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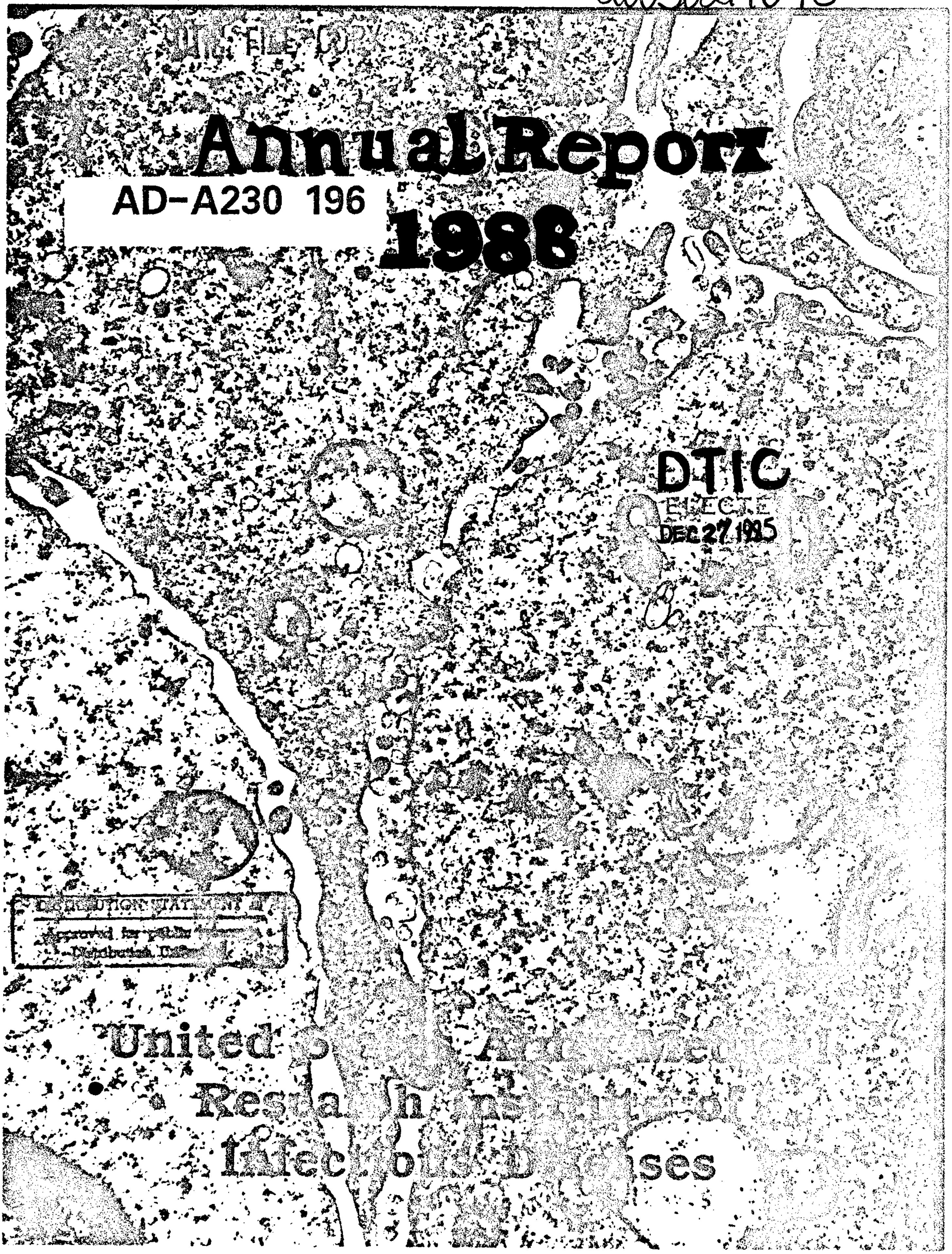

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United States Army Medical Research Institute of Infectious Diseases

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Author index - page viii

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A report of progress on the research program of the U.S. Army Medical Research Institute of Infectious Dieseases on Medical Defense Against Biological Agent (U) for Fiscal Year 1988 is presented.

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1

ANNUAL REPORT

Fiscal Year 1988

United States Army Medical Research
Institute of Infectious Diseases
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U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FREDERICK, MARYLAND 21701-5011

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES ANNUAL PROGRESS REPORT

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1 October 1988

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EDITOR'S NOTE

This FY 88 Annual Progress Report is a general review of research activities of the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, conducted on Medical Defense Against Biological Warfare Agents (U) under Projects 3M162770A870, 3M263763D807, 3M463750D809, 3S464758D847,3M162770A870, and 3M162770A871.

In conducting the research described in this report, the investigators adhered to the "*Guide for the Care and Use of Laboratory Animals*," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Commander's Foreword

The United States Army Medical Research Institute of Infectious Diseases (USAMRIID), a Department of Defense reference laboratory for disease agents of biological origin, has as its mission the medical defense of U.S. Armed Forces against biological warfare threats, as well as naturally occurring toxins and infectious agents of military importance that require special containment. To fulfill this mission, the USAMRIID staff develops strategies, products, information, procedures, and training in pursuit of effective countermeasures to potential biological threats. The countermeasures include vaccines, drugs, diagnostic reagents, and medical management methods to reverse or minimize the effects of a biological attack or an endemic disease and to preserve fighting strength.

USAMRIID's mission has expanded over the past four years, as mandated by the Vice Chief of Staff of the Army. This expansion comes as a result of Department of Defense recognition that the threat, and the necessity of an effective defense, demand immediate attention, effort, and resolve.

With the signing of the Intermediate-Range Nuclear Forces Treaty, and the current increased focus on both sides of the Atlantic on a chemical weapons treaty, there are many who believe that protection against biological warfare threats is becoming more important than ever in our contemporary history. The evidence has been mounting since 1976, when we received the first reports of use in Southeast Asia of the unknown toxic substances that were called "yellow rain." The most recent reports of use of chemical weapons come from Afghanistan and Iran. In fact, Valentin Falin, Director of the Soviet Press Agency Novosti, and numerous Soviet scientists have stated in open fora, such as the Annual Meeting of the American Association for the Advancement of Science, that the Soviet government will not endeavor to win a missile or conventional arms race with the U.S. They will, they state, use "asymmetrical means. . . .an example could be genetic engineering . . ." as they deem necessary.

We are gravely concerned that we will soon see proliferation of offensive biological warfare programs, as we have with chemical weapons programs, and limited weapon production by hostile states within a few years. In view of the Sverdlovsk incident in 1979, the alleged use of mycotoxins in southeast Asia and of chemical agents in Afghanistan and Iran, there is no doubt that increased emphasis on medical defense against biological warfare is needed.

USAMRIID's leadership role and its reputation in biological defense research are also extensive and highly visible internationally. USAMRIID coordinates formal medical defense measures with the North Atlantic Treaty Organization countries and member nations of The Technical Cooperation Program. The Institute is also consultant on biological defense programs for several other allied nations. Each year the Institute receives requests from talented postdoctoral candidates to spend time in the laboratories of internationally recognized USAMRIID investigators. In FY 88, USAMRIID hosted National Research Council postdoctoral fellows from the United Kingdom, the Republic of Korea, India, France, Sweden, and the People's Republic of China. The Institute also welcomed visiting scientists and dignitaries from counterpart organizations in the United Kingdom, the Federal Republic of Germany, the People's Republic of China, the Republic of Korea, and Sweden. By special request from the United States National Academy of Sciences, and with approval from the Department of Defense, a delegation of scientists from the Soviet Academy of Sciences visited USAMRIID in the spring of 1988.

In the event of war, USAMRIID would continue to be the lead U.S. laboratory for medical defense against BW threats, and, with other Army commands, would take action to: i) ascertain the nature of any suspected BW attack, ii) identify the disease-causing organism or toxin, iii) provide recommendations for rapidly implementing treatment, and iv) prevent additional casualties by vaccination or prophylactic drugs. USAMRIID's proficiencies in performing these and similar tasks have been tested and proven many times over in civilian and military emergency situations, for example: the outbreak of Korean hemorrhagic fever in Okinawa-based marines who had been training in Korea, the outbreak of eastern equine encephalitis that threatened the whooping crane population at Patuxent Wildlife Preserve in Maryland, the severe outbreak in 1988 of Rift Valley fever in west Africa, and the current outbreak of hemorrhagic fever in Yugoslavia. In these examples, USAMRIID scientists lead or are members of the specialized teams who pool expertise in infectious diseases and coordinate successful efforts that result in rapid and reliable diagnoses, and in some cases, countermeasures.

Defense Against Disease

Active immunization with vaccines is considered the ideal method of disease prevention, and has been the most effective method for controlling infectious diseases caused by conventional agents. Therefore, efforts in vaccine development receive high priority. Moreover, it may be possible with genetic engineering, to develop vaccines that can protect against an entire family of disease agents.

To provide additional broad-spectrum protection against the viruses, an antiviral drug development program was established in 1981. Through its auspices, thousands of compounds per year are screened for antiviral activity against a broad spectrum of exotic viruses. Research is also being pursued on immunopotentiators to use in tandem therapy with antiviral drugs.

The development of a medical countermeasure to a threat has historically been a long process, often requiring 14 years or more. Today, those research techniques, known under the umbrella term "biotechnology," offer the potential for reducing the total time required by four to five years. Biotechnology has been identified as one of the five major technology thrusts of the Army. These techniques are being applied in all Institute research programs and include recombinant DNA technology; DNA probes; manipulation of plasmids and genomes; hybridoma and monoclonal antibody production; sequencing studies; liposome microencapsulation technology; immunopotentiators and immunomodulators, including interferons and interleukins; and enzyme stabilization and immobilization techniques.

There is considerable importance placed on the rapid identification of an etiologic agent and diagnosis of disease. The primary objective of this work is the development of a small, deployable system capable of routinely producing diagnostic results that ordinarily require complex laboratory procedures and sophisticated equipment.

The Environmental Impact Statement

A two and one-half year study of the environmental impacts of the Biological Defense Research Program ended in April 1989 with the publication of the Programmatic Environmental Impact Statement, an exhaustively detailed document that discusses the potential risks and environmental effects of conducting research at the U.S. Army Medical Research Institute of Infectious Diseases, the U.S. Army Chemical Research, Development and Engineering Center, the U.S. Army Dugway Proving Ground, and more than 100 contractor sites. The report acknowledges that there is potential risk involved in the research, which requires the use of small quantities of infectious microorganisms and toxins for the development of vaccines and diagnostics to protect soldiers against disease. The risks are minimized, however, through training of personnel in safe laboratory techniques, extensive and redundant containment systems that prevent exposure of personnel or the environment to the research materials, and full compliance with the standards for laboratory safety, "Biosafety in Microbiological and Biomedical Laboratories," published by the National Institutes of Health and the Center for Disease Control.

The National Cooperative Drug Discovery Group

In 1987 the Institute received a 1.3-million dollar per year continuing annual grant from the National Institutes of Health (NIH) to screen compounds for antiviral activity against the human immunodeficiency virus (HIV). USAMRIID's unusual combination of scientific expertise in virology, knowledge of drug discovery and evaluation, and experience in biocontainment research have made the Institute an obvious and valued ally in the fight against AIDS. This cooperation with the NIH is exceedingly valuable in our efforts to discover new drugs effective against potential biological warfare agents, because it opens doors to large commercial inventories of compounds that might not otherwise be accessible.

Under USAMRIID direction, the National Cooperative Drug Discovery Group (comprising USAMRIID, the University of Arizona, the Southern Research

Institute, and Technassociates Incorporated) was formed to conduct research on HIV in cell culture systems to identify drugs that inhibit growth of the virus as well as specific viral functions, such as that of HIV reverse transcriptase.

The Drug Discovery Group has been successful: of the more than 50 drugs screened this year for antiviral activity, we have identified three that have antiviral activity in the murine AIDS model. One of these three has reached the stage where we anticipate that it can be ready for clinical trials in the near future. We have shown two additional drugs to be active against HIV reverse transcriptase in vitro, and have collected for testing over 150 drugs that have been reported to show an antiviral activity in other test systems.

Artificial Intelligence and Expert Systems

The development and application of computer artificial intelligence (AI) has opened new frontiers for the modification and manipulation of molecular configurations of therapeutic drugs. Analogs of effective drugs are being derived quite rapidly and more accurately than ever before. Once specific sites are identified for intervention, therapeutic drugs can be designed to react at these sites. For several years, we have given top priority to the application of computer artificial intelligence to many aspects of medical research and development programs, including vaccine and antiviral drug development and laboratory instrumentation.

We are currently using AI technology to analyze satellite imagery of vegetation growth, which is indicative of rainfall, in selected regions of Africa. This ability to measure vegetation growth and to infer rainfall has enabled us to predict mosquito activity in specific geographical areas, and thus, to predict outbreaks of vector-borne disease.

High-Hazard Evacuation and Patient Containment

USAMRIID has the capability of deploying a unique medical team to evacuate patients under stringent isolation procedures. Such procedures are essential for health care providers, and especially for patients who have, or are suspected of having a high-hazard disease, such as a hemorrhagic fever. The medical team places the patient in a transit isolator, which protects the patient from the environment, and prevents contamination of the environment by any infectious organism within the isolator, yet allows medical attendants access to the patient through glove ports. All transfers from the field, to the means of transport, to the patient isolation suites at USAMRIID, can be accomplished without breaking the containment barrier, and without risk of exposure.

USAMRIID and U.S. Air Force personnel maintain a high state of readiness for evacuation team deployment in joint exercises. There are cooperative quarterly fly-away training drills in which healthy volunteer service personnel are transported in maximum biocontainment in arctic, temperate, tropical, and desert climates.

Reference Laboratory

USAMRIID provides diagnostic services, diagnostic assays and reagents, monoclonal antibodies, DNA probes, and a variety of vaccines, toxoids, antitoxins, and antisera to federal, state, and local agencies, commercial organizations, and foreign governments.

Public Awareness and Information

Insofar as is possible, USAMRIID takes a pro-active stance on public awareness of and education about the BDRP. This year the Institute was in the public eye perhaps more than ever since its beginning in the 1960s. In addition to the attention brought about by the suit that resulted in a settlement requiring that an Environmental Impact Statement be published, USAMRIID stood as a sentinel and example for safety in biological research through two Congressional hearings. The first, in June, was before the Subcommittee on Postal Personnel and Modernization of the Committee on Post Office and Civil Service. The second, before the Senate Subcommittee on Oversight of Government Management, was held in July. In both of these hearings, it was through perseverance and testimony of the Commander of USAMRIID that the record was corrected and many false and irresponsible allegations laid to rest.

USAMRIID continues to welcome many foreign visitors and dignitaries within the framework of formal Memoranda of Understanding and Defense Exchange Agreements, and in connection with individual research collaborations. Visitors in 1988 included scientists from the United Kingdom, the Federal Republic of Germany, the People's Republic of China, France, the Republic of Korea, Sweden, Australia, the Netherlands and Brazil.

Vaccine and Drug Testing

We actively seek opportunities for large-scale testing in humans of proven vaccines and the antiviral drug ribavirin. These initiatives have led to mutually beneficial bilateral agreements with the People's Republic of China, the Republic of Korea, Argentina, and the Central African Republic. Under the aegis of these agreements, USAMRIID is able to collect the clinical evidence of drug or vaccine efficacy necessary for U.S. approval for its use, and, at the same time, aid friendly governments in fighting endemic diseases that are serious public health problems.

In conclusion, it should be emphasized that these products and information, although generated for defense against exotic agents with biological warfare potential, have broad application to both military and civilian medicine. USAMRIID has established itself as a center of excellence that fulfills a unique medical need by performing research on high-hazard agents that can be studied only at a small number of laboratories in the world.

AUTHOR INDEX

TABLE OF CONTENTS

ANNUAL PROGRESS REPORT - FY 88

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DAOG1519
2. DATE OF SUMMARY: 26 May 89
REPORT CONTROL SYMBOL: DD-DR&E(AR) 636

3. DATE PREV SUM'RY: 30 Oct 87
4. KIND OF SUMMARY: D. CHANGE
5. SUMMARY SCTY: U
6. WORK SECURITY: U
7. REGRADING:
8. DISB'N INSTR'N: CX
9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS12	AA	001
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11. TITLE (Precede with Security Classification Code): (U) Basic studies for the development of medical defensive countermeasures to toxins of biological origin

12. SUBJECT AREAS: 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE: 81 01
14. ESTIMATED COMPLETION DATE: 89 01
15. FUNDING ORGANIZATION: DA
16. PERFORMANCE METHOD: C. In-House

17. CONTRACT/GRANT
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18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	4.0	820
89	4.0	1170

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code): Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL: Huxsoll, D L
d. TELEPHONE NUMBER (include area code): 301-663-2833

20. PERFORMING ORGANIZATION
a. NAME: Pathology Division, USAMRIID
b. ADDRESS: Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR: Middlebrook J L
d. TELEPHONE NUMBER (include area code): 301-663-7211
f. NAME OF ASSOCIATE INVESTIGATOR (if available): Schmidt, J
g. NAME OF ASSOCIATE INVESTIGATOR (if available): Crosland, R

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MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code): (U) BW Defense; (U) Low Molecular Weight Toxins; (U)Microbial Toxins;(U) Biochemistry;(U) Therapy;(U) Lab Animals;(U) Guinea Pigs;(U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The technical objective of this work unit is to provide the research base for the development of protective modalities from toxins. Recent advances in molecular genetics have demonstrated that many toxins can be cloned and produced. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Our approach is to study any and all aspects of the toxin including detection, genetics, synthesis, elaboration, structure, composition, pharmacology, mechanism of action, pathogenesis, and sensitivity to drugs. We seek to develop novel means of protection from botulinum and marine toxins as well as mycotoxin. Some of these are synthetic vaccines, CRM-based vaccines, toxin-blocking drugs or toxin-reversing drugs.

25. (U) 8710 - 8809 Cross neutralization tests were run with rabbit antisera and various protections were defined. Monoclonal antibodies to pseudexin were prepared. Two of the antibodies neutralized the toxin. Several other of the monoclonal antibodies cross reacted with most elapid PLA_2 neurotoxins. Binding of taipoxin to PC-12 cells appears to be mediated by a specific receptor for this toxin. The phospholipase A_2 toxins, pseudexin A and B, were purified and sequenced. Epitope mapping of the pseudexins was begun and their physical properties described. Assays for cross-reacting material of botulinum toxin were developed. The drugs chloroquine, quinicrine, chlorpromazine, primacrine, and nicergoline were found to protect mice from several phospholipase A_2 neurotoxins and may be useful in man. Emetine was found to block the binding of T-2 to target cells and its molecular mechanism of action was defined.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M161102BS12: Science Base/Medical Defense against BW

WORK UNIT NO. S12-AA-001: Basic Studies of Conventional Toxins of Biological Origin and Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. L. Middlebrook, Ph. D.

ASSOCIATE INVESTIGATORS: R. Crosland, Ph. D.
J. Schmidt, Ph. D.
D. Leatherman

BACKGROUND

The biological warfare (BW) potential of toxins has long been recognized. The Army has had a research program directed towards the development of protective modalities, primarily vaccines. The program was driven by a combination of intelligence information and a knowledge of which toxins were potent enough and could be prepared in quantities sufficient for delivery. Basically, these considerations kept our list of candidate BW toxins rather short. With the emergence of molecular genetics as a real and practical technology, the number of potential threat toxins has become enormous. It is clearly impractical to attempt to study each and every toxin. Rather, a program has been designed which is flexible enough to accommodate new threats rapidly, while developing information with representative toxins to build general principles for protection. At present, we are studying botulinum toxin, tetanus toxin, several snake phospholipase neurotoxins, several snake postsynaptic toxins, ricin, mycotoxins, and diphtheria toxin.

SUMMARY

Cross-neutralization tests were run with rabbit antisera and various protections were defined. Monoclonal antibodies to pseudexin were prepared. Two of the antibodies neutralized the toxin. Several other of the monoclonal antibodies cross reacted with most elapid phospholipase A2 (PLA_2) neurotoxins. Binding of taipoxin to PC-12 cells appears to be mediated by a specific receptor for this toxin. The PLA_2 toxins, pseudexin A and B, were purified and sequenced. Epitope mapping of the pseudexins was begun and their physical properties described. Assays for cross-reacting material of botulinum toxin were developed. The drugs chloroquine, quinicrine, chlorpromazine, primacrine, and nicergoline were found to protect mice from several PLA_2 neurotoxins and may be useful in man. Emetine was found to block the binding of T-2 to target cells and its molecular mechanism of action was defined.

ECA Mechanisms of Action of Bacterial Exotoxins. Studies on Botulinum Toxin.

'RINCIPAL INVESTIGATORS: J. L. Middlebrook, Ph.D.
D. Leatherman

During the past year, we finished up the work on rabbit antisera and PLA_2 neurotoxins and established their serogroups. This information has been turned over to another scientist in the department and she is now establishing the detection tests for handoff to fielding agencies of the Army. We also investigated the cross-neutralizing capabilities of the various antisera and obtained some interesting results. While not yet complete, our data support the concept that it should be possible to develop vaccines that will protect from several toxins in a given serogroup. For example, the antisera to crotoxin neutralized three other PLA_2 neurotoxins in the serogroup, vegrandis, Mojave, and concolor toxins. Likewise, antisera to taipoxin, notexin, and *Naja naja atra* PLA_2 , three toxins in the elapid serogroup, cross neutralized textilotoxin, a fourth member. We intend to fully analyze the possible cross neutralizations and to apply this information towards efforts to develop cross-protective vaccines.

Monoclonal antibody work continued with considerable success. A battery of 15 lines was developed against pseudexin, two of which neutralized the toxin and most of which cross reacted with other elapid toxins. The cross reactions are now being defined in detail, although preliminary results suggest that these lines will be very useful to detect practically all elapid PLA_2 toxins.

In the search for cell lines which might be suitable for molecular mechanism of action studies, we radiolabeled three PLA_2 neurotoxins and studied their binding to PC-12 nerve cells. One of them, taipoxin, exhibited features of binding that appeared like receptor-ligand interactions. The binding was of high affinity, saturable, specific, and reversible. None of the other PLA_2 toxins blocked the binding, while unlabeled taipoxin did. These and further studies encouraged us to believe we identified a cell system that should be very useful in defining the mechanisms of action of taipoxin and possibly other PLA_2 neurotoxins.

Phage DNA from *Clostridium botulinum* type C was obtained and our oligonucleotide probe was used to prove that the structural gene for the toxin is on the phage.

During the past year, we continued to investigate the molecular mechanism of the interaction of T-2 mycotoxin with its ribosome receptor in intact cells. In previous studies conducted in this laboratory, numerous drugs were evaluated for their effects upon T-2 toxin association with mammalian cells. One of the drugs tested, emetine, significantly reduced T-2 toxin cell-association by a process that appeared to be non-competitive. Because T-2 toxicity (as measured by inhibition of protein synthesis) is proportionately dependent upon the extent of toxin-cell association, any agent that inhibits the association of toxin with cells is of considerable interest. The objective for the preceding year has been to determine the processes that are responsible for emetine-induced inhibition of T-2 toxin-cell association. The results are summarized below.

Emetine is an alkaloid drug which stabilizes translating ribosomes by inhibiting the elongation-termination stages of protein synthesis. This results in an accumulation of polyribosomes in intact mammalian cells. Pre-exposure of Chinese hamster ovary (CHO) cells to emetine resulted in a 60 - 80 % reduction in the association of T-2 toxin with the cells. Binding isotherms for T-2-cell association showed saturable curves for both emetine-treated and non-treated cells. Scatchard analysis of these data showed a reduction in the number of binding sites after emetine exposure from 1.5 x 10^6 sites/cell to 5.6 x 10^5 sites/cell. The emetine-induced reduction of T-2-cell association was time-, temperature-, and concentration-dependent. Upon removal of emetine by washing, its effect upon toxin-cell association was completely reversed. The return to maximum levels of toxin-cell association was significantly accelerated when the post-wash medium contained puromycin, which causes extensive breakdown of polyribosomes with a complete release of nascent peptides. In binding studies with ribosomes isolated from CHO cells, emetine failed to compete for T-2 binding to its ribosomal receptor. However, T-2 binding to ribosomes prepared from emetine-pretreated CHO cells was reduced 60% below the binding observed with ribosomes isolated from non-treated cells. These data suggest that the emetine-induced reduction of T-2-cell association represents a reduction in the number of accessible binding sites as a result of the drug's action upon polyribosomal populations in eukaryotic cells. The reduction in toxin association with cells containing emetine-stabilized polysomes is consistent with the observation of diminished toxin affinity for its site on polysomes in cell-free systems.

To confirm these observations, procedures are needed for evaluating polyribosomal populations from mammalian cells and correlating polysome profile data with toxin-cell association data. These procedures are presently under development and are being adapted to meet the specific experimental requirements of this project. Preliminary data from these correlation studies are encouraging and completion of this project is anticipated in 3 months.

MECB Protein Chemistry and Structure-function Studies of *Clostridium botulinum* Neurotoxins

PRINCIPAL INVESTIGATOR: J. Schmidt, Ph.D.

In an effort to purify further the mixture of PLA_2 isoenzymes called "pseudexins," I chromatographed peak V (from fractionation of crude venom on a mono-S column) in reverse-phase HPLC on a C18 column with gradients of TFA/acetonitrile. With this system, the individual isoenzymes were recovered in pure form, and it was also revealed that the mixture actually consisted of three proteins, not two as previously thought.

Pseudexins A, B, and C, individually purified by C18 HPLC, were placed in the amino acid sequencer and the first 28 - 34 residues were identified. The data were compatible with those from sequencing runs on mono-S peak V, and were sufficient to demonstrate that the three isoenzymes have homologous but unique structures.

I determined the complete amino acid sequences of pseudexins A and B, using about 3 - 4 mg of each isoenzyme, purified from fraction V by reverse-phase HPLC. The complete primary structures were assembled from N-terminal sequencing data and from sequencing of peptides generated by cleavage with clostripain, staphylococcal V8 protease, trypsin, chymotrypsin, and iodosobenzoic acid. Peptides so produced were purified by HPLC, analyzed in the sequencer, and placed in order in the overall sequence by comparison with structures of overlapping peptides.

In other work on pseudexins, I investigated the binding of anti-pseudexin monoclonal antibodies to native versus denatured pseudexins. Monoclone numbers 3, 8, and 9 bound to native pseudexins A and B on a nitrocellulose blot, but not to reduced/pyridylethylated pseudexins. Moreover, none of the above antibodies bound to the toxins on a western blot (i.e. toxins were reduced, subjected to sodium dodecylsulfate polyacrylamide electrophoresis, electrophoretically transferred to nitrocellulose, and immunostained). Apparently, the epitopes recognized by these monoclones are conformational in nature. In contrast, ascites number 5 was able to bind to denatured pseudexins, albeit weakly.

In an ongoing project, in collaboration with Dr. Lynn Siegel, antisera to peptides, based on sequences from the heavy and light chains of botulinum neurotoxins types B and E, were further evaluated by western blot, ELISA, and neutralization tests. All antisera bound to homologous peptide and also to homologous whole toxin. Different degrees of cross reactivity with heterologous serotypes and with tetanus toxin were found, depending not only on the peptide, but also on the individual rabbit in which a particular antiserum was raised. None of the peptide antisera neutralized toxin. Peptide antibodies were used to identify specific sequences in complex mixtures containing many different peptides, and to evaluate toxin production by organisms after exposure to mutagen.

In order to maintain our supplies of highly purified botulinum neurotoxins, a preparation of type E was grown in the fermenter and successfully purified. In addition, quantities of types B and C were also purified. Current supplies of purified botulinum neurotoxins are summarized in the following table:

Total mg*

Serotype	Purified (mg)	Used (mg)	On-Hand (mg)
A	36	3	33
B	112	21	91
C	41	7	34

* As of June 1988

MEGF Cellular Mechanisms of Actions of Militarily Relevant Toxins

PRINCIPAL INVESTIGATOR: Richard Crosland, Ph.D.

Antivenoms are the currently available agents for treatment of intoxication by snake venoms. The development of therapeutic strategies by more generally available drugs could improve treatment of envenomation by reducing the cost of the therapeutic agent, eliminating hypersensitivity reactions to antivenoms, and requiring less costly storage conditions. To these ends, the efficacies of the PLA_2 inhibitors chloroquine, chlorpromazine, dexamethasone, nicergoline, piracetam, primaquine, and quinacrine were investigated with respect to reducing the toxicity in mice of *Bungarus caeruleus* venom; *Bungarus multicinctus* venom and its neurotoxic components, a-bungarotoxin and b-bungarotoxin; *Crotalus durissus terrificus* venom and its neurotoxic component, crotoxin; and *Oxyuranus scutellatus* venom and its neurotoxic component, taipoxin; all of which have PLA_2 activity associated with them.

Venom ("venom" will hereinafter denote "venom" and "toxin") was administered i.p., followed immediately by an i.p. injection of drug. Lethality was measured 24 h later. The optimal protective dose of each drug with respect to each venom was first determined. Second, the drug-induced change in the LD_{50} of venom was measured. Third, the effect on lethality of the relative time of injection of drug with respect to the injection of venom was determined.

Chloroquine increased the LD_{50} of *B. caeruleus* venom, *B. multicinctus* venom, and b-bungarotoxin 16-, 5.0-, and 17-fold, respectively, while having no effect on the LD_{50} of the other venoms. Chlorpromazine also increased the LD_{50} of only the same three venoms 8.7-, 2.6-, and 3.8-fold, respectively. At high doses, dexamethasone caused a 3.5-fold increase in the LD_{50} of *O. scutellatus* venom and a 4-fold increase in the LD_{50} of taipoxin while having no effect on the LD_{50} of the other venoms. Nicergoline increased the LD_{50} of *B. multicinctus* venom 4.5- fold and the LD_{50} of b-bungarotoxin 3.9-fold while having no effect on the LD_{50} of the other venoms. Piracetam had no effect on any of the venoms. Primaquine increased the LD_{50} of *B. caeruleus* venom, *B. multicinctus* venom, and b-bungarotoxin 2.9-, 6.0-, and 3.9-fold, respectively, while having no effect on the LD_{50} of the other venoms. Quinacrine increased the LD_{50} of *B. caeruleus* venom 5.7-fold, the LD_{50} of *B. multicinctus* venom 10.5-fold and the LD_{50} of b-bungarotoxin 8.6-fold while having no effect on the LD_{50} of the other venoms.

The relationship between lethality and the time of injection of drug relative to the time of injection of venom was similar for all the drug-venom combinations tested. Protection from lethality was maximal when the drugs were administered immediately after the injection of a 100% fatal dose of venom. Protection was still present, although significantly reduced, when drugs were injected 15 min either before or after intoxication. With the exceptions of chloroquine and quinacrine, a 30-min pre- or postintoxication drug treatment offered virtually no protection from lethality.

