the other countries. This rapid decline in incidence from 1987-88 suggests an inter-epizootic period, and raises questions as to virus maintenance. The possible influences of immunity and climate on the intensity of RVF virus transmission are discussed.

405 RIFT VALLEY FEVER VIRUS TRANSMISSION IN RURAL NORTHERN SENEGAL: HUMAN RISK FACTORS AND POTENTIAL VECTORS. Wilson ML*, Chapman LE, Hall DB, Dykstra EA, Ba K, Zeller HG, Traore-Lamizana M, Hervy JP, and Linthicum KJ. Institut Pasteur, Dakar, Senegal; Centers for Disease Control, Atlanta, GA; ORSTOM, Dakar, Senegal; Yale University School of Medicine, New Haven, CT; and U.S. Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

Rift Valley Fever (RVF) is an acute, severe, vector-borne, viral zoonosis endemic throughout much of Africa. Studies in eastern and southern Africa indicate that intense transmission occurs sporadically in time and space, due in part to changing mosquito densities. Less is known of RVF epidemiology in West Africa. We undertook a retrospective cohort study of the semi-nomadic Pulaar people living in a rural settlement in northern Senegal. A sample of 279 people were bled and given a standardized questionnaire concerning activities. Serologic studies of 375 sheep and population estimates of potential mosquito vectors were also made. About 22% of people exhibited anti-RVF virus IgG; none had IgM. Seropositivity was similar between the sexes, increased markedly with age, and was uneven among camps (0%-37.5%). Sheep IgG prevalence averaged 30.1% overall (0.8% IgM), also varied among camps (0%-66.7%), but was spatially unlike that in humans. The only human risk factor that emerged was treating sick animals, and this held true independent of age and for both sexes. Mosquito abundance(CDC traps) varied seasonally with rainfall (>90% in 4 mo); species diversity was large (24 spp), dominated by *Aedes* and *Culex*. Results suggest that RVF is endemic in northern Senegal and that people are at considerable risk of infection.

406 SPATIAL ANALYSIS OF RIFT VALLEY FEVER VIRUS VECTOR HABITATS BY INTEGRATION OF SENSOR DATA FROM SPOT, LANDSAT TM, AND AIRBORNE IMAGING RADAR. Freier JE*, Linthicum KJ, and Angleberger DR. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Sensor data derived from SPOT, LANDSAT Thematic Mapper (TM), and airborne synthetic aperture radar (SAR) were analyzed in an integrated scheme to locate dambo mosquito breeding sites in central Kenya and to determine dambo flooding status on a real time basis. The increased spatial resolution of SPOT-1 panchromatic images combined with the enhanced spectral and radiometric resolution of LANDSAT-5 TM images provided improved mapping of dambo locations. TM images were enhanced by nearest-neighbor resampling, band ratioing, high-frequency filtering,and data transformation by using a principal components analysis. Thematic information of dambos was extracted by a supervised classification based on training sites selected from known dambo locations. After areas were classified according to dambo, probable dambo, possible dambo, and non-dambo categories, selected classes were

further refined in a post-classification comparison. Determination of dambo flooding status was made with an airborne SAR system capable of full polarimetric detection of L, C, and X radar bands. As SAR functions are independent of solar radiation, emitted microwaves can penetrate clouds or smoke to determine terrain features. The Kenyan dambo areas examined by SAR were the same as those studied in the satellite imagery analysis. The SAR system was configured with along track and across track resolutions of 1.6 and 2.4 meters, respectively. Data analysis concentrated on the LHH (horizontal-horizontal), LVV(vertical-vertical), LVH (vertical-horizontal), CHH, CVH, XVV, and XVH backscatter amplitude data with a 1.6 meter grid cell. Results indicated that the LHH channel was best for detecting flooded dambos. Overall, the integration of data from remotely sensed sources permits determination of important information about environmental conditions that could lead to an epizootic of Rift valley fever virus.

## Z: FILARIASIS CHEMOTHERAPY

407 *BRUGIA MALAYI* AND *ACANTHOCHEILONEMA VITEAE*: ANTIFILARIAL ACTIVITY OF TRANSGLUTAMINASE INHIBITORS *IN VITRO*. Rao UR*, Mehta K, Subrahmanyam D, and Vickery AC. College of Public Health, University of South Florida, Tampa, FL; University of Texas, MD Anderson Cancer Center, Houston, TX; and Ciba-Geigy Ltd. and Swiss Tropical Institute, Basel, Switzerland.

Enzyme inhibitors of known specificity can provide the basis for targeted drug delivery and can be used for the intervention of metabolic pathways that are essential for the survival of parasites. The effect of transglutaminase inhibitors on survival of adult worms, microfilariae and infective larvae of *Brugia malayi* was studied *in vitro*. The parasite viability was assessed by their ability to transform MTT into its blue formazan derivative. Monodansylcadaverine, a competitive inhibitor, and cystamine or iodoacetamide, active site inhibitors of transglutaminase, significantly reduced the parasite mobility and viability in a dose-dependent manner. Structurally related, inactive analogue of monodansylcadaverine, dimethyldansylcadaverine did not affect the mobility or survival of the parasites. Adult worms failed to release microfilariae when incubated in the presence of monodansylcadaverine or cystamine and this inhibitory effect on microfilaria release was concentration dependent. Similar embryostatic and macrofilaricidal effects of monodansylcadaverine were observed in *Acanthocheilonema viteae* adult worms. These studies suggest that parasite transglutaminase may act as a key regulatory enzyme in growth and survival of the parasites and thus could serve as a potential target for new therapeutic agents for treating filariasis.

408 DOSE RESPONSE OF THE BENZIMIDAZOLE CARBAMATE, UMF-078, AGAINST *BRUGIA PAHANGI* IN BEAGLES WITH INDUCED LYMPHATIC INFECTIONS. Dzimianski MT*, McCall JW, Elslager EF, Townsend LB, and Wise DS. Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA; Elslager Associates, Ann Arbor, MI; and College of Pharmacy, University of Michigan, Ann Arbor, MI.

UMF-078 is a benzimidazole carbamate with potent antifilarial activity. In earlier studies with the jird and canine models, UMF-078 was macrofilaricidal and microfilaricidal against *Acanthocheilonema viteae* and *Brugia pahangi*. In the present study, a dose titration was done using dogs with lymphatic infections of *B. pahangi*. Twenty-eight dogs infected 4 months earlier with 200 infective larvae of *B. pahangi* were selected for use and randomly allocated to 7 groups of 4 dogs each. One group served as a control while dogs in the remaining 6 groups were given UMF-078 IM at: 1) 50 mg/kg/day (MKD) for 3 days (x3), 2) 25 MKDx3, 3)12.5 MKDx3, 4) 6.25 MKDx3, 5) 50 MKDx2, and 6) 50 MKDx1. The dogs were bled for microfilarial counts prior to treatment and at weekly intervals thereafter until necropsy, which was 8 weeks after treatment began. The number of adult *B. pahangi* recovered from the control dogs ranged from 35 to 83 with an average of 63.8 worms. Posologies of 50 MKDx3 and 25 MKDx3 were curative against the macrofilariae; marked reductions were obtained with posologies of 12.5 MKDx3 (91%), 50

MKDx2 (92%), and 50 MKDx1 (85%). UMF-078 given at 6.25 MKDx3 was ineffective (38%). Only 50 MKDx3 was clearly microfilaricidal.

409 DIETHYLCARBAMAZINE AUGMENTS PLATELET-ACTIVATING FACTOR SYNTHESIS AND NEUTROPHIL ADHERENCE TO ENDOTHELIUM. Kanesa-thasan N*, Douglas J, and Kazura J. Case Western Reserve University, Cleveland, OH.

Diethylcarbamazine (DEC) inhibits prostaglandin production by microfilariae and pulmonary arterial endothelial monolayers (EM), consistent with inhibition of cyclooxygenase (CO). CO inhibitory drugs may augment synthesis of platelet-activating factor (1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine, PAF), a potent lipid mediator of neutrophil (PMN) adherence to EM. We therefore assessed the effects of DEC on stimulated PAF synthesis by EM and subsequent PMN adherence. EM pretreated with 1 $\mu g\ ml^{-1}$ DEC and stimulated with $10^{-7}$ M bradykinin demonstrated a 58% increase in PAF synthesis vs controls (M ± SD 637 ± 69 vs 404 ± 37 cpm $^3$H-PAF, respectively, $p=0.014$). Exposure of unstimulated EM to DEC had no effect on PMN adherence. In contrast, DEC pre-treated EM stimulated with bradykinin demonstrated a 24% increase in PMN adherence relative to EM not exposed to DEC ($p<0.05$). Prior loading of DEC-treated EM with 60 uM lyso-PAF, aprecursor of PAF, augmented binding of PMN to EM from 26 to 37%. These data suggest that vascular sequestration of PMN associated with DEC therapy is related in part to increased endothelial PAF synthesis.

410 LONG-TERM OUTCOME OF TREATMENT WITH DEC OR IVERMECTIN ON BANCROFTIAN FILARIASIS IN HAITI. Eberhard ML*, McNeeley DF, Addiss DG, Spencer HC, and Lammie PJ. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and St. Croix Hospital, Leogane, Haiti.

Control of lymphatic filariasis has been difficult and the long-term effectiveness of treatment is unclear. Data was collected on 7 groups of *W. bancrofti*-infected individuals treated with either DEC, ivermectin (IV), or both drugs. Two groups received 12 doses of DEC (6mg/kg) either daily or weekly, one group received a clearing dose of IV (20 mcg/kg) prior to 12 daily doses of DEC, and 4 groups received 200 or 400 mcg/kg IV preceded by a clearing dose of IV (20 mcg/kg). Two DEC groups were followed for 5 yr; two IV groups and one DEC group were followed for 2 yr. DEC (12 doses) provides better long-term mf clearance and reduction in mf numbers than does IV. When preceded by a 20 mcg/kg clearing dose of IV, daily DEC was equivalent to weekly administration in mf clearance and reduction in mf levels. No difference was detected between 200 and 400 mcg/kg IV nor between 400 mcg/kg as a single or divided dose. There is no evidence that IV kills adult *W. bancrofti*, but parasitological data at 2 yr indicates continued suppression of microfilaremia. These studies indicate that the benefit from treatment with either DEC or IV is much longer lasting than believed. Controlling lymphatic filariasis may hinge more on effective drug delivery than on the frequency of retreatments.

411 CHANGES IN CIRCULATING PARASITE ANTIGEN LEVELS AFTER DIETHYLCARBAMAZINE AND IVERMECTIN TREATMENT OF BANCROFTIAN FILARIASIS. Weil GJ*, Lammie PJ, Richards FO, Jr. and Eberhard ML. Washington University School of Medicine, St. Louis, MO; and Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

The purpose of this study was to measure changes in circulating parasite antigen levels and microfilaria counts after diethylcarbamazine (DEC) and ivermectin treatment of bancroftian filariasis in order to determine effects of these drugs on adult *Wuchereria bancrofti in vivo*. Thirty adult Haitians with microfilaremia (MF) were treated with 1 mg of ivermectin to reduce MF counts. Later, subjects were treated with either one or two 200 $\mu g/kg$ doses of ivermectin or with 12 daily 6mg/kg doses of DEC.

Microfilaremia was reduced by over 98% in all subjects 1 month after initiation of treatment. However, MF count recovery was significantly greater after ivermectin than after DEC 9 and 12 mo after treatment. Macrofilaricidal activity of these drugs was indirectly monitored by measuring circulating *W. bancrofti* antigen by enzyme immunoassay. Antigen levels fell by 75% after DEC and by 34% after ivermectin. These results suggest that low dose ivermectin followed by a standard course of DEC is a more effective macrofilaricidal regimen for *W. bancrofti* than either of the multidose ivermectin regimens used in this study.

412 COMPARATIVE TRIAL OF IVERMECTIN AND DIETHYLCARBAMAZINE GIVEN IN A CLEARING DOSE AND A SINGLE HIGH DOSE FOR TREATMENT OF LYMPHATIC FILARIASIS, HAITI. Addiss DG*, Eberhard ML, Lammie PJ, McNeeley MB, Lee SH, and Spencer HC. Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; Ste. Croix Hospital, Leogane, Haiti; and Division of Field Epidemiology, Epidemiology Program Office, Centers for Disease Control, Atlanta, GA

When ivermectin (IV) is given to Haitians with lymphatic filariasis in a clearing dose (20 μg/kg) followed by a high dose (200-400 μg/kg), long term suppression of microfilaremia (mf) occurs that is similar to that observed after 12-day diethylcarbamazine (DEC) treatment. However, single high-dose IV has not been adequately compared with single-dose DEC. To evaluate tolerance and long-term efficacy of various clearing dose/single high dose combinations of IV and DEC, 60 persons with mf counts of >100/ml were enrolled in the following groups of a 6-arm trial (numbers are doses for IV (mcg/kg) and DEC (mg/kg)): IV20/IV400; IV20/IV200; IV20/DEC6; DEC1/DEC6; placebo/IV200; and placebo/DEC6. Clearing dose was given on day 0, single high dose on day 5. On Day 10, groups receiving IV had significantly lower geometric mean mf (<1/ml) and fewer mf-positive persons (<20%) than did groups that received only DEC (>100 mf/ml and >90%, respectively). By 4-6 months, these differences narrowed as geometric mean mf increased in the IV groups. IV20 was associated with significantly more side effects (fever, headache, weakness, etc.) than either DEC1 or DEC6. These preliminary results suggest that the optimal clearing dose/single high dose regimen for long-term suppression of mf with the fewest side effects may include DEC. Data from the 1-year follow-up will be presented.

413 EFFICACY AND TOLERANCE OF SINGLE DOSE DIETHYLCARBAMAZINE VS. IVERMECTIN IN BANCROFTIAN FILARIASIS IN PAPUA NEW GUINEA. Greenberg J, Day K, Alpers M, and Kazura J*. Case Western Reserve University, Cleveland, OH; Papua New Guinea Institute of Medical Research, Goroka; and Imperial College, London, UK.

This is a 3 month progress report of a double-blind study comparing the efficacy of diethylcarbamazine (DEC) to ivermectin (IVR) in microfilaremic (mf) Papua New Guinea men. Two groups of 10 persons each (total 20) received DEC (either 1 mg and 6 mg/kg on days 1 and 5 or 6 mg/kg one time only); 3 groups of 10 persons each (total 30) received IVR (20 μg and 200 μg/kg on days 1 and 5; 20 μg and 400 μg/kg on days 1and 5; 220 μg/kg one time only). 14 days after drug administration, 20 persons had higher intensities of microfilaremia than the 30 other subjects (geometric mean of 1475 mf/ml compared to 4275 mf/ml pretreatment, a 65% decrease, in the former group; 2 mf/ml compared to 4462 mf/ml, a >99% decrease, in latter group, $p<0.001$). No differences between the groups in the severity of side effects were noted. At 3 months, the intensity of microfilaremia increased in all persons but remained lower in the group of 30 persons who experienced the greatest reduction of parasitemia at 14 days (mean of 564 mf/ml for the group of 20 persons vs 240 mf/ml for the group of 30 individuals). Follow-up at 6 months and unblinding of this trial prior to presentation will provide insights into the appropriate choice of chemotherapy for Bancroftian filariasis in an endemic area where transmission is high.

414 TOLERANCE, SAFETY AND EFFICACY OF ALBENDAZOLE FOR HUMAN LOIASIS: RESULTS OF A DOUBLE-BLIND, PLACEBO CONTROLLED TRIAL. Klion AD*, Massougbodji A, Horton J, Ekoue S, Lanmasso T, Ahouissou LN, Yetongnon J, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Faculte des Sciences de la Sante, Universite Nationale du Benin, Cotonou, Republique du Benin; and SmithKline Beecham Pharmaceuticals, Hertfordshire, England.

Conventional therapy for loiasis with diethylcarbamazine often requires multiple courses and may be associated with severe adverse effects in patients with high blood levels of microfilariae. The benzimidazole derivative, albendazole, has been used successfully to treat adult and larval stages of a wide range of nematodes with few adverse effects. In order to assess the filaricidal activity and clinical safety of albendazole in human loiasis, we conducted a double-blind, placebo controlled study in an endemic area in Bénin, Africa. Nineteen male patients with between 100 and 30,000 microfilariae/ml blood were randomly assigned to receive albendazole (200 mg; n=9) or placebo (n=10) twice daily for twenty-one days. In the albendazole group, microfilarial levels began to decrease at day 14 posttreatment, and by day 20 had fallen to a geometric mean of 32.2% of pretreatment levels. Mean microfilarial levels in the placebo group at day 20 were 91.7% of pretreatment values. There were no clinical adverse effects and geometric mean eosinophil levels remained unchanged in both groups. No hepatotoxicity, renal toxicity or hematologic abnormalities attributable to the drug were observed. Whether the slow microfilarial clearance in the albendazole group results from a gradual microfilaricidal activity of the drug or from a primarily macrofilaricidal effect will be clearer after the three (7/91) and six (10/91) months posttreatment evaluations.

## AA: ENTOMOLOGY

415 HOST-SEEKING BEHAVIOR IN THE YELLOW FEVER MOSQUITO INHIBITED BY A *NEUROPEPTIDE*. Brown MR*, Klowden MJ, and Lea AO. Department of Entomology, University of Georgia, Athens, GA; and Division of Entomology, University of Idaho, Moscow, ID.

The host-seeking behavior of a female mosquito, *Aedes aegypti*, is employed to locate a vertebrate host for ingestion of a blood meal. A blood meal provides nutrients for storage in the egg and for metabolism in the female. During blood digestion, the female generally no longer seeks another host. A decapeptide, Aea-HP-I, originally isolated from female heads, appears in the hemolymph of gravid mosquitoes at the same time host-seeking behavior is inhibited. The secreted peptide was identified with a radioimmunoassay, specific for Aea-HP-I. The peptide occurs in the neuroendocrine system of the brain and the midgut endocrine system as detected with immunocytochemistry. When the synthetic peptide is injected into sugar-fed females, it effectively inhibits host-seeking with a dose as little as 16.5 pmole; whereas a related peptide, differing by only one amino acid, is not inhibitory. These results indicate that a behavior critical for transmission of pathogens by mosquitoes may be subject to manipulation by genetic engineering or application of stable analogues to pest populations.

416 DETECTION OF ADENINE NUCLEOTIDES BY THE LABRAL APICAL RECEPTORS OF MOSQUITOES. Liscia A and Galun R*. The Department of Physiology, University of Cagliari, Cagliari, Italy; and Kuvin Centre for Infectious and Tropical Diseases, Hebrew University Medical School, Jerusalem, Israel.

It has been established experimentally that adenine nucleotides must be present in artificial saline diets in order to stimulate gorging by culicine mosquitoes. These compounds show the following descending order of potency: ADP > ATP = AMP = AMP - PNP and no activity for adenosine for *Culex pipiens*, and AMP-PNP > ATP > ADP >> AMP for *Aedes aegypti*. *Anopheles spp.*, studies suggest that they ingest saline avidly without any adenosine nucleotides. Two pairs of contact chemosensilla are found on the external surface of the tip of the labrum of female mosquitoes - the apical and subapical sensilla. As these sensilla

are the only ones present on the stylets that enter the artificial dies (or the host tissue), we tested their sensitivity to adenine nucleotides at a concentration range of $10^{-3}$ to $10^{-5}$ M by electrophysiological techniques. The sensitivity of the apical sensilla correlated very well with the behavioral response. In *C. pipiens* spike frequency was highest with ADP, followed by ATP, which equalled that of AMP. High activity was recorded also in response to AMP-PNP. No response to high concentration of ATP was recorded from the apical sensilla of *Anopheles*, which does not require adenine nucleotide signals for ingestion. A comparable study of sensitivity of the apical receptors of *A. aegypti* is currently being carried out.

417 INGESTION OF VARIOUS BLOOD CELLS BY MOSQUITOES. Vardimon-Friedman H*, Frankenberg S, and Galun R. Kuvin Centre for Infectious and Tropical Diseases, Hebrew University Medical School, Jerusalem, Israel.

While anopheline mosquitoes ingest plasma alone, *Culex spp.* and *Aedes spp* require the presence of blood cells in order to ingest the diet. The phago-stimulatory effect of the blood cells can be duplicated by the addition of adenine-nucleotides to plasma. Since activation of platelets releases ADP and ATP into the plasma, their effect on diet ingestion was quantified for the mosquitoes *Aedes aegypti* and *Culex univittatus*. About $10^6$ $mm^3$ inactivated platelets were required to induce ingestion by 80-90% of the mosquitoes of both species. Thrombin activation of the platelets reduces the effective does to less than 2 $x10^4$. For the same level of ingestion, $10^5$ RBC were required. The various types of leukocytes induce considerable ingestion at their normal concentration in blood. As more than $10^{-5}$ M of ATP or ADP are needed in order to induce high levels of ingestion, the ways by which the various blood cells create such concentrations of adenine nucleotides in the vicinity of the receptor site is discussed.

418 SALIVARY GLAND PROTEIN DEPLETION DURING BLOOD FEEDING IN ANOPHELINE MALARIA VECTORS. Klein TA* and Golenda CF. Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.

The reduction in total protein and saliva from the salivary glands during blood-feeding of anopheline mosquitoes on human hosts may have an important impact on the number of sporozoites injected during feeding.To assess total protein released from the salivary glands during blood-feeding by mosquitoes on human hosts, studies were conducted with 4 colonized species of anopheline vectors (*Anopheles gambiae, An.freeborni, An. stephensi* and *An. albimanus*). The time for colonized mosquitoes to feed to repletion in the laboratory was determined for each species. The salivary glands were removed within 1 hr of blood-feeding and assayed for total protein using a Comassie blue-based method. In all species, total salivary gland protein was significantly reduced in the salivary glands of mosquitoes that fed on humans compared to a cohort group of mosquitoes that were not blood fed. Depending on the species, total protein reduction for fed mosquitoes ranged between 28 and 49% compared to unfed mosquitoes. Saliva samples were subjected to polyacrylamide gel electrophoresis to compare proteins in the saliva of the vector anophelines. These data provide baseline information for subsequent studies that will be conducted with *Plasmodium falciparum* infected mosquitoes.

419 CHARACTERIZATION OF A PREFERENTIALLY EXPRESSED POLYPEPTIDE FROM THE HEMOLYMPH OF IMMUNE-ACTIVATED *AEDES AEGYPTI*. Beerntsen BT* and Christensen BM. Department of Veterinary Science, University of Wisconsin, Madison, WI.

The present studies were initiated to characterize an 84 kDa polypeptide that is preferentially expressed in the hemolymph of *Aedes aegypti* during wound healing and melanotic encapsulation reactions against filarial worms. A microsequence of this polypeptide was obtained and sequence data analysis by NBRF,

a protein database sequence program, showed no homology to any of the 26,000 known protein sequences. Degenerate oligonucleotide primers then were made and the Polymerase Chain Reaction used to gain nucleotide sequence data. Additional studies were done to determine if this polypeptide could be induced in mosquitoes exposed to parasites that develop normally into infective third-stage larvae. SDS-PAGE analysis of hemolymph from these mosquitoes as well as controls detected a polypeptide with a molecular weight similar to that of the 84 kDa polypeptide. Antibodies are presently being used in an attempt to determine the identity of this particular polypeptide. Characterization of the 84 kDa polypeptide will provide information regarding its possible role in wound healing and the immune response of mosquitoes against filarial worms.

420 ISOLATION OF *ANOPHELES GAMBIAE* RFLP FOR USE IN GENETIC AND CYTOGENETIC MAPPING. Romans PA*, Bhattacharyya RK, Colavita AC, D'cunha C, Graziosi C, Kew Y, and Seeley DC. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; and Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

Several genes affecting malaria refractoriness and other aspects of vector competence have been identified in *Anopheles gambiae*. However, their further study has been hampered by the lack of useful genetic/cytogenetic markers. Restriction fragment length polymorphisms (RFLP) are DNA-based genetic markers which have been exploited with great success in mapping and cloning human disease genes. We identified RFLP in *Anopheles gambiae* DNA by screening large unique sequence genomic clones against restriction endonuclease digested DNA's from a few individual mosquitoes. Approximately 85% of randomly chosen clones identify a frequent EcoR I RFLP, while over 90% identify a RFLP for one or more of EcoR I, Sal I, Hind III and BamH I. Cytogenetic locations of RFLP-marking clones are being determined by hybridization to a panel of DNAs amplified from microdissected polytene chromosome divisions, and, more precisely, by *in situ* hybridization to ovarian nurse cell polytene chromosomes. Conducting crosses and mapping with 10-15 well-spaced markers should enable us to localize any gene to a region on one of the five chromosome arms. Locations will be refined progressively by mapping with additional markers in the identified regions.

421 GENETIC MAP OF *AEDES AEGYPTI* WITH RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND MORPHOLOGICAL MARKERS. Severson DW*, Mori A, Helke DM, and Christensen BM. Department of Veterinary Science, University of Wisconsin, Madison, WI.

We have constructed a restriction fragment length polymorphism (RFLP) map of *A. aegypti* with an emphasis on saturation mapping the linkage group known to contain the primary genes associated with susceptibility of the mosquito to various filarial worm species. RFLP segregation analyses involved $F_2$-progeny of pairwise matings between laboratory strains of mosquitoes. With each segregating population, one of the parental strains carried morphological mutant markers. Segregation analyses involving morphological markers allowed us to orient our RFLP markers on the 3 *A. aegypti* linkage groups (chromosomes). Morphological markers utilized included: bronze body, red eye and sex (linkage group I); spot abdomen (linkage group II); and black-tarsi(linkage group III). We have identified RFLP markers for each of the *A. aegypti* chromosomes. Our research efforts continue toward development of a saturated RFLP linkage map (minimum average spacing of 5 cM between RFLP markers). This map will provide the mechanisms to ultimately isolate and characterize those genes that confer susceptibility to filarial worms including screening for quantitative trait loci (QTL mapping) and chromosome walking/jumping techniques.

422 DNA PROBES FOR THE MEMBERS OF THE *ANOPHELES PUNCTULATUS* COMPLEX IN AUSTRALIA AND COASTAL PAPUA NEW GUINEA. Burkot TR*, Beebe NW, Cooper L, Foley

D, Bryan J, Cooper RD, Hii J, and Packer M. Tropical Health Program, Queensland Institute of Medical Research, Bramston Terrace, Australia; Tropical Health Program, Department of Entomology, University of Queensland, St. Lucia Q, Australia; Army Malaria Research Unit, Ingleburn NSW, Australia; and Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea.

The members of the *Anopheles punctulatus* complex are the most important vectors of human malaria in the Southwest Pacific. They are also important vectors of filariasis caused by *Wuchereria bancrofti.* Allozyme analysis has revealed that there are at least 9 members in the complex. At least 3 isomorphic species are found in Australia with an additional 3 species found in the coastal regions of Papua New Guinea. Genomic libraries were made for 6 species in the complex and screened with DNA from homologous and heterologous mosquito species. Species specific DNA probes to distinguish these isomorphic species were found and sequenced. These probes can identify mosquito species in dot blots or squashes using fresh, frozen, dried or ethanol preserved material.The probes have a sensitivity of less than one nanogram. These probes will enable large scale investigations into the biology of these species to determine their relative importance in the transmission of human malaria and filariasis.

423 ESTIMATION OF THE LONGEVITY OF ADULT *AEDES AEGYPTI*, BASED ON THEIR RECUPERATION RATE AFTER APPLICATION OF ULTRA LOW VOLUME (ULV) INSECTICIDES. Reiter P* and Newton EA. Dengue Branch, Division Vector-borne Infectious Diseases, Centers for Disease Control, San Juan, PR; and Caribbean Epidemiology Center, Port-of-Spain, Trinidad, West Indies.

Vector longevity is a key factor in the transmission of vector-borne disease. Field estimates (e.g., by mark-recapture) are particularly difficult for low density species such as *Aedes aegypti.* We have devised a method based on the recovery of the adult population following Ultra Low Volume (ULV) adulticiding operations. The impact of ULV is limited to the period when the insecticide particles are airborne, generally a matter of minutes. The immature, aquatic stages of the mosquito are unaffected by the treatment, and adult populations return to pre-spray levels in a few days. Recuperation can be described by the differential equation:

$$\frac{dN}{dt} = \frac{(K-N)}{L}$$

where $N$ = the mosquito population; $t$ = time; $K$ = the carrying capacity for adult mosquitoes, and $L$ = the mosquito life span. The solution to this equation is:

$$N(t) = K(1 - re^{-t/L})$$

where $r$ is the level of reduction at time zero. By fitting this curve to data obtained in daily collections of adults during the post-spray recovery period we can obtain estimates of longevity. Twelve sets of treatment/recuperation data gave estimated life spans of 3.5 to 4 days (daily survival rates of 0.75 to 0.78). These values are consistent with survival rates obtained by other methods.

424 EPIDEMIOLOGIC EVALUATION OF TICK-BORNE INFECTIONS AMONG MILITARY PERSONNEL CONDUCTING FIELD TRAINING IN ARKANSAS Sanchez JL*, Yevich SJ, DeFraites RF, Fishbein DB, Greene NR, Dawson JE, Uhaa IJ, and Johnson BJ. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC; Viral and Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA; and Division of Vector-Borne Infectious Diseases, Fort Collins, CO.

To define the tick-borne disease threat to military personnel, an epidemiological and clinical prospective evaluation of 1200 soldiers was conducted. Exposures took place in areas of heavy tick infestation between May and September 1990 at Fort Chaffee, Arkansas. Sera were obtained one to two weeks before and two to six weeks after exposure, and a questionnaire probing state of health and vector exposure history was administered. Antibody titers against several etiologic agents of febrile illnesses were determined, to include: *Rickettsia rickettsii, R. typhi, Coxiella burnetii, Ehrlichia canis, Borrelia burgdorferi, Francisella tularensis,* and tick-borne viral agents. Overall seroconversion rates, indicated by a four-fold rise in titer, to *R. rickettsii* and *Ehrlichia spp.* were found to be 2.5% and 1.2%, respectively. An ehrlichia agent (*E. chaffeensis*), distinct from *E. canis,* was isolated from a febrile patient at Fort Chaffee, and 1.2% of the cohort of soldiers studied seroconverted to this agent. Risk of infection by *R. rickettsii* was clearly seasonal, with infection rates as high as 10.6% during May compared to a low of 0.2% during August. This correlated with *Amblyomma americanum* infection rates of 5.6% in May compared to <1% in August. A significant association between a history of tick attachment, seroconversion to *R. rickettsia,* and fever, chills, nausea/vomiting, was documented. Over 73% and 67% of seroconverters to *R. rickettsii* and *Ehrlichia spp.* respectively were asymptomatic. Personal protective measures were evaluated and use of permethrin in clothing was found to be protective.

## BB: MALARIA IMMUNOLOGY II

425 EVIDENCE FOR FUNCTIONAL REGIONS ON THE 175 K *PLASMODIUM FALCIPARUM* ERYTHROCYTE BINDING ANTIGEN. Sim KL*, Ware L, and Gross M. Walter Reed Army Institute of Research, Washington DC; Johns Hopkins School of Public Health, Baltimore, MD; and Smith Kline Beecham, King of Prussia, PA.

The gene encoding a putative *Plasmodium falciparum* merozoite receptor, a 175 kilodalton (K) erythrocyte binding antigen (EBA-175), has been cloned and sequenced. Antibodies to a 43 amino acid peptide, EBA-peptide 4, from the carboxyl third of EBA-175 have previously been shown to block the binding of EBA-175 to erythrocytes and inhibit the invasion of merozoites into erythrocytes. To determine if there are other functional regions on the EBA-175 molecule, EBA-175 was expressed in 9 overlapping fragments of 177 to 267 amino acid residues using an *Escherichia coli* high expression system. The expressed proteins were studied in immunoblot analyses with sera from adults from a hyperendemic malarious area of Indonesia, and with antibodies eluted from immune complexes formed when *P. falciparum* merozoites were cultured in the presence of sera from Aotus monkeys immune to reinfection with *P. falciparum* malaria. The hyperimmune monkey sera inhibit merozoite dispersal during schizont infected erythrocyte rupture, and may directly inhibit invasion of merozoites into erythrocytes, by reacting with exposed epitopes on merozoites. In addition to EBA-peptide 4, the hyperimmune human sera and the antibodies eluted from immune complexes identified one common region, EBA-10.3. Sera from mice immunized with EBA-10.3 recognized parasitized erythrocytes in an indirect fluorescent antibody test. Since EBA-10.3 is distinct from EBA-petide 4, these data suggest the identification of a second functional region on EBA-175. Work in progress to further characterize antibodies to EBA-10.3 will be reported.

426 SIALIC ACID INDEPENDENT BINDING OF SERA ANTIGEN OF *PLASMODIUM FALCIPARUM* TO HUMAN ERYTHROCYTES. Ziefer AA* and Perkins, ME. The Rockefeller University, New York, NY.

*P. falciparum* is dependent on sialic acids of glycophorin for invasion. For some strains, this is absolute but for others only a partial dependency is observed. Sialic acid independent invasion varies from 5%-50% of the rate into normal cells. The major merozoite surface glycoprotein MSA-1 (m.w. 198-200,000) and a second merozoite antigen EBA-175, have been shown to bind to the sialic acid residues of human erythrocytes. In the present study we demonstrate that the SERA antigen, a protein of 126 kd localized to the parasitophorous vacuole space, binds to erythrocytes independently of sialic acid. Its binding to

erythrocytes is low affinity as the protein is released by a single wash with isotonic buffer. The sialic acid independent binding of SERA may be responsible for alternate recognition of the erythrocyte during invasion.

427 CROSS-REACTIONS BETWEEN IDIOTYPES, *PLASMODIUM FALCIPARUM* DERIVED PEPTIDES, DINITROPHENYL AND β(2→6) POLYFRUCTOSAN Daniel-Ribeiro C*, Deslandes D, and Ferreira-da-Cruz MF. Department of Immunology, WHO Collaborating Center for Research and Training in Immunology of Parasitic Diseases, IOC - Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

In order to evaluate the role of the idiotype-anti-idiotype network in the polyclonal B-cell activation (PBA) associated with malaria we decided to study the cross-reactions between idiotype, *P. falciparum* $(NANP)_4$ and 307 peptides, the levan molecule, and the DNP heterologous antigens. We used a panel of nine monoclonal antibodies including one antibody (ABPC48 idiotype) directed against β(2 → 6) polyfructosan (a fragment of the levan molecule), five anti-idiotype antibodies specific to the ABPC48 idiotype, one antibody specific for DNP-TNP, one specific for phosphorlycholine (T15 idiotype) and finally one anti-T idiotype antibody. These monoclonal antibodies were tested by IRMA against the DNP molecule coupled to the bovine serum albumin (BSA), the 307 synthetic peptide that represents the epitope of a *P. falciparum* hepatic stage specific antigen and the $(NANP)_4$ synthetic peptide that corresponds to the repetitive epitope of the circumsporozoite surface. Besides the anti-TNP-DNP antibody, the ABPC48 and one anti-idiotype antibody (IDA 19) reacted with DNP-BSA (independently of the protocol used, employing ethanolamine or carbonate buffer). Two anti-idiotype antibodies were positive against the $(NANP)_4$ antigen and three reacted with the 307 antigen which was also recognized by the ABPC48. One of these antibodies (IDA 8) was positive to both *P. falciparum* peptides tested. These preliminary results suggest the existence of cross-reactions between plasmodial and other heterologous antigens and idiotypes. Therefore, malaria induced cross-reactive antibodies could act as auto- and/or hetero-antibodies explaining, at least partially, the malaria associated PBA phenomenon and could modulate the specific immune response during the course of the infection.

428 HUMAN ANTIBODY LEVELS TO R32LR CORRELATE WITH LYMPHOCYTE PROLIFERATIVE RESPONSES TO POLYMORPHIC *PLASMODIUM FALCIPARUM* CS PROTEIN T CELL EPITOPES. Mason CJ*, Adoyo MA, Klotz FW, Coyne PE, Holland CA, Icayan LO, Sherwood JA, Copeland RS, Koech DL, Strickland GT, Chulay JD, and Hoffman SL. Kenya Medical Research Institute, Nairobi, Kenya; Malaria Program, Naval Medical Research Institute, Bethesda, MD; Walter Reed Army Institute of Research, Washington, DC; US Army Medical Research Unit, Nairobi, Kenya; Walter Reed Army Medical Center, Washington, DC; US Army Medical Research Institute for Infectious Diseases, Ft Detrick, MD; and University of Maryland School of Medicine, Baltimore, MD.

The development and maintenance of high levels of antibodies directed against the repeat region of the *Plasmodium falciparum* circumsporozoite (CS) protein will require the development of vaccines capable of natural boosting with continued falciparum malaria exposure. The CS protein is known to contain several polymorphic T cell domains. *In vitro* lymphocyte proliferative responses to 18 CS protein derived peptides were studied in 116 adult male Kenyan residents of a malaria hyperendemic area. The 18 peptides included the following regions and variants of the CS protein: 123 to 142 (7G8), 151 to 170 (7G8), 326 to 345 (7G8, Wel, LE5), 351 to 370 (7G8, Wel), 361 to 380 (7G8, Wel, LE5,3D7), 368 to 390 (7G8), 371 to 390 (7G8, Wel, LE5, 3D7), and 378 to 398 (7G8 & di-alanine substituted). A total of 78 (67%) responded to at least one of the 18 peptides and 41 (35%), 34 (29%), 48 (41%), 55 (47%), and 36 (31%) responded to at least one variant of 326 to 345, 351 to 370, 361 to 380, 371 to 390, and 378 to 398 respectively. Antibodies to R32LR were measured by ELISA in these same 116 individuals. The mean absorbance was significantly higher in those individuals with lymphocyte proliferative responses to 123

to 142 ($p<0.001$) or 151 to 170 ($p<0.01$) or to at least one variant of 351 to 370, 361 to 380, 371 to 390, or 378 to 398 ($p<0.005, <0.01, <0.05, <0.001$) respectively (t-test, 2-tailed). These findings suggest that T-cell epitopes present on the CS protein are associated with antibody production in humans against the repeat region of the CS protein and support the inclusion of sequences from these regions in future experimental CS protein derived vaccines.

429 PROTECTIVE IMMUNITY OF RECOMBINANT *PLASMODIUM FALCIPARUM* SERA ANTIGEN EXPRESSED IN *SACCHAROMYCES CEREVISIAE*. Bathurst IC*, Inselburg J, Rossan RN, Kansopon J, Barr PJ. Chiron Corporation, Emeryville, CA.; Department of Microbiology, Dartmouth Medical School, Hanover, NH.; and Gorgas Memorial Laboratory, Panama City, Panama.

Defined regions of the serine-repeat antigen (SERA) of Honduras-1 strain of *Plasmodium falciparum* has been expressed in and purified from *Saccharomyces cerevisiae*. Two recombinant SERA antigens have been used previously for immunological studies in both rodents and Panamanian *Aotus* monkeys. Various muramyl tripeptide adjuvant systems have been compared with Freund's adjuvant in order to identify a formulation that will elicit protective immune responses. The SERA1 protein, that represents the amino-terminal region of the natural SERA molecule and contains the characteristic serine repeats, has also been used in vaccine cocktail trials to assess the effects of individual components on a multivalent malaria vaccine. The results suggest that a recombinant SERA antigen may be an effective component of a malaria vaccine.

430 FAILURE OF A SYNTHETIC VACCINE TO PROTECT *AOTUS LEMURINOS* AGAINST ASEXUAL BLOOD STAGES OF *PLASMODIUM FALCIPARUM*. Herrera S*, Herrera M, Corredor A, Rosero F, Clavijo C, and Guerrero R. Department of Microbiology, School of Health, Universidad del Valle, Cali, Colombia; and Parasitology Section, National Institute of Health, Bogata, Colombia.