I found that selected drugs currently in use in humans for other purposes significantly reduced the lethality in mice of some snake venoms and their presynaptic neurotoxic components if the drugs were administered soon before or after intoxication.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOG1522	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

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10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS12	AB	002
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13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
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17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
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19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Dalrymple, J M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7241
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Leppla, S H
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Schmaljohn C S

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Togavirus; (U) Flavivirus; (U) Bunyavirus; (U)Arenavirus (U)Anthrax; (U)Genes; (U)Antigen;(U)Diagnosis;(U)Lab Animals;(U)Mice;(U)RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To elucidate antigenic composition, replicative strategies, and specific gene functions for selected togaviruses, flaviviruses, bunyaviruses, arenaviruses and anthrax. To evaluate specific protein and nucleic acid constituents and gene products as potential diagnostic antigens, probes and immunogens with prophylactic potential. To develop methodology and provide a technical base for an improved BW defense program. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Characterize structural proteins and nucleic acid of selected pathogens by biophysical and biochemical techniques; identify the genome regions responsible for important antigenic determinants or diagnostic probes. Ultimately define the replication strategy for a better understanding of mechanisms for either deducing targets for chemotherapeutic intervention or inducing a protective immune response.

25. (U) 8710 - 8809 Hantaan viral genes coding for envelope glycoprotein antigens have been successfully expressed by using both the recombinant baculovirus and vaccinia virus systems. Animals immunized with the products of these expression systems have responded with the production of neutralizing antibodies. Recombinant vaccinia viruses expressing specific sequences from Rift Valley fever virus have refined requirements for future vaccines. Monoclonal antibodies to Crimean-Congo hemorrhagic fever (CCHF), vaccinia, and Hantaan viruses have been used to define major antigenic determinants and serve as useful diagnostic reagents for each of these viruses. The DNA sequence of *Bacillus anthracis* edema factor (adenylate cyclase) allowed identification of the enzymatically active site to be inactivated for vaccine development. Specific cellular sensitivity to anthrax lethal toxin was shown to be due to a post-binding event, and a requirement for extracellular calcium was shown for the expression of toxicity.

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PROJECT NO: 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO. S12-AB-002: Basic Studies on Conventional Agents of Biological Origin and Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

ASSOCIATE INVESTGATORS: C. S. Schmaljohn, Ph.D.
J. F. Smith, Ph.D.
S. H. Leppla, Ph.D.
J. R. Lowe, COL, Ph.D.
J. Arikawa, Ph.D.
Y. K. Chu, Ph.D.
S. F. Little, M.S.

BACKGROUND

The research concept being explored by the basic studies presented within this work unit includes an examination of the feasibility of employing biotechnology or molecular technology for the development of new and improved vaccines and/or diagnostic reagents. Although all of the agents under investigation represent real disease threats, the basic information developed and the strategies determined to be successful in the approach to vaccination or diagnosis should serve as valuable models for related disease-producing agents. Previous progress reports emphasized details of obtaining molecular clones and their DNA sequences, the generation of batteries of monoclonal antibodies, and the development of methods and techniques for the synthesis or expression of gene products. The state-of-the-art has progressed to the point that many of these clones, sequences, and monoclonal antibodies are now available, and experience with the associated technologies has increased the feasibility of obtaining similar reagents and information for other agents. In this report we report a continuation of the research for the investigation of the various methods for exploiting new information in the pursuit of improved disease prevention and control.

SUMMARY

Hantaan viral genes coding for envelope glycoprotein antigens have been expressed successfully by using both the recombinant baculovirus and vaccinia virus systems. Animals immunized with the products of these expression systems responded by producing neutralizing antibodies. Recombinant vaccinia viruses expressing specific sequences from Rift Valley fever virus (RVFV) have re-

fined requirements for future vaccines. Monoclonal antibodies to Crimean-Congo hemorrhagic fever (CCHF), vaccinia, and Hantaan viruses have been used to define major antigenic determinants and serve as useful diagnostic reagents for each of these viruses. The DNA sequence of *Bacillus anthracis* edema factor (adenylate cyclase) allowed identification of the enzymatically active site to be inactivated for vaccine development. Specific cellular sensitivity to anthrax lethal toxin was shown to be due to a post-binding event, and a requirement for extracellular calcium was shown for the expression of toxicity.

MEIA Molecular and Biological Characterization of Nairoviruses

PRINCIPAL INVESTIGATOR: J. F. Smith, Ph.D.

In FY 88 our studies focused on antigenic and biochemical analyses of the virus-specific proteins of CCHF virus and on a definition of the antigenic relationships among CCHF virus and heterologous Nairoviruses.

The CCHF virus-specific proteins were identified by electrophoresis of polypeptides solubilized from gradient-purified virions and nucleocapsid complexes, and by immunoprecipitation by polyclonal antisera of lysates of infected cells. The structural proteins consisted of two envelope glycoproteins, G1(80 kd) and G2 (37 kd); the nucleocapsid protein, NC (50 kd); a 240-kd polypeptide, L, assumed to be the viral polymerase; and two nonstructural proteins (82 and 120 kd). These two nonstructural proteins, as well as G1 and G2, contained predominantly high-mannose, asparagine-linked oligosaccharides. The 120-kd species shared determinants with both G1 and G2 and is probably a precursor polypeptide, which is postranslationally cleaved to form the mature glycoproteins.

Monoclonal antibodies to each of these proteins were produced from seven, independent hybridoma fusions. These fusions resulted in 464 positive hybridomas, 89 of which will used for cloning and further analysis.

These monoclones were analyzed by immunofluorescence assays with 13 strains of CCHF virus and 14 other Nairoviruses. The results indicate that epitopes residing on the NC protein are highly conserved among the CCHF viral strains, and, with few exceptions, are absent on heterologous Nairoviruses. In contrast, the reactions with G1 and G2 monoclones showed significant heterogeneity among the CCHF viral strains; many of these glycoprotein epitopes were conserved on other Nairoviruses. Apparently, antigenic cross-reactions observed previously with the *Nairovirus* genus monitor determinants on the viral envelope antigens, and purified NC complexes have proven to constitute a highly specific, serodiagnostic antigen in ELISAs.

Anti-G1 and anti-NC monoclones were evaluated for their ability to neutralize virus in vivo and to protect passively immunized infant mice. As expected, the anti-NC antibodies were inactive in both assays. Approximately half the anti-G1 monoclones had significant plaque-reduction titers, and some showed efficient protection in vivo. However, the correlation between these two assays was low, with some monoclones capable of neutralization, but not protection, and others showing protection without neutralization. An additional complication in designing protective immunogens is that the cross-reactivity studies have shown only limited conservation of these protecting epitopes on some strains of CCHF virus, suggesting that a monovalent vaccine may not provide protection against all strains of CCHF virus.

MEIB Analysis of Experimental Rift Valley Fever Vaccines

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

Vaccinia virus was evaluated as an expression vector for the generation of candidate human virus vaccines engineered to express a variety of genes coding for important antigenic determinants of viral pathogens of interest. Three separate strains of vaccinia virus were evaluated as possible parent vaccine strains, based on the fact that each has been used successfully in human smallpox vaccines.

Recombinant vaccinia vaccines were generated with each of the vaccinia strains containing a Rift Valley fever viral (RVFV) gene segment expressing both G1 and G2 envelope glycoproteins. Experiments were designed to reveal differences in expression or immunogenicity that could possibly result from the different vaccinia parent strains, which were safety- and immunogenicity-tested in mice. Of the three vaccinia strains examined (Connaught, Copenhagen, Lister) the Connaught-derived recombinants were of lower virulence for mice, yet elicited as good an immune response as any of those tested. Based on these data, the Connaught was selected as the parent vaccinia strain for future vaccinia recombination experiments and candidate human virus vaccine development.

A series of recombinant vaccinia viruses were generated containing truncated RVFV G2 gene segments. Two antigenic determinants important for protection were mapped to specific loci on the G2 gene and were conserved in most of of the truncated recombinants. After immunization of mice and subsequent challenge, responses varied from full to no protection, as the size of the G2 gene segment decreased. Interestingly, G2 segments that contained both of the antigenic determinants, previously shown to be important for protection, still failed to protect, suggesting that some regions near the carboxy terminus of the G2 protein are important for vaccinia viral expression of protective RFVF determinants.

A vaccinia viral parent strain, which could serve as the parent vaccinia for all recombinations generated for human vaccine development, has been evaluated in the Bioproductions Laboratory at USAMRIID. The original Connaught strain was passed through a series of plaque purifications in certified MRC-5 cells and one of the derivatives for further development. A plasmid was designed which contained homologous flanking sequences of the Connaught thymidine kinase (tk) gene with an engineered 150 base-pairs deletion to prevent possible regeneration of the entire tk gene, as well as a vaccinia promoter and the lac Z gene coding for beta galactosidase. Homologous recombination experiments with this plasmid DNA and the Connaught strain candidate vaccine virus resulted in a recombinant vaccinia virus that was thymidine kinase-negative and that expressed beta galactosidase.

MEID Molecular and Biological Characterization of Hantaviruses

PRINCIPAL INVESTIGATOR: C. S. Schmaljohn, Ph.D.

ASSOCIATE INVESTIGATORS: J. Arikawa, Ph.D.
J. M. Dalrymple, Ph.D.
Y.-K. Chu, Ph.D.

Monoclonal antibodies to the envelope glycoproteins of Hantaan virus were produced and characterized. Expression of Hantaan genes in eucaryotic vectors was used to develop diagnostic antigens and immunogens to Hantaan and Rift Valley fever viruses.

We produced a panel of 24 monoclonal antibodies to the envelope glycoproteins (G1 and G2) of Hantaan virus and examined them by plaque-reduction neutralization, hemagglutination inhibition, and indirect immunofluorescence. Competitive inhibition studies revealed nine distinct, partially overlapping, antigenic sites, two on G1 and seven on G2. Neutralizing epitopes and epitopes involved in hemagglutination were identified on both G1 and G2. The nine antigenic sites could be further divided into 13, based upon the serological cross-reactivity with viruses representative of each of the four known antigenic groups of Hantaviruses. These data have provided valuable insights into the antigenic relationships among Hantaviruses and have established a basis for understanding immune responses to Hantaan virus.

The M and S genome segments of Hantaan virus, which encode the G1 and G2 or the nucleocapsid (NC) protein, respectively, were inserted into two eucaryotic viral vectors: vaccinia virus and the baculovirus *Autographica californica* nuclear polyhedrosis virus (AcNPV). We were able to produce, in both expression systems, Hantaan proteins which were indistinguishable from authentic viral proteins by polyacrylamide gel electrophoresis.

We examined the baculovirus-expressed proteins as diagnostic antigen and were able to demonstrate, by ELISA fluorescent antibody, reactivity with a variety of rodent and human immune sera. In collaboration with LT COL J. LeDuc, we used the expressed NC antigen for Hantavirus screening assays. This expressed protein offers numerous advantages over authentic Hantaan antigen, including: (1) safety, because AcNPV infects only lepidopteran insect cells like moths and caterpillars, and not mammalian cells or dipteran insect cells like mosquitos. Therefore the baculovirus can be propagated under P2 containment rather than P3, as required for Hantaan; (2) expediency, in that the antigen can be produced at much greater levels per infected cell than authentic viral antigen and can be produced in only 3 days, compared to 7 - 10 for Hantaan virus; and (3) simplicity, in that the insect cultures inoculated with the baculovirus can be grown in spinner cultures at room temperature.

Expression of the entire M segment of Hantaan with AcNPV was used to produce both G1 and G2, and expression of portions of the M segment to generate only G1 or G2. These proteins are currently being tested as both diagnostic antigens and as immunogens. To date, we have been able to elicit neutralizing antibody to Hantaan in rabbits inoculated with cell lysates infected with the baculovirus construct containing the entire M segment, but not with recombinants expressing only one of the glycoproteins.

Unlike baculoviruses, vaccinia virus will infect humans and, therefore, vaccinia recombinants can be used as live-virus vaccines. Such vaccines allow production of proteins in the inoculated animal and, consequently, smaller immunizing doses are required. We prepared recombinants in two strains of vaccinia: the mouse-neurovirulent WR strain and the Connaught human-vaccine strain. Mice and hamsters were immunized with vaccinia-Hantaan recombinants expressing NC, both G1 and G2, or only G1 or G2. Antibody responses to both vaccinia and Hantaan were detected in mice, with the highest titers to Hantaan observed in animals immunized with the recombinant expressing both G1 and G2. The response to the Connaught vaccinia recombinant appeared as good or better that that to the live WR recombinant.

Although no animal model for disease caused by Hantaan virus has been discovered, a protection model for hamsters was developed by Dr. J. Dalrymple and Dr. Y.-K. Chu. Hamsters were immunized with each of the vaccinia-Hantaan recombinants and were subsequently challenged with Hantaan virus. Serum antibody titers to Hantaan and the presence of Hantaan antigen in the lungs of the animals were measured. The recombinants expressing G1 and G2 appeared to protect completely the animals from infection with Hantaan virus. The WR-vaccinia recombinant expressing only G1 also appeared to protect most of the animals from infection. No protection was observed with the other constructs. These results were very encouraging for development of a human-vaccinia vaccine, and we are pursuing that goal.

We also expressed the M genome segment of RVFV in AcNPV and have produced both G1 and G2 proteins from recombinants containing the complete segment, and G2 or a small nonstructural protein from recombinants containing portions of the M segment. The expressed proteins were examined as diagnostic antigen and were found to work well for detection of antibodies in human vaccinee sera or in mouse ascitic fluids, but high backgrounds were obtained with sera from African Rift Valley fever patients. More appropriate conditions for use of this antigen for screening samples from epidemic areas must be determined.

We immunized mice with the RVFV M segment recombinants and challenged the animals with RVFV. We demonstrated that, although the mice became infected with RVFV, they were protected from disease. We currently are repeating studies to determine if the expressed G2 protein only will be protective

MEDA Basic Research Studies for Protection Against Anthrax

PRINCIPAL INVESTIGATOR: S. H.. Leppla, Ph.D.

ASSOCIATE INVESTIGATORS: J. R. Lowe, LTC, Ph.D.
S. F. Little, M.S.

Nested deletions of the lethal factor (LF) and (PA) genes were obtained for use in DNA sequencing and for mapping antigenic and functional domains. Conditions were established for semi-random mutagenesis of the PA gene by the "TAB linker" method. The PA gene was expressed in *Escherichia coli* under control of the T7 promoter, and several deletions and fragments were obtained.

The cloned PA gene was transferred to *B. anthracis* strains by electroporation. The enhancement of PA synthesis by the bicarbonate ion in *B. anthracis* was shown to require the presence of a gene on plasmid pX01.

Aromatic amino acid-requiring mutants of *B. anthracis* obtained by transposon Tn*916* mutagenesis were shown to be avirulent and to protect guinea pigs when challenged by virulent *B. anthracis*

Pure LF protein was introduced into various cultured animal cells by osmotic lysis and by chemical conjugation to receptor ligands. Regardless of the mode of internalization, LF was toxic only to macrophages. Even in these, very high concentrations of LF were needed when PA was absent. This shows that the resistance of most cell types to the lethal toxin (PA + LF) did not result from a defect in internalization.

Mouse monoclonal antibodies to all three anthrax toxin components were analyzed. Neutralizing antibodies to LF and PA were found (7 of 77 PA antibodies, 7 of 62 LF antibodies). Several EF (edema factor) antibodies were found which competed with calmodulin for binding to the EF adenylate cyclase protein.

PRESENTATIONS

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Schmaljohn, C. S., J. Arikawa, S. E. Hasty, L. Rasmussen, H. W. Lee, P. W. Lee, and J. M. Dalrymple. 1988. Conservation of antigenic properties and sequences encoding the envelope proteins of prototype Hantaan virus and two virus isolates from Korean hemorrhagic fever patients. *J. Gen. Virol.* **69**:1949-1955.

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Takehara, K., M. K. Min, J. K. Battles, K. Sugiyama, V. C. Emery, J. M. Dalrymple, and D. H. L. Bishop. 1988. Identification of mutations in the M RNA of a candidate vaccine strain of Rift Valley fever virus. Submitted to *Virology*.

Watts, D. M., C. L. Bailey, N. T. Roberts, R. F. Tamariello, J. M. Dalrymple, and G, C. Clark. 1988. Maintenance and transmission of Keystone virus by *Aedes atlanticus* (Diptera:Culicidae) and the gray squirrel in the Pocomoke Cypress Swamp, Maryland. *J. Med. Entomol.* 25:793-500.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOG1526	2. DATE OF SUMMARY 26 May 89	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS12	AC	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) Basic studies for the development of medical defensive countermeasures to bioregulators

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	182
c. TYPE	d. AMOUNT	89	1.0	218
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Saviolakis, G A
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Bunner, D L
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Mice; (U) Therapy; (U) Mammalian Peptides; (U) Neurotransmitters; (U) Leukotrienes; (U) RA I; (U)Lab Animals

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To study the basic mechanisms of action and the physiological effects on host vital systems of mammalian low molecular weight peptides, such as neurohormones, known as putative peptide neurotransmitters, and leukokines. To develop therapeutic interventions for military personnel and to mitigate the adverse effects of these peptides, and to develop methods for their detection. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) I. - In vitro studies, using neural cell cultures, for characterization of peptide and non-peptide receptors, receptor regulation and interactions, post-receptor biochemical events, and evaluation of pharmacologic agonists and drugs. II. In vivo studies, using small animals, for evaluation of peptide systemic effects and pharmacokinetics after respiratory delivery.

25. (U) 8710 - 8809 The PC-12 rat pheochromocytoma cell line was continued to be used for the evaluation of peptide effects on catecholamine metabolism and interactions with the classical neurosecretors, nicotine, and K^+. The age of the culture and the cell phenotype were significant determinants of the response. In round, chromaffin-like cells, insulin, the interleukins $IL1\alpha$ and $IL1\beta$ and tumor necrosis factor α, but not dermorphin, directly increased catecholamine release and attenuated or inhibited the response to nicotine or K^+. Dermorphin only inhibited the response to nicotine or K^+. There were no effects of these peptides in elongated, neuron-like cells. The respiratory absorption of aerosolized insulin in three lipophilic vehicles was evaluated further in the rat and was found to be equipotent to i.v. insulin in causing hypoglycemia. Saline aerosols did not cause hypoglycemia.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO: S12-AC-003: Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin

PRINCIPAL INVESTIGATOR: G. A. Saviolakis, LTC, M.D.

ASSOCIATE INVESTIGATOR: D. L. Bunner, COL, M.D.

BACKGROUND

Small molecular weight endogenous peptides, such as neuropeptides and monokines, have major effects on physiological control of vital organ systems, such as cardio-respiratory, vascular, endocrine and immune systems, and on behavior. Due to their demonstrated potency, many of these peptides may be important as potential biological warfare agents. The purposes, therefore, of this work unit are: (a) to assess the pathophysiological effects of selected groups of peptides; (b) to develop methods for their detection; and (c) to develop therapeutic interventions to mitigate their effects on military personnel.

SUMMARY

In vitro studies. Rat PC-12 pheochromocytoma cells were continued to be used for studies of direct and modulated peptide effects on catecholamine release. The cell phenotype, which was dependent on the age of the culture, was a dominant determinant of the response. Coarse cultures (3-7 days old), composed mostly of elongated neuronal cells, were unresponsive. On the other hand, dense cultures, composed of round chromatin cells, responded only during the first part of the second week but not later. The responses to insulin, dermorphin, and several cytokines (interleukins la and lb, and tumor neurosis factor a) were studied. Except for dermorphin, all other peptides demonstrated a direct positive effect on catecholamine release. The response, however, to the natural neurotransmitter stimulus, nicotine, and to K^+-depolarization was attenuated or inhibited by all these above peptides. These results suggest that the evaluated peptides, which participate in the stress of inflammatory response, may function both as neurosecretors and neuromodulators. Studies designed to screen other peptides and to study the mechanism

of neuromodulatory effects have been planned.

In vivo studies. Studies to evaluate the pulmonary absorption of peptides were continued in collaboration with Dr. Creasia, Inhalation Toxicologist, assigned to Pathophysiology Division. In particular, the focus was on further characterization of the previously observed insulin effect. Three lipophilic vehicles, fusidic acid, azone, and glycerol were used to aerosolize insulin. A strong hypoglycemic effect was obtained with inhaled insulin in all three vehicles, but not with aerosols of insulin in saline solution. The biopotency of inhaled aerosolized insulin/vehicle was equal to that of intravenous insulin, and higher than the reported one following intranasal administration. These studies suggest that aerosolized peptides may be easily absorbed through the large surface area of the alveolocapillary membrane if delivered in a lipophilic vehicle. Further studies designed to evaluate the pulmonary absorption and pharmacokinetics of smaller peptides had been planned but were suspended because of the resignation of the one techician assigned to this program early in the year.

MEIIII Research efforts during the past year were focussed on the following:

In vitro studies. Evaluation of the effects of neuropeptides and cytokines on catecholamine secretion from PC-12 pheochromocytoma cells were continued. Insulin, the interleukins ILla, and ILlb, and tumor necrosis factor a directly increased catecholamine release, whereas dermorphin was inactive. Responses to the classical neurosecretors, nicotine and K^+, were inhibited, however, by these peptides. Peptide effects were obtained only with cells morphologically similar to rounded adrenal medulary (chromaffin) cells but not with cells differentiated into neurons.

In vivo studies. Animal studies aimed at studying the absorption and bioactivity of peptides from the lung. The absoption of insulin from the lung was characterized further and the potency of the effect was compared to that following intravenous administration. Aerosolized insulin was potent when delivered with three different lipophilic vehicles but not in saline solution. Inhaled insulin and intravenous insuline had similar hypoglycemic potencies.

PRESENTATIONS

Creasia, D.A., G. A. Saviolakis, and K. A. Bostian. 1988. Efficacy of inhaled insulin: effect of adjuvant. *Fed. Proc.* (In Press)

Ruwe, W. D., and G. A. Saviolakis. 1988. Sites in the brain of the rat and the rabbit involved in both fever and antipyresis. *Proc. Soc. Exp. Biol. Med.* (In Press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA302660	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AB	012
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced studies for the development of vaccines against rickettsia of potential BW threat

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	557
89	1.0	640

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Waag, D M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) McCaul, T F

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Q Fever; U) Coxiella burnetii; (U) Vaccines; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii* is perceived to have significant potential as a BW agent. The currently available vaccine is reasonably protective, but highly reactogenic. An efficacious yet more safe vaccine needs to be developed and stockpiled to protect at-risk US troops. The objective is to proceed with evaluation of a chloroform-methanol extracted residue (CMR) vaccine to assess feasibility for use in humans. Simultaneously a new generation subunit vaccine is being sought, which can be produced readily without the requirement for high containment laboratories. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Determine toxicity, safety, efficacy, and dose response to CMR vaccine. Extend animal model testing of CMR to identify and quantify humoral and cell-mediated immune responses. Proceed with human use evaluations if safety and efficacy are demonstrated. Define immunogenic subunits of *C. burnetii* to provide bases for development of a subunit vaccine.

25. (U) 8710 - 8809 Both phase I whole cells and chloroform-methanol-extracted residue are effective inducers of tumor necrosis factor and interferon. Therefore, components derived from *Coxiella burnetii* cells are potentially useful in stimulating potent biological response modifiers. Immunization of C57BL/10 J mice with phase I whole cells induced time- and dose- dependent immunosuppression without simultaneous increases in T suppressor cells in the spleen, but with the appearance of a new population of immunoglobulin-bearing thymocytes. Thus, the initial events in the induction of suppression may be related to activation of suppressor cells, rather than changes in the responder cell populations.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AB-012: Advanced Vaccine Development Studies on Rickettsia of Potential BW Threat

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATORS: D. M. Waag, Ph.D.
T. F. McCaul, Ph.D.

BACKGROUND

Coxiella burnetii is able to modulate the host immune response in positive and negative directions. These bivalent responses of the host may be both specific and non-specific. While animals injected with phase I cells show increased tumoricidal and bactericidal abilities, pathogenic reactions such as hepatomegaly, splenomegaly, liver necrosis, death, lymphocyte hyporesponsiveness, and antigen-specific negative modulation also occur. These adverse responses generated by the components of the immune suppressive complex (ISC) were the subject of the current study.

Inactivated phase I *C. burnetii* cells are effective in inducing the immunosuppressive activity in mice. Therefore, this activity is not the result of an infectious process, but of some microbial component. Phase I cells at low concentrations do not exert a toxic effect on lymphocytes cultured in vitro, but at high concentrations (>100 μg/ml), lymphocytes are killed. In fact, 5 μg of phase I whole cells (WCI) per ml as in-vitro recall antigen stimulates lymphocytes from saline-injected mice. However, when used as recall antigens with cultured lymphocytes from phase I (WCI) or reconstituted, phase I chloroform-methanol residue (CMRI)-injected animals, we observed substantial immunosuppression.

The CMRI is efficacious as a vaccine and non-toxic at high concentrations (>100 μg/ml) in vitro. Therefore, structural features of the ISC are responsible for the induction of suppression in vivo and the initiation of lymphocytosis in vitro.

The recognition of *C. burnetii* infection as a focal or regional health problem for individuals in the animal industry is well accepted. An important question regarding this zoonosis is the role of pets in the spread of Q fever to humans. Retrospective clinical and seroepidemiological data have lead to the hypothesis that this disease may be frequently involved in outbreaks of atypical pneumonia cases. Previously, in Nova Scotia, we initiated studies on the cause of pneumonia in 1979 and found that *C. burnetii* was the culprit in cases of atypical pneumonia. However, the exposure to cattle, sheep, and goats was not associated routinely as the cause of their infection. We have observed a significant number of cases of Q fever after the skinning of wild rabbits. Importantly, a recent report led to suspicion that exposure to parturient cats might explain discrete foci of outbreaks of Q fever in Nova Scotia. As a result of these observations, we designed a case-control study to determine the incidence and risk factors for acquisition of Q fever in Maritime Canada.

SUMMARY

In previous experiments, the phase I ISC was dissociated, but not inactivated, after extraction of phase I cells with chloroform-methanol. Also reconstitution of the phase I CMR with non-rickettsial chloroform-methanol extract (CME) and reagent grade lipids restored the ISC activity. The phase I lipopolysaccharide (LPS) alone or reconstituted with CME did not possess the properties of the ISC.

The expression of the ISC activity by different isolates of *C. burnetii* was tested. A survey of eight *C. burnetii* strains with structural variation in LPS indicated that phase I Ohio strain, phase I Henzerling, phase I Nine Mile, and phase I Nine Mile 514 expressed the ISC activity. Although the phase I, Nine Mile 514 strain carries the ISC, it is a semi-rough chemotype and does not express any rough LPS. Interestingly, the phase I cardiac valve-isolated, "KAV" and "PAV" strains, which express both the smooth (phase I), semi-rough, and rough (phase II), expressed significantly different properties of the ISC activity. Although organisms expressing the smooth and semi-rough LPS induce the ISC activity, not all organisms classified as phase I induce suppression in the murine system. Noteworthy was the diminution of ISC activity in strains possessing marked increase in the concentration of the rough LPS chemotype. Although some of these strains expressed both smooth and semi-rough LPS, they all expressed significant amounts of the rough LPS chemotype. Thus, the synthesis of LPS and expression of the ISC appear to be controlled coordinately and may reflect linkage on the chromosome, possibly under genetic control. The correlation between LPS structural variation and ISC may be related incidentally to the cell matrix receptor involved in anchoring the dithiothreitol-soluble and CME-active components.

Our studies indicate that exposure to the products of feline conception is a risk factor for acquisition of Q fever. The greatest risk is after exposure to stillborn kittens. Exposure to cat litters postpartem was the only other significant risk factor in the multivariate analysis. Several traditional risk factors for Q fever were present based on the univariate analysis, unadjusted for multiple comparisons. Individuals were at risk for the contraction of Q fever if they worked on a farm, slaughtered or dressed animals, and if they had contact with cat-, cattle-, sheep-, and tick-infested animals. Some of these risk factors obviously overlap, such as working on farms and being in contact with large domestic animals. Despite the weak association of this group of risk factors in the context of the overall study, in individual cases and from our knowledge of the biology, contact with cattle, sheep, and slaughter animals was the probable

means whereby some of our cases acquired Q fever.

The question of how cats are infected is paramount to breaking the cycle of spread to man. Our study suggests that cat-associated Q fever may be prevalent in cases of respiratory illness of unknown origin. More case-control studies should be performed to determine the role of cats in the spread of Q fever and the source of feline infection.

PRESENTATIONS

Waag, D., and J. C. Williams. 1987. Identification of suppressor cells induced following injection of C57Bl/O ScN mice with phase I *Coxiella burnetii* whole cells. Presented at the American Physiology Society Annual Meeting, Federation of American Societies for Experimental Biology, Washington, D. C., April.

Waag, D., J. C. Williams, K-L Amano, M. England, and J. Beveridge. 1987. Lack of correlation between the ability to induce in vivo and in vitro pathogenic reactions and LPS phenotypes of *Coxiella burnetii* strains. Presented at the American Society for Rickettsiology, Williamsburg, VA.

PUBLICATIONS

Amano, K I, J. C. Williams, S. R. Missler, and V. N. Reinhold. 1987. Structure and biological relationships of *Coxiella burnetii* lipopolysaccharides. *J. Biol. Chem.* **262**:1470-4747.

Her, G. R., S. Santikarn, V. N. Reinhold, and J. C. Williams. 1987. Simplified approach to HPLC precolumn fluorescent labeling of carbohydrates: N-(2-pyridinyl)-glycosamines. *Carbohdr. Chem.* **76**:129-139.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302664	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AD	014
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 0			

11. TITLE (Precede with Security Classification Code) (U) Advanced studies for the development of drugs against agents of biological origin

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	3.0	1125
89	3.0	1802

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Kende, M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7691
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Antiviral Drugs; (U) Pharmacology; (U) Viral Diseases; (U) Lab Animals; (U) Mice; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Identify effective drugs against viruses that are potential threats to military personnel; obtain data on toxicology, pharmacology, and metabolism of antiviral drugs and conduct preclinical and clinical studies to assess safety and efficacy in compliance with FDA regulations. Develop novel applications of drug delivery systems for antiviral chemotherapy. Develop adjuvants. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Assess efficacy of potential antivirals against viruses in tissue cultures and in rodent models for Rift Valley fever virus (RVFV) and VEE. Evaluate toxicity and pharmacology of promising compounds in preclinical protocols conducted in rodents and nonhuman primates. Provide technical support for clinical protocols. Evaluate state-of-the-art technologies for improved drug delivery. Perform animal studies to assess efficacy of immunopotentiating compounds and drugs combinations as potential antivirals or vaccine adjuvants.