The synthetic polymer, $SPf(66)_{30}$, corresponding to a hybrid protein containing small fragments of the 83K, 55K, 33K and CS antigens of *P. falciparum*, was studied to determine its protective capacity against malaria infection in *Aotus lemurinus* griseimembra monkeys. Two groups of 5 animals were immunized for 6 times with the polymer mixed either with Freund's adjuvants or Aluminum hydroxide. Two groups of 5 animals each, were used as controls and were immunized with saline solution mixed with the same adjuvants. Neither antimalarial nor anti-peptide antibodies developed after the 6 immunization doses. However, monkeys were challenged i.v. with $10^5$ *P. falciparum* parasites and parasitemia followed daily on blood smears. One monkey from each of the groups immunized using Freunds adjuvants (experimental and control) was protected. In those immunized using $Al(OH)_3$ one animal was protected in the experimental group but none in the controls. Anti-parasite antibodies developed during the infection but did not correlate with protection and failed to recognize $SPf(66)_{30}$ peptide in an ELISA test. Immunization with the Polymer did not boost natural antibodies present in two of the monkeys before the experiment. IFN was produced in some animals but was not correlated with protection. Possible causes for the failure of the peptide are discussed.

431 PROTECTION AGAINST MALARIA IN *AOTUS* MONKEYS IMMUNIZED WITH REC. BLOOD ANTIGEN FUSED TO A "UNIVERSAL" T CELL EPITOPE: IFN SERUM LEVELS AND PROTECTION. Herrera MA*, Rosero F, Herrera S, Caspers P, Rotmann D, Sinigaglia F, and Certa U. Department of Microbiology, School of Health, Universidad del Valle, Cali, Colombia; and Pharma Research Technology, F. Hoffmann-La Roche Ltd., Basel, Switzerland.

The major surface antigen p190 of the human malaria parasite *P. falciparum* contains non-polymorphic, immunogenic stretches of amino acids which are attractive components for a subunit vaccine against malaria. One such polypeptide, termed 190L, is contained in the 80 kDa processing product of p190 which constitutes the major coat component of mature merozoites. We describe here that immunization of *Aotus* monkey with 190L gives only poor protection against *P. falciparum* challenge. However, addition by genetic engineering of a "universal" T-cell epitope(CS.T3) to 190L improved immunity and as a result three out of four monkey were protected following challenge infection with blood-stage parasites. Neither antibody against the immunizing antigens or against blood-stage parasites, nor the capacity of the monkey's sera to inhibit *in vitro* parasite invasion correlated with protection. However, in contrast to sera from nonprotected monkeys, sera from protected animals contained elevated levels of interferon-γ (IFN-γ). These results suggest that IFN-γ is directly or indirectly involved in the process of asexual parasite control *in vitro*.

432 IMMUNOLOGICAL STUDIES OF RECOMBINANT POLYPEPTIDES BASED ON THE C-TERMINAL PROCESSING FRAGMENT OF GP195 EXPRESSED IN YEAST AND BACULOVIRUS SYSTEMS. Chang SP*, Hui GS, Gibson HL, Lee Ng CT, Yokota B, and Barr PL. University of Hawaii, Department of Tropical Medicine and Medical Microbiology, Honolulu, HI; and Chiron Corporation, Emeryville, CA.

The major merozoite surface protein (gp195) of *Plasmodium falciparum* has been studied for its potential as a blood stage malaria vaccine. Protection of monkeys has been achieved by immunization with purified, native protein and its natural processing fragments. We have expressed the gp195 gene in two eukaryotic expression systems, yeast and baculovirus, in an attempt to produce a recombinant polypeptide which closely resembles the native protein in conformation. A 42 kDaC-terminal recombinant polypeptide was produced intracellularly in yeast. This 42 kDa region was also secreted in insect cell cultures infected with a recombinant baculovirus construct. Differences were noted in the expression of epitopes recognized by monoclonal antibodies against native gp195. Recognition by mouse, rabbit and monkey anti-native gp195 antibodies will be compared for the two recombinant polypeptides. Both recombinant polypeptides induced antibodies which reacted with purified, parasite gp195 in an ELISA, with the surface of merozoites by immunofluorescence, and with the gp195 precursor molecule and several processing fragments by immunoblot. However, differences existed in titer against the native protein between antisera induced by the two recombinant polypeptides. The potential usefulness of these polypeptides in a blood stage malaria vaccine is under evaluation.

432 ANTIGENIC VARIATION IN *PLASMODIUM FALCIPARUM*. Brown GV*, Gooze L, Wycherley K, Woolish W, Southwell B, Leech JH, and Biggs BA. Immunoparasitology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; The Medical Service, San Francisco General Hospital and Dept of Medicine, University of California at San Francisco CA; and Division of Clinical Pharmacology and Experimental Therapeutics, University of California at San Francisco General Hospital, San Francisco, CA.

There are a number of mechanisms by which *Plasmodium falciparum* evades the host immune response. In other Plasmodial systems it has been shown that cloned parasites can express different antigens at the surface of the infected red cell but this has not been demonstrated for *P. falciparum*. Initially we prepared an anti-serum by immunizing a rabbit with an enriched parasite preparation and showed that this did not cross-react with other isolates. Clones of a doubly-cloned parasite isolate were examined serologically and biochemically for the expression of erythrocyte surface antigens. A large number of clones of non-parental phenotype were detected. Some of these did not express knobs and did not bind to endothelium whereas others clearly retained the property of cytoadherence but surface antigens did not cross react with the parental phenotype. We were able to raise an antiserum to one of these clones

and this serum did not react with the parental parasites. Thus we have been able to demonstrate that a clones line of *P. falciparum* can give rise to otherwise identical progeny that express antigenically distinct forms of an erythrocyte surface antigen. this demonstrates that antigenic differences on the surface of *P. falciparum*-infected erythrocytes can arise by antigenic variation of clonal parasite populations. The antigenic differences and other properties of sibling clones will be demonstrated including differences of the parasite-encodes protein, the *P. falciparum* erythocyte membrane protein 1 (PfEMP1).

434 ULTRASTRUCTURAL LOCALIZATION OF THE 145/102 KD ANTIGENS IN ASEXUAL BLOOD STAGES OF *PLASMODIUM FALCIPARUM*-INFECTED HUMAN ERYTHROCYTES. Wu LJ*, Liu EX, Li WL, and Miao WM. Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, China; Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China; and Department of Parasitology, Second Military Medical University, Shanghai, China.

Previous studies showed that monoclonal antibody (MAb) M26-32 could immunoprecipitate the 145/102 Kd antigens of *P. falciparum* and partially block the invasion of merozoites and inhibit the growth of *P. falciparum in vitro*. By IFA, it reacted not only with 8 isolates of*P. falciparum*, but also with *P. vivax* and *P. ovale*. Thus, it may be a potential tool in malaria diagnosis and protective immunity. In order to determine the precise location of the 145/102 Kd antigens of *P. falciparum*, FCC1/HN, *in vitro*, MAb M26-32 was used with protein A-gold label for immuno-electron microscopy. The results showed that the gold particles were present within the cytoplasm of ring forms, but absent on the surface of ring form-infected erythrocytes. As the parasite developed, more gold particles appeared within the cytoplasm of trophozoites and schizonts as well as in the cytoplasm of the infected erythrocytes. Gold particles were only associated with the cytoplasm of merozoites, but not with the rhoptries, micronemes and surface of the parasite. This study demonstrated that the 145/102 Kd antigens recognized by MAb M26-32 were the common antigens in cytoplasm of asexual blood stages of *P. falciparum* and some of them could be transported into the cytoplasm of the infected human erythrocytes.

435 TRANSGENIC MICE EXPRESSING HUMAN $B^S$ HEMOGLOBIN ARE PARTIALLY RESISTANT TO RODENT MALARIA. Shear HL*, Roth EF, Fabry ME, Costantini F, Pachnis A, and Nagel RL. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY; Department of Genetics, Columbia University, New York, NY; and Division of Hematology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.

The polymorphic frequency of the gene for $B^S$, which is involved in the generation of sickle trait and sickle cell anemia in the human population, is thought to be due to the enhanced resistance of sickle trait individuals to *Plasmodium falciparum* malaria. Epidemiological and *in vitro* evidence support this idea, however, evidence *in vivo* has been lacking. The generation of transgenic mice expressing high levels of human $B^S$ hemoglobin has allowed us to test this hypothesis *in vivo*. Transgenic mice expressing $B^S$ hemoglobin were produced by pronuclear co-injection of two linear constructs consisting of LCR-$B^S$ and LCR-*a* human globin genes. Transgenic mice and parental strains were infected by intraperitoneal injection of $10^6$ *P. chabaudi adami* or $10^5$ *P. berghei*-infected erythrocytes. The course of infection with *P. chabaudi adami* in transgenic mice was significantly delayed and diminished compared to controls. The course of *P. berghei* was also reduced significantly in the early phase of the infection, although not to as great a degree as with *P. chabaudi adami*. Since both of these parasites sequester, at least partially, the mechanism of protection may be similar to that of *P. falciparum*.

436 T CELL REGULATION OF SPLENIC IMMUNITY IN BLOOD STAGE *PLASMODIUM VINCKEI VINCKEI* MALARIA. Vinetz JM*, Kumar S, Torbett BE, Mosier DE, and Miller LH. Medical

Biology Institute, La Jolla, CA; and Malaria Section, Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD.

Protective immunity against blood stage *P. vinckei* malaria depends on cell mediated immunity, involving the interaction of $CD4^+$ T cells and a spleen modified by a previous malaria infection, described previously as the "malarial spleen". However, the precise mechanisms by which the spleen controls blood stage *P. vinckei* are not well understood. To define these mechanisms further, we used adoptive transfer of lymphocytes to immunodeficient mice to study the T cell regulation of splenic immunity in blood stage *P. vinckei* malaria. Two experiments suggest that T cells induce splenic effector cells. BALB/c nu/nu and SCID mice were twice infected with *P. vinckei* and chloroquine cured, and tnen reconstituted with unfractionated, syngeneic immune spleen cells. Challenge resulted in fulminant infection. Therefore, T cells are required to induce a malarial spleen. Next, we examined the role of T-derived lymphokines in blood stage immunity. Malaria-naive syngeneic or allogeneic spleen cells were transferred to CD4-depleted immune recipients. Naive syngeneic spleen cells failed to restore protection. In contrast, naive allogeneic spleen cells triggered protection.Therefore, once the malarial spleen has been induced, only T-derived cytokines, but not antigen-specific T cells, need to be present to initiate parasite killing. These results suggest that protective immunity to blood stage *P. vinckei* involves two distinct components: T cell-mediated induction of a malarial spleen; and T cell-derived cytokine activation of the malarial spleen for non-antigen-specific parasite killing.

437 NOVEL GENE ENCODING A LARGE *PLASMODIUM FALCIPARUM* SEXUAL STAGE SPECIFIC ANTIGEN CLONED BY EXPRESSION IN EUCARYOTIC (COS7) CELLS. Elliott JF*, Alano P, Carter R, Smith DK, Reed DG, and Bruce MC. Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada; Laboratorio di Biologia Cellulare, Istituto Superiore di Sanita, Roma, Italia; Division of Biological Sciences, University of Edinburgh, Edinburgh, UK; and Department of Immunology, University of Alberta, Edmonton, Alberta, Canada.

The *P. falciparum* sexual stage surface antigen Pfs230 is a promising candidate antigen for use in a transmission blocking vaccine against falciparum malaria. Monoclonal antibodies raised against the Pfs230 bind to reduction sensitive (i.e. higher order conformational) epitopes, perhaps explaining why it has not been possible to clone the 230 gene by expression in bacteria. As an alternate approach, we have attempted to clone the 230 gene by expressing falciparum libraries in eucaryotic (COS7) cells. A falciparum cDNA library of $2 \times 10^6$ recombinants was constructed from strain 3D7 gametocyte mRNA by the RNAse H method, using a BstXI linker strategy to insert the cDNA into the vector pJFE14DAF. The library was transfected into COS7 cells, and a pool of 13 different anti-Pfs230 monoclonal antibodies was used to select by 'panning' those COS cells which were transiently expressing all or part of the 230 antigen on their surface. After three successive cycles of antibody selection, a single clone (inset size 2350 bp) was highly enriched. On northern blots this DNA fragment cross-hybridized to a single ≈9 kb transcript which was detected only in gametocyte RNA and not in asexual stage RNA. The cDNA contained a single long open reading frame, beginning with an ATG near the 5' end and continuing throughout the entire 2350 bp insert. The deduced protein sequence showed a N-terminal signal sequence. Cross-hybridization to genomic libraries yielded a 3150 bp fragment which lies immediately 3' to the original cDNA and which shows the long open reading frame continuing throughout. The combined DNA sequence obtained thus far has the potential to code for a sexual stage protein of at least 200 kD.

## CC: MALARIA CHEMOTHERAPY II

438 ARE CURRENT MALARIA CONTROL STRATEGIES SUCCEEDING IN AFRICA? EXPERIENCES IN EVALUATION FROM TOGO. Breman JG*, Gayibor A, Fitzgibbon B, Toole MJ, Glikpo AK, Murphy KB, Sudre P, Bussell KE, and Karsa T. Malaria Branch, Centers for Disease Control, Atlanta, GA; International Health Program Office, Centers for Disease Control, Atlanta, GA; and Service du Paludisme, Service des Statistiques et Division d'Epidemiologie, Ministry of Health, Lome, Togo.

Malaria control strategies in Africa currently focus on reducing mortality in children by effective treatment of acute febrile illness. To determine if this approach is succeeding, countries have strengthened: 1) morbidity and mortality reporting, 2) drug efficacy surveillance, and 3) monitoring of treatment practices. A recent evaluation in Togo showed that in 1989, 730,162 consultations for malaria were recorded, a 140% increase from 1982. In 1989, 365 deaths from malaria and123 anemia-related deaths were recorded at the pediatric ward of the university teaching hospital in Lome, representing a 243% and 324% increase, respectively, from 1984. These increases may be due to a number of factors, including changes in reporting, parasite resistance to drugs, or an inadequate or poorly applied strategy. *Plasmodium falciparum* resistance to chloroquine was first noted in Togo in 1987. Families frequently treat febrile children at home, but with sub-optimal doses of drugs. These facts indicate that the current treatment strategy needs to be revised, better implemented, and more closely evaluated. The addition of anti-vector interventions aimed at protecting individuals and decreasing transmission might provide a needed complement to the Togo malaria control program.

439 QUININE TREATMENT OF SEVERE FALCIPARUM MALARIA IN AFRICAN CHILDREN. Pasvol G*, Newton CR, Winstanley PA, Watkins WM, White NJ, Elford BC, Marsh K, Peshu NM, Were JB, and Warrell DA . Kenya Medical Research Unit, Kilifi, Kenya; Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford UK; and St Mary's Hospital Medical School, London, UK.

Quinine remains the drug of choice for the treatment of chloroquine-resistant severe malaria. The pharmacokinetics and effectiveness of three dosage regimens of quinine severe examined in a group of 59 children with severe malaria randomised to receive high-dose intravenous or intramuscular (20 mg salt/kg loading, then 10 mg salt/kg 12 hourly) or low-dose intravenous quinine (10 mg salt/kg loading, then 5 mg salt/kg 12 hourly). In the group receiving the high-dose intravenous regimen, mean peak and trough quinine concentrations were consistently greater than 10 (range 6.2-22.1) and 6.5 (range 1.5-10.4) mg/1 respectively. The intramuscular regimen produced concentrations and times to peak concentrations similar to those in the high-dose intravenous group. The low-dose intravenous quinine regimen resulted in mean peak concentrations above 6 mg/1 (range 5.3-22.0) and mean troughs above 3.5 mg/1 (range 1.2-8.4). Judged by a number of criteria, the clinical response was better in patients receiving the high-dose than the low-dose intravenous regimen. No significant quinine toxicity was observed in any of the cases. The high-dose intravenous quinine regimen described here may be optimal for treatment of severe falciparum malaria in areas of chloroquine-resistance in Africa. The intramuscular regimen could provide a satisfactory alternative, where intravenous administration might be delayed or is impossible.

440 PEDIATRIC ANEMIA IN WESTERN KENYA: CAN EFFECTIVE MALARIA THERAPY REDUCE THE RISK OF ANEMIA AND BLOOD TRANSFUSION IN AFRICA? Lackritz EM*, Campbell CC, Ruebush TK, Adungosi J, and Were JB. Malaria Branch, Centers for Disease Control, Atlanta, GA; Siaya District Hospital, Siaya, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

To evaluate the epidemiology of pediatric anemia and develop strategies to limit anemia and blood transfusions in Africa, surveillance of pediatric patients was conducted in a rural Kenyan hospital from October 1989 to November 1990. We evaluated the association between anemia and malaria, other risk factors for anemia, and the impact of malaria therapy. Of the 2,433 inpatients, 28% (684) had hemoglobin (Hb) <5.0g/dl, and 20% (483) received blood transfusions. Malaria parasitemia was a major risk factor for severe anemia (Hb<5.0 g/dl) among inpatients ($p < .001$, etiologic fraction = 59%) and among the 3,017 outpatients ($p < .001$). Children <2 years of age were at increased risk for severe anemia ($p<.001$). Malnutrition, sickle cell disease, and hookworm infection were not associated with severe anemia. Malaria therapy was evaluated for 52 children with Hb 5.0-8.0 g/dl and *Plasmodium falciparum* parasite density >8,000/$mm^3$. The 27 children radically cured with pyrimethamine-sulfadoxine demonstrated a greater mean Hb increase (2.3 g/dl) than the 25 given chloroquine (1.5 g/dl, $p<.05$). Effective malaria therapy in young children improves hematologic recovery, thereby reducing the risk for severe anemia and blood transfusion.

441 HALOFANTRINE: AN OVERVIEW OF ITS USE IN CHILDREN. Boudreau EF*, Canfield CJ, and Horton RJ. Pharmaceutical Systems Incorporated, Gaithersburg, MD; and Smith Kline Beecham, UK.

Halofantrine has been studied extensively for treatment of malaria due to both *P. falciparum* and *P. vivax*. More than 2,200 patients have participated in clinical trials in 15 African countries, 2 South American countries and 3 Asian countries. The results of these studies are being compiled and will be submitted to the U.S. FDA during 1991.The overall *P. falciparum* cure rate is greater than 90%, although some areas in SE Asia recently report cure rates less than 60% for multi-drug resistant strains. Halofantrine is remarkably well tolerated; there have been no serious side effects. Clinical trial data on more than 500 children ranging in age from 4 months to 12 years who were treated with a 2% suspension have been analyzed and will be presented. After oral administration to children, halofantrine is routinely retained, and gastrointestinal side effects are minimal. Cure rates have been greater than 94% in children receiving 24 mg/kg of halofantrine in 3 divided doses at 6 hour intervals. Lower doses or single doses of halofantrine have been less effective. This drug is an important new weapon for treatment of acute malaria, especially in children where other drugs are either not well tolerated, or have lost their effectiveness due to development of resistance.

442 INTRAVENOUS IMMUNE GLOBULIN AS ADJUNCT TREATMENT IN SEVERE PEDIATRIC MALARIA. Taylor TE*, Molyneux ME, and Wirima JJ. College of Osteopathic Medicine, Michigan State University, East Lansing, MI; Liverpool School of Tropical Medicine, Liverpool, UK; and College of Medicine, University of Malawi, Blantyre, Malawi.

Even with optimal chemotherapy 33% of Malawian children with severe *P. falciparum* malaria die or develop neurologic sequelae. Intracerebral sequestration of parasitized red blood cells may contribute to the pathogenesis of this condition. This cytoadherence can be reversed *in vitro* and in squirrel monkeys by the addition of anti-malarial immune globulin. The clinical relevance of these findings was evaluated in a double-blind, placebo-controlled trial of intravenous immune globulin (IVIG), designed to determine if the addition of IVIG to standard therapy could decrease the rate of unsatisfactory outcomes to 10%. The IVIG (purified from local adult blood donors, IFAT 1:10,000) and placebo (albumin) were prepared to commercial standards by the Swiss Red Cross. The two treatment options were randomized and analyzed sequentially. Malawian children with *P. falciparum* parasitemia, and coma scores of 0-1 were included in the study. 400 mg/kg of IVIG or the placebo (4.4mg/kg) were administered over three hours, in conjunction with the loading dose (20 mg/kg) of quinine. All patients then received quinine (10 mg/kg) eight-hourly. The IVIG (n=17) and placebo (n=16) groups were well-matched in terms of known

risk factors. The study was terminated at this point because the proportions of unsatisfactory outcomes (10/17 vs 3/16) were sufficient to show that IVIG is not superior to placebo.

443 ADJUNCTIVE IRON CHELATION THERAPY SHORTENS DURATION OF COMA IN CHILDREN WITH CEREBRAL MALARIA. Gordeuk VR*, Thuma PE, Biemba G, Parry D, and Brittenham GM. Department of Medicine, MetroHealth Medical Center, Case Western Reserve University, Cleveland OH; Department of Pediatrics, Hershey Medical Center, Pennsylvania State University, Hershey PA; and Macha Hospital, Choma, Zambia.

To determine if adjunctive iron chelation therapy can shorten the duration of coma in cerebral malaria, we administered intravenous desferrioxamine B, 100 mg/kg/day, or placebo to 67 children. Patients were < 6 years of age with unarousable coma, asexual forms of *Plasmodium falciparum* > 5000/μL peripheral blood, more than 30 minutes since any previous convulsion, normal cerebral spinal fluid and glucose > 40 mg/dl. All patients were given quinine, 10 mg/kg, 8 hourly for 5 days and sulfadoxine/pyrimethamine as a single dose; adjunctive desferrioxamine B or placebo were administered in a prospective, randomized, double-blind design. Initially, children who received desferrioxamine B or placebo did not differ with respect to mean age, length of illness before presentation, prior treatment with chloroquine or herbal medicine, parasitemia or severity of coma (scored from 0 to 5 as in Quart J Med 1989;71:441). Mortality was 8.6% among children receiving desferrioxamine B and 15.5% among those given placebo ($p = 0.2$). In survivors, the time to recover from coma was examined using analysis of variance, and factors known to affect duration of coma (initial hemoglobin and glucose, convulsions during treatment, requirement for blood transfusion) were included as covariates. With mild coma (initially rated 3 to 4) no difference in duration of coma was observed. With severe coma (initially rate 0 to 2) the time to regain full consciousness was significantly reduced with desferrioxamine B therapy, from 54.8 ± 11.1 (mean ± SEM) to 26.5 ± 5.5 hours ($p=0.038$). Results suggest that adjunctive iron chelation therapy hastens recovery from coma in cerebral malaria.

444 EFFECTIVENESS AND TOLERANCE OF LONG-TERM MALARIA PROPHYLAXIS WITH MEFLOQUINE AMONG PEACE CORPS VOLUNTEERS. Lobel HO*, Bernard KW, Eng TR, Hightower AW, and Campbell CC. Malaria Branch, Centers for Disease Control, Atlanta, GA; Office of International Health, Department of Health and Human Services, Washington, DC; and Peace Corps Office of Medical Services, Washington, DC.

Because chloroquine (CQ) alone or in combination with paludrine no longer prevented *P. falciparum* infections among Peace Corps volunteers (PCVs) in Africa, mefloquine (MQ) was made available in September 1989. A surveillance system was instituted to measure the effectiveness and tolerance of each of the available regimens and to determine the mefloquine blood concentration necessary to prevent malaria. The incidence of *P. falciparum* among PCVs in West Africa between October 1989 and February 1991 was 3.1/100 person-months (pm) among users of CQ, compared with 2.0/100 pm among users of CQ and paludrine ($P > .05$), 1.4/100 pm among users of bi-weekly MQ ($P<.001$), and 0.4/100 pm among users of weekly MQ ($P < .001$). Based on the prophylaxis failure rates at various mefloquine blood concentrations, probit analysis was used to estimate the predicted probability of prophylactic efficacy at different mefloquine concentrations. No serious adverse drug reactions (ADRs) were reported. Minor self-reported ADRs occurred less frequently among users of MQ (39%) than among users of CQ (50%) ($P < .001$). The frequency of these ADRs decreased with prolonged use of MQ. These results indicate that weekly MQ prophylaxis is significantly more effective than biweekly dosing and that long-term MQ prophylaxis is well tolerated.

445 EMERGENCE OF MEFLOQUINE RESISTANT *PLASMODIUM FALCIPARUM* IN THAILAND: *IN VITRO* TRACKING. Wongsrichanalai C*, Webster HK, Wimonwattrawatee T, Sookto P, Chuanak

N, Wernsdorfer WH, and Thimasarn K. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Institute of Specific Prophylaxis and Tropical Medicine, University of Vienna, Austria; and Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Bangkok, Thailand.

Southeastern Thailand bordering Cambodia has been a focus of emerging antimalarial drug resistance. The malaria situation there provides a reliable indicator of future developments in other parts of Thailand and Southeast Asia. We have maintained a computerized data base on *in vitro* microtests of *Plasmodium falciparum* (PF) isolates randomly collected at malaria clinics from various parts of Thailand since 1984. Isolates from Borai, a border district in the Southeast, were grouped separately and their *in vitro* characteristics compared to those from "elsewhere" (other parts of Thailand combined). There was a progressive decrease in the 50% inhibitory concentration ($IC_{50}$) of mefloquine at Borai from 1984 to 1989 followed by an acceleration in degree and prevalence of resistance in 1990, during which the $IC_{50}$ (radioisotope technique) reached an average of 33.0 ng/ml (95% confidence interval = 20.9 - 45.1 ng/ml). Although halofantrine has not been used under the Thai National Malaria Control Program, halofantrine $IC_{50}$ values increased 5 folds from 1989 to 1990. $IC_{50}$s of the two drugs are highly correlated ($r = 0.90$, $P < 0.001$) suggesting cross-resistance. Mefloquine and halofantrine sensitivity patterns elsewhere remained unchanged. These observations identify southeastern Thailand as an epicenter of mefloquine and halofantrine resistance and forecast the spread of multidrug resistant PF malaria.

446 PROGUANIL/DAPSONE FOR MALARIA CHEMOPROPHYLAXIS ON THE THAI-CAMBODIAN BORDER. Shanks GD*, Suriyamongkol V, Timsaad S, Edstein MD, and Webster HK. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Royal Thai Navy Medical Department, Bangkok, Thailand.

The Thai-Cambodian border is a difficult area in which to provide adequate malaria chemoprophylaxis. In 1990-91, 438 Thai soldiers were randomized to receive proguanil (200 mg per day) combined with dapsone (4 or 12 mg per day) or weekly pyrimethamine/dapsone (12.5 mg and 100 mg). Men with G6PD (n=77) where given daily 100 mg doxycycline. Daily medication was given for two phases of 40 days each. Proguanil/ dapsone was equivalent to pyrimethamine/dapsone in falciparum malaria failure rates (10.3 vs 11.3%). Proguanil/dapsone was better than pyrimethamine/dapsone in preventing vivax malaria (1.6 vs 12.4%). Men receiving doxycycline had falciparum malaria (3.9%) and vivax malaria(1.3%) at low rates. Changing the dapsone component from 4 to 12 mg did not improve the daily proguanil/dapsone regimen. Approximately 1.3% of the men complained of medication side-effect during the 24000 medication days. Compliance averaged 69% for proguanil/dapsone, 70% for doxycycline and 74% for pyrimethamine/dapsone. Both compliance and drug metabolism played a role in prophylaxis failures based on plasma drug concentrations. Proguanil/dapsone did not demonstrate any hematologic toxicity. Proguanil/dapsone is not a useful alternative for chemoprophylaxis on the Thai-Cambodian border since both doxycycline and mefloquine have been demonstrated to be superior when tested in the same area (1989).

447 CIPROFLOXACIN TREATMENT OF DRUG-RESISTANT FALCIPARUM MALARIA. Watt G*, Shanks GD, Edstein MD, Pavanand K, Webster HK, and Wechgritaya S. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Surasinghanart Army Hospital, Aranyaprathet, Thailand.

New chemotherapeutic agents are urgently needed for the treatment of falciparum malaria caused by drug-resistant parasites. Fluoroquinolone antibiotics were effective against malaria *in vitro* and norfloxacin cured all 9 falciparum infections in adult patients from India. A randomized, open study of high dose ciprofloxacin (750 mg every 12h) in uncomplicated falciparum malaria was conducted in

Thailand. No patient completed the planned 1 week treatment course. Because of rising parasitemia (3 fold higher at 36h than on admission) and deterioration of clinical status, 3 individuals required quinine treatment 36h after commencing ciprofloxacin while the fourth was given quinine at 54h. The study was terminated early for safety reasons after only 4 ciprofloxacin and 4 control patients had been enrolled. Ciprofloxacin was well absorbed and efficiently entered erythrocytes; median plasma and red cell concentrations 90 min after the first dose were 4.0 (range = 3.7 - 6.8) and 5.1 (3.8 - 6.0) mcg/ml respectively. However, 50% inhibition of parasite growth *in vitro* required 6.6 mcg/ml (5.6-9.6). Ciprofloxacin should not be used alone to treat chloroquine-resistant falciparum malaria.

448 PROSPECTIVE TREATMENT TRIAL OF VIVAX MALARIA USING 25 MG/KG CHLOROQUINE BASE DEMONSTRATES RESISTANCE IN 10 (22%) CASES IN IRIAN JAYA. Murphy GS*, Basri H, Purnomo, Andersen EM, Bangs MJ, Gorden J, Arbani RP, Harjosuwarno S, Mount DL, Hoffman SL, and Sorensen K. US Naval Medical Research Unit, No.2, Jakarta, Indonesia; Malaria Division, National Institute of Health, Research and Development, Jakarta, Indonesia; Provincial Health Office, Irian Jaya, Indonesia; Malaria Branch, Centers for Disease Control, Atlanta, GA; and U.S. Naval Medical Research Institute, Bethesda, MD.

Several recent reports of chloroquine failing to cure vivax malaria have suggested that resistance may be emerging in *Plasmodium vivax* from the island of New Guinea. To test whether true resistance is present, we prospectively treated 45 human volunteers who contracted vivax malaria in Irian Jaya (Indonesian New Guinea) with 25 mg/kg chloroquine base over 3 days. All doses were witnessed, and volunteers who vomited or spit up any part of a dose were retreated. Volunteers were followed daily. Parasitemia was checked by blood smears at enrollment and on days 3, 7, 10, and 14. Whole blood chloroquine levels were measured by High Performance Liquid Chromatography (HPLC) on each of these days. All volunteers cleared their parasitemias by day 3. Ten (22%) developed *P. vivax* parasitemia by day 14 (3 by day 7, 3 by day 10, 4 by day 14). Of the 10 resistant cases, 6 were retreated with 25 mg/kg chloroquine base.Four of these (67%) developed parasitemia again during the next 14 days. Whole blood chloroquine levels were well above the level previously thought to confer protection for 9 of the 10 initial recurrences and all of the repeat recurrences. The levels were even higher in the samples prior to recurrence, confirming that parasitemia recurred in the presence of substantial amounts of chloroquine. This evidence confirms chloroquine resistance in *P. vivax* and suggests that treatment failures may be expected in Irian Jaya when standard doses of chloroquine are used.

449 EFFICACY OF ARTEMISININ (QINGHAOSU) FOR THE TREATMENT OF NON-COMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN COLOMBIA. Nicholls RS, Guerra MP, and Corredor A. Grupo de Parasitologia, Instituto Nacional de Salud, Bogota D.E., Colombia.

Artemisinin is a relatively new antimalarial compound obtained from the Chinese plant *Artemisia annua*. We studied the efficacy and side effects of artemether (Artemetheri-Kunming Pharmaceutical Factory-People's Republic of China), an artemisinin derivative, in patients with non-complicated *P. falciparum* malaria from several endemic zones in Colombia. Patients were admitted if their initial parasitaemia was at least 1000 parasites/μl and if there was no history of self administered anti-malaria medication during the previous 4 weeks. No children nor pregnant women were admitted into the study. The drug was administered via intramuscular injection under the following scheme: an initial dose of 160 mg followed by a daily dose of 80 mg on days 2-5. Parasite counts were done daily during the first 7 days. 12 patients were treated with an average initial parasitaemia of 10623 (1380-48150) or 0.25% (0.03-1.07%). 6 patients had no detectable parasitaemia 24 hrs after the initial dose and remained negative thereafter. In the other 6 patients a significant reduction (71-99%) was observed after 24 hrs. 4 of them had cleared parasitaemia by the 48 hr follow up and the remaining 2 had very low parasitaemia after 48 hr and had cleared parasitaemia by the 72 hr follow up. The only side effect reported by 5 patients was slight to moderate local pain at the site of injection. A very good antimalarial activity was observed in 2 patients who had

chloroquine-resistant malaria. Results suggest that artemisinin may be a highly effective and safe alternative for the treatment of *P. falciparum* malaria in Colombia. Further studies are needed to evaluate its efficacy in clinically complicated forms .

## DD: ARBOVIRUS VECTOR STUDIES

450 STABILIZED LA CROSSE VIRUS INFECTION IN *AEDES ALBOPICTUS*. Streit TG, Grimstad PR, and Craig GB, Jr. Vector Biology Laboratory, University of Notre Dame, Notre Dame, IN.

The recent spread of *Aedes albopictus* in the eastern half of the United States juxtaposes this mosquito with La Crosse encephalitis endemic areas. Questions arise concerning the ability of *Ae. albopictus* to join *Ae. triseriatus* in La Crosse virus (LACV)transmission cycles. To investigate vertical transmission of LACV, Peoria and Indiana LACV isolates were delivered to chipmunks via bite of infected mosquitoes. Viremic chipmunks and artificial membrane feeders were then used to infect strains of *Ae. albopictus* per orally. Following incubation of virus in the vector, mosquitoes refed upon suckling mice (SM). These parental mosquitoes oviposited and the eggs were reared to adults. $F_1$ progeny and subsequent generations of infected mosquito isolines were assayed for virus via transmission trials to SM, VERO cell culture assay, or IFA technique. Vertical transmission rates to $F_1$ individuals were low (≤10%). However, 36% of the infected $F_1$ mosquitoes exhibited stabilized LACV infection,demonstrated by virus transmission to ≤90% of their progeny. Stabilized infection was achieved in *Ae. albopictus* isolines derived from mosquitoes feeding on LACV infectious blood meal doses as low as 3600 pfu/ml chipmunk blood. Virus doses of similar titer failed to initiate infection in ≤90% of *Ae. triseriatus* tested. *Ae. albopictus* might well participate in vertical cycling of LACV.

451 GENETIC SELECTION OF A *FLAVIVIRUS* REFRACTORY STRAIN OF THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*. Miller BR* and Mitchell CJ. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, CO.

Inbred (isofemale) *Aedes aegypti* mosquito lines were derived that manifested a resistant or susceptible phenotype following ingestion of yellow fever virus; lack of virus movement from the midgut defined the resistant phenotype. Other flaviviruses, including dengue 1-4, Uganda S, and Zika viruses behaved in a similar fashion in the 2 mosquito lines. Crosses between the 2 lines produced progeny that were of intermediate susceptibility, indicating codominance; $F_2$ backcrosses to the parents yielded results consistent with a major controlling genetic locus and provide evidence of a second locus capable of modulating the phenotype of the major gene. The rapid selection necessary to fix the susceptible and refractory phenotypes support the hypothesis of a single major controlling locus. Viral movement across the midgut is likely to be governed by a single major gene and modifying minor genes or a group of closely linked genes. These inbred mosquito lines will be useful in discovering the molecular basis for Flavivirus resistance in *Ae. aegypti*.

452 NGARI VIRUS (*BUNYAVIRUS*, BUNYAVIRIDAE): REPLICATION IN AND TRANSMISSION BY *AEDES AEGYPTI*. Tammariello RF* and Linthicum KJ. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Ngari virus (Bunyamwera group) was first isolated in Senegal in 1979 from male *Aedes (Stegomyia) simpsonsi* mosquitoes reared from eggs collected in a forest. Subsequently, this virus has been isolated from West Africa from numerous species of *Aedes*, *Anopheles*, and *Culex*mosquitoes and from a sick sheep. However, little is known concerning the ecology of this virus, and vector competence studies have not been conducted. To assess the potential for Ngari virus to replicate in and be transmitted by *Aedes aegypti* (Rockefeller strain), mosquitoes were either allowed to feed on newborn mice that had been

inoculated with Ngari virus (strain 14-144, isolated in Senegal) 2 days earlier or were inoculated intrathoracically with the virus. After oral exposure to viremic mice (mean virus titer ingested = $10^{4.5}$PFU/mosquito) mosquito infection rates were 16% (4/25, mean virus titer = $10^{5.7}$PFU) and 20%(5/25, mean virus titer = $10^{5.5}$PFU) 7 and 13 days, respectively, after the infective bloodmeal. Dissemination rates, as determined by assaying legs, in infected mosquitoes were 100% at both 7 and 13 days after the infective bloodmeal. After intrathoracic inoculation, 100% (23/23) of the mosquitoes transmitted Ngari virus to newborn mice after an 8 day extrinsic incubation. The potential for transovarial transmission will be examined in the first and second ovarian cycle eggs. Adults reared from these eggs will be tested for the presence of virus. The studies demonstrated that *Ae. aegypti* is a competent laboratory vector of Ngari virus.

453 ROLE OF TICK SALIVA IN VIRUS TRANSMISSION. Labuda M*, Nuttall PA, and Jones LD. Institute of Virology, Bratislava, Czechoslovakia; and NERC Institute of Virology and Environmental Microbiology, Oxford, UK.

A factor produced in the salivary glands of feeding ticks enhances transmission of Thogoto (THO) virus. Studies were undertaken to determine whether saliva-activated transmission (SAT) occurs with other tick-borne viruses. Guinea pigs infested with uninfected ticks were inoculated with a virus mixed with a salivary gland extract (SGE). Extracts were derived from partially fed uninfected female ticks which were competent vectors of the respective virus. Six tick-borne viruses were tested: Central European encephalitis (CEE), Kadam (KAD), Dugbe (DUG), Kemerovo (KEM), Wad Medani (WM), and Dhori (DHO). None of the inoculated animals developed viremia. However, ticks were fed on animals inoculated with DHO or CEE viruses acquired virus [mean 25 and 40%, respectively]. The differences may reflect different infection strategies in the vertebrate host. Elucidation of the mechanisms of SAT is important in designing strategies for the control of tick-borne diseases.

454 HOST RESPONSE TO TICK SALIVA-ACTIVATED TRANSMISSION OF THOGOTO VIRUS. Jones LD and Nuttall PA*. NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, UK.

Thogoto (THO) virus is naturally transmitted by ticks but otherwise shows many characteristics of the influenza viruses. Previous studies showed that THO virus is transmitted from infected to uninfected ticks co-feeding on a guinea pig even though the animal does not develop a detectable viremia. "Non-viremic transmission" appears to be mediated by a protein that is secreted in tick saliva. Studies on the mechanism of saliva-activated transmission (SAT) indicate that a complex interaction between tick saliva, virus and vertebrate host occurs at the site of tick feeding where the tick delivers virus into the epidermal/dermal area of the host. Two approaches were adopted to examine this interaction: first - the spatial and temporal relationship between THO virus and the SAT factor, and second - the cellular involvement at the site of tick feeding. SAT was dependent on the site of inoculation of the SAT factor relative to the virus. However, when the SAT factor was inoculated into the animal up to 2 days preceding or 2 days following inoculation of the virus, SAT still occurred. Preliminary studies on the replication of THO virus in skin-associated cells indicate that the virus replicates in B and T lymphocytes, and macrophages, but not in Langerhans cells. Elucidation of the events in the skin following vector-borne transmission is crucial to our understanding arboviral diseases.