25. (U) 8710 - 8809 Among 10 different immunomodulators, 2 orally active compounds, AVS-1968 and 1018, were evaluated against Rift Valley fever (RVFV) and Banzi viruses. A wide safety margin, rapid interferon induction, marked virucidal activity, reduced frequency of administration, and therapeutic syngergism indicated the potential use of AVS-1018 for clinical trials. Stimulation of immunity was confined to the nonspecific response without effect on the specific antiviral reactivity. Inactivated *Coxiella burnetii* and hybrid human recombinant interferon-α consistently showed complete protection against RVFV and Banzi viral infections, but only *C. burnetii* evoked long-lasting protection between treatment and challenge. Better therapeutic synergism was evoked with an inducer than with the exogeneous interferon. A novel lipid composition of liposomes improved the distribution of ribavirin in the liver and the therapeutic efficacy in comparison with previously reported results.

DD FORM 1498 83 MAR   EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO: 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AD-014: Advanced Drug Development Studies Against Agents of Biological Importance

PRINCIPAL INVESTIGATOR: M. Kende, Ph.D.

BACKGROUND

Over the past few years at USAMRIID, several immunomodulators were identified that have broad-spectrum antiviral activity against "exotic" RNA viruses. This research program addresses the continued evaluation of these lead compounds for their application as broad-spectrum antiviral agents. The number of natural and synthetic products, as well 3S recombinant cytokines, which non-specifically activate the immune system and enhance host resistance to viral infections, has grown significantly. Particular attention has been given to compounds that are active orally. We are evaluating various approaches for the specific targeted delivery of these antivirals and immunomodulators for improvement of the therapeutic index. Our research activity is also directed at combination chemotherapy. Combinations of certain antivirals and immunomodulators have been shown to exhibit significant synergy due to their different modes of action.

SUMMARY

Results obtained in this reporting period gave additional indications on the usefulness of immunomodulators for the prevention and treatment of viral infections of military importance. Studies were initiated and performed to assess the amplification of virus-specific humoral and cellular immune responses by selected immunomodulatory substances with potent antiviral efficacy. Data obtained so far indicate that no amplification of virus-specific immune response occurred at times tested; however, kinetics of amplification as a function of time is still under study.

Advanced evaluation was completed of two immunomodulators, AVS 1018 and 1968, against viruses from three arboviral families. These studies revealed that oral admin'stration of these compounds has a sufficiently wide safety margin without causing side effects. One of the compounds, AVS 1018, induced peak titers of interferon much more rapidly than any other known inducers; therefore, we consider this antiviral to be particularly valuable for therapeutic administration. Studies also showed

that, with each inducer, the kinetics of interferon persistence was different; therefore, selection of an immunomodulator must include extensive characterization. Marked therapeutic synergism was also evident between these immunomodulators and ribavirin, and also between AVS 1018 and mouse interferon-γ. In comparison with the administration of interferon-α and -g, the combined use of an interferon-α/β inducer (AVS 1018) with exogenous interferon-γ, yielded better therapeutic synergism.

Formalin-inactivated extract of *Coxiella burnetii* had remarkable prophylactic efficacy against encephalitic infection caused by Banzi (flavi) virus. By administration of a single dose of inactivated microorganisms, complete protection persisted for at least 7 days prior to challenge.

Studies revealed that hybrid recom-binant human interferon-α was consistently very effective prophylactically and therapeutically against Rift Valley fever and Banzi viral infections in mice. In comparison with prophylactic treatment, therapeutically schedule, the dose required was considerably higher and the challenge level was more limited. At the present time, this antiviral compound is being evaluated in non-human primates for its activity against lethal yellow fever viral infection.

Efforts continued to identify immuno-modulator(s) that would be effective against arena (Pichinde) viral infection in guinea pigs, alone, or in combination of two immunomodulators, or with ribavirin. So far, none of the tested compounds was effective, neither alone nor in combined modality treatments. In present studies, because arenavirus was inhibited in vitro by interferon-γ, interferon-γ inducers (interleukin-2 and a germanium derivative) were employed Use of a second signal to promote interferon-γ induction is being explored. This may also be relevant for other, but not every, arboviral infection, as our recent, preliminary data suggest that Sindbis viral infection of macrophage cell line interferes ith interleukin-1 release. An impaired interleukin-1 response could affect specific and non-specific immune responses.

Liposome-mediated drug distribution studies indicate that, with a certain lipid composition, about 70% of encapsulated ribavirin can be recovered from the liver. In comparison with free drug, only liposome-encapsulated ribavirin was efficacious upon therapeutic administration. Homogeneous size distribution of the carrier was not a prerequisite for therapeutic efficacy.

PRESENTATIONS

Kende, M. 1988. Antiviral potential of biological response modifiers (BRM). Presented at the Second International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar Del Plata, Argentina, March.

Kende, M., and P. G. Canonico. 1988. Antiviral potential of biological response modifiers (BRM). Presented at the Second Symposium on Prevention and Treatment of Viral Infection, Bechyne, Czechoslovakia, June.

Kende, M., P. G. Canonico, M. Contos, and A. Gabizon. 1988. Liposomes in the therapy of Rift Valley fever virus infection. Presented at the UCLA Symposium, entitled "Liposomes in the Therapy of Infectious Diseases and Cancer," Lake Tahoe, CA, February.

Kende, M., W. L. Rill, M. J. Contos, and P. G. Canonico. 1988. Efficacy of a novel orally active immunomodulator, S-26308 against arbovirus infections. Presented at the 2nd International Conference on Antiviral Research, Williamsburg, VA, April.

Kende, M., P. G. Canonico, and T. R. Tice. 1988. Drug targeting strategies for the treatment of experimental Rift Valley fever virus (RVFV) infection. Presented at the International Conference on Pharmaceutical Sciences and Clinical Pharmacology, Jerusalem, Israel, May-June.

Kende, M., W. R. Rill, M. Derevjanik, M. Contos, and P. G. Canonico. 1987. Treatment of Rift valley fever virus RVFV) infection with biological response modifiers. Presented at the Annual Meeting of the International Society for Interferon Research, Washington, D. C., November.

Rankin, J. T., M. A, Ussery, M. Kende, and P. G. Canonico. 1988. Murine retrovirus models in AIDS drug development. Presented at the Second International Conference on Antiviral Research, Williamsburg, VA, April.

Ussery, M. A., J. T. Rankin, M. Kende, and P. G. Canonico. 1988. The efficacy of ribamidine, an analog of ribavirin, in two murine retrovirus models. Presented at the 7th Annual Meeting of the American Society for Virology, Austin, TX, June.

PUBLICATIONS

Burns, N. J., W. B. Barnett, J. H. Huffman, M. I. Dawson, R.W. Sidwell, E. DeClercq, and M. Kende. 1988. A newly developed immunofluorescence assay for determining the Pichinde virus inhibitory effects of selected nucleoside analogues. *Antiviral Res.* (In Press).

Kende, M., D. J. Ganjemi, W. Lange, D. A. Eppstein, J. Kreuter, and P. G. Canonico. 1988. Carrier-mediated antiviral therapy. *Appl. Virol. Res.* **1**:241-264.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOB6410	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AE	015
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89-01			

11. TITLE (Precede with Security Classification Code) (U)Advanced studies (non-system development) against infectious agents of biological origin

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	288
c. TYPE	d. AMOUNT	89	1.0	130
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Scott, G H
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Waag, D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Lab Animals; (U) Bacillus anthracis; (U) Guinea Pigs; (U) Mice; (U) Hamsters; RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Prophylactic vaccines, therapeutic agents, and immunomodulators developed to protect US troops against potential BW agents must be effective when the microorganism is presented as an aerosol. The objective is to evaluate animal models of aerosol-induced infections and toxemias. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Define and quantify the molecular aspects of clinical, pathological, and immunological changes that occur in vaccinated or treated animals when exposed to potential BW agents in aerosols.

25. (U) 8710 - 8809 In A/J mice, the phase I lipopolysaccharide (LPS) was nontoxic at a dose of 100 μg and elicited significant antibody titers and lymphocyte responses against LPS, and phase I and II cells. A single injection of 2.5 μg gave complete protection against virulent aerosol challenge without a detectable pre-challenge immune response. Studies of the antigenic structure of *Coxiella burnetii* morphological cell types indicate that antigenic shift occurred in conjunction with progression through the developmental cycle. The antigenic differences between the endospore and smaller cell variants, when compared to the other cell types, were so great that they were not recognized by the immune response.

DD FORM 1498, 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AE-015: Advanced Non-system Development Studies on Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATORS: G. H. Scott, Ph.D.
D. Waag, Ph.D.

BACKGROUND

Infection of laboratory animals and humans with virulent phase I *Coxiella burnetii*, the etiologic agent of Q fever, leads to progressive, but usually self-limiting, disease which is confirmed by the serological measurement of anti-*C. burnetii* antibodies. The distribution of Q fever is worldwide. About 5% of cases are chronic, involving liver granuloma or cardiac tissue (endocarditis). In acute disease, the temporal development of immunosuppression has been detected in mice and guinea pigs. In chronic disease of humans, an antigen-specific suppression circuit may be involved in the establishment and continuance of chronic Q fever. In order to learn more about the suppressor mechanisms, we are studying the mouse and guinea pig models more thoroughly. Our studies have centered around the immune suppressive complex (ISC) of *C. burnetii* and the immune mechanisms induced by this complex. The current phase I whole-cell vaccine is being replaced by a new candidate: chloroform-methanol extracted residue (CMR) from phase I whole cells. The CMR vaccine has been shown to be efficacious and non-reactogenic in animals, but is still difficult to produce from infected yolk sacs. Thus, an objective is to prepare a subunit vaccine by cloning the DNA of virulent *C. burnetii* and screening the clones for production of immunogenic proteins. This approach complements the biochemical approach of purifying immunogens and has the advantage of not requiring infectious microorganisms.

Vaccinogenic products generated via traditional fractionation procedures are being compared to recombinant DNA based vaccines. The A/J mouse is currently the animal model of choice for evaluating the efficacy of vaccines against virulent challenge.

SUMMARY

In previous studies we have tested the susceptibility of inbred strains of mice to infection by phase I *C. burnetii*. We are using the A/J strain as a model for testing the efficacy of Q fever vaccines.

Therefore, we tested the ability of vaccinated A/J mice to develop a protective immune response against virulent *C. burnetii* administered as airborne particles or injected parenterally.

Groups of A/J mice were injected i.p. with 10 μg of inactivated, phase I *C. burnetii* cells or with 10 μg of a chloroform-methanol extracted residue from phase I cells (CMRI). A control group was injected with sterile PBS. Three weeks later, mice from each group were challenged with virulent phase I *C. burnetii* by aerosol exposure and by i.p. injection. Mice injected with either vaccine survived a challenge dose that killed 100% of the non-vaccinated mice when it was administered by aerosol, and 60% when administered i.p. Vaccine-induced protection was also reflected by a post-challenge reduction in splenomegaly, and perhaps most importantly, in the reduction of viable *C. burnetii* in the spleens of vaccinated and challenged mice.

Antibody titers against the phase I lipopolysaccharide (LPS-I) were markedly increased in previously vaccinated animals that were challenged with viable phase I cells administered i.p.; by contrast, aerosol challenge did not stimulate anti-LPS-I titers. This failure of vaccinated mice to develop antibody titers against LPS-I when infected by airborne *C. burnetii* was unexpected and requires further study.

To facilitate the preparation of non-infectious diagnostic materials for interchange among different laboratories, we examined the feasibility of inactivating *C. burnetii* with gamma irradiation (^{60}Co) without altering its immunological and antigenic properties. The amount of irradiation necessary to reduce the number of viable *C. burnetii* by 90% ranged from 121K rads for purified phase II organisms to 60 K rads for purified phase I cells. *Coxiella burnetii* organisms in all three preparations survived 500 K rads, but none survived 1000 K rads.

The antigenic properties of the organisms were not significantly altered by 1000 K rads. Mouse protection studies indicated that irradiation and formalin-inactivated organisms provided vaccinated mice with similar levels of protection against lethal *C. burnetii* challenge. Electron microscopy indicated that 1000 K rads of gamma irradiation did not destroy the morphology of the organisms, and we have experienced no difficulty in using gamma-sterilized cells in enzyme-linked immunosorbent assays.

PRESENTATIONS

Scott, G. H., and J. C. Williams. 1986. Responses of inbred mice to *Coxiella burnetii* infection. Presented at the 6th National Conference of the American Society for Rickettsiology, Williamsburg, VA, September.

PUBLICATIONS

Scott, G. H., J. C. Williams, and E. H. Stephenson. 1987. Animal models in Q fever: pathologic responses of inbred mice to phase I *Coxiella burnetii*. J. Gen. Microbiol. 133:691-700.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
				DA302669	01 Oct 88	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER	
a. PRIMARY	63763A	3M263763D807		AH	017	
b. CONTRIBUTING						
c. CONTRIBUTING	DA LRRDAP, FY89- 01					

11. TITLE (Precede with Security Classification Code) (U) Advanced studies for the development of rapid diagnostic procedures on agents of biological origin

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	2.0	473
c. TYPE	d. AMOUNT	89	2.0	885
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7341
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meegan, J M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Bacterial Diseases; (U) Immunology; (U) Diagnosis; RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop, test, and perfect assays to rapidly detect and identify biological agents obtained from environmental samples or infected military personnel. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Antigen and early (IgM) antibody-capture enzyme immunoassays technology have been the approach adopted.

25. (U) 8710 - 8809 Antigen and early antibody (IgM) detection assays have been developed for the identification/diagnosis of disease caused by leptospira, and 14 arthropod-borne and hemorrhagic fever viruses. Monoclonal antibodies were developed, characterized, then tested for their ability to increase sensitivity when incorporated into these assays. Assays for Rift Valley fever virus (RVFV) optimized by this approach were field-tested during a major epidemic in Africa. A biotin-labeled, monoclonal-based assay was the most sensitive assay for direct detection of RVFV in febrile humans. For antibody assays, preliminary tests evaluated the use of synthetic peptides and expressed polypeptides as non-infectious, inexpensive replacements for viral antigens. Assays developed for a number of viruses were evaluated on epidemiologically relevant samples from Egypt, Pakistan, Korea, Argentina, and China.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO: 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO: 807-AH-017: Advanced Development for Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATOR: J. M. Meegan, CRD, Ph.D.
T. G. Ksiazek, LTC, D.V.M.
R. R. Graham, MAJ, D.V.M.
K. T. McKee, MAJ, M.D.
C. A. Rossi

BACKGROUND

The goal of this study is to develop and optimize rapid, simple tests for identifying agents of biological warfare potential or geographic importance. We anticipate that samples containing unknown agents will be received both as clinical specimens from acutely ill individuals and as environmental samples. Consequently, for each agent investigated, we have attempted to develop both antigen-capture immunoassays, for use on both clinical specimens and environmental samples, and immunoglobulin M assays, to measure transient antibodies produced by humans early in the course of infection, after antigenemia has passed.

In previous years, rapid assays to detect antigens and antibodies were developed for a number of militarily relevant viral diseases. After optimization, many of these assays were field-tested under Work Unit No. 809-EA-005. In all cases, the antibody assays worked will under field conditions, although the conditions are somewhat time consuming (approximately 4 h) and require skilled technicians and equipment not appropriate for use by minimally trained persons or in remote locations. Consequently, there is a need to simplify the test format and procedures. Antigen-detection assays also functioned well, but there is a need to increase their sensitivity. Incorporation of monoclonal antibodies into both the antibody and antigen assays holds promise as an effective means for simplifying assays, increasing their sensitivities , and shortening test time; however, such antibodies must be carefully selected and characterized for optimum reactivity. The focus of our studies has been to shorten the assay times and develop and characterize monoclonal antibodies to increase the sensitivity and to simplify test procedures.

SUMMARY

Antigen and early antibody (immunoglobulin M, IgM)-detection assays were developed to identify 14 arthropod-borne and hemorrhagic fever viruses, or to diagnose acute diseases due to infection with these viruses. In addition, an IgM-capture assay was developed and validated for leptospirosis. This assay was tested extensively in the laboratory by using acute and early convalescent human sera, sera from patients infected with any of a wide variety of pathogenic serovars of Leptospira. The assay was later successfully field tested in laboratories in Asia, South America, and the Caribbean. Assays to identify viral antigens or early IgM antibodies were evaluated on epidemiologically relevant serum samples from Egypt, Pakistan, Korea, Argentina, and China.

A bioengineered baculovirus, expressing hantaviral antigen, developed by collaborating investigators at USAMRIID, was evaluated as a possible antigen source for use in our rapid diagnostic assays for hemorrhagic fever with renal syndrome. Preliminary results indicate that the antigen reacts well with immune sera to prototype Hantaan virus, urban rat-associated Seoul virus, and newly defined Porogia virus, cause of a severe from of hemorrhagic fever with renal syndrome in the Balkan region of Europe. The antigen, however, reacted poorly with or not at all with immune sera to Puumala virus, cause of nephropathia epidemica of Scandinavia and Western Europe. The antigen is especially useful for measurement of immunoglobulin G, but suffers from non-specific reactions when used in the IgM assay, as it is currently formatted. Nonetheless, the ease with which this antigen can be produced in large volume and ints inherent lack of infectivity make it a promising candidate for incorporation into refined formats of our hantavirus assays. This and similar products are being further evaluated as safe and inexpensive sources of viral diagnostic antigens.

PRESENTATIONS

Antoniades, A., J. W. LeDuc, N. Mallovas, and K. Pappas. 1988. ELISA M capture for diagnosis of HFRS. Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece. September.

Cosgriff, T. M., C. M. Hsiang, J. W. Huggins, M. Y. Guang, J. I. Smith, Z. O. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, P. H. Gibbs, X. E. Gui, G. W. Yuan, and T. M. Zhang. 1988. Predictors of fatal outcome in the severe form of hemorrhagic fever with renal syndrome (HFRS). Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece. September.

Graham, R. R. 1988. Current trends in veterinary laboratory operations. Presented at the Brooke Army Medical Center Annual Meeting for Veterinary Officers, Fort Sam Houston, TX, May.

Huggins, J. W., T. M. Cosgriff, J. I. Smith, J. W. LeDuc, and J. M. Meegan. 1988. Intravenous ribavirin therapy for hemorrhagic fever with renal syndrome: Korean hemorrhagic fever (Korea) and epidemic hemorrhagic fever (Peoples' republic of China). Presented at the Annual Meeting of the Society of Armed Forces Medical Laboratory Scientists, Reno, NV, March.

Huggins, J. W., C, M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H. W. Lee. 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Huggins, J. W., C, M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, P. H. Gibbs, and D. D. Oland. 1988. Treatment of HFRS with high dose intravenous ribavirin. Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece, September.

Korch, G., J. Childs, G. Glass, and J. W. LeDuc. 1987. Epizootiology of hantaviruses in diverse habitats in Baltimore, MD, USA. Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Ksiazek, T. G., R. R. Graham, J. W. LeDuc, and C. J. Peters. 1988. Equine encephalitides in the Americas. Presented at the 125th Annual Meeting of the American Veterinary Medical Association, Portland, OR, July.

LeDuc, J. W. 1987. The epidiomiology of hantaviruses. 1987. Presented at the Uniformed Services University of Health Sciences Conference on Infectious Disease, Bethesda, MD, October.

LeDuc, J. W. 1987. Recent advances in the epidemiology and diagnosis of hantaviruses. Presented at the 29th Internation Colloquium on Hantaviruses, Antwerpen, Belgium, December.

LeDuc, J. W. 1988. Recent advances in the epidemiology and diagnosis of hantaviruses, cause of hemorrhagic fever with renal syndrome. Presented at U. S. Medical Department Course, "Medical Entomology in War and Contingency Operations, " San Antonio, TX, February.

LeDuc J. W. 1988. Hemorrhagic fever with renal syndrome in the Americas. Presented at the Second International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar del Plata, Argentina, March.

LeDuc, J. W. 1988. Recent advances in the epidemiology and diagnosis of hantaviral infections. Presented at the U.S.-Japan Cooperative Medical Sciences Program Meeting, Tokyo, Japan, July.

Mangiafico, J. A., J. L. Sanchez, L. T. Figueiredo, J. W. LeDuc, and C. J. Peters. 1987. Isolation from man of a newly recognized subtype of Cache Valley virus. Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

McKee, K. T., Jr. 1988. Hemorrhagic fever with renal syndrome. Presented at the Tri-Service Infectious Diseases Conferences, Homestead Air Force Base, FL, April.

Schmaljohn, C. S., J. Arikawa, J. M. Dalrymple, J. W. LeDuc, and A. Schmaljohn. 1988. Hantaan virus proteins expressed by vaccinia and baculovirus recombinants. Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece, September.

Soliman, A. K., B. A. M. Botros, T. G. Ksiazek, and J. C. Morrill. 1988. Murine typhus in rodents in Egypt. Presented at the XIIth International Congress for Tropical Medicine and Malaria, Amsterdam, The Netherlands, September.

Stapleton, J. T., J. W. LeDuc, L. N. Binn, and S. M. Lemon. 1987. Lack of neutralizing activity in fecal extracts following experimental hepatitis A virus in humans and owl monkeys. Presented at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, NY, October.

White, J. D., T. W. Geisbert, C. A. Rossi, and J. W. LeDuc. 1988. Electron microscopy of hantavirus isolates from Greece and Yugoslavia. Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece, September.

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Antoniades, A., and J. W. LeDuc, N. Acritidis, A. Alexiou-Daniel, A. Kyparissi, and G. A. Saviolakis. 1988. Hemorrhagic fever with renal syndrome in Greece: clinical and laboratory characteristics of the disease. *Rev. Infect. Dis.* (In Press).

Binn, L. N., W. H. Bancroft, K. H. Eckels, R. H. Marchwicki, D. R. Dubois, L. V. S. Asher, J. W. LeDuc, C. J. Trahan, and D. S. Burke. 1988. Inactivated hepatitis A virus vaccine produced in human diploid MRC-5 cells, pp. 91-93. *In* A. J. Zuckerman (ed.), Viral hepatitis and liver disease. Alan R. Liss, Inc., New York.

Botros, B. A. M., T. G. Ksiazek, J. C. Morrill, A. W. Salib, A. K. Soliman, R. McN. Scott, and A. Barakat. 1988. Rift Valley fever in Egypt 1986. Surveillance of sheep flocks grazing in the northeast Nile Delta. *J. Trop. Med. Hyg.* **91**:183-188.

Childs, J. E., G. E. Glass, G. W. Korch, R. R. Arthur, K. V. Shah, D. Glasser, C. Rossi, and J. W. LeDuc. 1988. Evidence of human infection with a rat-associated *Hantavirus* in Baltimore, Maryland. *Am. J. Epidemiol.* **127**: 875-878.

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Darwish, M. A., F. M. Feinsod, R. McN Scott, T. G. Ksiazek, B. A. M. Botros, I. H. Farrag, B. A. Tahir, and S. El Said. 1988. Arboviral causes of nonspecific fever and myalgia in a fever hospital patient population in Cairo, Egypt. *R. Soc. Trop. Med. Hyg.* (In Press).

Glass, G. E., J. E. Childs, G. W. Korch, and J. W. LeDuc. 1988. Association of intraspecific wounding with hantaviral infection in wild rats *(Rattus norvegicus). Epidemiol. Infect. 101*:459-472.

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Lemon, S. M., J. T. Stapleton, J. W. LeDuc, D. Taylor, R, Marchwicki, and L. N. Binn. 1988. Cell-culture-adapted variant of hepatitis A virus selected for resistance to neutralizing monoclonal antibody retains virulence in owl monkeys, pp. 70-73. *In* A. J. Zuckerman (ed.), Viral hepatitis and liver disease. Alan R. Liss, Inc., New York.

McKee, K. T., Jr., J. W. LeDuc, and C. J. Peters. 1988. Hantaviruses, pp. --. *In* R. B. Belshe (ed.), Textbook of human virology. (In Press).

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Peters, C. J., and J. W. LeDuc. 1988. Bunyaviridae, pp. --. *In* R. B. Belshe (ed.), Textbook of human virology (In Press).

Pinheiro, F. P., and J. W. LeDuc. 1988. Mayaro virus disease, pp. 137-150. *In* T. P. Monath (ed.), Epidemiology of arthropod-borne viral diseases. CRC Press, Boca Raton, FL.

Scott, R. McN., T. G. Ksiazek, J. K. Podgore, J. P. Burans, M. I. Moussa, J. N. Woody, A. A. Mohammed, M. A. Jama, and A. H. Farah. 1988. Continuing investigations of Dengue in northeast Africa. Submitted to *Lancet.*

Soliman, A. K., B. A. M. Botros, T. G. Ksiazek, I. Helmy, and J. C. Morrill. 1988. Seroprevalence of *Rickettsia typhi* and *Rickettsia conorii* infection among rodents and dogs in Egypt Submitted to *J. Trop. Med. Hyg.*

Xiang, C. M., M. Y. Guan, Z. M. Zheng, Z. A. Wu, X. Q. Ge, T. M. Zhang, G. H. Yuan, X. A. Gui, J. W. Huggins, T. M. Cosgriff, J. Smith, J. W. LeDuc, and J. M. Meegan. 1988. Study of antiviral specific therapy of epidemic hemorrhagic fever with ribavirin. *J. Exp. Clin. Virol.* (China) 2:47-51.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA302670	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AI	018
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) Advanced studies for the development of toxoids against toxins of potential BW threat

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0611 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	95 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	74
89	1.0	38

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Siegel, L S
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Microbial Toxins; (U) Vaccines; (U) Therapy; (U) Toxoids; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Evaluation and testing of toxoids and antitoxins for protection against botulinal neurotoxins. These neurotoxins are considered to have significant biological warfare potential and our at-risk forces should be immunized against them. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Obtain botulinum toxin serotypes A-G in partially or highly purified state. Evaluate toxoids prepared from such materials for protection of personnel against botulinum toxin poisoning. Simultaneously evaluate antitoxins produced against botulinal toxoids as suitable prophylactic or therapeutic agents for botulinal toxin poisoning.

25. (U) 8710 - 8809 We completed our study to evaluate the current botulism vaccine [botulinum pentavalent (ABCDE) toxoid]. We assayed more than 200 sera, drawn from individuals at various points in the immunization scheme. As a result of our investigation, the Institute immunization policy for botulinum toxoid was changed. We developed ELISAs, using purified type A or type B neurotoxin as the capture antigen, to assay for antibodies. Results from the ELISA were compared to neutralization test results for 186 serum samples for type A, and for 168 sera for type B. Statistically, the correlation coefficients for results from the two assays were high. However, due to the wide dispersion of values obtained, using ELISA test results to predict neutralizing antibody levels is unwarranted. In an ongoing project, we are evaluating synthetic peptides, produced according to known sequences of the neurotoxin molecule, for their efficacy as vaccines.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AI-018: Advanced Vaccine Development Studies on Toxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

BACKGROUND

Seven immunologically distinct neurotoxins (A, B, C_1, D, E, F, and G) are produced by strains of *Clostridium botulinum*. Types A, B, E, and F cause human botulism. Although there have been few well-documented human cases reported, types C_1, D, and G have the potential of producing toxic effects in man. Toxoids (chemically inactivated, but immunogenic, toxins) for each serotype are used to elicit immunity to these toxins. The botulinum toxoid used for human immunization was prepared by treating types A, B, C, D, and E toxins with formaldehyde and combining them to form a composite immunogen. This toxoid was manufactured by the Michigan Department of Public Health under contract to the Army in the late 1960s.

Antibodies capable of neutralizing protein toxins can be induced by synthetic peptides derived from those toxins. Such synthetic peptides are ideal candidiates for vaccines, as they can be produced at a lower cost and would be safer than toxoids (minimal risk of reversion to toxicity, lower risk of adverse reactions).

SUMMARY

To determine the immune status of persons receiving the current botulinum vaccine, Botulinum Pentavalent (ABCDE) Toxoid, and to evaluate the effectiveness of the vaccine, we surveyed immunized individuals for neutralizing antibodies to type A and to type B botulinum toxins. (The antibody response to type A correlates well with the response to types C, D, and E. Typically the response to type B is the poorest.) The assay for toxin-neutralizing antibodies is a mouse bioassay, employing a standard positive antiserum from the World Health Organization. We assayed more than 200 sera, drawn from individuals at various points in the immunization scheme. As a result of our investigation, the Institute immunization policy for Botulinum Pentavalent Toxoid was changed.

Neutralization tests are expensive, time-consuming, and cumbersome. Therefore, we developed ELISAs, using purified type A or type B neurotoxin as the capture antigen, to assay for antibodies. Results from the ELISA were compared to neutralization test results for 186 serum samples for type A, and for 168 sera for type B. Statistically,

the correlation coefficients for results from the two assays were high. However, due to the wide dispersion of values obtained, using ELISA test results to predict neutralizing antibody levels is unwarranted.

In an ongoing, collaborative project with Dr. Schmidt, we are evaluating synthetic peptides, produced according to known sequences of the neurotoxin mole-cule, for their efficacy as vaccines.

PRESENTATIONS

Siegel, L. S. 1987. Survey of immunized personnel for antibodies to A and B botulinal toxins using the neutralization test and ELISAs. Presented at the Annual Meeting of the Interagency Botulism Research Coordinating Committee, Madison, Wisconsin, November.

PUBLICATIONS

Siegel, L. S. 1988. Human immune response to botulinum pentavalent (ABCDE) toxoid determined by a neutralization test and by an enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* (In press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY		1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
		DA302668	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AG	019
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) Advanced studies for the development of immunotherapy against viral agents of BW threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	2.0	195
c. TYPE	d. AMOUNT	89	2.0	102
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Lassa Virus; (U) Viral Diseases; (U) Lab Animals; (U) Monkeys; (U) Guinea Pigs; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To select, acquire, and test immune plasma and globulin fractions for protective efficacy and safety in prophylaxis and therapy of hemorrhagic fever virus infections that pose special problems for U.S. Forces sent to those areas where these diseases are endemic. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Specific immune plasma is obtained by plasmapheresis from convalescent patients after naturally occurring infections with Lassa virus, Argentine hemorrhagic fever virus (Junin), and Ebola virus. Plasma units are tested by current blood bank procedures and for presence of protective (neutralizing) antibodies. Criteria are established for optimal therapeutic administration of the final products. Alternate strategies for acquiring high titered antibody are developed and tested.

25. (U) 8710 - 8809 Lassa-immune plasma collection in Liberia was expanded by inclusion of two additional health-care facilities in the routine plasmapheresis program. Additional plasma units (310) were obtained, bringing the total inventory of high-quality, lassa-immune plasma units from Liberia to 817. Screening for HIV-1 antibody continued, but no positive donors were identified. Among 131 newly identified prospective donors, 9 were excluded for hepatitis B surface antigen reactivity, plus 2 for surrogate markers for non-A, non-B hepatitis. From Sierra Leone, 135 additional units were received; 96 had acceptable titers, bringing the total from this region to 326. A pilot lot of processed plasma was prepared from representative units of Liberian plasma. Chemical sterilization with tri (n-butyl) phosphate preceded aerosil precipitation and QAE chromatography to ensure redundancy in removal of adventitious viruses in the final product. The resulting monomeric IgG retained all expected neutralizing activity in vivo, and conferred protection to guinea pigs challenged with either Liberian or Sierra Leone Lassa strains. A dose-seeking study in primates will proceed clinical trials in patients naturally exposed to Lassa fever in endemic foci.

DD FORM 1498 83 MAR   EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AG-019: Advanced Immunotherapy Studies Against Potential BW Viral Agents

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

BACKGROUND

Lassa fever is a viral disease of considerable public health importance in regions of West Africa, particularly Liberia, Sierra Leone, and Nigeria, where several thousand cases are believed to occur annually. While serological data suggest that subclinical cases may occur, the case-fatality ratios among hospitalized cases are still high, variously estimated at 14 to 22% in Sierra Leone and 13 to 14% in Liberia. To increase survival rates, passive immunization of acutely ill patients is employed frequently. One of many problems in evaluating plasma efficacy is the variable quality of the plasma infused. One objective of this study is the identification of Lassa-convalescent patients to be recruited as plasma donors. Through this process, a pool of optimal donors has been identified, and guidelines for identifying new donors established. The feasibility of preparing IgG by chromatography for intravenous treatment of human Lassa fever is established. However, there is concern that adventitious agents present in the original plasma pool might not be removed by processing, and thus contaminate the final product. The viruses causing AIDS (human immunodeficiency virus, HIV); and hepatitis A, B, and non-A, non-B are of principal concern. This work unit will try to determine the safety and efficacy of various plasma fractioning methods for preparation of monomeric IgG and for removal of these adventitious agents. The availability of high-titer plasma has facilitated the formal testing of passive immunization with plasma and globulin products for treatment of Lassa fever.

SUMMARY

Lassa-immune plasma collection efforts in Liberia were expanded by inclusion of two additional health care facilities in the routine plasmapheresis program. Three hundred and ten additional plasma units were thus obtained, bringing the total inventory of Liberian high-quality, Lassa-immune plasma units to 817. Screening in a Good Laboratory Practices-certified contract laboratory for HIV-1 antibody continued, but no virus-positive donors were identified. Among 131 newly identified prospective donors, nine were excluded for hepatitis B surface antigen reactivity and two for surrogate markers for non-A, non-B hepatitis. From Sierra

Leone, 135 additional units were received; 96 had acceptable titers, bringing the total from this region to 326. A pilot lot of processed plasma was prepared from representative units of Liberian plasma. Two stages in the processing of monomeric IgG from plasma were formally demonstrated to remove adventitious agents. By means of a "spiking" study in which 8 $\log_{10}$ $TCID_{50}$ was deliberately added to plasma before processing, HIV−1 infectivity was demonstrably removed by both chemical sterilization with tri (n-butyl) phosphate, and QAE chromatography. Thus, two redundant sterilization techniques plus the exclusion of all individual plasma units with potentially contaminated virologically prior to the actual processing, reduced the possibility of virological contamination of the final product to practically zero. The resulting monomeric IgG retained all expected neutralizing activity in vivo, and conferred protection to guinea pigs challenged with either Liberian or Sierra Leone Lassa strains. A dose-seeking study in primates will precede clinical trials in patients naturally exposed to Lassa fever in endemic foci. The plan is to prepare separate immune globulin preparations and seek IND approval for their use in Liberia.

In addition, a battery of monoclonal antibodies derived from rats immunized by inoculation of live Lassa virus is presently being screened to identify those with virus-neutralizing or anti-cell activities. The therapeutic potential for these monoclonal antibodies will be explored, with the goal of eventually substituting these for immune globulin prepared from convalescent plasma, which is becoming increasingly difficult and prohibitively expensive to obtain and process.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA308926	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AL	020
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced studies for the development of immunomodulators/enhancers against infectious agents of potential BW threat

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defence

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	615
c. TYPE	d. AMOUNT	89	1.0	190
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Anderson, A O
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Wood, O
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Mice; (U)Vaccines;(U)Microorganisms;(U)Aerosols;(U)Lab. Animals;(U)Hamsters;(U)GuineaPigs(U)RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Prophylactic vaccines, therapeutic age :, and immunomodulators developed to protect US troops against potential BW agents must be effective when the microorganism or toxin is presented in an aerosol. The objective is to determine the safety, efficacy, and dose response of prophylactics, therapeutics, and immunomodulators against an airborne challenge. Emphasis is on how effective products induce protection and why ineffective ones fail. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Define and quantify the molecular aspects of clinical, pathological, and immunological changes that occur in vaccinated or treated animals when exposed to potential BW agents in aerosols. Apply advanced methodology to determine how a prophylactic or therapeutic induced protection; or, conversely to analyze why a product failed to instill protection.

25. (U) 8710 - 8809 Infectious particles that retain infectivity upon passage through the gastrointestinal tract may use mucosal lymphatic tissues as portals of entry as 60% of aerosolized particles (less than 0.1 μm diameter) enter the gastrointestinal tract. Exposure of mice to drinking water containing live Rift Valley fever virus (RVFV) (2H-501 and T-1 strains) duplicated the time-course and dose response of an aerosol challenge. It is therefore feasible to use the oral route for immunization with live-attenuated strains of RVFV. Accelerated leukocyte traffic into injection sites and regional lymph nodes was induced by recombinant neutrophil-activating protein (rNAP-1), which was also shown to be identical to the 8-Kd T-lymphocyte chemotactic factor (TCF) isolated from PHA-conditioned media. A family of recombinant *E. coli* enteric vaccine vectors for RVFV and Dengue virus was obtained, and studies have been initiated into the utility of the X3730 strain of *Salmonella* as vectors for mucosal and systemic priming. Neuronal transmission of Venezuelan equine encephalomyelitis virus to olfactory bulbs of the brain occurs in immune and non-immune mice if no secretory immunity is present in the nasal mucosa.

DD FORM 1498 83 MAR   EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807:	Industrial Base BW Vaccines/Drugs
WORK UNIT NO. 807-AL-020:	Advanced Development Studies on Immunomodulators/Enhancers
PRINCIPAL INVESTIGATOR:	A. O. Anderson, COL, M.D.
ASSOCIATE INVESTIGATORS:	M. Vahey, Ph.D. M.L. Pitt, Ph.D. C. York, B.S.

BACKGROUND

Many natural infections are initiated at mucosal surfaces; mucosae are potential portals of entry in the event of a biological warfare attack with known or unknown toxins, bacteria, or viruses. We conduct basic and applied research on mechanisms of induction, regulation, and effector functions of secretory antibody and cellular immunity at mucosal surfaces for protective immunity, particularly against aerosol-disseminated viruses, bacteria, and toxins. The information so gained will provide useful information for drug development, vaccines, and immunomodulators for respiratory mucosal protection. In these studies the potential for oral immunization with live-attenuated Rift Valley fever (RVF) virus, effects of recombinant cytokines on leukocyte migration in lymphatic tissues, development of mucosal expression vectors, and neuropathogenesis of Venezuelan equine encephalomyelitis (VEE) were explored.

SUMMARY

Tracer studies showed that 60% of radiolabeled, killed viral particles passed through the gastrointestinal tract between 2 and 6 h after aerosol exposure. The gut has not been studied as a portal of entry for RVF virus. RVF viral strains: Entebbe, T-1, and ZH-501, did not appreciably lose infectivity in tap water over 3 days. Mice left without water overnight and then exposed for 24 h to drinking water containing 10^3, 10^4, and 10^5 PFU of ZH-501 RVFV per ml exhibited dose-related mortality with no deaths at 10^3, 20% mortality at 10^4, and 90% mortality at 10^5 PFU/ml. We estimate that each mouse consumed around 8 ml of virus-containing water. All mice exposed to an oral suspension of the T-1 strain of RVF virus developed titers by 14 days post exposure. Aerosol immunization with live T-1 RVF virus elicited similar development of neutralizing antibodies by day 14.

Molecularly defined agents that increase migration of antigen-reactive lymphocytes into lymphatic tissues will be useful in the development of immunomodulators for

enhanced specific or non-specific immunity. With others, we showed that recombinant neutrophil-activating protein (rNAP-1), which is identical to the 8-Kd lymphocyte chemotactic factor (TCF) isolated from conditioned media of PHA-simulated T cells, produced a dose-related increase in emigration of circulating lymphocytes into regional lymph nodes. In the local injection site, NAP-1 was chemotactic for lymphocytes at low doses but for neutrophils at high doses. Receptor sites for NAP-1 were fewer on T-cells than on neutrophils. Therefore, the effective dose ranges of chemotaxins were sufficiently separated to permit local concentration of NAP-1 to be a regulating factor for leukocyte selection or distinction between immunity and inflammation.

A family of recombinant *Escherichia coli*-based enteric vaccine vectors were generated by using the pMG plasmid to exploit ampicillin selection and secretion of antigen coded by G1-G2 cDNA. Recombinants for RVF virus were obtained but were unstable. SDS-polyacrylamide gel electrophoresis indicated some deposition of recombinant RVF viral antigen in the periplasmic space.

We received the X3730 *Salmonella* strain system with the *E. coli* vector, PYA, from Dr. Roy Curtiss (Washington University). We will use this stable strain to build RVF virus/enteric vaccine strains. In collaboration with the National Cancer Institute, cDNA libraries g-gtll were used to 1) screen with conventional antibodies to isolate TGF-b growth factor receptor clones and also 2) to use a binding "bioassay" to characterize positive clones in vitro. This collaboration involved Dr. Jonathan Keller of the NCI.

Aerosol challenge with VEE virus resulted in encephalitis in mice, whether or not they were passively or actively immunized with convalescent serum or C-84 formalin-inactivated VEE vaccine. Neuronal transmission of VEE directly from nasal mucosa to olfactory bulbs in systemically immune mice was consistent with previous observations of binding of labeled VEE to lateral olfactory tracts and optic nerves, and communicating fibers within the pyramidal system in frozen sections of normal mouse brain. Classical virus-isolation techniques were used to confirm this receptor-mediated transmission. Non-immune and passively immunized Swiss mice were exposed to an aerosol of Trinidad donkey strain VEE; viral accumulation and replication proceeded in the following order: nasal turbinates, olfactory bulbs, blood, and cerebrum. Passive immunization with convalescent mouse serum prevented viremia and appearance of high VEE viral titers in the cerebrum but did not reduce infection of the nasal turbinates or olfactory bulbs after aerosol challenge. Foci of VEE antigen were detected by immunohistochemistry in the olfactory nuclei, basal ganglia, hypocampus, amygdala, and mammilothalamic tracts. The pattern of antigen distribution was not identical with the in-vitro binding assay but the regions of viral antigen accumulation were in sites of termination of tracts labeled in the in-vitro assay. Taken together, these data strongly suggest that uptake and distribution of VEE in olfactory tracts occurs after aerosol exposure. This could explain the inability of circulating antibodies to protect against aerosol-acquired VEE.

Further studies with the in-vitro assay for VEE neurovirulence suggested that molecular moieties of the host receptor for VEE virus might be determined in western blots of normal mouse brain proteins. A unique band of approximate mass 17 Kd, bound (biotinylated or metabolically radiolabeled VEE) as a single band in normal mouse brain homogenate. Since the band was specifically inhibited by non-labeled VEE virus, it is possible that the 17-Kd moiety is a component of the VEE neurotropism receptor.

PRESENTATIONS

Anderson, A. O. 1988. Lymphocytes: development and biology. Presented at the University of Pennsylvania, Philadelphia, PA, February.

Anderson, A. O. . 1988. Physiologic functions of lymphatic tissues. Presented at the University of Pennsylvania, Philadelphia, PA, February.

Anderson, A. O. . 1988. Organization of the lymphatic system. Presented at the Laboratory of Microbiology and Immunology, National Institutes of Health, Bethesda, MD, March.

Anderson, A. O. 1988. Cross regulation between mucosal and peripheral immunity. Presented at the 18th Annual Joint Immunobiology Meeting, Harper's Ferry, WV, May.

Anderson, A. O. 1988. Sociobiology of lymphocytes and mononuclear cells. Presented at the Roswell Park Memorial Institute, Department of Health, State of New York, Buffalo, April.

A. O. Anderson, and J. Ward. 1988. Cell biology of lymphocyte recirculation: implications for AIDS. Presented at the Laboratory of Microbiology and Immunology, National Institutes of Health, Bethesda, MD, April

Anderson, A. O., O. Wood, L. Fischbach, and M. L. M. Pitt. 1987. Mucosal priming alters pathogenesis of Rift Valley fever. Presented at the Centenary Symposium of the Institute Pasteur, Molecular Biology and Infectious Diseases, Paris, France, October.

Pitt, M. L. M. 1988. Transdiaphragmatic traffic of peritoneal mononuclear cells to lung interstitium and bronchus associated lymphatic tissues. Presented at the 18th Annual Joint Immunobiology Meeting, Harper's Ferry, WV, May.

Pitt, M. L. M. 1988. Macrophage traffic and the peritoneal microenvironment. Presented at the Department of Genetics, Stanford University School of Medicine, Stanford, CA, June.

Pitt, M. L. M., and A. O. Anderson. 1988. Adjuvant effects on peritoneal macrophage traffic to the lung. Presented at the Annual Meeting of the Federation of American Societies for Experimental Biology, Los Angeles, CA, May. Abstract was published in The FASEB Journal 2:A1259,1988.

Pitt , M. L. M., and Anderson, A. O. 1988. Oral Infectivity of Rift Valley fever virus (RVFV) in A/J mice. Presented at the 11 th International Convocation on Immunology. State University of New York, University of Buffalo, The Ernest Witebesky Center for Immunology. Immunology and Immunopathology of the Alimentary Canal, Buffalo, NY, June.

PUBLICATIONS

Anderson, A. O. 1988. Immunophysiology of lymphocytes, organization of lymphatic tissues, pp. . *In* J. J. Oppenheim and E. Shevach (ed.), Immunophysiology. The role of cells and cytokines in immunity and inflammation. Oxford University Press, NY (In Press).

Anderson, A. O., and J. M. Ward. 1988. Endocytic stripping of ligands from lymphocytes in high endothelial venules (HEV): implications for immunomodulation vs viral pathogenesis. *Adv. Exp. Med. Biol.* (In Press).

Anderson, A. O., L. F. Snyder, M. L. Pitt, and O. L. Wood. 1988. Mucosal priming alters pathogenesis of Rift Valley fever. *Adv. Exp. Med. Biol.* (In Press).

Anderson, A. O., O. L. Wood, A. D. King, and E. H. Stephenson. 1987. Studies on anti-viral mucosal immunity using the lipoidal amine adjuvant avridine. *Adv. Exp. Med. Biol.* 216B:1781-1790.

Larsen, C. G., A. O. Anderson, E. Appella, J. J. Oppenheim, and K. Matsushima. 1988. Identity of the chemotactic cytokine for T-lymphocytes with neutrophil activating protein (NAP-1): a candidate interleukin-8. Submitted to *Science.*

Pitt, M. L. M., and A. O. Anderson. 1988. Direct transdiaphragmatic traffic of peritoneal macrophages to the lung. *Adv. Exp. Med. Biol.* (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA305650	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AK	022
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced studies for the development of immunotherapy against toxins of potential BW threat

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8410	8901	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	3.0	451
89	3.0	662

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Hewetson, J F
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Solow, R
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Military Medicine; (U) Mycotoxins; (U) Lab Animals; (U) Mice; (U) Diagnosis; (U) RA I; (U) Monkeys; (U) Toxins

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop methods for the field detection of mycotoxins in biological and environmental samples. To develop prophylactic and/or therapeutic agents for soldiers exposed to these toxins. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Use immunoassays to detect mycotoxins in biological/environmental samples. Develop monoclonal and polyclonal antibodies for use as prophylactic agents. Develop stable toxin-protein conjugates for possible vaccine use.

25. (U) 8710 - 8809 The T-2 metabolites, HT-2 and T-2 tetraol, can be readily detected by immunoassay in urines after T-2 exposure, and a potentially fieldable ELISA kit compared favorably with a standard RIA. Saxitoxin and/or saxitoxin derivatives were detected by an ELISA immunoassay in clinical specimens from victims exposed to an outbreak of paralytic shellfish poisoning, demonstrating for the first time the presence of these toxins in tissues. Goat antisera to brevetoxin (PbTx-3) protected rats exposed to toxic doses of PbTx-3. After a report of Red Tide, levels of PbTx-3 in field samples of marine clams and oysters were determined by RIA and the results compared favorably to a standard mouse bioassay. Microcystin-LR was not directly cytotoxic to non-parenchymal endothelial cells, but hepatocytes treated with microcystin-LR produced metabolites or mediators that induced permeability changes in endothelial cells.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AK-022: Advanced Immunotherapy Studies Against Toxins

PRINCIPAL INVESTIGATOR: J. F. Hewetson, Ph.D.

ASSOCIATE INVESTIGATOR: R. Solow, CPT, Ph.D.

BACKGROUND

Small-molecular-weight toxins that lead to death or illness upon contact or ingestion are of interest to the Army because of their potential as biological warfare agents. The toxins we are investigating include T-2 mycotoxin and its metabolites, saxitoxin and its derivative, brevetoxin and its derivatives, and microcystin. T-2 toxin is a mycotoxin occurring in moldy grain. It causes alimentary toxic aleukia in man and domestic animals, and has been implicated in biological warfare incidents in Southeast Asia and Afghanistan. Saxitoxin is a potent neurotoxin which acts on the sodium channel. It has been responsible for many cases of paralytic shellfish poisonings. Brevetoxin is a polyether toxin which acts on the sodium channel (at a different site than saxitoxin). This toxin has been responsible for mass fish kills and human health problems. Microcystin, a cyclic peptide found in blue-green algae, is a potent hepatotoxin. It has been responsible for many deaths among livestock. The need for rapid, reliable, quantitative detection methods and therapeutic antidotes is well established. Detection methods available include variations of the radioimmunoassay (RIA) and the enzyme-linked immunosorbent assay (ELISA). The ELISA is most appropriate for field use, as it does not employ radioisotopes. Adaptation of ELISA technology for toxin detection and defining treatment effective therapies for toxicosis are the major goals of this research.

SUMMARY

The T-2 metabolites, hydroxy T-2 (HT-2) and T-2 tetraol, can be detected by RIA and ELISA in rat urines after intramuscular, oral, or dermal exposure of the animals to the toxins. A potentially fieldable ELISA kit was tested with some of the urines; the results compared favorably with a standard RIA.

An outbreak of paralytic shellfish poisoning in Guatemala provided clinical material to confirm the validity of a saxitoxin ELISA and membrane assay for toxin detection in field samples. Four animal species have been immunized with saxitoxin conjugates to produce high-

titer antisera for immunoprophylaxis studies.

Goat antisera to brevetoxin (PbTx-3) protected rats exposed to lethal doses of the toxin. After a report of the occurrence of red tide, these antisera were used in a competitive RIA to determine levels of PbTx-3 in field samples of marine clams and oysters. The assay compared favorably to a standard mouse bioassay.

The flavonoid, silymarin, was an effective protective agent against microcystin-LR-induced toxicity in vitro. Microcystin-LR was not directly cytotoxic to non-parenchymal endothelial cells, but hepatocytes inoculated with microcystin-LR produced metabolites or mediators that induced permeability changes in the endothelial cells.

PRESENTATIONS

Hewetson, J. F., J. E. Beheler, and R. W. Wannemacher, Jr. 1987. T-2 tetraol and HT-2 detection in urines of rats and monkeys exposed to T-2 toxin. Presented at the Annual Meeting of the American Society of Pharmacology and Experimental Therapeutics, Honolulu, HA, December.

Hewetson, J. F., S. E. Hall, J. I. Smith, and J. E. Beheler. 1988. Comparison of an ELISA and sodium channel assay for detection of paralytic shellfish poisoning in clams and clinical specimens from intoxicated individuals. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology, Las Vegas, NV, May.

Hewetson, J. F., R. W. Wannemacher, Jr., and R. J. Hawley. 1988. Detection of T-2 mycotoxin and its metabolites in urines of exposed rats. Comparison of a potentially fieldable kit with a laboratory assay. Presented at the Army Science Conference, Westpoint, NY, June.

Pace, J. G., N. A. Robinson, and M. A. Poli. 1987. In vitro measurement of intrinsic clearance predicts hepatic extraction ratio of PbTx-3. Presented at the 1st International Symposium on Red Tides, Takamatsu City, Japan, November.

Solow, R., K. A. Mereish, C. W. Anderson, Jr., and J. Hewetson. 1989. Effect of microcystin-LR on cultured rat endothelial cells. Presented at the Annual Meeting of the Society of Toxicology, Atlanta, GA, February-March.

Templeton, M. J., M. A. Poli, and R. D. LeClaire. 1987. Effectiveness of an antibody to Florida red tide dinoflagellate toxin PbTx-2 in conscious rats. Presented at the International Symposium on Red Tides, Takamatsu City, Japan, November.

Templeton, C. B., M. A. Poli, R. D. LeClaire, and R. Solow. 1988. An antibody to prevent effects of brevetoxin poisoning in conscious rats. Presented at the Army Science Conference, Westpoint, NY, June.

PUBLICATIONS

Chanh, T. C., E. P. Reed, R. I. Huot, M. R. Schick, and J. F. Hewetson. 1988. Anti-idiotype antibodies against a monoclonal antibody specific for the trichothecene mycotoxin T-2. Submitted to *Biophys. Biochem. Res. Commun.*

Hewetson, J. F., J. C. Pace, and J. E. Beheler. 1987. Detection and quantitation of T-2 mycotoxin in rat organs by radioimmunoassay. *J. Assoc. Off. Anal. Chem.* **70**:654-657.

Hewetson, J. F., R. W. Wannemacher, Jr., and R. J. Hawley. 1988. Detection of T-2 mycotoxin metabolites in urines of exposed rats. Comparison of a potentially fieldable kit with a laboratory assay. Submitted to *Proc. Army Sci. Conf.*

Hewetson, J. F., R. W. Wannemacher, and R. J. Hawley. 1988. Immunodetection of T-2 metabolites in urines of rats after dermal, oral, or intramuscular exposure to T-2. Submitted to *Fund. Appl. Toxicol.*

Poli, M. A. 1988. Metabolism and excretion of brevetoxin PbTx-3 in rats and isolated rat hepatic cells. *Toxicon* **26**:36.

Poli, M. A. 1988. Laboratory procedures for detoxification of equipment and waste contaminated with brevetoxins PbTx-2 and PbTx-3. *J. Assoc. Official Anal. Chemists* (In Press).

Templeton, C. B., and M. A. Poli. 1988. Cardiorespiratory effects of brevetoxin PbTx-2 in conscious rats. *Toxicon* **26**:43.

Templeton, C. B., M. A. Poli, and R. D. LeClaire. 1988. An antibody to prevent the effects of brevetoxin poisoning in conscious rats. Submitted to *Proc. Army Sci. Conf.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA303917	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AC	001
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY	89 -01		

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Q Fever

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 04	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	225
c. TYPE	d. AMOUNT	89	1.0	250
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Waag, D M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Q Fever (U) Coxiella burnetti; (U) Vaccines; (U) Medical Defense; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii*, the causative agent of Q fever, is considered by most experts to have those properties which make it a potential candidate for BW. The existing vaccine to protect US troops against this threat is reasonably effective, but causes sterile abscesses in previously sensitized individuals. This institute has a broad program to improve the current vaccine. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) This work unit is dedicated to the transfer of research-level vaccine production technology to conditions for pilot-scale processing of the Q fever vaccine. Definition of the pilot-scale conditions will permit the orderly production of large volume lots of vaccine that are safe and efficacious.

25. (U) 8710 - 8809 Paired sera collected from volunteers were evaluated before and after vaccination with 30 μg of IND 610 Q fever vaccine. The reciprocal titers of antibodies in an enzyme-linked immunosorbent assay were compared with those from an immunoblot assay. At least 17 proteins were identified as potential candidate diagnostic antigens. All of the individuals produced antibodies in one or more subsets of these proteins. Individuals did not consistently sero-convert to all of these proteins, rather each sero-conversion was to a selected subset of these proteins. The new candidate chloroform-methanol-residue vaccine will be delivered to USAMRIID in January 1989, when it will be evaluated in phase I safety testing.

DD FORM 1498 63 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORKUNIT NO. 809-AC-001: Vaccine, Q Fever

PRINCIPAL INVESTIGATOR: J. C. Williams, CDR, Ph.D.

ASSOCIATE INVESTIGATORS: G. F. Meadors, LTC, M.D.
D. M. Waag, Ph.D.

BACKGROUND

Coxiella burnetii, the causative agent of Q fever, is considered by most experts to have those properties which make it a potential candidate for biological warfare. The existing formaldehyde-inactivated, phase I whole cell vaccine is reasonably effective, but exhibits undesirable effects in previously sensitized individuals. A broad program is ongoing to develop and produce improved subunit vaccines.

SUMMARY

Individuals at risk of acquiring Q fever in the laboratory were evaluated by measurement of humoral antibody titer (ELISA), cell-mediated response (in vitro lymphocyte proliferation), and delayed hypersensitivity (skin test) before and after administration of a formaldehyde-inactivated, phase I vaccine (IND 610). The criteria used were that individuals positive by at least two of the tests would not be vaccinated. Vaccine was not given to 46% of the tested individuals. Of those receiving vaccine, no adverse reactions were noted. The responses before and after vaccination were evaluated to develop experimental values that may be predictive of protective immunity. No correlation between skin test data and either humoral or cell-mediated responses could be established. However, about 80% and 65% of humoral antibody and cell-mediated responses were two standard deviations (95% confidence interval) above the pre-vaccination mean values. The low conversion rate (80% and 65%) is probably due to the low tolerable dose of the phase I vaccine.

The pilot lot of the chloroform-methanol residue (CMR) vaccine for *C. burnetii* has been produced. Sterility tests and composition evaluations are ongoing. Pre-clinical testing will commence in FY88.

PRESENTATIONS

Waag, D., and J. C. Williams. 1987. Identification of suppressor cells induced following injection of C57Bl/O ScN mice with phase I *Coxiella burnetii* whole cells. Presented at the American Physiology Society Annual Meeting, Federation of American Societies for Experimental Biology, Washington, D. C., April.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA305651	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AN	002
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 89 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Clinical Study

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	175
c. TYPE	d. AMOUNT	89	1.0	245
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Cosgriff, T M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2997
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Popovic, N A
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Hack, D C

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Vaccines; (U) Volunteers; (U) High Containment Medical Care; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) (1) Monitor immunizations of persons where work places them at risk of exposure to vaccine agents; enter data on safety and immunogenicity of experimental and licensed vaccines into computer data base. (2) Maintain facilities to transport and treat patients under conditions of total (P-4) biohazard containment to prevent the possible spread of highly pathogenic microorganisms/toxins to medical personnel or the environment. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Reactions to immunizations are carefully monitored to assess safety in humans. Immune responses are measured by conventional serology, skin-testing, and lymphocyte studies. Maximum containment facilities and fully trained medical personnel are maintained in a constant state of readiness to transport, treat, and evaluate persons who have been exposed to highly contagious and virulent pathogens.

25. (U) 8710 - 8809 Computer data entry for the Special Immunizations Program has again progressed rapidly during the last year, with entry of pre-1983 data. Work is in progress to combine all computer files related to immunization at USAMRIID into one central file. Extensive review of immunization data is on-going. The review allows us to assess the reactogenicity, immunogenicity, and efficacy of our vaccines. The high-containment facilities are supplied with state-of-the-art medical equipment. An improved system of radio communications for facility personnel is under study. Medical personnel undergo continuous training in high-containment techniques, as well as participate in organized drills and alerts.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3S464758D847:	Medical Defense Against Diseases of BW Importance
WORK UNIT NO. 847-AN-002:	Vaccine, Advanced Development
PRINCIPAL INVESTIGATOR:	T. M. Cosgriff, COL, M.D.
ASSOCIATE INVESTIGATORS:	A. J. Galloway, CPT, M.D. F. J. Malinoski, MAJ, M.D.

BACKGROUND

As part of the task of developing vaccines to meet the biological warfare threat, Phase I and Phase II clinical trials of candidate vaccines are conducted by the medical Division. If these trials demonstrate that a vaccine is safe and immunogenic, Phase III studies are conducted in larger numbers of volunteers and, when possible, in endemic areas.

SUMMARY

Q-fever Vaccine. During fiscal year 1988, Q-fever vaccine, inactivated, freeze-dried, NDBR 105, continued to undergo clinical testing. Over 180 volunteers have participated in the study to date. We have observed no significant adverse reactions, and no cases of laboratory-acquired disease have occurred among vaccinated persons. No correlation is apparent among skin-test results and measurements of cellular and humoral immunity, before or after immunization.

Chikungunya Vaccine. During fiscal year 1988, chikungunya viral vaccine: live, attenuated, dried TSI-GSD-218, was tested in an additional 11 volunteers, bringing the total immunized to date to 40. No significant adverse reactions occurred in any volunteer. Minor complaints have been equally common in vaccinated and control groups. The vaccine has been uniformly effective in eliciting neutralizing antibody in all recipients.

Tularemia Vaccine. During fiscal year 1988, 18 volunteers participated in initial safety and efficacy trials of *Francisella tularensis* vaccine (live, TSI-GSD-213, Lot 1R) Nine of these volunteers received the vaccine and nine received a placebo. All volunteers who received the vaccine developed a characteristic local lesion at the site of inoculation and experienced a rise in antibody titers. Three of the nine who received the vaccine had evidence of transient liver dysfunction. Clinical trials have been suspended indefinitely pending further evaluation of the effects of the vaccine on liver function.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA305993	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	BA	004
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Antiviral Drug, Ribavirin

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	375
89	1.0	430

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Huggins, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7691
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Ribavirin; (U) Antiviral Drug; (U) Sandfly Fever; (U) Virus; (U) Prophylaxis; (U) Treatment; (U) RA I; (U) Volunteers

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop the drug ribavirin as an antiviral for treatment of viral diseases of military importance. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Perform clinical trials to evaluate efficacy and toxicity of prophylactic drugs in accordance with regulatory requirements.

25. (U) 8710 - 8809 A research protocol for a prospective, prophylactic, clinical study in volunteers evaluating the efficacy of ribavirin against dengue 2 virus was completed. Studies evaluating ribavirin prophylaxis against dengue viremia in rhesus monkeys failed to show ribavirin therapy effective in reducing viremia. On the basis of these results, the clinical protocol has been placed on hold until additional animal studies are performed.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-BA-004: Ribavirin

PRINCIPAL INVESTIGATOR: J. W. Huggins, Ph.D.