455 COMPARATIVE INFECTIONS OF EPIZOOTIC AND ENZOOTIC STRAINS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS IN *AMBLYOMMA CAJENNENSE* TICKS. Linthicum KJ*, Gordon SW, and Monath TP. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Although the maintenance cycle of epizootic strains of Venezuelan equine encephalomyelitis (VEE) virus is unknown, enzootic strains are thought to be maintained in a mosquito [*Culex* (*Melanoconion*) spp.] - rodent cycle. Recent experimental evidence, demonstrating that a I-A variant of the virus can orally infect *Amblyomma cajennense* larval ticks and be transmitted transstadially to nymphs, adults and vertebrate hosts for at least 171 days, suggests that ticks could play a role in the maintenance of epizootic viral strains. To compare the potential for enzootic strains of VEE virus to infect *A. cajennense*, larval ticks were fed on guinea pigs inoculated with an enzootic strain of variant I-E (68U201, SMB-4 Vero-1) or guinea pigs inoculated with an epizootic strain of variant I-A (Trinidad donkey SMB-4, CE-14, Vero-1). Peak viremias were $10^{5.2}$ and $10^{7.3}$PFU/ml in guinea pigs infected with enzootic and epizootic viral strains, respectively. At drop-off, ticks feeding on enzootic- and epizootic virus-infected hosts had mean viral titers of $10^{2.5}$ and $10^{3.9}$PFU, respectively. While epizootic virus was recovered from 84% (210/250) of larval ticks up to 16 days post-infective bloodmeal (PIB), enzootic virus recovery rates declined rapidly from 19/20 (95%) at drop off to 0/10 by 16 days PIB. Epizootic virus was found in 12/3,006 (0.4%) unfed nymphs (mean titer =$10^{2.9}$PFU) 63 days PIB and 4/400 (1%) of the adults (mean titer =$10^{3.6}$PFU) 131 days PIB. No enzootic virus was recovered from 4,600 unfed nymphs tested 63 days PIB. Although the epizootic strain of virus replicated in larval ticks and was transstadially transmitted, the enzootic strain did not replicate in larvae or nymphs. These studies indicate that *A. cajennense* ticks are unlikely to be involved in the maintenance cycle of enzootic strains of VEE virus.

456 VIRUS ISOLATIONS FROM MOSQUITOES CAPTURED IN TWO PERUVIAN AMAZON LOCATIONS; IQUITOS AND ANDOAS. Need JT* and Phillips I. Entomology Department, U.S. Naval Medical Research Institute Detachment, Lima, Peru and Virology Department, U.S. Naval Medical Research Institute Detachment, Lima, Peru.

Although numerous arboviral epidemics (including but not limited to yellow fever, dengue, VEE, EEE, SLE, Mayaro, Oropouche, Guama, Oribora, Itaqui, Marituba, and Carapara-Apeu fevers) are recorded from the Peruvian Amazon, their geographic range is incompletely known and very little is known about the arthropod vectors. The NAMRID Iquitos laboratory in the central Amazon Basin is well situated to act as a surveillance site for such arboviruses. In 1988, we began collecting mosquitoes using standard methods for arbovirus isolation. In 1990, mosquitoes from an additional site, Andoas, were included in the study. To date, nearly 25,000 mosquitoes of 9 different genera have been captured. 7255 mosquitoes (533 pools) have been tested for virus. Five of 20 pools of mosquitoes captured in Iquitos during the early stage of a 1990 outbreak of dengue yielded dengue type 1 virus. In addition, 32 virus isolations preliminarily identified by hyperimmune grouping ascitic fluids as belonging to Groups A, B, Bunyamwera or Tacaribe have been made in C636 cells and 45 isolates have been made in in Vero cells. We are still awaiting test results of 47 other isolates.

457 WEST NILE AND SINDBIS ANTIBODIES IN BIRDS AND RODENTS IN THE NILE DELTA REGION OF EGYPT. Nashed NW*, Main AJ, Tewfik SA, Metwally SA, and Hanafi HA. Medical Zoology Div., US Naval Medical Research Unit Number 3, Cairo, Egypt.

As a corollary to a seroprevalence survey of school children (3% positive for West Nile virus antibodies) in the Bilbeis area northeast of Cairo, intensive monthly mosquito, rodent, and bird collections were begun in 1990 in 3 villages. Of 31,434 mosquitos (7 species), tested all were negative for West Nile and Sindbis viruses. Most (97%) of the mosquitoes caught and tested were *Culex pipiens*, a suspected vector of both West Nile and Sindbis viruses in Egypt. ELISA antibodies against West Nile and Sindbis viruses were present in 10% and 8.5%, respectively, of 1324 bird sera. Twenty of 47 species captured were positive for either or both viruses. The highest prevalences of West Nile antibody were in rock doves (21%) and in white wagtails and house sparrows (14% each). The highest prevalences of Sindbis virus

antibody were in white wagtails (14%) and in house sparrows (8%). Among 261 peridomestic rodents captured, antibodies against West Nile and Sindbis were present in sera of 11.5% and 4.6%, respectively, including *Mus musculus* (26% and 41%, respectively) and *Rattus spp.* (15% and 0%, respectively). Neutralization assays to confirm the above ELISA antibody test results are pending. Studies are ongoing to better understand the epidemiology of arboviral diseases in Egypt.

458 DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND *CULICOIDES VARIIPENNIS* VECTOR COMPETENCE FOR BLUETONGUE VIRUS. Tabachnick WJ* and Robertson MA. Arthropod-Borne Animal Diseases Research Laboratory, USDA-ARS, Laramie, WY.

Although the *blu* locus has been identified as controlling oral susceptibility of *Culicoides variipennis* to infection with bluetongue virus, it is likely that there are other loci in this species with the same function. Studies of these genetic factors would be facilitated by the availability of a genetic map with numerous genetic markers. We have constructed a DNA genomic library of *Culicoides variipennis*. DNA fragments from this library, labeled with radioisotope, have been used as probes to screen individual *Culicoides variipennis* genomic DNA which has been digested with restriction enzyme, electrophoresed and southern blotted.We have detected several restriction fragment length polymorphisms (RFLPs) in several *Culicoides variipennis* laboratory colonies and field populations. RFLPs are being screened for their usefulness in studies of vector competence loci. We have also used random oligonucleotide primers to amplify random *Culicoides variipennis* DNA fragments using polymerase chain reaction (PCR). RFLPs isolated from the genomic library appear to be more suitable for genetic studies of vector competence than random amplified polymorphic DNA (RAPD) markers. The application of DNA markers to genetic studies of insect vector competence for arboviruses will be outlined.

## EE: KINETOPLASTIDA IMMUNOLOGY

459 PROPOSED MECHANISM OF ACTION FOR NORMAL HUMAN SERUM ON AFRICAN TRYPANOSOMES. Ortiz JC* and Seed JR. Department Parasitology, School Public Health, University North Carolina, Chapel Hill, NC; and Department of Epidemiology, School Public Health, University North Carolina, Chapel Hill, NC.

Using a single parental clone (TXTat, *Trypanosoma b. gambiense*), we have selected human serum sensitive (HsS) and human serum resistant (HsR) clones using continuous *in vivo* passages in the absence of any immunological pressure. Changes in HS susceptibility are always predictably associated with changes in the trypanosome's surface coat (VSG). One dimensional SDS-PAGE showed that the MWs of VSGs isolated from HsS clones are consistently different from those of HsR ones. Uncoated insect procyclics transformed from these bloodstream clones are always HsR, thus the presence of VSG appears to be directly associated with HS sensitivity, while resistance must be associated with a change, the absence of, or the inability of VSG to bind to the trypanolytic factor (TF) in HS. Additionally, we hypothesize that movement across the plasma membrane, endocytosis, and processing of the TF are necessary steps in trypanolysis. Inhibitors of cell surface receptor movement (ie.local anaesthetics, membrane stabilizers, microtubule/microfilament inhibitors) were used as evidence that this step takes place. Receptor endocytosis and phagolysosomal processing were also shown to be involved in the mechanism of action of TF on the trypanosome. Our experiments demonstrate that VSG and VSG-associated processes strongly determine the susceptibility to normal human serum.

460 A MEMBRANE 60 KDA GLYCOPROTEIN OF INVASIVE FORMS OF *TRYPANOSOMA CRUZI* PRESENTS PUTATIVE LYMPHOCYTE EPITOPES. Villalta F*, Howard SA, and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

The identification and characterization of surface molecules of invasive forms of *T. cruzi* and the definition of their relevant T and B cell epitopes are important for molecular immunological intervention in Chagas' disease. A 60 kDa *T. cruzi* trypomastigote glycoprotein was purified from trypomastigote membranes by preparative isoelectrofocusing and Superose gel filtration using a FPLC system. This 60 kDa acidic glycoprotein is present in the membrane of trypomastigotes but not in epimastigotes. This molecule is recognized by antibodies produced during human chagasic infection. Specific IgG against the purified 60 kDa glycoprotein increased the uptake of trypomastigotes and parasite killing by macrophages. We found that the *T. cruzi* 60 kDa glycoprotein is able to specifically activate primed lymphocytes, since there was a significant increase of $^{3}$H-thymidine incorporation by spleen cells obtained from CBA mice primed with the purified 60 kDa glycoprotein with respect to control values. The *T. cruzi* 60 kDa glycoprotein did not stimulate unprimed spleen cells, indicating that this effect is specific and is not due to a polyclonal activation. We conclude that the major acidic *T. cruzi* 60kDa surface glycoprotein is capable of priming and activating lymphocytes and that the host mounts a specific immune response against this surface glycoprotein of *T. cruzi* trypomastiogtes facilitating their uptake and destruction by macrophages.

461 HUMAN PBMC PROLIFERATE AND SECRETE CYTOKINES IN RESPONSE TO *TRYPANOSOMA CRUZI* INFECTION *IN VITRO* Van Voorhis WC* and Barrett LK. Division of Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA.

*Trypanosoma cruzi*, the protozoan agent of Chagas' disease, causes massive polyclonal lymphocyte proliferation during the acute infection. The proliferating lymphocytes do not appear to be directed to *T. cruzi* antigens. It is possible this aberrant proliferative response is responsible for the lack of containment of *T. cruzi* and the breakdown of tolerance, leading to autoimmune antibodies and T cells observed in chronic disease. We are studying this response *in vitro* by infecting human PBMC with *T. cruzi*. 15-20% of B and T lymphocytes undergo blast transformation after infection with *T. cruzi* CL trypomastigotes, with peak proliferation occuring by day 6. By immunofluorescent cytometry, lymphoblasts from these cultures are 33% B cells (CD19+) and 51% T cells (CD3+), and approximately half of the T cells are CD4+ and half CD8+. Many lymphoblasts express MHC class II (69%) and IL-2R p55 (68%), suggesting both B and T lymphoblasts express these molecules. Anti-MHC class II and anti-IL-2R p55 mAb significantly inhibit the proliferative response of PBMC to T. cruzi. The mRNA for cytokines IL-1β IL-2, IL-5, IL-6, IFN-γ, and TNF-α are detected after *T. cruzi* infection of PBMC, peaking on day 3. No IL-4 or IL-10 mRNA are detected. Large quantities of bioactive IL-1 and IL-6 are found in the supernatants of infected PBMC. Monocytes, infected in the absence of lymphocytes, assume activated morphology and accumulate mRNA for IL-1β, TNF-α, and IL-6. This *in vitro* model of infection with *T. cruzi* gives insight into the interaction of the immune system with the parasite and increases the understanding of immune manifestations of the acute and chronic Chagas' disease.

462 EPITOPE IDENTIFICATION OF INFECTION-ENHANCING ANTIBODIES AGAINST THE *TRYPANOSOMA CRUZI* NEURAMINIDASE. Prioli RP*, Ortega-Barria E, Mejia JM, and Pereira M. Division of Geographic Medicine, New England Medical Center Hospitals, Boston, MA.

We have previously produced and characterized polyclonal and monoclonal (TCN-2) antibodies against the neuraminidase of *Trypanosoma cruzi*. Now we report on the identification of a synthetic peptide that contains the epitope recognized by these antibodies. The dodecamer peptide D-S-S-A-H-G-T-P-S-T-P-A, termed TR, was deduced from the DNA sequence of the long tandem repeat domain located in the C-terminus of the neuraminidase. Polyclonal anti-neuraminidase and TCN-2 antibodies reacted with TR in a dose dependent manner, but not with BR (Y-S-V-D-D-G-E-T-W-E), a synthetic peptide derived from the N-terminus, cysteine-rich domain of the enzyme, indicating the specificity of the binding. Polyclonal

anti-TR antibodies were developed and their biological activities compared to that of TCN-2. Anti-TR antibodies identify the same molecular polymorphism previously observed with TCN-2 and were able to immunoprecipitate active enzyme. Further evidence suggesting that anti-TR and TCN-2 recognized similar sequences in the neuraminidase molecule was obtained by immunofluorescence which demonstrated that both antibodies detected, in the same fashion, the differential expression of their epitopes in intra- and extracellular trypomastigotes and in the different developmental forms. Taken together, these results indicate that the epitope recognized by TCN-2 and by the polyclonal anti-neuraminidase antibodies is present within the unit of the tandem repeat domain and confirm that the unit is an integral part of some of the isoforms of the neuraminidase molecule.

463 IMMUNOGENETICS AND IMMUNE MECHANISMS IN RESISTANCE TO ACUTE *TRYPANOSOMA CRUZI* INFECTION IN MICE. Powell MR*, Theodos CM, and Wassom DL. Department of Zoology and Biomedical Sciences, Ohio University, Athens, OH; Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Pathobiological Science, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

Infection of man or laboratory mice with *Trypanosoma cruzi* results in disease that ranges from asymptomatic to fatal. By using inbred strains of mice, we recently demonstrated that at least one gene in the MHC and at least one gene in the genetic background can control the outcome of acute infection with the Brazil strain. Sera from mice which express background genes associated with susceptibility (C3H), regardless of the expression of resistant (H-2$^q$) or susceptible (H-2$^k$) MHC genes, contained IgG1, IgG2a, IgG2b, IgG3 and IgM antibodies which bound numerous parasite antigens on western blots. Conversely, sera from mice which share background genes associated with resistance (B10) but express either the resistant H-2$^q$ or the susceptible H-2$^k$ haplotypes, contained only IgG2b and IgM antibodies. Cytokine profiles also differed. Con-A stimulated lymph node cells from susceptible C3H mice produced higher levels of IL-2 than B10 mice which express background genes associated with resistance. None of the lymph node cell preparations produced appreciable amounts of IL-4. Con-A stimulated spleen cells, however, produced predominantly IL-4 and little IL-2 regardless of the mouse strain tested. These data demonstrate clear differences in immune responses during acute infection which may be related to the host's ability to survive infection.

464 LIPOPHOSPHOGLYCAN MAY BE A TRANSMISSION BLOCKING VACCINE FOR LEISHMANIASIS. Sacks DL*, Warburg A, Pimenta PF, Perkins PV, and Lawyer P. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; and Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.

The ability of antibodies against *Leishmania major* lipophosphoglycan (LPG) to inhibit the development of promastigotes within *P. papatasi* midguts was studied. LPG was purified from *L. major* logarithmnic phase promastigotes, emulsified in RIBI adjuvant, and used to immunize BALB/c mice which had been subcutaneously infected 4 weeks previously with *L. major* amastigotes. *P. papatasi* female flies were membrane fed on $10^6$ amastigotes/ml in heparinized blood from LPG immunized infected mice or infected controls. In several experiments, 100% of flies in each group dissected 3 days after feeding demonstrated active promastigote infections within abdominal midguts containing partially digested bloodmeals. Six days post-feeding, after bloodmeals in all flies had been passed, 82% of control flies displayed abdominal and/or thoracic midgut infections whereas only 18% of flies fed on bloodmeals containing anti-LPG antibodies continued to be infected. Similar infection rates were observed in flies dissected on day 12. In a subsequent experiment, the loss of infection in 78% of the experimental group was not observed until day 12, at which time only 23% of the control flies had lost their infections. Promastigotes recovered from day 3 midguts of flies fed on LPG immunized mice could be stained with

FITC-anti-mouse Ig, whereas midgut promastigotes recovered from control flies could not. The data suggest that the expression of the LPG coat by midgut promastigotes prevents recognition of the parasite by anti-leishmanial antibodies contained within an infective bloodmeal, and that the presence of surface reactive anti-LPG antibodies inhibits the development of infection within the fly.

465 IDENTIFICATION OF *LEISHMANIA DONOVANI* ANTIGENS EXPRESSED ON OR RELEASED FROM THE SURFACE OF INFECTED MACROPHAGES. Melby PC* and Darnell B. Department of Medicine, The University of Texas Health Sciences Center, San Antonio, TX.

Host defense against the intracellular parasite *Leishmania* is mediated by cellular mechanisms. *Leishmania* antigens displayed on the surface of the infected macrophage are available for recognition by T cells which can lead to macrophage activation and parasite killing. To identify such antigens, P388D1 mouse macrophage-like cells were infected with *L. donovani* amastigotes which had been metabolically labeled with $^{35}$S-methionine. Free parasites were removed by washing and the infections allowed to proceed for another 4-16 hrs. Released $^{35}$S-labeled proteins were precipitated from cell-free culture supernatants, subjected to SDS-PAGE, and identified by silver stain and autoradiography. A large number of released proteins were detected by silver stain, but only a few antigens (presumably of parasite origin) were identified by autoradiography. Radiolabeled antigens associated with the surface of the infected macrophage were also identified. Macrophage membranes isolated by adherence to cationic beads were solublized and subjected to SDS-PAGE, silver staining and autoradiography. A large number of diverse radiolabeled antigens were associated with the isolated membranes. The autoradiographic pattern was distinct from that of solubilized $^{35}$S-labeled amastigotes suggesting that there was selective accumulation of certain parasite antigens on the surface of the infected macrophage, or that there was rapid turnover of amastigote proteins with subsequent incorporation of free radiolabel into macrophage proteins. Studies to identify the kinetics of this process and to further characterize the antigens are in progress.

466 INHIBITION OF NITRIC OXIDE SYNTHESIS FROM L-ARGININE RESULTS IN DECREASED HOST RESISTANCE TO *LEISHMANIA MAJOR* AND SEVERE CACHEXIA. Evans TG*, Thai L, and Hibbs JB. Division of Infectious Diseases, University of Utah, Salt Lake City, UT.

Experimental evidence suggests that the synthesis of nitric oxide from L-arginine is an important effector mechanism in host defense against intracellular pathogens. This cytokine-induced response can be blocked by the use of N8-monomethyl-L-arginine (L-NMMA), a potent inhibitor of nitric oxide synthesis, and has been shown to worsen *L. major* (Lm) infections *in vivo* when inoculated at the site of infection. To further evaluate the L-arginine:NO pathway *in vivo* 20 BALB/c mice were infected in the footpad with $10^5$ stationary phase Lm promastigotes and were maintained on feeding and drinking water consisting of either distilled water, an average of 30 mg per day of N-LMMA (1.5 g/kg) or 12 mg $NH_4$ acetate taste control (0.6 g/kg). N-acetyl cysteine (NAC) was also orally administered in another group at 6.5 mg (0.32 g/kg) to maintain intracellular sulfhydral levels in Lm-infected mice. Mice in the N-LMMA group showed marked wasting compared to controls (p=.005) after five weeks, despite the fact that we saw no wasting in control uninfected mice drinking the same diet in another experiment. In addition, the N-LMMA group had significantly larger lesion size (p=.002), and regional node parasite load (p=.025) than any of the other three groups. There was no protective effect of NAC on Lm infection, with an actual tendency toward worsening of the infection. Inhibition of nitric oxide generation by the oral feeding of N-LMMA resulted in cachexia and worsened Lm infection. This link between infection, nitric oxide synthesis inhibition, and cachexia is not previously described. We could find no benefit in ameliorating the infection by increasing sulfhydral stores through the ingestion of NAC .

467 CD4+ T LYMPHOCYTES EXPRESSING CELL SURFACE TNF CAN MEDIATE CONTACT-DEPENDENT MACROPHAGE ACTIVATION FOR ANTILEISHMANIAL DEFENSE. Sypek JP* and Wyler DJ. Division of Geographic Medicine and Infectious Diseases, Tufts University School of Medicine, New England Medical Center Hospitals, Boston, MA.