BACKGROUND

Although most viral diseases are not associated with high mortality and are self-limiting, certain disease outbreaks, such as the influenza pandemic of 1918-19, can be associated with great loss of life. Exotic viral diseases, about which we know very little and with which we are presently unable to cope, occur in many areas of the world. Many viruses with the potential for inducing illness of high morbidity and mortality remain endemic in certain areas of the world; notable examples are Rift Valley fever, Lassa fever, and Ebola fever in Africa; Argentine and Bolivian hemorrhagic fevers in South America; and hemorrhagic fever with renal syndrome in Asia. Numerous other examples are easily cited. Broad-spectrum antiviral agents would be welcome insurance against the threat of viral outbreaks during military operations. A number of compounds recently have been approved by the Food and Drug Administration for the prevention or treatment of viral diseases. These compounds have extremely narrow spectra of activity. In contrast, the new antiviral drug, ribavirin, appears to provide broad-spectrum activity. Our studies are to evaluate in humans the efficacy of putative antivirals against viruses of military importance.

SUMMARY

Studies evaluating ribavirin prophylaxis against dengue viremia produced by experimentally infecting primates failed to demonstrate any reduction in viremia. Based on this information, the protocol was not implemented, pending additional preclinical studies.

PRESENTATIONS

Malinoski, F. J., S. Hasty, J. M. Dalrymple, and P. G. Canonico. 1988. Prophylactic ribavirin treatment of dengue type 1 infection in rhesus monkeys. Presented at the Second International Conference on Antiviral Research, Williamsburg, VA. Abstract is published in *Antiviral Res.* 9:131.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA303505	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 30 Oct 87	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISS'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	EA	005
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE *(Precede with Security Classification Code)* Rapid diagnosis system for potential BW agents

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 03	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER		88	1.2	200
c. TYPE	d. AMOUNT	89	1.2	227
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS *(include zip code)* Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER *(include area code)* 301-663-2833	d. TELEPHONE NUMBER *(include area code)* 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)* Meegan, J
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* Nuzum, E

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Vaccines; (U) Medical Defense; (U) Viral Diseases; (U) Immunological Reagents; (U) Antigens; (U) Rapid Diagnosis; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) To develop, standardize, then conduct field tests of developed, rapid diagnosis assays. To support extramural contracts in rapid diagnosis. To assess availability and suitability of commercial reagents for use in rapid detection assays for viruses affecting U.S. military personnel. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Employ immunoassay methods to develop and evaluate rapid assays for diagnosis of viral diseases of natural or BW threat to the military. When possible, test these assays at field laboratories using epidemiologically relevant samples. Develop and supply for extramural rapid diagnosis projects standardized reagents, safety-tested and efficacy-tested.

25. (U) 8710 - 8809 Testing of rapid identification/diagnosis assays for Korean hemorrhagic fever (KHF) was extended by applying newly available bioengineered expression products which greatly simplified test reagent production. The KHF system has been shown, with relevant field samples from our repository, to meet or surpass the previous test, which used inactivated authentic viral proteins. These and similar products are being further refined as a safe and inexpensive source of viral diagnostic antigens in current and emerging technology assays. Diagnostic support of efficacy trials for chikungunya and Argentine hemorrhagic fever vaccine is continuing. A genus-specific assay for leptospirosis has been further tested in Asia, Australia, and South America and data continue to suggest this assay is a rapid and effective means of diagnosis. Technical evaluation of contractor-developed assays were carried out for the rapid identification system. Consultation and reagents continue to be provided to extramural contractors funded by USAMRDC, USAMMDA, and USACRDEC.

DD FORM 1498, 83 MAR — EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809:	Development of Drugs and Vaccines Against Diseases of BW Importance
WORK UNIT NO: 809-EA-005:	Rapid Identification and Diagnosis System
PRINCIPAL INVESTIGATOR:	J. W. LeDuc, LTC, Ph.D.
ASSOCIATE INVESTIGATORS:	T. G. Ksiazek, LTC, D.V.M. J. M. Meegan, CDR, Ph.D. R. R. Graham, MAJ, D.V.M. K. T. McKee, Jr., MAJ, M.D. C. A. Rossi

BACKGROUND

The objectives of this study are to conduct field tests of rapid diagnosis/identification assays; to evaluate clinical specimens to establish is rapid diagnosis can best be achieved by detecting antigen or early immunoglobulin M (IgM) antibody; and to provide a technical support base for USAMRIID, the U. S. Army Medical Materiel Development Activity (USAMMDA), and the U. S. Army Chemical Research Development and Engineering Command (USACRDEC) internal and extramural programs aimed at developing novel rapid detection/identification systems. The main technology used is the enzyme immunoassay. Field tests were conducted in areas of the world where the viruses or bacteria are endemic. In earlier studies, assays were transported to OCONUS laboratories in developing countries, and testing revealed they were durable, rapid, simple, sensitive, and reproducible.

Our main goal is to detect antigen in clinical or environ-mental specimens within 2 to 3 h. Because the first indication that a disease is present might be an infected serviceman, and because some diseases do not produce a detectable antigenemia during clinical disease, we made an attempt to develop rapid IgM antibody-detection assays for each agent. In previous years, we established that assays for antigen detection and, in some cases, early IgM antibody can rapidly diagnose patients presenting with a number of militarily relevant diseases.

SUMMARY

Field tests of rapid identification/diagnosis assays for Hantaan virus, cause of hemorrhagic fever with renal syndrome, were continued in Korea, and extended to study sites in Greece and Yugoslavia, where an especially severe form of the disease exists. Results continue to indicate that, at admission, diagnosis of the complex of diseases is best established by demonstration of specific IgM antibody. In addition, information was obtained to suggest that the assay works well with a number of viruses closely related to

prototype Hantaan, but is less sensitive in the diagnosis of nephropathia epidemica due to Puumala virus, a more distantly related *Hantavirus*.

Field evaluations of the leptospirosis assay were conducted in Barbados, Brazil, Korea, Australia, Malaysia, Thailand, and with sera from several locations in the United States. The test was found to detect rapidly specific anti-*Leptospira* IgM antibodies in sera obtained 4-7 days after onset of illness in acutely ill patients. These antibodies may persist for 1 year or more; consequently, measurement of both IgM and IgG antibodies on at least two serum samples is required to confirm the diagnosis. Nonetheless, a presumptive diagnosis can be made on a single serum sample obtained early in the disease course when effective antibiotic therapy can be implemented.

An extensive epidemic/ epizootic of Rift Valley fever (RVF) occurred in October 1987 in Mauritania and Senegal. This outbreak offered us the opportunity to validate under field conditions our rapid diagnosis assays for this RVF virus with a large collection of authentic human and animal specimens. We were able to evaluate the antigen-capture immunoassay and compare it with standard virus isolation and identification procedures and found that the antigen-capture immunoassay was 30% as sensitive, detecting antigen in 24 of 82 human serum samples from RFV virus was isolated, and 97% as specific, with two false-positive sera out of 61 RVF virus-negative samples. Although the sensitivity of the antigen-capture immunoassay was low in comparison with traditional virus isolation and identification methods, this shortcoming was more than offset by its speed and simplicity. Traditional procedures require at least 1 week for isolation and identification and require the use of cell culture or laboratory animals, as well as other, more sophisticated resources, while the immunoassay requires less than 3 h and minimum equipment.

PRESENTATIONS

Antoniades, A., J. W. LeDuc, N. Malisiovas, and K Pappas. 1988. ELISA M capture for diagnosis of HFRS. Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece, September.

Cosgriff, T. M., C. M. Hsiang, J. W. Huggins, M. Y. Guang, J. I. Smith, Z. O. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, P. H. Gibbs, X. E. Gui, G. W. Yuan, and T. M. Zhang. 1988. Predictors of fatal outcome in the severe form of hemorrhagic fever with renal syndrome (HFRS). Presented at the First International Symposium on Hantaviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece, September.

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Huggins, J. W., C, M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H. W. Lee. 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

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Xiang, C. M., M. Y. Guan, Z. M. Zheng, Z. A. Wu, X. Q. Ge, T. M. Zhang, G. H. Yuan, X. A. Gui, J. W. Huggins, T. M. Cosgriff, J. Smith, J. W. LeDuc, and J. M. Meegan. 1988. Study of antiviral specific therapy of epidemic hemorrhagic fever with ribavirin. *J. Exp. Clin. Virol.* (China) **2**:47-51.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA311563	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 30 Oct 87	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AK	007
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP FY	89- 01		

11. TITLE (Precede with Security Classification Code) Vaccine, Chikungunya

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	300
c. TYPE	d. AMOUNT	89	1.0	340
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lupton, H W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7241
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Cole, F E
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ramsburg, H H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Vaccines; (U) Volunteers; (U) Chikungunya; (U) Clinical Trials; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop and test a live, attenuated chikungunya vaccine for prophylactic treatment of at-risk military personnel. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Conduct preclinical tests to evaluate vaccine safety and efficacy. Conduct animal and/or in vitro studies to demonstrate cross protection against heterologous viruses. Design and conduct clinical trials and laboratory tests to evaluate vaccine safety and efficacy.

25. (U) 8710 - 8809 Phase I clinical evaluation of the CHIK vaccine (IND-2426) continued. Seven groups have been studied, comprised of 21 vaccine recipients and 19 placebo controls. Seronegative volunteers were given 10^5PFU of vaccine or placebo s.c. Minor clinical abnormalities were seen in few principals and controls. All vaccinees seroconverted, with peak PRN_{80} titers occurring by day 28. IgM levels increased after 10 days, peaking by day 21. Low level, transient viremias were seen in 6 of 18 vaccinees. Clinical trials are continuing. Mutagen-attenuated Rift Valley fever master and production seeds and candidate vaccine,[ZH-548 MP12 strain] are in final testing at The Salk Institute. Genetic stability studies of the RVF vaccine are in progress. Serial passage studies in two- to four-day-old lambs, designed to evaluate reversion to virulence of the vaccine virus, were not possible due to the low viremia which occurred in this sensitive host. Genetic stability during four serial passages in cell culture was demonstrated. All cell culture passages of the virus also remained non-lethal for mice and did not lose their ability to protect against challenge with lethal RVF virus. Studies in progress will determine if the vaccine viral strain will remain non-lethal for mice after mouse passage of high-titer brain tissue.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809:	Development of Drugs and Vaccines Against Diseases of BW Importance
WORK UNIT NO. 809-AK-007:	Vaccine. Chikungunya
PRINCIPAL INVESTIGATOR:	H. W. Lupton, COL, D.V.M.
ASSOCIATE INVESTIGATORS:	F. E. Cole, Jr., Ph.D. H. H. Ramsburg, M.S.

BACKGROUND

Chikungunya (CHIK), an arthropod-borne alphavirus, which produces a dengue-like illness in man, is found throughout Africa, Southeast Asia, the western Pacific, and India, where it often causes epidemics. Despite its widespread geographic distribution, individual strains of CHIK are closely related antigenically, thereby allowing a vaccine to provide broad-spectrum protection against heterotypic strains of this virus as well as against the antigenically related viruses of O'nyong nyong, Mayaro, and Ross River. Using CHIK strain 15561, a southeast Asian, human isolate from a mild case of CHIK fever, we developed a vaccine by a series of plaque-to-plaque passages in certified MRC-5 cells. Master and production seeds and vaccine were produced and preclinically tested in compliance with both the Good Laboratory Practices and the Good Manufacturing Practices Regulations. An Investigational New Drug submission was approved by the Office of Biological Research and Review, Food and Drug Administration, in July 1986 (IND 2426). Phase 1 clinical trials are in progress.

Rift Valley fever virus was confined to sub-Saharan Africa, rarely causing serious illness in man, until 1975, when deaths occurred in South Africa. In 1977, the virus spread to Egypt where an increased incidence of lethal human disease made self-evident the need for a more effective vaccine. In 1982, investigators at USAMRIID and the University of Alabama, Birmingham, received a grant from the U.S./Israel BARD Fund to initiate a program to develop a live, attenuated vaccine by passage of the virus in the presence of mutagenic chemicals. A candidate vaccine was developed, and master and production seeds and several lots of candidate vaccine were prepared in vaccine-quality MRC-5 cells by The Salk Institute. Field trials are being conducted in South Africa and Senegal.

SUMMARY

Phase I clinical evaluations of the new live, attenuated CHIK vaccine (CHIK 181/Clone 25) continued. Seven groups of volunteers, comprised of 21 principals and 19 placebo controls were used in a double-blind study. Seronegative volun-

teers were given placebo or 10^5 PFU of CHIK vaccine s.c. Minor clinical abnormalities were seen occasionally in principal and control groups. All vaccinated volunteers seroconverted, as detected by the 80% plaque-reduction serum neutralization test. Peak titers occurred on day 28 post vaccination (range: 1:80 to 1:2560). Immunoglobulin M levels rose after 10 days and peaked on day 21. Low level, transient viremias were seen in 6 of 18 vaccine recipients. Clinical trials are continuing.

Mutagen-attenuated, ZH-548, MP12 strain, Rift Valley fever master and production seeds and several immunofluorescence and pathology studies from the monkey neurovirulence study were reported last year. Low levels (< 4.3 $\log_{10}$/ml) of virus were demonstrated in the spleen of one of five monkeys in the candidate vaccine group, and in one of five in the production seed group in contrast to two of five that received the Smithburn vaccine and five of five parent virus recipients. Virus was not detected in brains, spinal cords, or livers of monkeys receiving candidate vaccine or production seed; whereas 1.7 $\log_{10}$/ml of virus were seen in the cord of one monkey that received the Smithburn vaccine and > 4.4 $\log_{10}$/ml in all five that received parent virus. In the parent virus group, 2.7 $\log_{10}$/ml of virus were found in the liver of one monkey, < 2.7 $\log_{10}$/ml from the lymph nodes of three of five tested, while > 5.1 $\log_{10}$/ml were seen in the brains of all five monkeys.

Studies to determine the genetic stability of the live, attenuated Rift Valley fever candidate vaccine are in progress. Serial passage studies in two- to four-day-old lambs, designed to evaluate reversion to virulence of the candidate vaccine, were not possible due to the low viremia that occurred in this extremely sensitive host. However, genetic stability during four serial passages in cell culture has been demonstrated, as measured by plaque size and morphology, which remained unchanged. Moreover, all four cell culture passages of the vaccine viral strain remained non-lethal for mice (defined as the killing of <10% of mice inoculated with varying dilutions) and did not lose the ability to protect against challenge with lethal Rift Valley fever virus. Studies in progress will determine if the vaccine viral strain will remain non-lethal for mice after mouse passage of high-titer brain tissue harvested from mice inoculated with tenfold dilutions of the vaccine virus or tenfold dilutions of samples of the four subsequent serial cell culture passages.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA314901	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DL-DR&B(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
14 June 88	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AM	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89-01			

11. TITLE (Precede with Security Classification Code) Vaccine, Tularemia

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
88 06	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	160
c. TYPE	d. AMOUNT	89	1.C	140
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Galloway, A K
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7655
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Francisella tularensis; (U) Tularemia; (U) Vaccine; (U) Military Medicine; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct Phase I (safety and tolerance), Phase II (efficacy), and Phase III (field trials) testing of experimental vaccines for prophylaxis of diseases of unique military importance, particularly those with potential as biological warfare threats. A Literature survey has been done. DTIC search has been filed in the 1498 file. Continued efforts from DA313616.

24. (U) Experimental vaccines which have undergone immunogenicity and safety testing in preclinical studies will be studied in rigorous clinical trials after extensive scientific and ethical reviews.

25. (U) 8806 - 8809 Phase I testing of *Francisella tularensis* vaccine, live, TSI-GSD-213, Lot 1R was begun. During fiscal year 1988, 18 volunteers participated in initial safety and efficacy trials. Nine of these volunteers received the vaccine and 9 received placebos. All volunteers who received the vaccine developed a characteristic local lesion at the site of inoculation and had a rise in antibody titer by hemagglutination. A lymphocyte transformation assay was developed with whole cell antigen. Lymphocyte transformation data also suggested that the vaccine is immunogenic. Three of the 9 volunteers who received the vaccine had evidence of transient liver dysfunction. Clinical trials have been suspended indefinitely pending further evaluation of the effects of the vaccine on liver function.

DD FORM 1498 83 MAR   EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-AM-003: Vaccine, Tularemia

PRINCIPAL INVESTIGATOR: A. K. Galloway, MAJ, M.D.

BACKGROUND

Francisella tularensis, the causative agent of tularemia, is considered a potential candidate for biological warfare. A live, attenuated strain of the organism has been in use as a vaccine since the early 1960s. New lots of the vaccine, however, have been produced for use in individuals at risk of infection.

SUMMARY

Phase I testing of *Francisella tularensis* vaccine, live, TSI-GSD-213, lot IR, was begun. During fiscal year 1988, 18 volunteers participated in initial safety and efficacy trials. Nine of these volunteers received the vaccine and nine received placebos. All volunteers who received the vaccine developed a characteristic local lesion at the site of inoculation and had rises in their antibody titers (determined by hemagglutination). A lymphocyte transformation assay was developed with whole-cell antigen. Lymphocyte transformation data also suggested that the vaccine was immunogenic. Three of the nine volunteers who received the vaccine had evidence of transient liver dysfunction. Clinical trials have been suspended indefinitely, pending further evaluation of the effects of the vaccine on liver function.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA313526	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D.CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	AG	004
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY-89 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Argentine hemorrhagic fever

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	511
89	1.0	590

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lupton, H W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2405
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Barrera Oro, J G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Cole, F E

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Junin Virus (U) Vaccines; (U) Lab Animals; (U) Monkeys; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop and test a live, attenuated Junin virus vaccine prepared against Argentine hemorrhagic fever for prophylactic treatment of at-risk personnel. A literature search has been done. DTIC search is filed in the 1498 file.

24. (U) Conduct preclinical tests to evaluate vaccine safety, immunogenicity, and stability. Conduct animal and in vitro studies to determine vaccine cross protection against heterologous Arenaviruses. Evaluate the vaccine constituents and production processes with goals of improved titer, stability, and immunogenicity. Design and conduct clinical and field trials to evaluate vaccine safety and efficacy.

25. (U) 8710 - 8809 Phase I clinical trials to determine the safety and immunogenicity of the live, attenuated, Canoid #1 Junin (JV) (C #1) vaccine continued. We observed seroconversion in 112 of 117 (96%) C #1 vaccinees. This compares favorably with the 100% seroconversion rate seen in 70 volunteers studied last year. Based on these data, the "marginal humoral immunogenicity" credited to C #1 vaccine last year was incorrect. Sera from early phase I tests will be retested to document the inadequacy of the earlier assay system. Neurovirulence tests on 3 recently produced lots of C #1 were completed; all lots passed. The ability of the C #1 vaccine to induce cross-protection against Machupo virus (causative agent of Bolivian hemorrhagic fever) was studied in rhesus monkeys. Our results strongly suggest that C #1 will provide excellent protection against disease caused by Machupo virus. More than 7200 volunteers have been identified and enrolled for the Candid #1 double-blind, placebo-controlled field efficacy trial to begin in October 1988. An ecological study to evaluate the prevalence and dynamics of JV among rodent populations in the endemic areas is proceeding well. More than 1800 total captures of *Calomys, Akodon,* and *Oryzomys* species have yielded 17 antigen-positive and 12 antibody-positive (1 with both) animals.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3S464758D847:	Medical Defense Against Diseases of BW Importance
WORK UNIT NO. 847-AG-013:	Advanced Vaccine Development Studies on Viruses of Potential BW Threat
PRINCIPAL INVESTIGATOR:	H. W. Lupton, COL, D.V.M.
ASSOCIATE INVESTIGATORS:	K. T. McKee, Jr., MAJ, M.D. F. E. Cole, Jr., Ph.D. J. G. Barrera Oro, M.D.

BACKGROUND

A United Nations Development Project jointly conducted by U.S. and Argentine investigators resulted in development of Candid #1 strain Argentine hemorrhagic fever (Junin virus) vaccine at USAMRIID. Master seed, production seed, and vaccine were produced and preclinical testing was completed in compliance with Good Laboratory Practices and Good Manufacturing Practices Regulations, as well as with vaccine requirements for the United States and Argentina. Preclinical data were documented in an Investigational New Drug (IND) submission. Clinical protocols were approved and vaccine has been administered to small groups of research volunteers at USAMRIID since October 1985. Preliminary data from Phase I clinical studies indicate that the vaccine is safe and immunogenic. Field testing of the vaccine will be conducted at the Instituto Nacional de Estudios sobre Virosis Hemorragicas in Pergamino, Argentina, over the next 3 years. Adequate experimental design has necessitated a small safety trial in seropositive individuals, a larger-scale safety trial, and an efficacy trial. The efficacy trial will be a double-blind, placebo-controlled study in >6500 individuals selected from a population with a disease incidence of >44 cases per 10,000 individuals over two endemic seasons. Thus, a threefold reduction in disease will demonstrate vaccine efficacy with 95% confidence and an 80% power.

SUMMARY

Phase I clinical trials to determine the safety and immunogenicity of the live, attenuated, Candid #1 Junin viral vaccine continued. An additional 121 volunteers were studied, bringing the total to 191 (92 Americans and 99 Argentinians). Using a recently developed, sensitized C'PRN test (S-C'PRNT) to evaluate response to the Candid #1 vaccine, we observed seroconversion in 112 of 117 (96%) vaccinees. These results compare favorably with the 100% seroconversion rate seen in the 70 volunteers studied last year by means of C'PRNT, ELISA, fluorescent antibody, and lymphocyte transformation. In addition, we re-

evaluated duration of immunity by using the SCPRNT and IgG ELISA. Although the positive responses by ELISA decreased gradually from 53/58 (91%) at 6 months to 3/10 (30%) at >21 months, the S-CPRNT demonstrated 64/64 positive responses at 6 months post vaccination, which persisted in 9/10 (90%) at >21 months. As indicated, the ELISA yielded 50 to 60 % false negatives and will therefore not be used routinely in the future, unless a more sensitive assay is developed. Based on these data, the "marginal humoral immunogenicity" credited to this vaccine in earlier assays appears incorrect. Sera from the first phase I tests will be retested to determine if suboptimum early results were a reflection of an inadequate assay system. Neurovirulence tests on three recently produced lots of Candid #1 have been completed; all lots passed.

More than 7200 volunteers have been identified and enrolled for the Candid #1 double-blind, placebo-controlled field efficacy trial scheduled to begin 3 October 1988. We expect that 300-500 of these individuals will be excluded on medical or other entry exclusionary criteria, yielding at least the required 6500 sample size (half in each group) calculated to be necessary to give 90% efficacy at 80% power over a 2-year follow-up.

An ecological study to evaluate the prevalence and dynamics of Junin virus among rodent populations in the endemic areas by trap-out and mark-recapture techniques is proceeding well. More than 1800 total captures of Calomys, Akadon, and Oryzomys species have yielded 17 antigen-positive and 12 antibody-positive (one with both) animals; analysis by macro- and micro-habitat, trap location, and gastric contents are ongoing.

The ability of the Candid #1 vaccine to induce cross-protection against Machupo virus (causative agent of Bolivian hemorrhagic fever) was studied in rhesus monkeys. Two dosage groups were used: six animals received 3 PFU of Candid #1 and six received 103 PFU, s.c. Immunization resulted in significant levels of homologous PRN antibody in 11/12 animals, and Machupo virus-neutralizing antibody in 3/12. After subcutaneous challenge with 103 PFU of Machupo virus, 12/12 normal challenge controls developed erythematous rashes, hemorrhagic nasal discharges, and bloody diarrhea. All became anorectic and dehydrated. Machupo viremia peaked at 4-7 $\log_{10}$ PFU/ml at 17 days post challenge. This coincided temporally with modest peak elevation in serum enzymes, prolonged prothrombin and partial prothrombin times, decreased coagulation factor levels, increased FDP levels, and decreased platelet counts. All 12 of these animals died between days 17-42 post challenge. In contrast, all clinical parameters of the vaccinated, Machupo-challenged animals remained normal. Viremia occurred in two out of six given only 3 PFU of C #1, which ranged from 75-775 PFU/ml for several days. The remaining 10 animals showed no viremia from days 1-27 post challenge, and survived in good health. These results strongly suggest that Candid #1 will provide strong cross-protection against disease caused by Machupo virus.

PRESENTATIONS

Barrera Oro, J. C., R. Kenyon, J. Meegan, K. McKee, C. MacDonald, F. Cole, H. Lupton, and C. J. Peters. 1987. The immune response to Candid #1 (C#1): a live-attenuated Junin virus vaccine against Argentine Hemorrhagic Fever (AHF). Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December..

Barrera Oro, J. C., H. W. Lupton, P. B. Jahrling, J. Meegan, R. H. Kenyon, and C. J. Peters. 1988. Cross-protection against Machupo virus with Candid #1 live-attenuated Junin virus vaccine. I. The postvaccination-prechallenge immune response. Presented at the Second International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region. Mar del Plata, Buenos Aires, Argentina. March.

Lupton, H. W., P. B. Jahrling, J. C. Barrera Oro, and C. J. Peters. 1988. Cross-protection against Machupo virus with Candid #1 live-attenuated Junin virus vaccine. II. Post-challenge virological and immunological findings. Presented at the Second International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region. Mar del Plata, Buenos Aires, Argentina. March.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA305652	01 Oct 88	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	AN	002
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY-89 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Clinical Study

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1	9
c. TYPE	d. AMOUNT	89	1	34
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Cosgriff, T M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2997
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Galloway, A J
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Malinoski, F J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) RA I (U) Vaccines; (U) Volunteers; (U) Phase I, Phase II, and Phase III Clinical Trials

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct Phase I (safety and tolerance), Phase II (efficacy), and Phase III (field trials) testing of experimental vaccines for prophylaxis of diseases of unique military importance, particularly those with potential as biological warfare threats. A literature search has been done. DTIC search has been done.

24. (U) Experimental vaccines which have undergone immunogenicity and safety testing in preclinical studies will be studied in rigorous clinical trials after extensive scientific and ethical reviews.

25. (U) 8710 - 8809 Studies have been conducted on Q-fever, Chikungunya and tularemia vaccines. Results to date indicate that these vaccines are safe and immunogenic.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3S464758D847: Medical Defense Against Diseases of BW Importance

WORK UNIT NO. 809-AN-002: Vaccine, Advanced Development

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, COL, M.D.

ASSOCIATE INVESTIGATORS: A. J. Galloway, CPT, M.D.
F. J. Malinoski, MAJ, M.D.

BACKGROUND

As part of the task of developing vaccines to meet the biological warfare threat, Phase I and Phase II clinical trials of candidate vaccines are conducted by the medical Division. If these trials demonstrate that a vaccine is safe and immunogenic, Phase III studies are conducted in larger numbers of volunteers and, when possible, in endemic areas.

SUMMARY

Q-fever Vaccine. During fiscal year 1988, Q-fever vaccine, inactivated, freeze-dried, NDBR 105, continued to undergo clinical testing. Over 180 volunteers have participated in the study to date. We have observed no significant adverse reactions, and no cases of laboratory-acquired disease have occurred among vaccinated persons. No correlation is apparent among skin-test results and measurements of cellular and humoral immunity, before or after immunization.

Chikungunya Vaccine. During fiscal year 1988, chikungunya viral vaccine: live, attenuated, dried TSI-GSD-218, was tested in an additional 11 volunteers, bringing the total immunized to date to 40. No significant adverse reactions occurred in any volunteer. Minor complaints have been equally common in vaccinated and control groups. The vaccine has been uniformly effective in eliciting neutralizing antibody in all recipients.

Tularemia Vaccine. During fiscal year 1988, 18 volunteers participated in initial safety and efficacy trials of *Francisella tularensis* vaccine (live, TSI-GSD-213, Lot 1R) Nine of these volunteers received the vaccine and nine received a placebo. All volunteers who received the vaccine developed a characteristic local lesion at the site of inoculation and experienced a rise in antibody titers. Three of the nine who received the vaccine had evidence of transient liver dysfunction. Clinical trials have been suspended indefinitely pending further evaluation of the effects of the vaccine on liver function.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA308927	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	BA	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 89 -01			

11. TITLE (Precede with Security Classification Code) (U) Antiviral Drug, Ribavirin

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	89 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	1.0	827
89	1.0	899

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Huggins, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7691
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antiviral; (U) Ribavirin; (U) Chemotherapy; (U) Junin Virus; (U) Hantaan Virus; (U) Hemorrhagic Fever with Renal Syndrome; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop the drug ribavirin as an antiviral for the treatment of viral diseases of military importance. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Conduct Phase III clinical trials appropriate for the development and FDA approval of ribavirin for the prophylaxis or treatment of serious viral infections. Establish liaison with medical authorities in appropriate areas to study the diseases. Design clinical trials and obtain appropriate U S and host country study clearances for conducting human trials. Conduct clinical trials to evaluate efficacy and toxicity in accordance with regulatory requirements.

25. (U) 8710 - 8809 Analysis of the results of a clinical trial of the efficacy of ribavirin in treatment of clinically ill patients with the Chinese variant of hemorrhagic fever with renal syndrome was completed and a statistical summary prepared. Preparation of the materials, including supporting documentation, for the clinical section for a New Drug Application to be filed with the FDA was completed. Three additional patients were treated with ribavirin at U.S. military facilities.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3S464758D847:	Medical Defense Against Diseases of BW Importance
WORK UNIT NO. 847-BA-003:	Ribavirin
PRINCIPAL INVESTIGATOR:	J. W. Huggins, Ph.D.

BACKGROUND

Ribavirin (l-β-D-ribofuranosyl-1,2,3-triazole-3-carboxamide), a nucleoside analogue with a close structural resemblance to guanosine, has been found to inhibit significantly a broad spectrum of both DNA and RNA viruses. Ribavirin is the only antiviral agent which has been shown to have efficacy in the therapy of respiratory syncytial virus (HSV) infection. Other clinical studies have shown that ribavirin is effective for treating of Lassa fever in man. In these studies, patients with an admission viremia of ≥ 105-6 TCID) ml were found to have a ≥ 73% case fatality unless treated with intravenous ribavirin within the first 6 days of illness when the mortality rate was reduced 8%, compared to 43% in those patients treated after day 6. This work unit expands these clinical trials by examining the efficacy of ribavirin in two other militarily relevant, viral diseases: hemorrhagic fever with renal syndrome (HFRS) and Argentine hemorrhagic fever (AHF).

SUMMARY

Geographically diverse but clinically similar human diseases caused by Hantaan viruses and characterized by fever, hemorrhage, and renal damage in five overlapping phases are known collectively as hemorrhagic fever with renal syndrome (HFRS). Existing therapy consists of intensive fluid and electrolyte management and treatment of symptoms. In a prospective, randomized, double-blind, placebo-controlled, clinical trial, patients were administered intravenous ribavirin or placebo. Mortality was significantly reduced among ribavirin, compared to placebo-treated, patients when comparisons were adjusted for baseline risk estimators of mortality [total serum protein and AST (SGOT)] using a stepwise logistic procedure [p=0.047 (two-tailed)]. Treatment initiated by the fourth day of fever showed maximum drug intervention with reduction in kidney damage, a major component of the disease. Ribavirin treatment decreased serum creatinine, proteinuria, duration, and magnitude of hypertension, and edema; and improved serum sodium regulation and total urinary sodium excretion. Ribavirin therapy also reduced the duration of hypotension; and decreased maximum white blood cell counts and hemorrhagic manifestations by increasing platelets, and decreasing petechiae and ecchymosis. Ribavirin shortened the duration of each post-febrile clinical phase, with significant effects on the duration of hypotensive

and oliguric phases, while resulting in an earlier onset of the polyuric phase. The only significant side effect was a reversible anemia, and reticulocytoses was not impaired. The results of this study show that intravenous ribavirin therapy at appropriate doses has provided the first effective drug therapy for early treatment of HFRS.