Tumor necrosis factors are highly homologous proteins secreted by either stimulated macrophages or T cells which exert a variety of biological functions that include tumor cytotoxicity and macrophage activation. A form of TNF anchored to the plasma membrane (mTNF) of macrophages and T cells also has been shown to mediate antitumor cytotoxicity *in vitro*. In our studies of host defense to cutaneous leishmaniasis, we identified a distinct mechanism of macrophage activation for antileishmanial defense that involves direct cell contact between effector $CD4^+$ T cells and *Leishmania*-infected macrophages. The mechanism is novel in that it does not depend on lymphokine secretion by the effector T cell and induces no cytotoxic effects in target macrophages. These contact-mediated effects are antigen-specific and genetically-restricted. A cloned $CD4^+$ *Leishmania*-specific $T_H1$ hybridoma line ("1B6") that can exert cell contact-dependent effects synthesizes the mTNF (Mr 50-60 kd) and expresses mTNF on its surface. 1B6 cells enriched for mTNF ($TNF^+$) or depleted for mTNF ($TNF^-$) were isolated by FACS; only $TNF^+$ but not $TNF^-$ cells could induce antimicrobial effects in infected macrophages, effects that were blocked by anti-TNF antibody. Similarly, $TNF^+$ $CD4^+$ lymph node lymphocytes isolated from mice with resolving footpad infections of *L. major* can exert antileishmanial effects, effects that were also blocked by anti-TNF antibody. We propose that mTNF on $CD4^+$ effector cells may allow for an effective and physiologically distinctive means by which activation signals are targeted to macrophages in an antigen-specific and genetically-restricted fashion.

468 THE KINETICS OF MITOGENIC AND ANTIGENIC RESPONSES IN EXPERIMENTAL MURINE LEISHMANIASIS. ASSOCIATION BETWEEN LYMPHOPROLIFERATION, TNF PRODUCTION, AND RESOLUTION OF THE DISEASE. Karagouni EE and Dotsika EN*. Hellenic Pasteur Institute, Athens, Greece.

Lymphoproliferation following mitogen or antigen stimulation have been shown to be major indicators of effector immune functions. In this study we have determined the lymphoproliferative capacity of mice undergoing either incurable (BALB/c + *Leishmania major*) or curable (BALB/c + *L. infantum* and CBA + *L. major* leishmaniasis. Spleen and lymph node cells from these mice were stimulated *in vitro* with Con A (10 μg/ml) or crude soluble extract ($2x10^5$ *L. major*, $1x10^6$ *L. infantum*) from *Leishmania* promastigotes or purified gp 63 glycoprotein (0.1 μg/ml). Supernatants from these cultures were tested for TNF activity. We observed a rapid loss of the mitogenic and antigenic lymphoproliferative responses in BALB/c mice infected with *L. major*, along with minute amounts of TNF in the corresponding supernatants. In contrast, BALB/c mice infected with *L. infantum* and CBA mice infected with *L. major* has lymphoproliferative responses to Con A equivalent to normal uninfected mice. Substantial antigenic responses were sustained through the course of the infection. Moreover, the supernatants of these cultures had significant amounts of TNF. Our findings can be interpreted as showing that the susceptibility of BALB/c mice to *L. major* infection is due either the the absence of TNF producer cells or to the lack of the appropriate signal to provoke production of TNF that would activate macrophages to promote the resolution rather than the development of fatal disease.

## SYMPOSIUM: MALARIA VACCINES

S7 THE DESIGN OF MULTIPLE ANTIGEN PEPTIDE (MAPS) VACCINES BASED ON RESPONSES OF SPOROZOITE-IMMUNIZED VOLUNTEERS. Nardin E*. New York University School of Medicine, New York, NY.

The demonstration that protection against sporozoite transmitted malaria could be induced in man, as well as monkeys and rodents, provided the initial impetus for research on sporozoite-induced immune mechanisms and vaccine development. The first synthetic malaria vaccine to undergo Phase I and Phase II trials based was based on a peptide representing the 3' repeat region of the circumsporozoite protein of *Plasmodium falciparum*, $(NANP)_3$, conjugated to tetanus toxoid. The immune response to this first generation synthetic vaccine demonstrated the limitations of a foreign protein carrier that lacked parasite-derived T cell epitopes. Our recent studies have investigated the Multiple Antigen Peptide system (MAPs) as an entirely synthetic peptide vaccine that does not require a protein carrier. MAPs containing T and B cell epitopes of the *P. berghei* CS protein induced high levels of antisporozoite antibodies and protected immunized mice against viable sporozoite challenge. A *P. falciparum* MAPs vaccine was constructed containing equimolar ratios of $(NANP)_3$ and a T cell epitope located in the 5' repeat region of the the CS protein that was identified by a CD4+ T cell clone isolated from a *P. falciparum* sporozoite immunized volunteer. The MAPs containing the *P. falciparum* CS repeats were antigenic for human T cells *in vitro* and induced high levels of antisporozoite antibodies in three out of four inbred strains of mice. The use of MAPs containing immunodominant B cell epitope(s) in combination with multiple well-defined T cell epitopes should make it feasible to construct vaccines which may overcome genetic restrictions and induce high levels of T cells for humoral as well as cellular immunity.

S8 INDUCTION OF CYTOLYTIC T CELL RESPONSES BY THE REPEATLESS *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN MOLECULE INCORPORATED INTO LIPOSOMES. White K, Gordon D, Gross M, Richards RL, Alving CR, Ballou WR and Krzych U*. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

Protective immunity induced with irradiated sporozoites is believed to depend in part on circumsporozoite (CS) protein-specific T cell responses, evaluation of which has been a formidable task in testing for an effective anti-malaria vaccine. We and other investigators have demonstrated CS protein-specific cytolytic T cells (CTL) following immunization with irradiated sporozoites and CS protein expressed in bacterial vector systems. Furthermore, it has been shown that the molecular context of an antigen during immunization, i.e. native vs denatured, as well as the mode of its administration, i.e. alone or incorporated into a carrier system, can profoundly affect the type and the specificity of the effector T cells arising upon immunization. In the present study, we tested an alternative method of CTL induction by incorporating recombinant CS repeatless *P. falciparum* protein (RLF) into liposomes {L(RLF)}. We chose the RLF molecule in order to eliminate the dominant anti-repeat antibody response that might obscure reactivities to otherwise potentially dominant T cell sites. Anti-CS protein-specific cytolytic responses were tested in B10.Br mice immunized with L(RLF) against a panel of target cells, including *P. falciparum* 368-390 peptide-pulsed L cells, *P. falciparum* CS protein-transfected cell line, (LPF), and appropriate controls. The data demonstrate that although no detectable CTL responses were observed in spleen cell cultures obtained from mice immunized with RLF or liposomes alone, CTL responses were recorded in cultures obtained from mice immunized with L(RLF). In summary, our results demonstrate that generation of CTL responses can be accomplished by incorporation of vaccines into liposomes, suggesting that the route of antigen processing/presentation might ultimately be responsible for induction of specific effector T cells.

S9 IMMUNOGENICITY OF NEW FORMULATIONS OF SYNTHETIC PEPTIDE SPF66 VACCINE. Ballou WR*, Gordon DG, Alving CR, and Sadoff JC. Department of Immunology, Walter Reed Army Institute of Research, Washington DC.

A major objective of our research program is the development of methods for enhancing the immunogenicity of subunit malaria vaccines using novel antigen delivery systems and adjuvants. Recently, successful immunization of primates and humans against *Plasmodium falciparum* malaria was reported using SPf66, a novel low molecular weight (12-25kD) synthetic peptide polymer vaccine. However, large doses of alum adjuvanted vaccine (1-2 mg/dose) were required, and antibody titers were generally low. To further evaluate the potential of this vaccine candidate, we had several lots of SPf66 prepared under GLP/GMP. The characteristics of the commercially manufactured polymer and the results of immunogenicity studies in experimental animals using alternative formulations suitable for human use will be discussed.

S10 EXPRESSION IN *E. COLI* AND IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* MSA-2. Anders RF, Dyer S, Kemp DO, Pye D, Wu MJ, Smythe JA, Marshall VM, Kemp DJ, Goss N, Woodrow GC, and Coppel RL. The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia.

Merozoite surface antigens (MSA) are prime candidates for inclusion in a vaccine against malaria asexual erythrocytic stages. Two MSA (MSA-1 and MSA-2) have been identified on merozoites of *P. falciparum*, both attached to the merozoite membrane by a C-terminal glycosyl phosphatidylinositol moiety. MSA-1 is a large polypeptide (≈200 kDa) which has not been expressed as a full-length recombinant protein. In contrast, MSA-2 is a relatively small polypeptide (≈28 kDa) which we have been able to express as a near full-length protein in *E. coli*. Like MSA-1, MSA-2 exhibits a structural dimorphism and genes representative of the two forms of MSA-2 have been expressed both as GST fusion proteins and as near full-length proteins with 13 residue N-terminal extension which includes 6 histidine residues. The MSA-2 of *P. falciparum* cloned line 3D7 expressed with the N-terminal histidines has been isolated in high purity by affinity chromatography on a Ni resin followed by reverse-phase HPLC and anion-exchange chromatography. The purified recombinant MSA-2 was used to immunize rabbits and several different inbred strains of mice and antibody responses were measured by ELISA using recombinant antigens and indirect fluorescence microscopy using fixed parasite smears. Antibody responses were poor in rabbits and also in BALB/c(H-$2^d$) and C57Bl/6 (H-$2^b$) mice, but good responses to the homologous MSA-2 were seen in CBA (H-$2^k$), BALB/c (H-$2^k$), and CSL Swiss outbred mice. The specificity of the antibody responses of the responder mice varied with BALB/c (H-$2^k$) and CSL Swiss strongly recognizing epitopes in the conserved regions of MSA-2 whereas the response in CBA mice was largely directed against variable regions of MSA-2. It appears likely that the antibody responses of humans immunized with MSA-2 will also vary with respect to titer and specificity.

S11 PFS25 TRANSMISSION BLOCKING VACCINES. Kaslow DC*, Bathurst I, Isaccs S, Keister DB, Moss B, and Barr PJ. Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Pfs25, a cysteine-rich, 25-kilodalton sexual-stage surface protein of *Plasmodium falciparum*, is a target of antibodies that block transmission of malaria from vertebrate host to mosquito vector. Recombinant vaccinia virus (vSIDK), expressing Pfs25, induces transmission blocking antibodies (TBAb) in mice and Aotus monkeys. MHC-disparate congenic mouse strains immunized with vSIDK elicit transmission blocking antibodies, demonstrating that the ability to develop TBAbs is not genetically restricted in mice. Recombinant Pfs25 synthesized by secretion in yeast elicits TBAbs in mice immunized in conjunction with a muramyl tripeptide adjuvant or alum. Despite having only partial conformational integrity, and

lacking glycosylation sites and glycosylphosphatidyl-inositol anchor, the yeast-derived product still induced full transmission blocking immunity in Aotus monkeys.

S12 ROLE OF ADJUVANT FORMULATIONS BASED ON COPOLYMERS IN THE DESIGN OF A MALARIA PEPTIDE VACCINE. Millet P*, Kalish ML, Olsen M, Grady KK, Collins WE, and Hunter RL. Malaria Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA

This study was performed to define safe adjuvant formulations able to elicit a protective immune response against malaria parasites in association with vaccine candidates based upon peptides and recombinant proteins. The working hypotheses were 1) vaccine preparations consisting of selected copolymer adjuvants and/or nontoxic immunomodulators such as detoxified lipopolysaccharide (LPS) can induce a protective isotype of IgG antibody, and experiments performed in mice demonstrated that adjuvant formulations modulated the isotyope of the antibody. While all adjuvant formulations induced a high immune response directed against the peptide vaccine in ELISA, some formulations reacted poorly or not at all against *P. cynomolgi* sporozoites in an indirect fluorescent antibody test. In a comparison between copolymers L141 and L121, only the copolymer L121, with or without LPS, induced antibodies able to inhibit the *in vitro* development of *P. cynomolgi* sporozoites in primary cultures of rhesus monkey hepatocytes. These results have been used to design a rhesus monkey vaccine trial.

S14 MALARIA SPOROZOITE VACCINE PROVIDES MINIMAL PROTECTION DESPITE INDUCTION OF HIGH LEVELS OF ANTIBODIES. Hoffman SL*, Edelman R, Bryan J, Schneider I, Davis J, Sedegah M, Gordon DM, Hollingdale MR, Gross M, Paparello S, and Jones T. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

The *Plasmodium falciparum* circumsporozoite (CS) protein vaccine, R32NS18I, which includes 30 copies of NANP and 2 copies of NVDP, the major and minor tandem repeats of the *P. falciparum* CS protein, fused to 81 amino acids from the non-structural protein of influenza A, administered with monophosphoryl lipid A, cell wall skeleton of mycobacteria, and squalane (Detox™) as adjuvant, was evaluated for safety, immunogenicity, and efficacy in 12 volunteers. One volunteer developed a reaction at the injection site after the second dose and declined further immunization. The 11 other volunteers tolerated the 3 doses of 1230 mg vaccine, but most complained of sore arms similar to after typhoid immunization. Two weeks after the third dose of vaccine, eight of the eleven volunteers had indirect fluorescent antibody titers against air-dried *P. falciparum* sporozoites > 1:1024 (1:1024, n=l; 1:2048, n=l; 1:4096, n=4, 1:8192, n=2). Two weeks after the third dose of vaccine the volunteers were challenged by the bite of five *Anopheles stephensi* mosquitoes infected with sporozoites of the 3D7 clone of the NF54 strain of *P. falciparum* in two groups. In the first group one of five controls and one of 4 immunized volunteers did not develop parasitemia; the uninfected immunized volunteer had an IFA titer of 1:4096. In the second group all six controls developed parasitemia, while a volunteer with an IFA titer of 1:4096 did not develop parasitemia. In addition a volunteer with an IFA titer of 1:4096 had a delay of 2 days, and a volunteer with an IFA titer of 1:8192 had a delay of 5 days in developing blood stage parasitemia as compared to the 6 non-immunized controls challenged on the same day. These data indicate that subunit vaccine-induced antibodies against the CS protein can reduce and in some cases prevent effective sporozoite invasion of hepatocytes, but that there is no correlation between absolute level of antibodies measured by IFAT and resistance to infection. Work is now underway to characterize the antibodies responsible for reducing sporozoite invasion of hepatocytes, and then to develop an assay that will discriminate between such functional and non-functional antibodies, and to develop vaccines that primarily produce these antibodies.

S15 FIELD TRIAL OF A RECOMBINANT *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE (CS) PROTEIN CONJUGATE VACCINE IN THAI SOLDIERS. Brown AE, Singharaj P, Webster HK, Pipithkul J, Gordon DG, Boslego JW, Krinchai K, Su-archawaratana P, Wongsrichanalai C, Hollingdale MR, Ballou WR, Wittis JS, Cryz SJ, and Sadoff JC. U.S. Army Medical Component, Armed Forces Institute for Medical Sciences, Bangkok, Thailand.

A randomized, double blind, placebo controlled field trial of a falciparum sporozoite vaccine (R32ToxA) was carried out in Thailand to assess safety, immunogenicity, and efficacy (Phase Ib, IIb). The vaccine consists of a recombinant protein derived from the central repeat region of the circumsporozoite protein of *Plasmodium falciparum* conjugated to exotoxin A from *Pseudomonas aeruginosa*. Three doses of R32ToxA or a control vaccine were given to 191 Thai soldiers at 0, 2, and 4 months in a malaria-free area. Following immunization, the soldiers were deployed as part of their regular duties to a malarious area on the Thai-Cambodian border. The soldiers were closely monitored and malaria parasitemias promptly treated. The vaccine was well tolerated. Immunogenicity will be determined in terms of specific antibody (IgG and IgM) and cellular immune responses. Assessment of efficacy will include a comparison of the incidence of *P. falciparum* infection in subjects grouped by serum concentration of specific antibody. These results will be presented and discussed in terms of continued efforts to develop a malaria sporozoite vaccine.

S16 IMMUNOGENICITY AND EFFICACY OF A *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE VACCINE IN A MALARIA ENDEMIC AREA OF KENYA. Sherwood JA*, Copeland RS, Taylor KA, Abok K, Ruebush TK, Ondolo HAO, Were JBO, Oloo AJ, Githure JI, Mason CJ, Wirtz RA, Schneider IP, Gordon DM, Ballou WR, Hollingdale MR, Gross M, Wittis JS, Sadoff JC, and Roberts CR. U.S Army Medical Research Unit-Kenya; and Walter Reed Army Institute of Research, Washington, DC

A phase II immunogenicity and efficacy study of a recombinant circumsporozoite (CS) repeat vaccine conjugated to toxin A (R32ToxA) was conducted in an area western Kenya endemic for falciparum malaria. 78 male volunteers age 18-30 years were paired (38 pairs) such that they could share the same house during the study. At weeks 0, 8 and 24, one of each pair was vaccinated with 400 mcg R32ToxA and the other with recombinant hepatitis B vaccine. After the third immunization, volunteers were radically cured with a course of quinine sulfate and doxycycline and followed for 6 months. Volunteers switched sleeping positions in houses weekly. Four mornings per week, indoor resting mosquitoes were collected, speciated, dessicated and later tested by ELISA for *P. falciparum* antigen. Three mornings per week, indoor resting mosquitoes were collected, speciated, dissected for sporozoites, and later tested by ELISA for *P. falciparum* antigen. Volunteers were visited daily for illness and given treatment for malaria if indicated by symptoms and a positive malaria smear. Irrespective of symptoms, malaria blood slides were made weekly. Before and after vaccination, blood samples were taken for urinalysis, blood count, blood chemistry and anti-sporozoite antibody tests, and lymphocytes were obtained for proliferative assays. Results will be discussed.

## AMERICAN COMMITTEE ON MEDICAL ENTOMOLOGY

S17 OVERVIEW OF VECTOR MANAGEMENT; THE PROBLEMS. Arata AA. Vector Biology and Control Project/Agency for International Development, Arlington, VA.

The participants in this ACME symposium will present the newest vector control techniques being developed. But to achieve successful disease control, we will have to employ them in concert with diagnosis, surveillance and treatment. In most endemic areas there are no clear policies stating the objectives of vector-borne disease control programs to guide the role of vector control. Who in the developing countries is going to be able to employ the new methods of vector population reduction? Who is going to pay for them? Methods for disease control and/or prevention must be cost effective but,

as yet, we have paid little attention to methods for economic evaluation based on sound data collection and analysis. Finally, other developmental sectors must be brought into strategies for vector-borne disease control not only for the obvious ecological reasons but for economic support as well. The control of vector-borne diseases should not be supported only by donors and underfinanced and understaffed ministries of health but also by economic development programs in agriculture, forestry,mineral exploitation and water management that contribute to, and suffer from, endemic vector-borne diseases.

S18 THE FUTURE IS HERE: THE EMBATTLED INSECTICIDE ARSENAL. Jany W. American Cyanamid, Lahaska, PA.

The insecticide arsenal for public health is aging poorly. New products for agricultural vector control are not finding their way into public health vector control program. Discovery and development programs for public health products are years away from contributing significant alternatives. Environmental and regulatory requirements are forcing many of our older products into early retirement. Those that remain have more label restrictions and their applications are in some cases significantly reduced by state and federal regulations and guidelines. Meanwhile, the insects that vector diseases, reduce our effectiveness at work or reduce our enjoyment of free time are still here. Diseases such as the encephalitides, dengue, malaria and yellow fever are still claiming lives in what seems to be a never-ending battle. What products will the public health industry use in the future for insect control? What forces are acting both in the insecticide industry and federal and state governments to cause the decline in insecticide availability for public health? What can the public health industry do, if anything, to influence these forces now and in the future?

S19 REPELLENTS: AN IMPASSE FOR DISEASE VECTORS OR HUMAN HOSTS? Bowen MF. SRI International, Menlo Park, CA.

Topically-applied insect repellents afford an essential means of personal protection against disease-bearing insect pests,particularly in those instances in which other means of insect control are absent or impractical. For the past 35 years, the most effective commercial and military repellent formulations (and thus the ones most commonly used) have been those containing N.N.diethyl-m-toluamide (DEET) as the active ingredient. Toxicological problems (including significant skin irritation, penetration and storage, circulatory absorption, incomplete excretion, and neurotoxic effects) as well as other drawbacks associated with its usage leave reasonable doubt that DEET will pass the more stringent requirements now demanded by Federal regulations. Unfortunately,an equivalent alternative to DEET does not exist. This absence is not due to lack of empirical effort. Tens of thousands of compounds have been screened for use as potential insect repellents over the last 3 decades yet none have been found to be as effective as DEET. A major reason for this failure is our poor understanding of the mechanisms by which repellents affect insect behavior. Our knowledge of how DEET affects peripheral sensory receptor function in mosquitoes provides insight into the mode of action of repellent compounds and can guide the rational future development of alternative repellents.