PRESENTATIONS

Huggins, J. W. 1987. China trials with ribavirin [intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS)]. Presented at the Symposium, Joint Immunology Boards and Viral Diseases Panels, U.S. - Japan Cooperative Medical Sciences Program, U.S. Department of State, Monterey, CA, December.

Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H.W Lee. 1987. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, December.

Huggins, J. W., C. M. Hsiang, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T. M. Zhang, and H.W Lee. 1988. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the Second International Conference on Antiviral Research, Williamsburg, VA, 1987. *Antiviral Res.* 9:131.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302626	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 30 Oct 87	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AP	131
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for development of field ecology data on arboviruses of military importance

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	1.0	100
c. TYPE	d. AMOUNT	89	1.0	96
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Linthicum, K J
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2775
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Logan, T M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Turell, M J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases (U) Arthropod Transmission; (U) Entomology; (U) RA 1; (U) Lab Animals (U)Gerbils

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Identify arthropods and vertebrates associated with the maintenance and transmission of medically important arboviruses to man and define ecologic and environmental factors influencing the ability of arthropods to transmit viruses. Information will allow for development of methods for predicting relative risk of military personnel to arbovirus infections and for designing specific vector control strategies. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Ecologic and environmental factors relating to an arthropod's ability to transmit viruses are studied under natural and controlled environments.

25. (U) 8710-8809 We analyzed and evaluated data from the advanced very high resolution radiometer (AVHRR) on the National Oceanic and Atmospheric Administration's (NOAA) polar-orbiting meteorological satellites to determine the key ecological parameters leading to and associated with a Rift Valley fever (RVF) epidemic/epizootic in Senegal and Mauritania. The data from the AVHRR on NOAA satellites are being used to monitor and predict the potential for RVF viral activity in 15 regions in five countries in sub-Saharan Africa. Breeding habitats of RVF vector species are being mapped in enzootic areas in Kenya by analyzing computer-enhanced images and data from the thematic mapper instrument on Landsat satellites. A species of mosquito (*Aedes fowleri*) associated with RVF enzootic regions in Senegal, was tested in the laboratory and has been shown to be a competent vector of RVF virus. A species of mosquito (*Aedes excrucians*) found in areas in Sweden associated with Ockelbo virus was found to be a competent vector of this virus.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M162770A870: Risk Assessment, Prevention, and Treatment of Infectious Diseases

WORK UNIT NO. 870- AP-131: Exploratory Studies for Development of Field Ecology Data on Arboviruses of Military Importance

PRINCIPAL INVESTIGATOR: K. J. Linthicum, MAJ, Ph.D.

ASSOCIATE INVESTIGATORS: T. M. Logan, CPT, Ph.D.
M. J. Turell, Ph.D.
S. W. Gordon, CPT

BACKGROUND

Previous research has found that Rift Valley fever (RVF) virus epidemics/epizootics occur only during periods of exceptionally heavy rainfall. Field ecology studies have demonstrated that the link between rainfall and RVF outbreaks are floodwater *Aedes* mosquitoes which breed in discrete, well-defined habitats known as dambos, and serve as endemic/enzootic vectors of the virus. Remote sensing technology has been shown to be a useful tool for monitoring RVF viral activity in areas where it is endemic. Attempts to determine the vector competence of arthropods that may be involved in the ecology of the disease have been limited because of the unavailability in the laboratory of a sufficient number of specimens.

SUMMARY

Digital data and imagery from the advanced very high resolution radiometer (AVHRR) on board the National Oceanic and Atmospheric Administration's (NOAA) polar-orbiting, meteorological satellites have been evaluated to determine the key ecological parameters leading to and associated with a RVF epidemic/epizootic in Senegal and Mauritania. The affects of human intervention in the area of the outbreak, previously unknown, have been documented by remote sensing techniques. Data from the AVHRR on NOAA satellites are also being used to monitor and predict the potential for RVF viral activity in 15 regions in five countries in sub-Saharan Africa. Breeding habitats of RVF vector mosquitoes are being mapped in enzootic areas in Kenya by analyzing computer-enhanced images and data from the Thematic mapper instrument on Landsat satellites and the high resolution instruments on SPOT satellites. The feasibility of employing various types of remote sensing imagery to monitor other diseases ecologically linked to rainfall and other meteorological parameters is being assessed in study sites in Africa and South America.

Aedes fowleri, a mosquito associated with ground pools in Senegal, has been experimentally infected with RVF virus and has been found to be a competent vector of the virus. *Aedes albopictus*, a mosquito species recently introduced into North and South America, has also been found to be a competent vector of RVF in the laboratory. *Phlebotomus duboscqi*, a sand fly found in RVF

endemic areas in Africa, was shown to be a competent vector of RVF virus after both intrathoracic inoculation and oral exposure. This is the first demonstration that a sand fly could be involved in the ecology of RVF transmission. Two African tick species, *Rhipicephalus appendiculatus* and *Hyalomma truncatum* were experimentally exposed to RVF virus by intracoelomic inoculation (IC) and feeding. *Rhipicephalus appendiculatus* was found to be an incompetent vector. *Hyalomma truncatum* was found to be a competent vector after intracoelomic inoculation. The virus also passed transstadially from nymph to adult stage. This is the first report of infection, replication, and transmission of RVF virus in any tick species.

PRESENTATIONS

Linthicum, K. J. 1988. Use of remote sensing to monitor the threat of vector-borne diseases. Presented to 1988 U.S. Army Medical Entomology Training Course, San Antonio, TX, February.

Linthicum, K. J., C. L. Bailey, F. G. Davies, A. Kairo, and T. Logan. 1988. Observations on the horizontal distribution of ground pool *Aedes* pupae within a flood dambo in Kenya. Presented at the Annual Meeting of the American Mosquito Control Association, Denver, CO, February.

Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker. 1988. Use of remote sensing to predict the threat of vector-borne diseases to military deployment. Presented at the 1988 Army Science Conference, Fort Monroe, VA, June.

Linthicum, K. J., T. M. Logan, C. L. Bailey, D. M. Watts, and D. J. Dohm. 1987. Experimental infection of Dugbe virus (family Bunyaviridae, genus *Nairovirus*) in five North American and one African ixodid ticks. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Logan, T. M. 1988. Crimean-Congo Hemorrhagic fever. 1988. Presented to 1988 U.S. Army Entomology Training Course, San Antonio, TX, February.

Turell, M. J., C. L. Bailey, M. F. Faran, K. Linthicum, and L. Patrican. 1987. Competence of selected African mosquito species for Rift Valley fever virus. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene in Los Angeles, CA., November-December.

PUBLICATIONS

Faran, M. E., W. S. Romoser, R. G. Routier, and C. L. Bailey. 1988. The distribution of Rift Valley fever virus in the mosquito *Culex pipiens* as revealed by viral titration of dissected organs and tissues. *Am. J. Trop. Med. Hyg.* (In Press).

Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker. 1988. Use of remote sensing to predict the threat of vector-borne diseases to military deployment. *Proceedings of the 1988 Army Science Conference.* (In Press).

Linthicum, K. J., C. L. Bailey, F. G. Davies, C. J. Tucker. 1988. Use of satellite remote sensing imagery to predict Rift Valley fever virus activity in Kenya, pp. . *In* Viral diseases in Africa. Proceedings of the Scientific, Technical and Research Commission of the Organization of African Unity Symposium on Viral Diseases in Africa Affecting Plants, Animals and Man. Nairobi, Kenya. (In Press).

Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker. 1988. Use of remote sensing to monitor the threat of vector-borne diseases. *Proceedings of the U.S. Army Medical Department Biennial Medical Entomology Training Course*, pp. 64.

Linthicum, K. J., F. G. Davies, A. Kairo, and C. L. Bailey. 1988. Rift Valley fever virus disease in Kenya, pp. . *In* viral diseases in Africa. Proceedings of the Scientific, Technical and Research Commission of the Organization of African Unity Symposium on Viral Disease in Africa Affecting Plants, Animals and Man. Nairobi, Kenya. (In Press).

Logan, T. M., K. J. Linthicum, C. L. Bailey, D. W. Watts, and J. P. Moulton. 1988. Experimental transmission of Crimean-Congo hemorrhagic fever virus (Family Bunyaviridae, Genus *Nairovirus*) by *Hyalomma truncatum* Koch. *Am. J. Trop. Med. Hyg.* (In Press).

Turell, M. J., and J. R. Beaman. 1988. Vector competence of a Houston, Texas strain of *Aedes albopictus* for Rift Valley fever virus. *J. Am. Mosq. Cont. Assoc.* 4:94-96.

Turell, M. J., M. E. Faran, M. Cornet, and C. L. Bailey. 1988. Vector competence of Senegalese *Aedes fowleri* (Diptera: Culicidae) for Rift Valley fever virus. *J. Med. Entomol.* 25:262-266.

Watts, D. M., T. G. Ksiazek, K. J. Linthicum, and J. Hoogstraal. 1988. Crimean-Congo hemorrhagic fever, pp. . *In* Epidemiology of arthropod-borne viral diseases, T. P. Monath (Ed.), CRC Press, Inc. (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA313365	01 Oct 88	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	623105A	P6231050H29	AE	035
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- C1			

11. TITLE (Precede with Security Classification Code) Advanced studies for the development of therapeutics against primate retroviruses

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 05	91 04	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	0.5	100
c. TYPE	d. AMOUNT	89	2.0	275
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Aids; (U) Drug Development; (U) Animal Models; (U) Immunomodulators; (U) HIV; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. To develop prophylactic and therapeutic approaches to AIDS. This work may provide important medical benefits to soldiers at risk for this disease. A literature search has been done. DTIC search has been filed in the 1498 file.

24. Primate models for human AIDS are developed, with simian immunodeficiency viruses and related lentiviral strains. Virological, immunological, and pathology parameters of infection establish staging criteria of disease severity analogous to human AIDS. At various stages, the efficacy of intervention with antiviral drugs, immunomodulators, and candidate vaccines is assessed.

25. 8710 - 8809 Two simian immunodeficiency viral (SIV) strains were selected for model development. The PBj strain, isolated from sooty mangabes, initially infected 6 of 6 rhesus monkeys, but killed only one of the animals acutely. However, upon subsequent passage, this virus killed 5 of 6 juvenile rhesus monkeys within 14 days. Lethality was related to dehydration with fulminant diarrhea, rather than immunosuppression; however, the model has potential application for rapid indications of treatment efficacy against lentiviruses. A second SIV strain, E11-S, infected all rhesus monkeys inoculated; all are expected to die in 12-14 months, after passing through stages analogous to human AIDS. Virology assays based on replication in primary human peripheral blood leukocytes, as indicated by reverse transcriptase and p-24 antigen expression, have been adapted to both the PBj and E11-S strains. Immunology assays, based on ELISA and the western blot, are being adapted. A clinical trial for AZT in the PBj model is being developed.

DD FORM 1498, 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. P6231050H29: Medical Protection Against Retrovirus

WORK UNIT NO. H29-AE-035: Advanced Studies for the Development of Therapeutics Against Primate Retroviruses

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

BACKGROUND

The importance of research leading to the treatment and prevention of acquired immune deficiency syndrome (AIDS) is universally acknowledged. The development of realistic animal models for AIDS is critical to this effort. We are in the process of developing models in primates and plan to use these animals to test antiviral drugs, immunotherapy regimens, and candidate vaccines. Preliminary to such testing, the models must be sufficiently well characterized with respect to quantitative measurements of virology, immunology, clinical, and pathology parameters to validate them as authentic models of human AIDS. The development of staging criteria for disease progression, analogous to the Walter Reed staging system (1) used to grade human immunodeficiency viral (HIV-1) infections is an immediate goal of our model development research. Since the initial isolation of an authentic lentivirus, now known as simian immunodeficiency virus (SIV), from a rhesus macaque at the New England Primate Research Center, there has been a rapid expansion of SIV isolates, model systems, and data. Much of this is very relevant to HIV infections in humans, and many of the recently developed models are potentially more useful than the HIV chimpanzee model for testing immunization and therapeutic strategies.

SUMMARY

A variant of SIV (SMM/PBj), isolated from a chronically infected pigtailed macaque at the Yerkes primate Research Center, was shown in previous studies to produce acutely fatal disease in pigtail macaques. The present study extends investigation of SMM/PBJ pathogenesis to rhesus monkeys. Six rhesus monkeys (three juveniles plus three adults) were inoculated iv with supernatant fluid contained three tissue culture infectious doses (3 $TCID_{50}$) from human peripheral blood lymphocyte (HPBL) cultures co-cultivated with spleen cells from the original pigtailed macaque (PBj). All animals seroconverted, but only one rhesus (a juvenile, #4EN) died acutely (day 10). Homogenized tissues from 4EN were passaged to additional monkeys in an attempt to increase lethality. Five of six juveniles receiving 100 $TCID_{50}$ of 4EN spleen or lymph node developed acute disease; four died (days 8 - 10), one recovered (after hydration), and one remained asymptomatic, as did three of three adults. Clinical disease was charac-

terized by diffuse, severe lymphadenopathy with 5 days of inoculation and severe diarrhea beginning 10 to 3 days before death. Anorexia,lymphopenia (1000/mm^3), and mild hypoalbuminemia preceded onset of diarrhea by 24 h, while all other hematology and clinical chemistry parameters remained within normal limits. Aerobic and anaerobic blood cultures were negative, and there was no evidence of opportunistic viral or bacterial agents in gastrointestinal or lymphoid tissues by light or electron microscopy, or in feces by culture, ELISA, or electron microscopy. Necropsy revealed two- to tenfold increases in all lymphoid tissues; most severely affected were spleen, mesenteric lymph nodes, and gut-associated lymphoid tissues. In the gastrointestinal tract, there was severe multifocal to patchy villus blunting and fusion, with crypt hyperplasia in the small intestine, and disorganization of mucosal epithelial structure in the stomach and colon; there was no evidence of ulceration. Viral burdens, as measured by infectivity and p24 antigen in peripheral blood mononuclear cells, serum, and tissues, are being correlated with disease severity, as are concentrations of cytokines. The role of soluble mediators in pathogenesis of SIV, and the possible beneficial effects of cytokine antagonists in reduction of SIV disease are being investigated.

A second SIV strain (E11-S), originally isolated from pigtailed macaques, was also studied. This SIV strain infects all rhesus and cynomolgous macaques inoculated with 10,000 $TCID_{50}$ of virus. Death occurred in 12 to 14 months, and all animals passed through stages of disease progression analogous to human AIDS, although the time course was accelerated. A titration is being planned to determine the minimal infectious and lethal doses of this viral seed, prior to initiation of challenge studies for drug and vaccine efficacy trials. In addition, two infectious, molecular clones are now available for in-vivo primate testing. For both PBj and E11-S isolates, standard virology and immunology assays have been adapted to measure critical events in the pathogenesis of these infections. Likewise, immunohistochemistry and in-situ hybridization are being developed to define critical target tissues with more precision.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302630	26 May 89	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AA	130
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89-01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for the development of medical defensive countermeasures to toxins of biological origin

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER				
c. TYPE	d. AMOUNT	88	2.0	611
e. KIND OF AWARD	f. CUM/TOTAL	89	2.0	357

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Friedlander A M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) York, C G
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Hines, H B

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Low Molecular Weight Toxins; (U) Rapid Detection; (U) Vaccines; (U) Lab Animals; (U)Mice; RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Elucidate pathogenesis of intoxications induced by aerosols, to include determination of the sequence of events leading to protective immunity. Data obtained will provide the basis for evaluation of prophylactic and therapeutic regimens developed to protect deployed US forces. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Develop animal models and define the clinical, pathological, and immunological changes during intoxications. Characterize immune defenses within the respiratory tract. Information is used to provide basis for efficacy of vaccination and therapy procedures.

25. (U) 8710 - 8809 Analytic methods, including gas chromatography-mass spectrometry and high-pressure liquid chromatography, have been optimized for the detection of mycotoxins, saxitoxin and neosaxitoxin, and the brevetoxins. Several of the low molecular weight toxins, including mycotoxin (T2), saxitoxin, and microcystin, alter arachidonic acid metabolism in cultured cells. Testing of our current mobile aerosol chamber has demonstrated minimal leakage from the nose-only exposure system into the Hazelton cabinet during an aerosol exposure. Preliminary designs of a gas-tight, mobile aerosol exposure cabinet have been developed.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AA-130: Exploratory Studies for the Development of Medical Defensive Countermeasures to Toxins of Biological Origin

PRINICIPAL INVESTIGATOR: A. M. Friedlander, COL, M.D.

ASSOCIATE INVESTIGATORS: J. L. Middlebrook, Ph.D.
H. B. Hines, Ph.D.
S. M. Naseem, Ph.D.
C. G. York, M.S.

BACKGROUND

Medical defense, in the form of of therapeutic drugs or vaccines to protect against a potential biological warfare agent, must be effective when the agent is delivered by aerosol. Our investigations are designed to define the respiratory toxicity of potential agents, to identify and characterize commercially available equipment that will simplify the conduct of such studies, to develop new equipment when necessary, and to develop analytical chemistry methodologies for potential toxin threats.

SUMMARY

Gas chromatography-mass spectrometry (GC-MS) methods for analysis of mycotoxins in urine have now been developed with detection limits in the 1 ng/ml range.

A high-performance liquid chromatography (HPLC) assay for saxitoxin and neosaxitoxin has been developed and used for their direct detection in urine at the 0.1 and 1 mg/ml range, respectively. The assay has also been useful for determining the stability of saxitoxin and other gonyautoxins under storage conditions. Additional studies of oxidation products of saxitoxin and urinary metabolites of saxitoxin have begun.

Mass spectrometry and HPLC have been developed to detect brevetoxins in the 0.1 to 5 mg/ml range, respectively. In-vitro metabolism of brevetoxin by rat hepatocytes has been observed and is being investigated.

Continuing studies have demonstrated that certain low molecular weight toxins (T2, saxitoxin, and microcystin) affect regulation of arachidonic acid metabolism and inflammation in alveolar macrophages and hepatocytes.

The present mobile aerosol chamber has been tested and demonstrated to show minimal leakage

from the nose-only exposure system into the contained environment of the Hazelton cabinet. Preliminary designs of a gastight mobile exposure cabinet have been submitted.

PRESENTATIONS

Naseem, S. M., H. B. Hines, D. A. Creasia, and K. A. Mereish. 1988. Comparative effects of toxins on arachidonic acid release and metabolism in cultured rat hepatocytes and alveolar macrophages. Presented at the Annual Meeting of the Federation of American Societies for Experimental Biology and Medicine, Las Vegas, Nevada, May.

Hines, H. B., M. A. Poli, and J. G. Pace. 1987. The characterization of brevetoxin PbTx3 hepatic metabolites. Presented at the Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, Detroit, Michigan, October.

PUBLICATIONS

Poli, M.A., C. B. Templeton, J. G. Pace, and H. B. Hines. 1988. Investigations of the brevetoxins. *In* Proceedings of the American Chemical Society Symposia (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DAOG3810	01 Oct 88	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AB	150
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for the development of medical defensive countermeasures to infectious agents of biological origin

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
80 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	11.0	2308
c. TYPE	d. AMOUNT	89	11.0	2415
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division,USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Linthicum, K J
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Liu, C T
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Turell, M J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Arboviruses; (U)Anthrax (U) Vaccines; (U) Lab Animals; (U) Mice (U) Rats; (U) Guinea Pigs; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the disease spectrum of arboviruses to include vector and reservoir competence, pathogenesis of viral strains, and therapy and immunoprophylaxis. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Longitudinal epidemiological studies correlate clinical manifestations with seroconversions and identify sites for in-depth ecological studies to recover viral strains from reservoirs. Ecological and genetic factors relating to vector and reservoir competence are studied under controlled conditions.

25. (U) 8710-8809 In laboratory studies we demonstrated that, in Pichinde virus-infected animals, capillary permeability to albumin was inhibited to such an extent that effective tissue perfusion with nutrients became questionable for maintaining life. A project was started to study unique biochemical changes in plasma from Pichinde virus-infected animals to determine criteria for early and rapid diagnosis of arenaviral infection in man. For the first time in laboratory studies, a sandfly (*Phlebotomus duboscqi*) was shown to be a competent vector of Rift Valley fever (RVF). Egyptian and Senegalese strains of RVF virus were found to form reassortants with the S and M segments of RNA in mosquitoes. Mosquito inoculation with certain toxins was found to be an efficient bioassay system. In laboratory studies *Aedes albopictus* mosquitoes were found to be a competent vector for RVF virus. For the first time in laboratory studies, Venezuelan equine encephalitis virus was shown to replicate in a tick, and be transmitted vertically to subsequent stages, and horizontally to a vertebrate host during feeding.

DD FORM 1498 83 MAR   EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AB-150: Exploratory Studies for the Development of Medical Defensive Countermeasures to Infectious Agents of Biological Origin

PRINCIPAL INVESTIGATOR: K. J. Linthicum, MAJ, Ph.D.

ASSOCIATE INVESTIGATORS: M. J. Turell, Ph.D.
C. T. Liu, Ph.D.
T. M. Logan, CPT, Ph.D.
S. W. Gordon, CPT

BACKGROUND

A number of highly pathogenic viruses, with invertebrate and vertebrate vectors, are known to be potential threats to United States military personnel in endemic areas. Many of these viruses cause severe human disease, mainly hemorrhagic fevers, which include Rift Valley fever (RVF), Crimean-Congo Hemorrhagic fever (CCHF), and Dugbe. In most cases, little is known about the epidemiology and life cycles of these viruses or about the pathogenesis, therapy, or immunoprophylaxis of the viral diseases. The main emphasis of this work unit is to evaluate the natural threat and impact of this group of highly pathogenic viruses by accurately establishing the incidence and distribution of apparent and unapparent infections and determining epidemiological, ecological, and agent-related factors influencing endemic viral activity; and to alter the highly pathogenic nature of these agents by defining the pathogenic mechanism involved in the disease processes and developing therapeutic measures to intervene in hemorrhagic fever.

SUMMARY

For the first time in laboratory studies, a sand fly (*Phlebotomus duboscqi*) was shown to be a competent vector of RVF after both oral exposure and intrathoracic inoculation. Also,for the first time, in laboratory a tick (*Hyalomma truncatum*) was shown to be a competent vector of RVF after intracoelomic inoculation.

In laboratory studies, we demonstrated that in Pichinde virus-infected strain 13 guinea pigs, capillary permeability to albumin and albumin returning to the circulation were inhibited to such an extent that effective tissue perfusion with nutrients became questionable for maintaining life. A project was started to study unique biochemical changes in plasma from Pichinde virus-infected

animals to determine criteria for early and rapid diagnosis of arenaviral infection in man.

Two *Aedes* mosquito species, *Ae. fowleri* and *Ae. albopictus*, were found to be competent vectors of RVF virus after oral exposure. The stage of *Anopheles albimanus* at the time of infection with RVF virus was found to be critical for this mosquito's ability to transmit this virus.

In the laboratory we demonstrated that environmental temperature significantly affected the ability of *Aedes* mosquitoes to transmit Ockelbo virus. In field studies we showed, for the first time, that ducks and geese are naturally infected with Ockelbo virus and that *Ae. excrucians* is a competent vector of this virus.

MGLJ — Studies of Arbovirus Infection, Dissemination, and Transmission in Vectors

PRINCIPAL INVESTIGATOR: K. J. Linthicum, MAJ, Ph.D.

ASSOCIATE INVESTIGATOR: T. M. Logan, CPT, Ph.D.
S. W. Gordon, CPT

AND

MGLM — Experimental Transmission of CCHF Virus by Ticks

PRINCIPAL INVESTIGATOR: T. M. Logan, CPT, Ph.D.

ASSOCIATE INVESTIGATOR: K. J. Linthicum, MAJ, Ph.D.
S. W. Gordon, CPT

Laboratory studies have demonstrated that several species of *Hyalomma* ticks are competent vectors of CCHF virus after intracoelomic (IC) inoculation of the virus. Transstadial and horizontal transmission of CCHF virus has been found to occur in orally exposed *H. truncatum* and *H. impeltatum*. Dugbe virus replicated in and was transmitted by several African and North American species of ticks after IC inoculation. For the first time in a tick species, Venezuelan equine encephalomyelitis virus has been shown to replicate in and be transmitted by *Amblyomma cajennense*, a principally neotropical species.

The role that particular species of ticks play in the ecology of CCHF virus in nature is determined, in part, by specific aspects of their life cycle. Only a few of the 29 species of ticks from which CCHF virus has been isolated in nature have had their life cycles studied in depth. The life cycle of *H. impeltatum* has been documented in the laboratory by closely following specimens from the egg to the adult stage. The life cycle required an average of 108 days to complete and statistics measured include weight and size, pre-feeding periods, feeding and premolt intervals, and oviposition and incubation parameters.

Hyalomma truncatum ticks were found to be competent vectors of RVF virus after IC inoculation. The virus passed transstadially from the nymph to the adult stage. This was in contrast to *Rhipicephalus appendiculatus*, which was shown to be an incompetent vector.

Based on a study of field-collected adult *Ae. mcintoshi* (a mosquito involved in the enzootic cycle of RVF) given a blood meal and analyzed immunocytochemically at different times post-blood meal, the RVF mosquito tissue tropisms in *Ae. mcintoshi* appear to be similar to those found in *Culex pipiens*. However, the dynamics of tissue infection in the African species appear to be different. Specimens intrathoracically inoculated with RVF virus, allowed to go through at least two cycles of blood-feeding/oviposition, and then prepared for immunocytochemical examination revealed four instances of single RVF antigen-positive eggs in different individuals, indicating that Ae. mcintoshi eggs can become infected with virus.

MGLC Pathophysiology of Arenavirus Disease

PRINCIPAL INVESTIGATOR: C. T. Liu, Ph.D.

We demonstrated that over-all capillary permeability to albumin was not significantly increased in Pichinde virus-infected strain 13 guinea pigs. Despite development of pulmonary edema in infected animals, the definite absence of systemic edema post infection further supports this conclusion.

Studies were designed and performed to study the transport of labeled albumin in a reversed direction compared to capillary leakage from the circulation to the interstitium. Results suggest that in Pichinde-infected animals, effective tissue perfusion with nutrients be come questionable for maintaining life.

Techniques were developed for exercising strain 13 guinea pigs on a treadmill. Long-term daily exercise decreased the animal growth and metabolic rates without marked changes in rectal and body surface temperatures. Monitoring effects of exercise on Pichinde virus-infected strain 13 guinea pigs served as a model for studying pathogenesis of arenavirus-induced hemorrhagic fevers under stress conditions.

An assay system for measuring tissue Na-K-Mg-H ATPase was developed to ascertain the locus of action producing death in arenaviral infection. A separate study was initiated to find unique biochemical plasma changes in Pichinde virus-infected guinea pigs and ultimately to establish specific criteria for early and rapid diagnosis of arenaviral infection in man.

A buffered, polyethylene glycol solvent mixture for dissolving a potent leukotriene antagonist for treating Pichinde virus-infected guinea pigs was injected into animals to determine their tolerance for the antagonist. Although the animals' growth rates were inhibited, they appeared active and gained body weight above pre-injection level.

MGLK — Factors Affecting Vector Competence of Mosquitoes for Rift Valley Fever Virus

PRINCIPAL INVESTIGATOR: M. J. Turell, Ph.D.

Aedes fowleri, a mosquito associated with ground pools in Senegal and other places in West Africa, was shown to be a competent vector for RVF virus. *Aedes albopictus*, the "tiger mosquito" recently introduced into North and South America, was also shown to be a competent vector of RVF virus.

In laboratory studies, using a precisely controlled environmental chamber, we showed that environmental temperature significantly affected the ability of several *Aedes* species to transmit Ockelbo virus. In field studies in Sweden, we demonstrated, for the first time, that ducks and geese are naturally infected with Ockelbo virus and that *Ae. excrucians* and *Ae. cinereus* are competent vectors of this virus.

We determined that inoculation of mosquitoes is an efficient bioassay system for certain classes of toxins and antitoxins. This may allow mosquito inoculation to replace mouse inoculation for selected studies.

We demonstrated, for the first time, that *Phlebotomus duboscqi* is a competent vector of RVF virus. These results are in contrast to the failure of a neotropical species to transmit RVF after oral exposure in laboratory studies.

We found that the stage of *Anopheles albimanus* at the time of infection with RVF virus is critical to this mosquito's ability to transmit this virus. Mosquitoes infected as larvae were efficient transmitters of RVF virus, while those infected as adults were unable to transmit virus.

The ability of an Egyptian and a Senegalese strain of RVF virus to form reassortants with S and M segments of RNA in *Culex pipiens* mosquitoes was demonstrated. These mosquitoes then transmitted the reassortants, as well as the parental types, to hamsters by bite.

PRESENTATIONS

Turell, M. J., C. L. Bailey, M. F. Faran, K. Linthicum, and L. Patrican. 1987. Competence of selected African mosquito species for Rift Valley fever virus. Presented at the 1987 Annual Meeting of the American Society of Tropical Medicine and Hygiene in Los Angeles, CA, November-December.

Romoser, W. S., K. Lerdthusnee, L. A. Patrican, K. J. Linthicum, D. Dohm, C. L. Bailey. 1987. An immunocytochemical study of Rift Valley Fever virus tissue tropisms in selected African mosquitoes. Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Linthicum, K. J., T. M. Logan, C. L. Bailey, D. M. Watts, and D. J. Dohm. 1987. Experimental infection of Dugbe virus (Family Bunyaviridae, Genus *Nairovirus*) in five North American and one African ixodid tick. Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Logan, T. M., K. J. Linthicum, C. L. Bailey, D. M. Watts, and J. Moulton. 1987. Transstadial transmission of Crimean-Congo Hemorrhagic fever virus in orally infected *Hyalomma truncatum* with observations on virus replication in *Rhipicephalus appendiculatus*. Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene. Los Angeles, CA, November-December.

Romoser, W. S., K. Lerdthusnee, K. J. Linthicum, D. Dohm, and C. L. Bailey. 1988. Additional evidence for Rift Valley fever virus dissemination via cells at the foregut-midgut junction. Presented at the Annual Meeting of the American Mosquito Control Association.