S20 SUCCESSFUL CONTROL OF TSETSE FLIES USING ATTRACTANTS. Brady J. Imperial College, London, UK.

In West Africa, colored, pyethroid-impregnated traps have been deployed along a riverine 'gallery forest' at ca. 10 per linear km. and have suppressed tsetse populations by >95%. In Kenya,locally made 1 $m^3$ blue cloth traps baited with crude synthetic oxodour (acetone + fermented ox urine deployed at 2 $km^2$ have done even better (ca. 99% reduction). In Zimbabwe, G.A. Vale et al. made the crucial discovery of the importance of host odour for several species of tsetse. Working with Overseas Development

Administration scientists in London (using split GC-EAG analysis), they revealed the key components of ox odour to be: $CO_2$, various ketones, octenol, and phenols that are breakdown products of urine. Traps and targets in Zimbabwe are now routinely baited with acetone, 1-octen-3-ol,4-methylphenol and 3-n-propylephenol (released at around 500 mg $h^{-1}$, and 400, 800 and 100 µg $h^{-1}$, respectively). Current Zimbabwe targets consist of 1.5 x 0.5 m black cloth screens supported vertically on a wire frame just above ground level, baited with this odour and sprayed evenly, with 500 ml of 0.1% deltamethrin. Earlier versions included side panels of black mosquito netting to 'hit' the flies that circled without landing. This netting deteriorated much faster than the cloth, however, and is now omitted. This target technology was first tested on a large scale in 1984 over a 600 $km^2$ triangular area of uninhabited bush in the Zambezi valley, which contained ca. 5000 flies $km^{-2}$. One side had been cleared of flies by spraying, one was partially protected by the Zambezi river (500 m wide), the third by a dense belt of targets. Through the rest of the area, targets were deployed at 4 per $km^2$, being resprayed and rebaited every 3 months. Average mortality inflicted was 2% $day^{-1}$, which reduced the population by >99.9% with 6 months, so that a test herd of oxen placed in the centre of the area acquired no trypanosome infections. The targets killed tsetse far more effectively that other insects, and apart from some mortality of tabanids and muscoids the ecological impact was minimal. These targets are now used routinely elsewhere in Zimbabwe, and are due to be the main control method in a four-country, EEC-funded control programme.

S21 THE ROLE OF BIOLOGICAL CONTROL IN THE INTEGRATED CONTROL OF MOSQUITOES. Lacey LA. USDA, ARS, APO, NY.

A multitude of environmental and financial concerns have necessitated the increased use of noninsecticidal means of mosquito control along with a more judicious use of conventional insecticides. Integrated vector control (IVC) comprises several components of which biological control (BC) may play variable roles ranging from none at all to a key role. The viable options for BC have been expanded over the past 15 years with the discovery and/or further development of several pathogens (*Bacillus spp., Lagenidium*, etc.) parasites (mermithid nematodes) and predators (larvivorous fish, *Toxorhynchites spp., Macrocyclops spp.*). These organisms have been used in both inoculative and inundative strategies. In most cases, they were used in an inundative or augmentative manner in conjunction with other interventions. In addition to the efficacy of potential BC agents in a given habitat,their effects on the environment, particularly on indigenous natural enemies and compatibility with other IVC components must also be considered. Ultimately, the cost:benefit ratio will determine the feasibility of using BC agents in IVC programs. The utilization of community participation in control programs has already resulted in the cost effective use of BC agents in certain developing countries.

S22 VACCINES AGAINST ARTHROPODS. Kay BH. Queensland Institute of Medical Research (QIMR), Brisbane, Australia.

Vector vaccines have been used to vaccinate hosts against bloodsucking and tissue invading arthropods, in order to reduce transmission, fecundity and/or survival. Vaccination protocols against both insects (mosquitoes, flies, lice) and ticks have involved inoculation of whole body extracts to recombinant antigens contained in bacteria. This paper reviews QIMR studies done with *Aedes aegypti* in reducing susceptibility by 20-50% with respect to the alphavirus Ross River and the flavivirus Murray Valley encephalitis, and comments on recent Australian progress with respect to veterinary pests. Finally, it compares the usefulness and practicability of this approach with respect to human and veterinary problems.

S23 GENETIC APPROACHES TO MALARIA CONTROL: HOW LONG IS THE ROAD? Gwadz RW. NIAID, National Institutes of Health, Bethesda, MD.

The concept of malaria control through replacement of vector populations with populations of the same species unable to transmit the parasite is based on the early observations that most mosquitoes do not transmit malaria. Later workers showed that vector susceptibility was an inherited characteristic under genetic control. We are now developing the capacity to move genes not only within but between species and genera. We are also identifying a variety of genetically-controlled refractory mechanisms from within and outside of mosquitoes which can interfere with development and transmission of the parasite. The greatest challenge may now lie in developing a better understanding of the population structure of vector species and the evolution of strategies for replacing these populations with less pernicious forms. The tools of molecular biology should greatly facilitate both laboratory and field studies. Similar efforts are being directed at the vectors of filariae and arboviruses.

S24 APPLICATION OF REMOTE SENSING TECHNOLOGY TO VECTOR CONTROL. Washino K. University of California, Davis, CA.

Remote sensing and geographic information systems technologies were combined to predict the spatial and temporal population dynamics of the western malaria mosquito, *Anopheles freeborni*, in California rice fields during two separate years. These technologies show promise for addressing the problem of malaria vector control in other rice-growing regions throughout the world. Progress on studies to explain mechanisms involved is presented.

S26 COMMUNITY INVOLVEMENT IN THE PREVENTION OF CHAGAS' DISEASE IN BOLIVIA. Bryan RT, Centers for Disease Control, Atlanta, GA.

Recent estimates suggest that 90 million people, or 25% of the total population of Latin America, are at risk for Chagas' disease. Although other modes of transmission (congenital and blood transfusion) exist, it is primarily a vector-borne affliction of the rural poor, and its persistence in man is closely linked to socioeconomic conditions. In Bolivia, where 64% of the population resides in economically depressed rural areas, Chagas' disease is a major public health problem. Approximately 1.2 million (20%) of Bolivia's 6 million inhabitants are infected with *Trypanosoma cruzi*, and of these, about 300,000 (5% of total population) exhibit manifestations of Chagasic heart disease. The Bolivian Ministry of Health (MPSSP) and the USAID child Survival Project (CCH) have jointly planned and initiated the MPSSP/CCH National Chagas' Disease Control Program, which combines house improvements,residual insecticide applications, and education. Community involvement forms the foundation of this program. Regional Medical Supervisors (RMSs), hired and trained by the program, oversee several operational teams, each composed of a team supervisor,health promoter, house inspector, carpenter, plasterer, and roofer. These individuals are selected from participating communities and are trained in their specific duties by RMSs and other Program educators. The initial phase of this program begin in January 1991 with the selection of pilot sites in the departments of Cochabamba, Chuquisaca, and Tarija. Community organization and baseline epidemiologic studies have been completed. House improvements, currently underway for approximately 1,000 homes,should be completed by October, when insecticide application will occur. A program evaluation and follow-up epidemiologic surveys are planned for early 1992.

S27 COMMUNITY-BASED PREVENTION AND CONTROL OF DENGUE/DENGUE HEMORRHAGIC FEVER. Gubler DJ. Centers for Disease Control, Fort Collins, CO.

Epidemics of dengue/dengue hemorrhagic fever became an increasingly important public health problem worldwide during the past 10 years. Increasingly, more frequent and larger epidemics and an

expanding distribution of the severe and fatal form of disease have been caused by a combination of lack of effective mosquito control, increasing urbanization in the tropics and increased air travel. Recommended methods of *Aedes aegypti* control have not been effective in stopping or preventing this changing epidemiology. In recent years, it has become increasingly clear that new approaches to prevention and control of epidemic dengue/dengue hemorrhagic fever are required. This paper will review recent progress in developing community-based, integrated approaches to prevention and control. The program in Puerto Rico will be discussed as a model for this approach.

## SYMPOSIUM: MODELS OF VECTOR-BORNE DISEASES

S36 MODELS FOR STUDYING EFFECTS OF DRUGS ON MALARIA. Singer B. Yale University, New Haven CT.

Drug resistance is the result of complex interactions between migration patterns of human populations, genetics of malaria parasites, and regimens. We present hybrid transmission/genetics models as coherent framework for understanding the development of drug resistance and projecting the potential consequences of diverse control strategies. Malaria in the Brazilian Amazon, Northern Thailand, and Indonesia are specific contexts that will constrain or model specifications.

S40 THE ECOLOGICAL INTERACTIONS INVOLVED IN THE TRANSMISSION OF LYME DISEASE. Sandberg S and Awerbuch T. Framingham State College, Framingham, MA; and Harvard University, Boston, MA.

Since 1975, and the first report of epidemic Lyme disease, the infection has spread rapidly throughout much of the United States, Europe, and Asia. A deer tick, *Ixodes dammini*, serves as the vector of Lyme disease in the northeastern United States. Its complex 2-year life cycles and a unique range of hosts provides this tick with the capacity to perpetuate the infectious agent, the spirochete, *Borrelia burgdorfei*. We developed a model of the life cycle of the tick and the prevalence of the spirochete based on quantitative representation of the various biological processes affecting the force of transmission of the agent of Lyme disease. This model consists of Leslie matrices that represent the seasonal interaction of the vector tick with environmental factors and with various hosts in each of its developmental stages. Simulations were run using a spreadsheet. As a result we obtained the exponential growth of the tick population, the seasonal distribution of ticks at the various stages, the stable state distribution, and the prevalence of infection.

## SYMPOSIUM: CELL SURFACE CYSTEINE-RICH PROTEINS

S42 ADHESION RECEPTORS OF THE IMMUNE SYSTEM AND SUBVERSION BY PARASITES. Staunton D. Harvard Medical School. Boston, MA.

Adhesion molecules play a central role in the functions of the immune system. These molecules direct cell-cell interactions critical for antigen presentation, lymphocyte activation, localization, migration, and effector/target functions at the site of inflammation. The integrin family of adhesion receptors is involved in the cell-cell and cell-matrix interactions of a wide variety of cell types and is also exploited as receptors for invading intracellular parasites. A second family of adhesion receptors of the immune system is the immunoglobulin superfamily, which includes the antigen-specific receptors of T and B lymphocytes and molecules such as CD4 and ICAM-1 which serve as receptors for HIV and rhinoviruses respectively. The third family of adhesion proteins of the immune system is the selections which contain N-terminal lectin domains and help regulate leukocyte binding to endothelium at inflammatory sites. A common theme in pathogenesis of parasitic infections is the subversion of these adhesion receptors as means to adhere to and invade the host.

S43 ADHERENCE LECTIN GENE FAMILY OF *ENTAMOEBA*. Mann B. University of Virginia, Charlottesville, VA.

The galactose lectin of *E. histolytica* is a heterodimeric glycoprotein consisting of a 170 kDa and 35 kDa subunits linked by disulfide bonds. This lectin functions in mediating adherence to colonic mucins and epithelia as well as other target cells and affinity-purified native lectin has been shown to be a protective antigen in an animal model of amebiasis. The derived amino acid sequence of the cloned 170 kDa subunit suggests that it is an integral membrane protein with a cysteine-rich extracellular domain. The 170 kDa subunit is resistant to protease digestion unless first reduced and alkylated, implicating a role for the cysteines in the protease resistance of the lectin, which may be important for parasite survival in the gut. No carbohydrate-binding domain can be identified based on identity to the conserved carbohydrate-binding domains of the C or S-type lectins, bacterial lectins, or plant lectins. The 170 kDa subunit is encoded by a gene family. Two of the members of this family are 87.6% homologous at the amino acid level. The majority of these differences are concentrated in the first 380 amino acids. The number and the position of the cysteine residues are conserved in both gene products with one exception. A comparison of the similarities and differences between individual members of the gene family should provide valuable information regarding the functional activities of the lectin.

S44 CYSTEINE-RICH PROTEINS OF *GIARDIA LAMBLIA*. Nash T. National Institutes of Health, Bethesda, MD.

The surface of the trophozoite form of *Giardia lamblia* is covered by cysteine-rich proteins which undergo antigenic variation. These proteins termed variant-specific surface proteins (VSPs) are antigencially distinct, vary in molecular weight from 35 KDa to 200 kDa, contain between 11-12% cysteine with common cys-x-x-cys sequences, and have a common carboxy-terminal region. Some VSPs also contain repetitive sequences. The epitopes which can be expressed in a clone are characteristic of its lineage and differ among some isolates. In a majority of instances, isolates unable to express a particular epitope, lack the gene encoding for it. The rate of expression of epitopes is both isolate and epitope dependent and ranges from 1 appearance for every 6-13 generations. The number of VSP genes in one isolate has been estimated between 130-150 with 4 genes/VSP. Antigenic variation occurs *in vivo* in humans, gerbils and mice, however, cyclical waves of parasites of certain types have not been observed. Although the biological significance of VSPs are unknown, certain VSPs are more resistant to digestion by the intestinal proteases trypsin and alpha chymotrypsin suggesting VSPs may allow for survival in the small intestine.

S45 STRUCTURAL AND FUNCTIONAL STUDIES OF THE HIV gp 120 AND TRYPANOSOME VARIANT SURFACE GLYCOPROTEINS. Wiley D. Harvard University, Cambridge, MA.

Molecular genetics and proteolytic experiments have been used to help define the CD4 binding regions of the HIV-1 gp120 molecule. A molecule missing 41% of the gp120 amino acids has been constructed that retains CD4 affinity. Two VSG variants from *Trypanosoma brucei* have been crystallized and their structures determined by X-ray crystallography. Despite no significant amino acid sequence homology, the molecules share a common three-dimensional structure.

S46 CONSERVATION OF THE CYSTEINE-RICH DOMAIN IN THE CARBOXYTERMINAL REGION OF THE MAJOR MEROZOITE SURFACE ANTIGEN OF *PLASMODIA*. Long C. Hahnemann University, Philadelphia, PA.

Our laboratory had previously described a monoclonal antibody with significant passive protective activity against challenge infection with the erythrocytic stages of the rodent malarial parasite, *Plasmodium yoelii*. The epitope recognized by this antibody was subsequently localized to the carboxy-terminal, cysteine-rich domain of the major merozoite surface antigen (MSA-1) and was shown to depend on disulfide bond formation. Since some isolates of *P. yoelii* do not express the epitope recognized by the protective antibody, we have used the polymerase chain reaction to obtain DNA sequences encoding the 3' portion of the MSA-1 antigen using DNA preparations derived from a number of strains and species of rodent plasmodia. The postulated amino acid sequences derived from this analysis were compared with the published sequences of the homologous region from *P. chabaudi* and two alleles of *P. falciparum*. When this data is aligned, it is apparent that nine of ten cysteine residues in this region are remarkably conserved between all the plasmodia examined. This conservation, coupled with the finding that the carboxy terminal fragment of the MSA-1 protein remains with the merozoite after invasion of the next erythrocyte, suggests that the cysteine-rich domain plays an important role in the biological function of the malaria parasite.

S47 GAMETE CYSTEINE-RICH SURFACE PROTEINS OF *PLASMODIA*. Kaslow D. National Institutes of Health, Bethesda, MD.

Pfs25 and Pgs25 are cysteine-rich, 25-kilodalton sexual-stage surface proteins of *P. falciparum* and *P. gallinaceum*, respectively, and targets of antibodies that block transmission of malaria from vertebrate host to mosquito vector. The genes encoding both of these antigens have been cloned. The deduced amino acid sequence revealed a striking feature: the presence of four tandem epidermal growth factor (EGF)-like domains. EGF-like domains have been found in a wide range of membrane bound or extracellular proteins, are cysteine rich, and depend on proper disulfide bond formation for structural integrity. Although the function of Pfs25 and Pgs25, and the mechanism by which antibodies to these proteins block the appearance of oocysts in the mosquito midgut wall are still unknown, when fed to mosquitoes with a transmission blocking monoclonal antibody, parasites appear to accumulate on the bloodmeal side of the peritrophic membrane.

## SYMPOSIUM: ADVANCES IN THE EE STAGES OF MALARIA PARASITE

S48 *IN VITRO* CULTIVATION AND RODENT EE SPECIFIC ANTIGENS. Sinden RE*, O'Dowd C, Suhrbier A, Winger LA, and Couchman A. Imperial College, London, England.

Following expected statements denying the importance or even the existence of immunity to the liver stages, it has been established beyond reasonable doubt that exoerythrocytic (EE) parasites are the targets of specific cytotoxic T cells, of non-specific factors and even antibody responses of the immunized or infected host. The malarial parasites *Plasmodium berghei* and *P. yoelii* have played significant roles in these analyses, not least because they offer highly comparable in vivo and *in vitro* techniques which in combination offer unusual opportunities to develop specific reagents and analyze specific questions with precision. This paper will describe methods for high density culture of the EE parasites of *P. berghei* from both normal and irradiated sporozoites. In these cultures the "normal" growth of the parasite can be analyzed with considerable precision. The properties of monoclonal antibodies raised against these EE cultures will be outlined,and the distribution and expression of one liver stage-specific antigen Pbl-1 as seen by CLSM in HepG2 cells infected with irradiated or non-irradiated sporozoite will be described. The effects of passive immunization with anti-Pbl-1 antibody, and active immunization with recombinant Pbl-1 polypeptides will be discussed. From these (and other) analyses it has emerged that it is prudent to consider carefully the roles of liver stage antigens in the analysis of the effective immunity engendered by the irradiated sporozoite, and in natural populations.

S49 SPECIFIC HUMAN HEPATOCYTE RECEPTORS FOR SPOROZOITE INVASION AND EXPRESSION OF *PLASMODIUM FALCIPARUM* EE ANTIGENS. Hollingdale MR*, Zhu J, Sina B, Sakhuja K, van Pelt J, and Shoemaker J. Biomedical Research Institute, Rockville, MD; and the University of Nijmegen, Nigmegen, The Netherlands.

*Plasmodium falciparum* sporozoites only invade a few types of cells *in vitro*, and develop into mature exoerythrocytic (EE) parasites only inhuman hepatocytes, and rarely in Aotus hepatocytes. Such specificity is presumably mediated by specific hepatocytes receptor-sporozoite ligand interactions. Specific human hepatocyte 55 and 20kd receptors have been identified that may be recognized by a 16kd *P. falciparum* sporozoite surface antigen (SHEBA, sporozoite hepatocyte binding antigen). Results of cloning both the human receptors and SHEBA will be discussed. After invasion, EE parasites express three groups of antigens that have been identified. The first represents sporozoite antigens that continue to be detected during part or all of EE development but may not be synthesized. These include CS protein, CSP-2, CSP-3, a 140kd antigen that may be analogous to *P. yoelii* SSP-2, and SHEBA. The second group represents antigens found in both EE and red blood stages, and includes many merozoite antigens. The third group represents antigen specific to the EE stage and thus far only one *P. falciparum* antigen LSA-1 (liver stage-specific antigen) has been identified. Immune responses to these antigens may be mediated by antibodies that inhibit sporozoite invasion or agglutinate EE merozoites, and CD4+ and CD8+ cytotoxic T cells directed against EE antigens expressed in association with Class I on the EE-infected hepatocyte. Future anti-malarial vaccines will likely include EE- associated epitopes that elicit some or all of these responses.

S50 AMINO ACID SEQUENCE HOMOLOGY BETWEEN THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX* AND THE MCH CLASS I ANTIGEN: CHANCE OR DESIGN. Millet P*, Chizzolini C, Pieniazek NJ, Charoenvit Y, Jones TR, Hoffman SL, and Collins WE. Centers for Disease Control, Atlanta, GA; and Naval Medical Research Institute, Bethesda, MD.