Linthicum, K. J., C. L. Bailey, F. G. Davies, A. Kairo, T. Logan. 1988. Observations on the horizontal distribution of ground pool *Aedes* pupae within a flooded dambo in Kenya. Presented at the Annual Meeting of the American Mosquito Control Association.

Patrican, L. A., and C. L. Bailey. 1987. Influence of immune blood in the infectivity of *Culex pipiens, Aedes fowleri*, and *Aedes mcintoshi* mosquitoes with Rift Valley fever virus. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Romoser, W. S., K. Lerdthusnee, M. E. Faran, K. J. Linthicum, L. A. Patrican, and C. L. Bailey. 1988.Immunocytochemical and ultrastructural studies of Rift Valley fever virus in selected African mosquito species. Presented at the XVIII International Congress of Entomology, Vancouver, Canada, July.

Romoser, W. S., K. Lerdthusnee, K. J. Linthicum, D. J. Dohm, and C. L. Bailey. 1988. Additional evidence for Rift Valley fever virus dissemination via cells at the foregut-midgut junction. Presented at the Annual Meeting of the American Mosquito Control Association, Denver, CO, February.

Rossi, C. A., M. J. Turell, and C. L. Bailey. 1987. Characterization mutant strains of Rift Valley fever virus. Presented at the 1987 Annual Meeting of the American Society of Tropical Medicine and Hygiene in Los Angeles, CA, November-December.

Liu, C. T. 1988. Body surface areas and K values in strain 13 guinea pigs with different weights and ages. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology and Medicine, Las Vegas, NV, May.

Pinter, G. G., C. T. Liu, and C. J. Peters. 1988. Capillary permeability and lymphatic hemodynamics in Pichinde virus-infected guinea pigs. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology and Medicine, Las Vegas, NV, May.

Logan, T. M. 1988. Crimean-Congo Hemorrhagic Fever. Presented to 1988 U.S. Army Medical Entomology Training Course, San Antonio, TX, February.

PUBLICATIONS

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA308918	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AD	131
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for the development of vaccines against infectious agents of potential BW threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	8.0	1729
c. TYPE	d. AMOUNT	89	8.0	1867
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Kenyon, R H
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Crumrine, M H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Vaccines; (U) Lab Animals; (U) Guinea Pigs; (U) Monkeys; (U) RA I;(U)Rodents

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To isolate, study, and characterize agents of potential BW threat. To obtain immunogens that elicit protective immunity and to devise effective regimens to protect US military personnel in the field. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Naturally occurring and laboratory-derived strains are molecularly and biologically characterized and assessed for virulence or attenuation in susceptible animal models. Attenuated strains are tested for ability to induce cross-reactions with virulent strains. Protective immunity elicited by inactivated antigens is determined.

25. (U) 8710 - 8809 Cross-protective relationships among newly isolated, Lassa-like (LV) viruses, including LCM strains, revealed immunological enhancement with certain combinations, and may impact on the design of LV vaccines. Aerosol infectivity studies of Junin virus established parameters for testing vaccine efficacy against aerosol challenge. Congo-Crimean hemorrhagic fever virus (CCHF) animal model development now includes SCID mice. In anthrax studies, protective antigen (PA) was tested in combination with the adjuvant, "Tri-mix," as a prototype vaccine in guinea pigs; complete protection against *B. anthracis* spores and ELISA titers >20,000 were elicited. Differentiation and sporogenesis of cell variants of *C. burnetii* were shown to be regulated by host cell factors; avirulent strains were more susceptible to non-immunological, host-microbicidal factors, and the distribution of *C. burnetii* variants within infected cells also correlated with virulence. An attenuated Rift Valley fever (RVF) viral strain (T-1) immunized mice exposed orally and conferred partial protection against aerosol challenge. The genetic basis for anaphylaxis to formalin-inactivated RVF vaccine in mice was also studied. VEE virus bound directly to oligodendrogliocytes, resulting in direct invasion of olfactory bulbs, thus avoiding passive immunization and incomplete immunization by formalin-inactivated VEE vaccines.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AD-131: Exploratory Vaccine Development Studies on Conventional Agents of Potential BW Threat

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

ASSOCIATE INVESTIGATORS: A. O. Anderson, COL, M.D.
M. H. Crumrine, LTC, Ph.D.
A. M. Friedlander, COL, M.D.
R. H. Kenyon, Ph.D.

BACKGROUND

Basic studies on the pathophysiology and immunology of conventional agents of potential biological warfare threat are essential to the systematic development of protective vaccines. Among the arenaviruses, four are significant human pathogens. Lassa virus causes severe, often fatal Lassa fever in tens of thousands of patients in West Africa annually. Junin and Machupo viruses are associated with the Argentine and Bolivian hemorrhagic fevers, respectively, and have the documented potential to cause devastating outbreaks in South America. Lymphocytic choriomeningitis virus (LCMV) is distributed world-wide and has caused significant morbidity in human populations naturally exposed to aerosols from infected animals. All these viruses are highly infectious via the aerosol route and have the demonstrated potential to cause explosive outbreaks under artificial conditions. The aerosol potentials for anthrax and Q-fever (caused by *Coxiella burnetii*) are also well documented. An understanding of the protective immune mechanisms, especially mucosal and cellular immune responses responsible for recovery and protection against acute disease, are prerequisite for the development of effective vaccines and therapeutic measures, and are the focal points for this research.

SUMMARY

Cross-protective relationships among newly isolated, Lassa-like viruses, including LCMV strains in monkeys and guinea pigs, revealed immunological enhancement with certain combinations, and may impact of the design of Lassa viral vaccines. Aerosol infectivity studies of Junin virus established parameters for testing vaccine efficacy against aerosol challenge. Congo-Crimean hemorrhagic fever (CCHF) animal model development was expanded to include SCID (severe, combined immunodeficiency) mice. In anthrax studies, protective antigen (PA) was tested in combination with the adjuvant "Tri-mix" as a prototype vaccine in guinea pigs; complete protection

against *Bacillus anthracis* spores and ELISA titers > 20,000 was elicited. Differentiation and sporogenesis of cell variants of *C. burnetii* were shown to be regulated by host cell factors; avirulent strains were more susceptible to non-immunological, host microbicidal factors and the distribution of *C. burnetii* variants within infected cells also correlated with virulence. An attenuated Rift Valley fever viral (RVFV) strain (T-1) immunized mice exposed orally and conferred partial protection against aerosol challenge. The genetic basis for anaphylaxis due to formalin-inactivated RVFV vaccine in mice was also studied. Venezuelan equine encephalomyelitis (VEE) virus was shown to bind directly to oligodendrogliocytes, resulting in direct invasion of olfactory bulbs, thus circumventing passive immunization and incomplete immunization by formalin-inactivated VEE vaccine.

MGLD Characteristics of Virulent and Attenuated Junin Virus Infections

PRINCIPAL INVESTIGATOR: R. H. Kenyon, Ph.D.

We have developed a technique to estimate the numbers of lymphocytes recognizing Junin viral antigen in the blood of human vaccinees. By limiting-dilution analysis, we estimated the frequency of Junin antigen-recognizing cells to be from 1 in 6,000 to 1 in 30,000 by the end of the second month post vaccination. The numbers of these cells began to decline after the second month post vaccination. We detected between 1 in 12,000 to 1 in 75,000 cells in the few individuals we monitored after 1 yr post vaccination. An individual with clinical Argentine hemorrhagic fever 30 years ago showed a frequency of 1 reactive cell in 40,000.

We infected rhesus monkeys with Junin virus by an aerosol route. The disease pattern was similar to that seen when monkeys were infected peripherally. There were very high levels of α-interferon in these monkeys, but no γ-interferon was detected

Studies continued for the search to find an animal model for CCHF. We investigated the pathogenesis of CCHF virus in suckling mice inoculated i.p. All mice died by day 7 post inoculation; virus was detected, by plaque formation and immunofluorescence techniques, predominantly in liver throughout the infection, and in the brain late in infection. The most commonly observed lesions in liver tissue sections were necrosis of hepatocytes and extramedullary hematopoietic tissue throughout the infection, and vasculitis and meningoencephalitis of brain and meninges late in infection. Adult SCID mice were also infected with CCHF virus and 20% died from the infection. Virus was found predominantly in liver, lungs, brain, and adrenals beginning on day 13. Viral titers increased until death of the mice on days 24 to 35, or until resolution of the infection. We also infected rhesus, African green, and patas monkeys with CCHF virus. About half the monkeys became viremic only on days 3 to 5, but none showed signs of illness.

MGDA Exploratory Research for the Protection Against Anthrax

PRINCIPAL INVESTIGATOR: M. H. Crumrine, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: J. W. Ezzell, Ph.D.
B. E. Ivins, Ph.D.

Enzyme-linked immunosorbent assays have been developed for detection of antibody to purified *Yersinia pestis* F1 antigen and a surface carbohydrate antigen of *Francisella tularensis* in sera from patients and vaccinees. Monoclonal antibodies to the *B. anthracis* polysaccharide, which are highly specific for anthrax vegetative cells, were produced as ascites fluid (approximately 200 ml) and conjugated to fluorescein and horseradish peroxidase. The monoclonal antibodies have proven to be valuable in identifying strains of *B. anthracis* with no cross reactions with other *Bacillus* species. "Tri-mix" is a new immunological adjuvant from Ribi Immunochem Research, and consists of monophosphoryl lipid A, trehalose dimycolate, and the cell wall skeleton of *B. globigii* (BCG) tubercle bacillus. The new adjuvant was tested with PA as a prototype vaccine in guinea pigs. A single injection of the mixture provided complete protection 10 weeks later to a challenge of 7,000 B. anthracis Ames spores and elicited anti-PA ELISA titers averaging > 20,000.

MGBI *Coxiella burnetii* Genetic and Cellular Aspects

PRINCIPAL INVESTIGATOR: A. M. Friedlander, COL, Ph.D.

ASSOCIATE INVESTIGATORS: J. C. Williams, Ph.D.
T. McCaul, Ph.D.

Differentiation and sporogenesis of cell variants of the obligate intracellular, bacterial parasite, *C. burnetii*, are thought to be regulated by host cell factors. To study this phenomenon, we infected 14 different cell lines with virulent and avirulent strains of *C. burnetii*. In continuous cultures, avirulent strains were eventually eliminated, while virulent strains persisted indefinitely. This may indicate that the avirulent strain is more readily affected by non-immunological host microbicidal factors. These preliminary experiments also revealed that all cell variants, including endospores, are present in cultured cells. In companion experiments we studied the factors controlling persistence and chronicity of in-vivo infections. Electron microscopy revealed that organisms in infected mouse spleen cells reside, not only in specific intracellular phagolysosomes, as reported by others, but also in the cytosol proper. These initial observations also showed that the *C. burnetii* cell variants differed in their location within the infected cell and in their eventual disposition. Improved understanding of the determinants of cell variation of *C. burnetii* will help in the development of improved vaccines.

MGBA Mucosal Immunity: Response to Aerosol Challenge with Toxins, Microbes, and other Biological Agents; Immunomodulators and Mucosal Immunity

PRINCIPAL INVESTIGATOR: A. O. Anderson, COL, M.D.

ASSOCIATE INVESTIGATORS: M. T. Vahey, Ph.D.
M. L. M. Pitt, Ph.D.

Oral infectivity of RVFV in A/J mice was studied. This virus is hepatotropic, neurotropic and is both arthropod- and aerosol-transmissible. Our previous studies with formalin-inactivated RVFV established that both peripheral and mucosal immunity are required for complete protection, especially after aerosol exposure. In current studies, we have been testing the feasibility of developing a live-attenuated oral vaccine for Rift Valley fever. Both wild-type (ZH-501) and attenuated (T-1) RVFV strains were stable in water up to 3 days. Mice ingesting water containing graded doses of ZH-501 had dose-related mortality. Mice exposed to T-1 in water did not become ill. Compared to unvaccinated controls, there was a prolonged mean time-to-death in mice orally exposed to 10^5 PFU per ml of T-1 after aerosol challenge with 10 LD_{50} of ZH-501. These data suggest that there is a potential for developing an oral RVFV vaccine.

Recombinant DNA technology was used to analyze the genetic basis of immunoglobulin isotype (IgE vs IgA) commitment in a mouse model of anaphylactic allergy to formalin-inactivated Rift Valley fever vaccine. All C3H/Hej mice primed i.p. with RVFV vaccine (NDBR-103) died upon secondary exposure to the vaccine, while C3H/HeJ, Balb/cByJ, BD/F1, and Swiss-Webster mice were resistant. Total RNA was isolated from the lung, large intestine, spleen, peripheral lymph nodes, mesenteric lymph nodes, and mediastinal lymph nodes of five mouse strains, including C3h/H3J, C3H/OuJ, Balb/cByJ, BD/F1, and Swiss-Webster. Slot blots and Northern gel blots were run on the RNA samples. Preliminary interpretation of the results indicate that RVFV vaccination increased e-chain expression in the lungs of C3H/HeJ mice but not C3H/OuJ; and there was a reciprocal relationship between a-chain mRNA and e-chain mRNA expression in all tissues of these strains.

Multiuse probes for in-situ detection of dengue 2 viral genomic products and of RVFV genomic and replicative products have been developed. Both vector systems have been characterized for incorporation of either radioisotope or biotin analogs. Analyses of cultured-cell extracts show cross hybridization to these probes.

In frozen sections of mouse brain, VEE virus binds specifically to the oligodendrogliocytes that sheath extra- and intracerebral nerve tracts associated with cranial nerves 1 and 2, and communicating fibers of the pyramidal system. Live, Trinidad-donkey-strain VEE aerosols produced direct infection of the olfactory bulbs a brain by neural transmission in non-immune and passively immunized mice. A 17-Kd polypeptide from normal mouse brain homogenate has been shown to bind specifically to labeled VEE in Western blots. this binding could be blocked by pretreating the homogenate with unlabeled VEE.

PRESENTATIONS

Barrera Oro, J. G., H. W. Lupton, P. B. Jahrling, J. Meegan, R. H. Kenyon, and C. J. Peters. 1988. Cross-protection against Machupo virus with Candid #1 live-attenuated Junin virus vaccine. I. The post vaccination prechallenge immune response. Presented at the 2nd International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar del Plata, Argentina, March.

Cosgriff, T. M., D. B. Parrish, R. M. Lewis, P. B. Jahrling, J. W. Huggins, and C. J. Peters. 1987. The original hemorrhagic fever revisited: hemostatic derangement in rhesus monkeys infected with yellow fever virus. Presented at the 29th Annual Meeting of the American Society for Hematology, Washington, D. C., December.

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Jahrling, P. B., R. W. Trotter, J. C. Barrera Oro, H. W. Lupton, T. M. Cosgriff, and C. J. Peters. 1988. Cross-protection against Machupo with Candid #1 live-attenuated Junin virus vaccine. Presented at the 7th Annual Meeting of the American Society for Virology, Austin, TX, June.

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McCaul, T. F., C. E. Snyder, Jr., and J. C. Williams. 1988, Immunocytochemical detection of an immunogenic protein of *Coxiella burnetii* by monoclonal antibodies. Presented at the 9th European Congress on Electron Microscopy, York, U.K., September.

McCaul, T. F., H. A. Thompson, and J. C. Williams. 1988. Ultrastructural studies of sporulation of *Coxiella burnetii* Presented at the Annual Meeting of the American Society of Rickettsiology and Rickettsial Diseases, Santa Fe, NM, April.

McCaul, T. F., and J. C. Williams. 1988. Immunocytochemical detection of antigenic determinants of *Coxiella burnetii.* Presented at the 88th Annual Meeting of the American Society for Microbiology, Miami Beach, FL, May.

McCaul, T. F., and J. C. Williams. 1988. Ultrastructural localization of carbohydrate determinants of lipopolysaccharide of *Coxiella burnetii* (Phase I) by monoclonal antibodies. Presented at the Annual Meeting of the Electron Microscopy Society of America, Milwaukee, WI, August.

PUBLICATIONS

Anderson, A. O. 1987. Physiology of lymphocytes and mononuclear cells. Organization of the lymphatic system (Book Chapter).

Anderson, A. O., L. F. Snyder, M. L. Pitt, and O. L. Wood. 1987. Mucosal Priming alters pathogenesis of Rift Valley fever. Submitted to Proc. 9th Internatl. Conf. Lymphatics Tissues and Germinal Centres in Immune Reactions.

Anderson, A. O., and J. M. Ward. 1987. Endocytic stripping of ligands from migrant lymphocytes in high endothelial venules (HEV): implications for immunomodulation vs viral pathogenesis. Submitted to Proc. 9th Internatl. Conf. Lymphatics Tissues and Germinal Centres in Immune Reactions.

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Kenyon, R. H., and C. J. Peters. 1988. Actions of complement on Junin virus. *Rev. Infect. Dis.* (In Press).

Lewis, R. M., T. M. Cosgriff, B. Y. Griffin, J. Rhoderick, and P. B. Jahrling. 1988. Immune serum increases Arenavirus replication in monocytes. *J. Gen. Virol.* **69**:1735-1739.

Lewis, R. L., J. C. Morrill, P. B. Jahrling, and T. M. Cosgriff. 1988. Hemorrhagic fever virus replication in monocytic cells. *Rev. Infect. Dis.* (In Press).

McCaul, T. F., C. E. Snyder, and J. C. Williams. 1988. Immunocytochemical detection of an immunogenic protein of *Coxiella burnetii* by monoclonal antibodies. *Inst. Phys. Conf. Ser. No. 93.* **3**:553-554.

McCaul, T. F., and J. C. Williams. 1988. Ultrastructural localization of carbohydrate determinants of lipopolysaccharide of *Coxiella burnetii* (Phase I) by monoclonal antibodies, p. 374. *In* (G. W. Bailey, ed.) Proceedings of the 46th Annual Meeting of the Electron Microscopy Society of America. San Francisco Press, Inc., San Francisco, CA.

McCaul, T. F., and J. C. Williams. 1988. Antigenic differences among *Coxiella burnetii* morphological cell types revealed by post-embedding immunoelectron microscopy. Submitted to *Infect. Immun.*

Peters. C. J., C. -T. Liu, G. W. Anderson, Jr., J. C. Morrill, and P. B. Jahrling. 1988. Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted. *Rev. Infect. Dis.* (In Press).

Pitt, M. L. M., and A. O. Anderson. 1987. Direct transdiaphragmatic traffic of peritoneal macrophages to the lung. Submitted to Proc. 9th Internatl. Conf. Lymphatics Tissues and Germinal Centres in Immune Reactions.

Rosato, R. R., F. F. Macasaet, and P. B. Jahrling. 1988. Enzyme-linked immunosorbent assay detection of immunoglobulins G and M to Venezuelan equine encephalomyelitis virus in vaccinated and naturally infected humans. *J. Clin. Microbiol.* 26:421-425.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA302650	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AF	135
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for the development of immunotherapy against toxins of potential BW threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	89 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	7.0	1332
89	7.0	1293

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Wannemacher, R W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Franz, D R
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Pace, J G

22. KEYWORDS (Precede EACH with Security Classification Code) (U)Military Medicine;(U)Low Molecular Weight; RA I Toxins;(U)T-2 Toxin;(U) Saxitoxin;(U) Rapid Detection;(U) Lab Animals;(U) Mice;(U) Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop the ability to detect toxins in biological samples; to study the mechanisms of action; and to develop and evaluate biologics and selected compounds for prevention and treatment of diseases induced by toxins of military importance. A literature search has been done. DTIC search has been filed in the 1498 file.

24 (U) Develop new technology for fermenter-type production of sufficient toxin for isolation, purification, alteration, and detection studies; Use HPLC and mass spectrometry (MS) plus immunology to detect agents and their metabolites in biological fluids. Immunogenicity of various antigens will be utilized to make experimental toxoids/vaccines.

25. (U) 8710 - 8809 Saxitoxin, like T-2 toxin, had a higher toxicity by aerosol than parenterally. Both *in-vivo* and *in-vitro* studies indicate that aerosol exposure to these toxins does not alter lung function. Microcystin-LR is an acute hepatic toxin which causes hemorrhagic shock and secondary cardiogenic effects. When injected in the mouse, liver took up 40% - 60% of radiolabeled microcystin-LR. Very little of the microcystin was metabolized in liver perfusion system. Microsystin appears to have marked effects on cellular membranes, which can result in release of mediators that may be responsible for the cellular destruction. Several drugs that stabilize cell membranes were protective against microcystin in isolated heptocyte systems. Of the low molecular weight toxins which have been evaluated to date, only the tricothecenes and ionophores (A23187 and valinamyacin) were skin irritants. Soap and water was an effective therapy for their decontamination.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M16277A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AF-135: Exploratory Immunotherapy Studies onToxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: R. W. Wannemacher, Jr., Ph.D.

ASSOCIATE INVESTIGATORS: D. L. Bunner, COL, M.D.
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BACKGROUND

A number of low molecular weight toxins are potential biological warfare agents. In order to develop a medical defense against these toxins, it is necessary to study their toxicity, pathophysiology, and pharmacokinetics. This information will be used to develop rational therapies and means of detection. Our earlier studies were done on a component of "yellow rain," i.e., the trichothecene mycotoxins. Work on these toxins is being phased down and the technology developed is being used to study other small-size toxins that are also potential biological defense warfare agents. These include the marine toxins (saxitoxin, tetrodotoxin, and brevetoxin), blue-green algal toxins (microcystin and anatoxin a), and coral toxins (palytoxin).

SUMMARY

Monkeys were injected i.v. with $[^3H]$-T-2 mycotoxin, most of the radioactivity appeared in the urine and feces at the end of 5 days. In urine, the major metabolites were 3'OH HT-2 and T-2 tetraol. In guinea pigs exposed to $[^3H]$ -T-2 toxin by the aerosol route, 93% of the radiolabel was metabolized and excreted in the urine and feces. The major urinary metabolites were T-2 tetraol, a more -polar unknown, and glucuronide conjugates. In the urine of monkeys exposed i.v. to sublethal doses of T-2 mycotoxin, metabolites of this toxin were detected by immunoassays and mass spectral analysis. Again, 3'OH HT-2 and T-2 tetraol were the major metabolites. In contrast to the T-2 toxin, in-vivo distribution and metabolism studies of $[^3H)$-verrucarin A and verrucarol in guinea pigs showed that 51 and 46%, respectively, of the total radiolabel was excreted in the urine and feces by day 9. This suggests a slow rate of metabolism and excretion of the macrocyclic trichothecenes, which may explain the higher toxicity of some of these compounds.

At low doses in the monkey, T-2 mycotoxin caused emesis and marked reduction in food intake. In-vitro T-2 had multiple effects on cell membrane

mechanisms. In-vivo rat studies indicated that T-2 and T-2 tetraol were equally effective in inhibiting protein synthesis and acute toxicity. This is in contrast to in-vitro data which suggest that T-2 mycotoxin is 1000 times more potent than tetraol in inhibiting protein synthesis. These data all emphasize the multi-organs effects of T-2 toxin and its metabolites. They also raise the question as to whether inhibition of protein synthesis is the major mechanism of action of this group of toxins.

The brevetoxins produced some severe cardiorespiratory alterations as well as some centrally mediated effects. About 55% of this toxin was extracted during in-vitro liver perfusion studies. This suggests a lower rate of hepatic metabolism which would leave more toxin available for effects on other peripheral and central tissues. Brevetoxin was poorly absorbed through skin.

Intravenous injection of microcystin resulted in marked liver lesions. These ultrastructural changes correlated with biochemical changes, such as increased serum-liver specific enzymes, decreased P:O ratios and destabilization of desmosomal proteins. These effects were not observed when microcystin was added to isolated mitochondrial preparations or hepatocytes. This toxin, when added to hepatocytes, did cause a disruption of the attachment matrix, followed by loss of cytoplasmic enzymes, but not membrane-associated enzymes. This effect was blocked by cholic acid, suggesting bile acids may protect. Microcystin inhibited the uptake of cholic and taurocholic acids, which suggests that these acids share the same transport sites. When applied to the skin in dimethylsulfoxide, microcystin was not absorbed as rapidly as T-2 mycotoxin. The results of the in-vivo skin toxicity of microcystin agree with the in-vitro absorption rates.

Saxitoxin and tetrodotoxin were absorbed through the skin at much slower rates than T-2 mycotoxin. Brevetoxin, saxitoxin, tetrodotoxin, and microcystin were all inactivated by a 30-min exposure to 0.1% sodium hypochlorite. This is a 50-fold lower concentration than that required to inactivate T-2 mycotoxin. Tetrodotoxin was inactivated by high and low pHs, while microcystin was relatively stable.

MCHB

PRINCIPAL INVESTIGATOR: R. W. Wannemacher, Jr., Ph.D.

ASSOCIATE INVESTIGATORS:

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When [^{3}H]-T-2 mycotoxin was injected i.v. into monkeys, 95% of the radioactivity appeared in the urine and feces by day 5. At a dose of 14 μg of T-2 per kg, two out of six monkeys developed emesis, and all reduced their food intake by 50%. At doses of 0.32 and 0.5 mg/kg, T-2 mycotoxin produced emesis and decreases in food intake but had little effect on platelet function. T-2 metabolites were detected in the urine of the latter two groups of monkeys by immunoassay and mass spectrum analysis. Sensitivity but not specificity appeared to be equivalent for the two techniques.

Previous work with T-2 mycotoxin aerosol showed that inhalation of T-2 was at least 10 times more toxic than T-2 administered i.v. to the rat and mouse.
This work has now been extended to the guinea pig in which inhalation of T-2 aerosol was found to be twice as toxic than T-2 given intravenously. Rats exposed to T-2 toxin via aerosol or i.v. challenge did develop metabolic acidosis but had normal blood pCO_2 and elevated pO_2. T-2 metabolites can produce significant hemoglobin abnormalities, which, in part, may be the reason for the marked elevation in pO_2 seen during toxemia.

A 5-min pre-exposure of L-6 cells to T-2 mycotoxin (0.04 ng/ml) resulted in a significant reduction in protein synthesis and decreased uptake of glucose and calcium. A concentration of 4 ng of T-2 toxin per ml was required to cause a loss of cellular LDH. Thus, we concluded that T-2 mycotoxin had multiple effects on cell membrane transport and, at high concentrations, impaired cellular membrane integrity. These effects occurred rapidly, within 10 min of exposure to T-2.

A new class of macrocyclic trichothecene mycotoxins (myrotoxin B) was found to be 100 times more toxic than T-2 mycotoxin in the mouse bioassay, and 20 times more potent as a skin irritant. Thus, myrotoxin B appears to have the highest toxicity of the trichothecene mycotoxins evaluated to date.

Infusion of brevetoxin (PbTx-2) into the conscious rat resulted in a dose-related decrease in respiratory rate, core and peripheral temperatures, and heart rate but had no effect on arterial blood pressure or blood gases. An antibody against PbTx-2 offered some protection against the respiratory effects of this toxin.

Saxitoxin, tetrodotoxin, and microcystin were absorbed at a much slower rate through the skin in the presence of dimethylsulfoxide (DMSO) than was T-2 mycotoxin. Brevetoxin was poorly (less than 1%) absorbed through the skin in the presence of DMSO.
All of the newer toxins were inactivated by 1% sodium hypochlorite. Tetrodotoxin was rapidly inactivated at high or low pH, while microcystin was relatively stable.

A number of computer programs have been adapted for use with various analytical instruments and personal computers. They will be valuable tools in literature surveys, data acquisition and storage, visual aid production, and data analysis.

MGHC

PRINCIPAL INVESTIGATOR: J. G. Pace, Ph.D.

ASSOCIATE INVESTIGATORS: N. A. Robinson, CPT. Ph.D
W. L. Thompson, M.S.

T-2 toxin had no effect on liver, heart, or blood adenine nucleotide concentrations. In-vivo rat studies showed that T-2, tetraol, and several protein synthesis inhibitors caused rapid and prolonged inhibition of protein synthesis, leading to death.

In-vivo distribution and metabolism studies of radiolabeled verrucarin A and verrucarol in guinea pigs showed that 51 and 46%, respectively, of the total radiolabel was excreted in urine and feces by day 9. Three urinary unknown metabolites were isolated and are being identified by mass spectrometry.

Fate and distribution of T-2 toxin, given by aerosol, was studied in guinea pigs. The total deposition of T-2 was 27 + 4%. By 14 days, 92.9% of the radiolabel was metabolized and excreted in the urine and feces. The major urinary metabolites were T-2 tetraol, a more-polar unknown, and glucuronide conjugates.

Fate and distribution of T-2 in the primate model showed the toxin was metabolized and eliminated mainly as 3'-OH T-2 and tetraol. We have determined the pharmacokinetic parameters.

In-vitro, de-epoxy T-2 and tetraol were one to two logs less toxic than the parent compounds in the protein synthesis inhibition assay.

In-vitro liver perfusion studies showed that brevetoxin (PbTx-3) was extracted (E=0.55) by liver, metabolized to five polar unknowns, and eliminated via bile. In-vitro and in-vivo clearance rates were in good agreement, suggesting the perfusion model can be used to predict pharmacokinetic parameters for PbTx-3.

Microcystin, added to attached hepatocytes, caused a rapid disruption of the attachment matrix, followed by loss of cytoplasmic enzymes, but not membrane-associated enzymes. This effect was blocked by cholic acid, suggesting bile acids may protect. Microcystin inhibited the uptake of cholic and taurocholic acids, which is consistent with the hypothesis that the toxin and bile acids share the same transport system. Microcystin had no apparent effect on a number of other established tissue culture cell lines.

In-vitro microcystin had no effect on mitochondrial swelling or respiration, which suggests that the toxin is not an ionophore. In-vivo rat studies showed microcystin caused liver enlargement, cloudy swelling of mitochondria and rough endoplasmic reticulum, endoplasmic reticular whorls, and loss of desmosomes. These ultrastructural changes correlated with biochemical changes, such as increased serum liver-specific enzymes, decreased P:O rations, and destabilization of desmosomal proteins.

MGCC

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

No additional studies have been done on the effects of treatment with a candidate drug, 3,4-diaminopyridine, for the treatment of botulinum intoxication. The earlier work has been submitted for publication.

PRESENTATIONS

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG3815	01 Oct 88	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	146
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for the development of generic medical defensive countermeasures to agents of biological origin

12. SUBJECT AREAS 1503 Defense; 0605 Clinical Medicine; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	89 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
88	2.0	444
89	2.0	454

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Ussery, M A
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7691
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Pifat, D Y
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Gabrielsen, B

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U)Antiviral Drugs; (U)Pharmacology; (U)Viral Diseases; (U)Lab Animals;(U) Mice;(U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct laboratory studies to develop novel antiviral drugs by identifying potential targets for pharmacologic intervention. These drugs are needed to treat soldiers who become exposed to virulent viral agents. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) Describe mechanisms of action and metabolism of new drugs and provide analytical support for drug analysis. Perform structure-activity analyses to identify new analogs for synthesis. Apply approaches of molecular virology and cell biology to define the molecular basis of virus binding, uptake, uncoating, replication, and maturation.