The repeat region of the circumsporozoite (CS) protein of many isolates of *P. vivax* is characterized by the repeated sequences DRADGQPAG or DRAAGQPAG. The amino-acid sequence AGDR, contained within each sequence, as been shown to be a protective epitope by passive transfer to Saimiri monkeys of a monoclonal antibody (Mab), NVS3, specifically directed against this amino-acid sequence. Using the FASTA program to search the SWISS-PROT, release 17 (March 1991), data base for the occurrence of the sequence AGDR, we found major histocompatibility complex (MHC) class I-A,B,C. Indeed, the Mab NVS3 recognized a major band with relative molecular weight of 45-KDa on hepatocyte extracts from Saimiri and Aotus monkeys. An anti-class I Mab (W6/32) enhanced with number of schizonts of *P. vivax* developing *in vitro* with Saimiri hepatocytes up to 10 times. In 1 experiment, the Mab NVS3 facilitated the development of *P. falciparum* sporozoites in Aotus monkey hepatocytes. These studies suggest the participation of the MHC class I antigen in the penetration/development process of sporozoites of malaria parasites and raise questions about the role of the sequence PAGDR on both the MHC class I molecule and the CS protein of most isolates of *P. vivax*.

S51 NATURAL IMMUNITY TO EE STAGES. Vanderberg JP*. New York University, New York, NY.

The type and degree of inflammatory response is dependent upon species and age of the host, as well as species of parasite. Young rats injected with *P. berghei* sporozoites had weak inflammatory responses,whereas older rats mounted a strong response. Immunohistological studies showed that sporozoites released substantial quantities of CS protein into hepatocyte cytoplasm. Sporozoites induced a generalized cellular inflammation within the liver of the host, which first became evident at around 4 hrs and progressed to the formation of granulomas by 24 hrs. However, sporozoites that invaded and then proceeded to develop further did not appear to be targets until the initiation of a second wave of inflammation at around 40 hrs. Similarly, BALB/c mice mount a striking response soon after sporozoite

injection. However, these mice mount considerably less of a response against *P. yoelii*. This difference may relate to the fact that BALB/c mice are about 2,000 times more susceptible to *P. yoelii* than to *P. berghei* sporozoites. The rapid hepatic changes induced by invasive *P. berghei* sporozoites also have a deleterious effect on concomitantly injected *P. yoelii* sporozoites.However, once the *P. yoelii* sporozoites have successfully invaded hepatocytes, their subsequent development is no longer susceptible to the inflammatory changes induced by *P. berghei* sporozoites.

S52 INHIBITION OF MALARIA LIVER STAGES BY CD8+ T CELL CLONES. Rodrigues MM, Nussenzweig RS, and Zavala F*. New York University, New York, NY.

Immunization of mice (Balb/c) with irradiated *P. yoelii* sporozoites induces cytotoxic T cells which recognize an epitope contained within the amino acid positions 277-288 of the CS protein. CD8+ T cell clones specific for this epitope were derived from sporozoite immunized mice and their cytotoxic activity assesses in *in vitro* and *in vivo* assays. The adoptive transfer of clones YA23 and YA26 into naive mice conferred protection to a challenge with sporozoites. The protection conferred by these clones is stage and species specific. By measuring the plasmodial ribosomal RNA in the liver of sporozoite-infected mice,it was determined that these clones inhibited, in a dose dependent manner, the development of liver stages of the parasite. When adoptively transferred into naive mice, T cell clones YB8 and YB15 had a significantly lower and no anti-parasite activity respectively. Recent studies revealed that "protective" and "non-protective" clones have identical *in vitro* cytotoxic activity and that their pattern of lymphokine production does not correlate with their anti-parasite activity. Histological studies revealed that T cells from "protective"and "non-protective" clones are capable of reaching the liver incomparable amounts, however their pattern of migration within the liver parenchyma is strikingly different. While "protective" T cells are capable of "associating" with the parasitized hepatocytes in significant numbers, the "non-protective" T cells seem unable to establish this connection. These results will be discussed in light of ongoing comparative studies of "protective" and "non-protective" clones with regard to the expression of surface molecules involved in antigen recognition and cell adhesion.

S53 IDENTIFICATION OF T CELLS RECOGNIZING PREERYTHROCYTIC AND EXOERYTHROCYTIC STAGE ANTIGENS IN HUMAN VOLUNTEERS IMMUNIZED WITH IRRADIATED SPOROZOITES. Krzych U*, Jareed T, Seguin M, Lyon J, Hollingdale MR, and Ballou WR. Walter Reed Army Institute of Research, Washington, DC; and Biomedical Research Institute, Rockville, MD.

Immunization with radiation attenuated sporozoites induces protective immunity characterized by humoral and cellular responses directed against epitopes on the circumsporozoite (CS) protein and, presumably,other preerythrocytic stage antigens. These antigens thus represent the universe of potential candidates stimulating protective effector mechanisms. We obtained peripheral blood lymphocytes from human volunteers immunized with large numbers of irradiated *P. falciparum* sporozoites and examined their T cell repertoire using purified recombinant malaria proteins (CS and MSA- 1 fragments E, J and P, synthetic peptides from CS and LSA-1, and intact parasitized erythrocytes. Positive responses were recorded to recombinant CS protein and MSA-1 fragment E as well as ring infected erythrocytes, but not to synthetic CS peptides. Conversely, a standard 51Cr release assay revealed functional cytolytic T cells (CTL) specific for cells labeled with a previously identified CS peptide epitope (a.a. 368-390) that were present for a brief time after immunization. Additional data concerning the existence of proliferative and cytotoxic T cells directed against other non-CS protein antigens will be discussed.

S54 CHARACTERIZATION OF AN INHIBITORY MONOCLONAL ANTIBODY AGAINST *PLASMODIUM YOELII* LIVER STAGE PARASITES. Charoenvit Y*, Mellouk S, Sedegah M, Leef MF, de la Vega P, and Hoffman SL. Naval Medical Research Institute, Bethesda, MD.

After inoculation, malaria sporozoites enter hepatocytes where they develop for several days before rupturing and releasing merozoites that invade erythrocytes. This liver stage of malaria is an attractive target for vaccine development since the host is asymptomatic during this period. To identify liver stage antigens, we immunized mice with a suspension of hepatocytes infected with *P. yoelii* liver stage schizonts, and produced a series of monoclonal antibodies. One of the antibodies NYLS3, and IgG1, recognizes liver and blood stage parasites, but not sporozoites, by indirect fluorescent antibody test. This MAb also recognizes a 17 kD protein in extracts of blood stage parasites by SDS/PAGE immunoblotting analysis. It does not recognize *P. berghei liver* stage parasites. When this MAb was added to hepatocyte cultures 24 hours after sporozoites were added to the cultures, it inhibited schizont development by 45%. The inhibitory effects reached 80% when mineral oil activated peritoneal macrophages were also added to the cultures. This antibody has been used to identify a *P. yoelii* gene fragment encoding a portion of the protein, and the sequence information used to produce an oligonucleotide probe for identification of its *P. falciparum* analogue. These preliminary findings suggest that in addition to antibodies against circulating sporozoites, and cytotoxic T lymphocytes and cytokines against infected hepatocytes, there may be a role for antibodies that recognize antigens of the surface of malaria infected hepatocytes in protective immunity against the pre-erythrocytic stages of malaria.

S55 ULTRASTRUCTURE OF EE PARASITES USING ELECTRON AND CONFOCAL LASER SCANNING MICROSCOPY. Aikawa M*, Atkinson CT, and Hollingdale MR. Case Western Reserve University, Cleveland, OH; and Biomedical Research Institute, Rockville, MD.

Mice immunized with a peptide representing a modification of the repeat region of *Plasmodium falciparum* EE-specific antigen LSA-1 developed antibodies that specifically recognized a novel 230 kd *P. berghei* EE-specific antigen LSA-2. Peptide immunized mice were protected to *P. berghei sporozoite* challenge, and spleen cells from immunized mice killed *P. berghei* EE infected mouse hepatocytes. Thus, LSA-2 is the first EE-specific anti-malarial vaccine. Preliminary immunoelectron microscopy studies suggested LSA-2 was localized on the *P. berghei* EE parasitophorous vacuole membrane (PVM). Using mouse antisera to LSA-2 we further studied the three dimensional structure of the PVM within infected host cells using immunoelectron microscopy and confocal laser scanning microscopy at 3, 24, and 50 hours after sporozoite invasion.Fluorescent label was not detected at 3 hours, but was associated with the cytoplasm of 24-hour old EE parasites. Specific labelling of the PVM was not found by immunoelectron microscopy until 50 hours when numerous vesicles and finger-like projections of the PVM were found in the cytoplasm of infected cells. Labelled vesicles were often isolated and located at the periphery of the infected hepatocyte. Confocal microscopy demonstrated that these vesicles formed discontinuous chains that extended 3-10 μm away from the parasite. These structures appear to be similar to the membranous clefts of *Plasmodium*-infected erythrocytes and may be important in the trafficking of host or parasite proteins within infected hepatocytes.

S56 EXOREYTHROCYTIC DEVELOPMENT OF FALCIPARUM MALARIA EE PARASITES IN A SCID MOUSE MODEL. Sacci JB, Jr, Schriefer ME, Resau JH, Wirtz RA, Detolla LJ Jr, Markham R, and Azad AF. University of Maryland School of Medicine, Baltimore, MD; Walter Reed Army Institute of Research, Washington, DC; and Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

Research on the exoerythrocytic (EE) stages of human malaria parasites has been hindered by the lack of an easily available suitable animal model. We report a novel approach to produce mature EE-stage *Plasmodium falciparum* parasites using severe combined immune deficient (SCID) mice with transplanted human hepatocytes. Transplantation of human hepatocytes into SCID mice (SCID hu-hep), their subsequent infection by intravenous route with *P. falciparum* sporozoites and the development of mature liver stage merozoites was achieved. The maturation of merozoites in the transplanted hepatocytes was

monitored by immunofluorescent staining for the expression of MSA-1 and LSA-1, and, additionally, the decrease in reactivity of parasites to an anti-circumsporozoite protein monoclonal antibody. These studies clearly demonstrated the presence of parasites displaying merozoite specific antigens. The SCID hu-hep model can serve as a source of human malaria liver stage parasites, decreasing the need for non-human primates. Utilization of this model will facilitate characterization of EE stage antigens and the assessment of stage-specific chemotherapeutic agents and candidate vaccines.

## AMERICAN COMMITTEE ON ARTHOPOD-BORNE VIRUSES

S57 VENEZUELAN HEMORRHAGIC FEVER: A NEW ARENAVIRAL DISEASE OF MAN. Manzione M, Betancourt A, Godoy O, Salas R, Pineda E, and Paublini HR*. Ministerio de Sanidad y Asistencia Social and Instituto Nacional de Higiene, Caracas, Venezuela.

Venezuelan Hemorrhagic Fever (VHF), a previously unrecognized disease, was discovered in 1989 during an epidemic of dengue hemorrhagic fever (DHF) was occurring in Venezuela. The onset was insidious with fever general and malaise pharyngitis progressing to gastrointestinal symptoms, muscle pains and followed by gastrointestinal haemorrhage (hematemesis and melena) and severe hypotension. Leukopenia and thrombocytopenia were outstanding laboratory findings. Most deaths occurred 10 to 14 days after onset. A temporary alopecia and partial deafness were noted during convalescence. VHF occurs in rural areas of Guanarito (Estado Portuguesa) and most patients were agricultural workers. The disease caused 23 deaths and serious disease in 19 additional patients. The age group ranked from 3 to 55 years with the highest risk in the 15 to 24 years of age. The highest fatality rate was also observed in this last age-group. Males were more frequently affected.

S58 VENEZUELAN HEMORRHAGIC FEVER : A SEVERE MULTISYSTEM HEMORRAGIC ILLNESS CAUSED BY A NEWLY RECOGNIZED ARENAVIRUS. Salas RA, Manzione N, Tesh RE*, Rico-Hesse R, Pacheco ME, Ramos B, Taibo ME, Lorenzo B, Bruzual R, and Shope R. Ministerio de Sanidad y Assistencia Social and Instituto Nacional de Hygiene, Caracas, Venezuela; Yale University School of Medicine, New Haven, CT; Universidad Central de Venezuela, Caracas, Venezuela; and Disease Assessment Division, US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, MD.

Three arenaviruses (Lassa, Junin and Machupo) have been associated with severe hemorrhagic disease in humans. Recently, a fourth arenavirus, designated Guanarito virus, was isolated from a fatal case of hemorrhagic fever in Venezuela. Serologic and molecular characterization of Guanarito virus indicates that it is a new member of the family Arenaviridae. Clinical and laboratory findings on 14 confirmed cases (9 fatal) of Guanarito virus infection (Venezuelan hemorrhagic fever) will be presented. Patients ranged in age from 6 to 54 years; the sex ratio was identical. All patients were residents of rural areas in the plains (llanos) of southwestern Venezuela. Evidence of Guanarito virus infection in 2 rodents collected in houses of fatal cases suggests that the transmission mechanism of Venezuelan hemorrhagic fever is similar to that of the other rodent-borne arenaviruses.

S59 DEVELOPMENT OF ANIMAL MODELS FOR VENEZUELAN HEMORRHAGIC FEVER VIRUS USING GUINEA PIGS AND RHESUS MONKEYS. Jahrling PB*, Geisbert TW, Hall WC, and Salas R. Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Pathology Associates, Inc., Frederick, MD; and Instituto Nacional de Higiene, Caracas, Venezuela.

Guanarito virus (GUA) is a newly recognized arenavirus associated with Venezuelan Hemorrhagic Fever (VHF). To develop an animal model for VHF, we initially tested the susceptibility of guinea pigs and rhesus monkeys to GUA infection. Results: 4 of 4 rhesus monkeys inoculated IM with GUA were

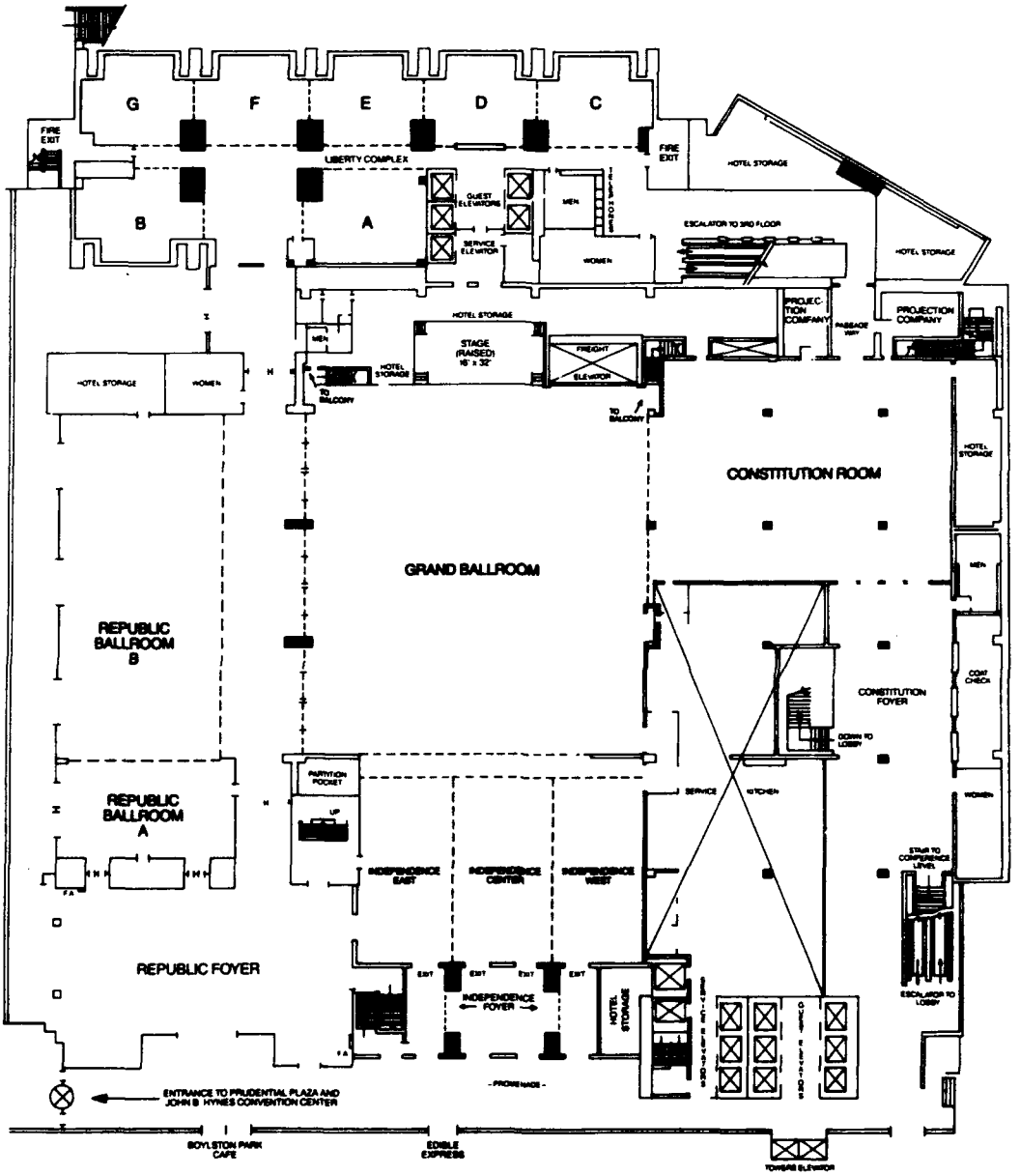
infected, developing transient viremia, and seroconverting within 11 days of inoculation; however, only 1 animal died (day 20). Only this animal exhibited prolonged PTT, decreased platelets, decreased HCT, and elevated LDH, BUN and creatinine. In contrast to monkeys, inbred strain 13 guinea pigs uniformly died, 11 to 14 days after inoculation. By immunohistochemistry and electron microscopy, GUA viral antigens were concentrated in gastrointestinal epithelium, lymphoid tissues of multiple organs, fixed tissue macrophages, epithelium of lungs and bronchi, endothelium of multiple organs, and occasionally in hepatocytes. In a cross challenge study,guinea pigs immunized with JUN vaccine failed to resist challenge with GUA. Surprisingly, LCM-immunized guinea pigs did resist challenge, despite the absence of a pre-challenge antibody titer to GUA. Conclusions: The guinea pig model for GUA should be developed further, to provide insight into pathogenesis, treatment, and immunization against the agent of VHF and related arenaviruses.

S61 A LONGITUDINAL STUDY OF JUNIN VIRUS ACTIVITY IN RODENTS FROM THE EPIDEMIC AREA OF ARGENTINE HEMORRHAGIC FEVER. Mills JN*, Ellis BA, Calderon GE, Maiztegui JI, Ksiazek TG, McKee KT, Peters CJ, and Childs JE. Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD; Instituto Nacional de Estudios sobre Virosis Hemorragicas, Pergamino, Argentina; and Disease Assessment and Medical Divisions, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

Junin Virus (JV) activity in rodent populations was monitored for 29 months at 2 mark-recapture grids located in agricultural fields and adjacent roadsides and fence lines in the epidemic area of Argentine Hemorrhagic Fever (AHF). Blood and oral swabs taken from rodents captured at 5-week intervals were analyzed by enzyme immunoassay (ELISA)for JV antigen. *Calomys laucha* and *C. musculinus* were the most frequently captured rodents, making up 47% and 35% of captures, respectively. Of the 29 Ag-positive rodents, 26 were *C. musculinus* and 3 were *C. laucha*. Males were more frequently positive than females, and old adults were more frequently positive than younger individuals. Positive rodents were temporally and spatially aggregated on both grids. Seasonal peaks in prevalence were correlated with maxima in density of *Calomys*. At peak prevalence 21% of captured *C. musculinus* were Ag-positive, including 5 rodents which were Ag-negative on previous capture. Most positive *C. musculinus* were captured in roadsides and fencelines; all positive *C. laucha* were from crop fields. Transmission of JV by infected *C. musculinus* may occur more frequently to rodent species of more stable border habitats, rather than to *C. laucha* which prefers crop fields. These conclusions have implications for attempts to control AHF through manipulations of the environment.

# AUTHOR INDEX

## PLAZA LEVEL, 2ND FLOOR



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