25. (U) 8710 - 8809 Seven hundred and twenty compounds were tested for in-vitro antiviral activity against RVFV; 23 compounds showed antiviral activity. Active compounds are undergoing further development, including additional in vitro testing and in vivo evaluation in mouse models. The antiviral program against dengue viral infection has continued with the evaluation of 320 new compounds; 11 of these compounds were identified with activity against dengue 1, 2, and/or 4. Fourteen compounds have been tested against Rauscher leukemia virus and 5 compounds in the LP-BM-5 mouse AIDS model. Five active antivirals and 3 active immunomodulators have been identified. Two natural product alkaloids have in-vivo activity against Japanese encephalitis virus. An active compound undergoing advanced development, AVS-206, was shown to be approximately 5 times less toxic than ribavirin in rhesus monkeys.

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PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 8 71-AH-146: Exploratory Development Studies Seeking Generic Medical Defensive Countermeasures Against Agents of Biological Origin

PRINCIPAL INVESTIGATOR: M. A. Ussery, MAJ, Ph.D.

ASSOCIATE INVESTIGATOR: D. Y. Pifat, Ph.D.
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BACKGROUND

This research program has focused on the discovery and development of agents or procedures that will be effective against a broad range of "exotic" RNA viruses. The program maintains an extensive antiviral drug screening effort. The larger number of compounds evaluated are obtained through no-cost technical exchange agreements with the private sector, while others are synthesized based on known leads. Initial testing consists of in vitro assays against a battery of RNA viruses as well as rodent models. Viruses in the Institute-based screen include dengue, Rift Valley fever, Crimean-Congo, and Venezuelan equine encephalomyelitis. Animal models include two retroviruses in support of the Institute's AIDS program.

Other approaches being evaluated are the targeting of antiviral agents to specific tissue sites. This approach involves the synthesis of compounds that have an inherent capacity to concentrate in specific organs. This approach also involves coupling of antivirals to carriers, which will promote the site-specific delivery of the drug. We are also studying the molecular basis of drug action to identify potential targets for drug interactions, as well as for assessing the potential for toxicity

SUMMARY

Seven hundred and twenty compounds were tested for in-vitro activity against Rift Valley fever virus (RVFV). Twenty-three compounds showed antiviral activity (in-vitro therapeutic indices of 5 or more). Active compounds are undergoing further development, including additional in-vitro and in-vivo evaluation in mouse models. Ten compounds were tested in vivo against RVFV or Venezuelan equine encephalomyelitis viral infections of mice. One compound showed activity against RVFV. Two previously iden-

tified active compounds (ribavirin and AVS 79) were synergistic in their in-vivo activity against RVFV. The antiviral program against dengue viral infections has continued with the evaluation of 370 new compounds. Eleven of these were identified that had activity against dengue 1, 2, and/or 4.

Fourteen compounds have been tested in vivo against Rauscher leukemia virus and five compounds against the LP-BM5 mouse AIDS virus in support of the Institute's AIDS Drug Development Program. Five active antivirals have been identified as well as three active immunomodulators. AZT and MVE-2 were additive in their effect when given in combination. In addition, much background work on the pathogenesis of these two diseases has been accomplished. For example, after 12 weeks in the mouse AIDS model, the natural killer cell activity was depressed and could not be stimulated with poly ICLC (an effective stimulator of normal spleen cells).

Several analogues of the antivirally active Amaryllidaceae alkaloids, narciclasine and lycoricidine, were synthesized (or obtained from naturally occurring precursors), characterized, and submitted for testing. One of these, isonarciclasine, produced the highest therapeutic indices yet observed in this series. Efficacy against bunya- and flaviviruses was retained, while toxicity in cell culture was decreased by a factor of several hundred. Narciclasine and pancratistatin, two naturally occurring members of this class of alkaloids, were effective in Japanese encephalitis virus-infected mice, inducing 100% survival within a relatively narrow dose range. To our knowledge, these are the first compounds to exhibit such high efficacy in vivo. They are equally as effective when administered to Punta Toro virus-infected mice. In the latter case, effects were comparable to, or better than, ribavirin.

Ten, hitherto unreported/unsynthesized analogues of the known antiviral compounds, tiazofurin, selenazofurin, and ribavirin amidine, were synthesized, purified, characterized, and submitted for antiviral testing. Several of these analogues contain the amino acids glycine, arginine, or glutamine as substituents of the amidine or carboxamide moieties of the parent compounds.

A series of ribavirin and tiazofurin analogues were prepared and tested in which the ribofuranosyl moiety was replaced by assorted deoxyribofuranose sugars. Antiviral activity, as compared to the parent compounds, was lost.

Several analogues of 4-acetyl-4-phenylpiperidine (AVS 999) were prepared, purified, and submitted for testing in an attempt to follow up on the HIV activity of the parent compound.

The antiviral compound 1-ß-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine (AVS #206) was compared to ribavirin in terms of its effects on hematological parameters in adult rhesus monkeys. A ribavirin regimen of 60 mg/kg on day 0, followed by 20 $mg^{-1}/kg^{-1}/day^{-1}$ for 10 days caused a marked decrease in hematocrit, hemoglobin, and red blood cell counts, as well as an increase in platelet and reticulocyte counts. Under identical conditions and with the same regimen, AVS U206 caused no measurable decrease in red blood cell parameters; no other deleterious effects were observed.

Further experiments were conducted to titrate AVS-206 in adult rhesus monkeys. Doses of AVS-206 ranging from 60 mg/kg to 400 mg/kg on day 0 and 30 mg/kg to 200 $mg^{-1}/kg^{-1}day^{-1}$ for 10 days were inoculated into groups of adult rhesus monkeys. Data revealed that, like ribavirin, the compound AVS-206 was capable of causing anemia, elevating platelet counts, and increasing reticulocyte counts. AVS-206 was approximately five times less erythrotoxic than ribavirin for adult rhesus monkeys. In addition, the anemia, reticulocytosis, and increase in the number of platelets seen with the highest doses appear to be slightly delayed when compared to those seen with ribavirin.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOG3811	2. DATE OF SUMMARY 01 Oct 88	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
30 Oct 87	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AK	139
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY89- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory studies for the development of rapid diagnostic procedures against agents of biological origin

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	89 01	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		88	2.0	366
c. TYPE	d. AMOUNT	89	2.0	349
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Knauert, F K
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ezzell, J W

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Immunology; (U) Lab Animals; (U) Rats; (U) Biotechnology; (U) RA I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop technology for rapid diagnosis and identification of BW agents in the military clinical and environmental sphere. Field diagnosis will enhance the medical protection of US military personnel. A literature search has been done. DTIC search has been filed in the 1498 file.

24. (U) To develop and refine state-of-the-art nucleic acid probes and other methods for virus detection and identification.

25. (U) 8710 - 8809 Rift Valley fever virus nucleic acid probes labeled with ^{32}P progressed to field testing after a large outbreak in West Africa. The assay successfully detected viral RNA directly in the sera of patients; however, antigen detection and virus isolation were still more sensitive. Other non-isotopic means of labelling probes continue to be explored but signal-to-noise ratio and sensitivity remain obstacles with currently available technologies. Emerging nucleic acid amplification strategies are being explored; specifically, means of adapting polymerase chain reaction (PCR) techniques to RNA viruses are being sought. Further application of engineered viral polypeptides from novel expression systems are being pursued with collaborating scientists based at USAMRIID and through extramural contract arrangements. Antigens produced in this manner have so far offered safe and efficient sources of noninfectious diagnostic materials for current and emerging assay systems. Further experience is still needed to gauge the potential for wide application.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AK-139: Exploratory Development Studies Seeking Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATOR: F. K. Knauert, Ph.D.

BACKGROUND

Recent technological advancements have made nucleic acid hybridization an attractive alternate method for detecting viruses and other pathogens in clinical and environmental samples. We used a cloned sequence complementary to the M segments RNA of Rift Valley fever (RVF) virus to develop such an assay, and have compared its usefulness to current procedures for detecting and identifying pathogens. Under well-controlled laboratory conditions, we can routinely detect 0.5 x 10^4 PFU of RVF virus replicated in cell culture; however, when the procedure is applied to clinical specimens from infected humans or animals, the sensitivity of the assay is significantly less. We suspect that the loss of sensitivity is due to nonspecific adsorption of cellular proteins and nucleic acids that compete with target viral RNA for limited binding sites. We have made progress in previous years in modifying our procedures to alleviate these problems, but we have yet to reach our goals for assay sensitivity.

SUMMARY

We continued to improve the sensitivity of the nucleic acid hybridization assay by testing clinical specimens from infected humans or animals. We found that the most reproducible method for preparing serum samples was to pretreat them with polyethylene glycol, and to resuspend and digest the collected precipitate in a proteinase K solution. This method was validated with serum collected from experimentally infected monkeys and was found to be sensitive and specific, with virus isolation as a criterion.

An epidemic of RVF occurred in October 1987 in Mauritania and Senegal in West Africa which provided a unique opportunity for us to test our hybridization assay on a large collection of authentic human serum samples, allowing a realistic evaluation of the diagnostic potential of this technique as compared to standard virological isolation procedures and our antigen-capture immunoassays. The RVF virus [^{32}P]-nucleic acid probes successfully detected viral RNA directly in the sera of patients infected during the epidemic. This procedure was less sen-

sitive than either direct virus isolation or antigen detection by the immunoassay; however, a significant number of specimens were positive by the hybridization assay. These results indicate that nucleic acid hybridization assays are of value as rapid diagnostic procedures for agents of biological warfare potential.

Non-isotope methods of labeling nucleic acid probes continue to be investigated, but signal-to-noise ratio and sensitivity remain obstacles. Emerging nucleic acid amplification strategies are being explored; specifically, means of adapting polymerase chain reaction techniques to RNA viruses are being sought. Further application of engineered viral polypeptides from novel expression systems are being pursued in collaboration with other scientists at USAMRIID and through extramural arrangements. Antigens produced in this manner have, so far, offered safe and efficient sources of noninfectious, diagnostic materials for current and emerging assay systems. Further experience is still needed to gauge the potential for wide application.

PRESENTATIONS

Knauert, F. K., and B. A. Parrish. 1987. Detection of Rift valley fever viral (RVFV) RNA in immune complexes. Presented at the 36th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Los Angeles, CA, December,

APPENDIX A

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UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FY 88

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APPENDIX B

CONTRACTS, GRANTS, MIPRS, AND PURCHASE ORDERS IN EFFECT

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FY 88

CONTRACT NUMBER	TITLE, INVESTIGATOR, INSTITUTION
PO-84-PP-4861	The Role of Plasmids and Bacteriophages in Toxigenicity of *Clostridium botulinum* and characterization of converting bacteriophages. M. W. Eklund, U.S. Department of Commerce, Seattle, WA.
DAMD-17-85-C-5224	Diagnosis and Management of Trichothecene Toxicosis in the Swine Model. W. B. Buck, University of Illinois, Urbana, IL.
DAMD-17-85-C-5241	Pathophysiology and Toxicokinetic Studies of Blue-green Algae Intoxication in the Swine Model. V. Beasley, University of Illinois, Urbana, IL.
DAMD-17-85-C-5167	Use of Recombinant DNA Techniques for the Production of a More Effective Anthrax Vaccine. D. L. Robertson, Brigham Young University, Provo, UT.
DAMD-17-85-C-5212	Genetic and Physiological Studies of *Bacillus anthracis* Related to Development of an Improved Vaccine. C. B. Thorne, University of Massachusetts, Amhearst, MA
DAMD-17-85-C-5226	Rift Valley Fever Virus: Molecular Biologic Studies of the M Segment RNA for Application in Disease Prevention. M. Collett, Molecular Genetics, Minnetonka, MN.
DAMD-17-85-C-5283	Functional Consequences of Chemical Modification of the Saxitoxin Binding Site on Neuronal Sodium Channels. B. K. Krueger, University of Maryland, Baltimore, MD.

DAMD-17-85-C-5171	Characterization of the *P. brevis* Polyether Neurotoxin Binding Component in Excitable Membranes. D. G. Baden, University of Miami, Coral Gables, FL.
DAMD-17-85-C-5204	Metabolism, Mass Spectral Analysis and Mode of Action of Trichothecene Mycotoxins. C. J. Mirocha, University of Minnesota, St. Paul, MN.
DAMD-17-85-C-5232	Genetically-engineered Poxviruses and the Construction of Live Recombinant Vaccines. E. Paoletti, New York Department of Health and Health Research, Albany, NY.
DAMD-17-85-C-5276	Development of Systems for Delivery of Antiviral Drugs, W. Shannon, Southern Research Institute, Birmingham, AL.
DAMD-17-85-C-5285	Therapeutic Approaches to the Treatment of Botulism. L. L. Simpson, Jefferson Medical College, Philadelphia, PA.
DAMD-17-86-C-6044	Enhancement of Antiviral Agents Through the Use of Controlled Release Technology. T. R. Tice, Southern Research Institute, Birmingham, AL.
DAMD-17-85-C-5266	Human Hybridomas for Exotic Antigens. M. Cohn, The Salk Institute for Biological Studies, La Jolla, CA.
DAMD-17-85-C-5274	Chemical Synthesis *Coxiella burnetii* Lipopolysaccharides: Structural Characterization, Chemical Synthesis and Immunogen. V. N. Reinhold, Harvard School of Public Health, Boston, MA.
DAMD 17-85-C-5280	Mechanism of Action of Tetanus Toxin. M. Klempner, New England Medical Center Hospitals, Boston, MA.
DAMD-17-86-C-6041	Synthesis of Nucleoside Analogues with Potential Antiviral Activity against Negative Strand RNA Virus Targets. R. D. Walker, Birmingham University, Birmingham, England.
DAMD-17-86-C-6002	Synthesis of Nucleoside Mono- and Dialdehydes as Antiviral Agents. J. P. Neenan. Rochester Institute of Technology, Rochester, NY.

DAMD-17-86-C-6001	Rare 2-Substituted Purine Nucleosides. V. Nair, University of Iowa, Iowa City, IA.
DAMD-17-86-C-6012	Chiral Acyclic Nucleosides: Potential Broad Spectrum Antivirals. E. Abushanab, University of Rhode Island, Kingston, RI.
DAMD-17-86-C-6011	Synthesis Laboratory for USAMRIID Selection Panel. J. A. Secrist, III, Southern Research Institute, Birmingham, AL.
DAMD-17-85-C-5274	Drug Development against Viral Diseases of Military Importance. J. A. Secrist, II. Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6161	A Core Facility for the Study of Neurotoxins of Biological origin. L. L. Simpson, Jefferson Medical College, Philadelphia, PA.
DAMD-17-86-C-6055	Cloning Sequencing and Structural Manipulation of the Enterotoxin D and E Genes from *Staphylococcus aureus*. J. J. Iandola, Kansas State University, Manhattan, KS.
MIPR 86-MM-6502	Rapid Screening and Structural Characterization of Biological Toxins. M. Ross, Naval Research Laboratory, Washington, D. C.
DAMD-17-86-G-6011	Hemorrhagic Fever with Renal Syndrome (HFRS) (Korean Hemorrhagic Fever). H. W. Lee, Korea University College of Medicine, Seoul, Korea.
DAMD-17-86-C-6162	Development of New Immunogens and a Controlled Release Delivery System for Oral Immunization against Staphylococcal Enterotoxin B. T. R. Tice, Southern Research Institute, Birmingham, AL.
DAMD-17-86-G-6002	Double Blind, Placed Controlled Clinical Trial of Ribavirin Therapeutic Efficacy in the Treatment of Epidemic hemorrhagic Fever. C-M. Hsiang, Hubei Medical College, Wuhan, Peoples' Republic of China.
DAMD-17-86-C-6118	Biology of Immunomodulators. J. D. Gangemi, University of South Carolina, Columbia, SC.

DAMD-17-86-C-6154	Evaluation of Immune Response Modifying Compounds Utilizing Virus-Specific Human T Lymphocyte Clones. M. Cohn, Georgetown University, Washington, D. C.
DAMD-17-86-C-6117	Efficacy and Mode of Action of Immune Response Modifying Compounds against Alphaviruses and Flaviviruses. P. S. Morahan, Pennsylvania College of Medicine, Philadelphia, PA.
DAMD-17-86-C-6121	Screening of Immunoenhancing Drugs with Antiviral Activity against Members of the Arena-, Alpha-, and Adenoviridae. P. A. LeBlanc, University of Alabama, Birmingham, AL.
DAMD-17-86-C-6166	Effects of Immunomodulatory Drugs on T Lymphocyte Activation and Function. C. Tsoukas, Scripps Clinic and Research Foundation, La Jolla, CA.
DAMD-17-86-C-6056	Mechanisms of Action of Clostridial Neurotoxins on Dissociated Mouse Spinal Cord Neurons in Cell Culture. G. Bergey, University of Maryland at Baltimore, MD.
DAMD-17-86-C-6134	Biosystematics of *Aedes* (Neomelaniconion). T. J. Zavortink. University of San Francisco, San Francisco, CA.
DAMD-17-86-C-6061	Crotoxin: Structural Studies, Mechanism of Action and Cloning of its Gene. I. I. Kaiser, University of Wyoming, Laramie, WY.
DAMD-17-86-C-6060	Synthesis and Testing of Tetrodotoxin and Batrachotoxin Antagonists. L. Toll, SRI International, Menlo Park, CA.
DAMD-17-86-C-6062	Receptor Binding and Membrane Transport of Botulinum Toxins. J. R. Dankert, University of Central Florida, Gainesville, FL.
DAMD-17-86-C-6057	Development and Testing of an In Vitro Assay for Screening of Potential Therapeutic Agents Active Against Sodium Channel Neurotoxins. G. B. Brown, University of Alabama, Birmingham, AL.

DAMD-17-86-C-6058	Mass-Screening of Curarimimetic Neurotoxin Antagonists. J. Schmidt, State University of New York, Albany, NY.
DAMD-17-86-C-6160	Mechanism of the Presynaptic Neurotoxin Tetanus Toxin. T. B. Rogers, The University of Maryland, Baltimore, MD.
DAMD-17-86-C-6063	Structure-Function Relationship of Hydrophyiidae Postsynaptic Neurotoxins. A. Tu, Colorado State University, Fort Collins, CO.
DAMD-17-86-C-6120	Development of Methods for Carrier-Mediated Targeted Delivery of Antiviral Compounds Using Monoclonal Antibodies. M. I. Dawson, SRI International, Menlo Park, CA.
DAMD-17-86-C-6013	Research in Drug Development Against Viral Diseases of Military Importance (Biological Testing). W. Shannon, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6119	Combination Chemotherapy Using Immune Modulators and Antiviral Drugs Against Togaviruses and Bunyaviruses. S. Baron, Medical Branch, University of Texas, Galveston, TX.
DAMD-17-86-C-6028	Determination of the In Vitro and In Vivo Activity of Compounds Tested Against Punta Toro Virus. R. O. Sidwell, Utah State University, Logan, UT.
DAMD-17-86-C-6107	Research in Drug Development for Therapeutic Treatment of Neurotoxin Poisoning: Studies of Conotoxins. R. Almquist, SRI International, Menlo Park, CA.
DAMD-17-87-C-7007	The Mechanism of Action of Ribavirin on Bunyavirus Infected Cells. J. L. Patterson. Children's Hospital Corporation, Cincinatti, OH.
DAMD-17-86-C-6042	Drug Development Against Viral Disease (Biological Testing). G. Tignor. Yake University, New Haven, CT.

PO-86-PP-6811	Dermorphin as a Behavioral and Autonomic Modulator. G. Feuerstein, Uniformed Services University of the Health Sciences, Bethesda, MD.
PO-86-PP-6813	The Regulation of a Post-Translational Peptide Acetyltransferase: Strategies for Selectively Modifying the Biological Activity of Neural and Endocrine Peptides. W. R. Millington, Uniformed Services University of the Health Sciences, Bethesda, MD.
PO-86-PP-6814	Regulatory Peptides: Behavioral and Neurochemical Effects. A. H. Barrett, Uniformed Services University of the Health Sciences, Bethesda, MD.
DAMD-17-87-C-7069	Diagnosis and Prevention of Infection by Phlebotomus Fever group Viruses. D. H. L. Bishop, Natural Environmental Research Council, Swindon, U.K.
DAMD-17-86-C-6001	Rare 2-Substituted Purine Chemistry. V. Nair, University of Iowa, Iowa City, IA
DAMD-17-86-C-6239	Research, Development, and Delivery of Second Generation Fiber Fluorescent Immunoassay Instruments. T. R. Glass, ORD, Cambridge, MA.
DAMD-17-86-G-6016	Epidemiology and Epizootiological Investigations of Hemorrhagic Fever Viruses in India. P. M. Tukei, Virus Research Center (KMRI), Nairobi, Kenya.
DAMD-17-86-G-6032	Epidemiological and Epizootiological Investigation of Filoviruses in the Central African Republic. A. J. Georges, Pasteur Institute, Paris, France.
DAMD-17-86-C-6133	Studies of Infection and Dissemination of Rift Valley Fever Virus in Mosquitoes. W. S. Romoser, Ohio University, Athens, OH.
DAMD-17-86-C-6173	Immunological Techniques for Detection of Fungal and Dinoflagellate Toxins. F. S. Chu, University of Wisconsin, Madison, WI.
DAMD-17-86-C-6234	Synthetic Vaccines for the Control of Arenavirus Infections. M. Buchmeier, Scripps Clinic and Research Foundation, La Jolla, CA.

DAMD-17-87-C-7090	Active Antitoxic Immunization Against Ricin Using Synthetic Peptides. A. K. Judd, SRI International, Menlo Park, CA.
DAMD-17-87-C-7007	In Vitro and In Vivo Measurement of Percutaneous Penetration of Low Molecular Weight Toxins of Military Interest. B. W. Kemppainen, Auburn University, Al.
DAMD-17-87-C-7111	Interferon Inducers Against Infectious Diseases. J. Bello. Roswell Park Memorial Institute,Buffalo, NY.
DAMD-17-87-C-7101	Epidemiology of Hantavirus Infectious in Baltimore. J. E. Childs, Johns Hopkins University, Baltimore, MD.
DAMD-17-87-C-7140	Potential Vaccine for Anthrax. R. Doyle, University of Louisville, Louisville, KY.
DAMD-17-87-C-7005	Immunologic Approach to the Identification of Vaccines to Various Toxins. T. Chanh. Southwest Foundation for Biomedical Research. San Antonio, TX.
DAMD-17-87-C-7114	Development of a Toxic Knowledge System. H. L. Trammel, University of Illinois, Urbana-Champaign, IL.
DAMD-17-87-C-7019	Freshwater Cyanobacteria (Blue-Green) Toxins: Isolation and Characterization. W. Carmichael, Wright State University School of Medicine, Dayton, OH
DAMD-17-87-C-7002	Dinoflagellate Toxins Responsible for Ciguatera Food Poisoning. D. M. Miller, Southern Illinois University, Carbondale, IL.
DAMD-17-87-C-7014	Production of Antigens and Antibodies for Rapid Diagnosis of Arbovirus Diseases. R. E. Shope, Yale University, New Haven, CT.
DAMD-17-87-C-7001	Binding Assays for the Quantitative Detection of *P. brevis* Polyether Neurotoxins in Biological Samples and Antibodies as Therapeutic Aids. D. G. Baden. University of Miami, Miami, FL.

DAMD-17-87-C-7155	Mode of Action of Membrane Perturbing Agents: Snake Venom Cardiotoxins and Phospholipases A. J. Fletcher, Hahnemann University, Philadelphia, PA.
DAMD-17-87-C-7210	Marine Biotoxins: Laboratory Culture and Molecular Structure. P. J. Scheuer, University of Hawaii at Manoa, Honolulu, HI.
PO-87-PP-7809	Marine Toxins: Automatic Toxicology and Therapeutic Strategies. G. Feuerstein, Uniformed Services University of the Health Sciences, Bethesda, MD.
DAMD-17-87-C-7093	Toxin Production and Immunoassay Development. I. Palytoxin. D. C. Vann, Hawaii Biotechnology Group, Inc., Aiea, HI.
DAMD-17-87-C-7154	Expression of Yellow Fever Antigens and Infectious Virus from Cloned cDNA. C. Rice, Washington University School of Medicine, St. Louis, MO.
DAMD-17-87-C-7137	Peptide Transport Through the Blood-Brain Barrier. W. M Partridge, Regents of the University of California, Los Angeles, CA.
DAMD-17-87-G-7003	Ecology and Epidemiology of Crimean-Congo Hemorrhagic Fever Virus Transmission in the Republic of Senegal. J. P. Digoutte, Institute Pasteur de Dakar, Senegal.
DAMD-17-87-C-7051	Tetrodotoxin Immunoassays. P. Grothaus, Hawaii Biotechnology Group, Inc., Aiea, HI
DAMD-17-87-C-7123	Research Pathology and Special Techniques Support Services. W. C. Hall, Pathology Associates, Inc., Ijamsville, MD.
PO-87-PP-7852	The 3-D Structures of some Diarrhea-Causing Bacterial Toxins. M. Sax, Veterans Administration, Iowa City, IA.
DAMD-17-87-C-7110	Determinants of Infectivity of Pathogens in Vector Ticks. A. Spielman, Harvard University, Boston, MA.

PO-87-PP-7853	Microvascular Physiologic and Anatomic Responses of the Guinea Pig to Experimental Arenavirus Infection. M. Katz, Veterans Administration. Iowa City, IA.
DAMD-17-87-G-7019	Seroepidemiological Survey for Congo-Crimean Hemorrhagic Fever and Hantaan Virus. A. Antoniades, Aristotelian University of Thessaloniki, Thessaloniki, Greece.
PO-87-PP-7825	Immunoassay Procedures for Fiber Optic Sensors. F. S. Ligler, Naval Research laboratory, Washington, D. C.
DAMD-17-8C-7094	Site-Specific Antagonists. C. -Y. Kao, Research Foundation of SUNY, New York.
DAMD-17-87-C-7257	Mechanisms of Action of Low Molecular Weight Toxins in the Cardiovascular System. W. T. Woods, University of Alabama, Birmingham, AL.
DAMD-17-87-C-7233	T Cell Responses to Arenavirus Infections. G. A. Cole, University of Maryland, College Park, MD.
DAMD-17-87-C-7188	Neurotoxin and Epitope Structural Studies. D. S. Hunt, University of Virginia, Charlottesville, VA.
DAMD-17-87-C-7259	Molecular Strategy for the Construction of a Genetically Engineered Vaccine for Venezuelan Equine Encephalitis Virus. R. E. Johnston, Raleigh, NC.
DAMD-17-87-C-7135	Genetic Engineering of *Clostridium difficile* Toxin A Vaccine. S. Muldrow, Spielman College, Atlanta, GA.
DAMD-17-87-C-7176	Diagnosis and Prevention of Infection by Nairoviruses. P. A. Nutall, Natural Environment Research Council, London, England.
DAMD-17-87-C-0004	Molecular Characterization of Attenuated Junin Virus Variants. V. Romanowski, Universidad Nacional de la Plata, La Plata, Argentina.
DAMD-17-87-G-7005	World Reference Center for Arboviruses. R. E. Shope, Yale University, New Haven, CT.

PO-87-PP-7829	Development and Evaluation of Supercritical Fluid Chromatography (SFC) and SFS-Mass Spectrometry for Analysis of Trichothecenes, Marine Toxins and Neurotoxins. R. D. Smith, U. S. Department of Energy, Richland, WA.
PO-87-PP-7858	Development and Evaluation of Immunomodulators of Hematopoietic and Immunologic Mechanisms. G. G. Tsoukos, Uniformed Services University of the Health Sciences, Bethesda, MD.
PO-87-PP-7857	Collaborative Research Program on Seafood Toxins: II. S. W. Page, U. S. Food and Drug Administration, Washington, D.C.
PO-88-PP-8804	IgG Subclass and Isotope Specific Immunoglobulin Responses to Lassa Fever and Venezuelan Equine Encephalomyelitis: Natural Infection and Immunization. R. J. M. Engler, Uniformed Services University of Health Sciences, Bethesda, MD
PO-88-PP-8815	Antiviral Drugs: Molecular Modeling and QSAR. K. B. Ward, Naval Research Laboratory, Washington, DC.
PO-88-PP-8819	Protein Toxins: Preparation, Analysis and Molecular Modeling. K. B. Ward, Naval Research Laboratory, Washington, DC.
PO-88-PP-8820	Small Molecular Toxins: Crystallization, X-ray Analysis and Modeling. K. B. Ward, Naval Research Laboratory, Washington, DC.
PO-88-PP-8835	1988 U.S. Army Summer Faculty Research and Engineering Program – USAMRIID. G. G. Outterson, Scientific Services Program, Batelle, Research Triangle Park, NC.
PO-88-PP-8837	Investigation of Toxins and Venoms by Novel MS Techniques. T. Krishnamurthy, Chemical Research and Development Center, Aberdeen Proving Ground, MD.

PO-88-PP-8854	Relating Remotely Sensed Satellite Imagery to Ecologically Linked Disease Outbreaks in Africa and South America. C. J. Tucker, Laboratory for Terrestrial Physics - Code 623, National Aeronautics and Space Administration/Goddard Space Flight Center, Greenbelt, MD.
PO-88-PP-9902	Animal Vaccine Trials: VEE and RVF Viruses, R. G. Breeze, Plum Island Animal Disease Center, Greenport, NY.
PO-88-14-7002	Detection and Mapping of Rift Valley Fever (RVF) Disease Vector Habitats in Kenya, Africa and Along the Senegal River. V. G. Ambrosia, TGS Technology, Inc., National Aeronautics and Space Administration/Ames Research Center.
DAMD-17-88-C-8008	Novel Treatments for Botulism: Development of Antagonists for Identified Steps in the Action of Botulinum Neurotoxins. J. O. Dolly, Imperial College of Science Technology, United Kingdom.
DAMD-17-88-C-8011	Synthesis and Biological Evaluation of Brain-specific Anti-RNA Viral Agents. M. Brewster, Pharmatec, Inc., Alachua, FL.
DAMD-17-88-C-8199	Optical Assays for Microbial Infections. D. M. Maul, Biostar Medical Products, Inc., Boulder, CO.
DAMD-17-88-C-8037	Molecular Biological Approaches to Disease Prevention and Diagnosis. M. M. Sveda, Bionetics Research, Inc., Rockville, MD.
DAMD-17-88-C-8095	Stereospecific Total Synthesis of Radiolabeled Microcystin. C. Jennings-White, SRI International, Menlo Park, CA.
DAMD-17-88-C-8133	*Coxiella burnetii* Vaccine Development - Lipopolysaccharide Structural Analysis. V. N. Reinhold, Harvard College, Holyoke Center, Cambridge, MA.
DAMD-17-88-C-8149	Antigen and Genome Detection of Arena-, Bunya-, and Filovirus Infections. A. Bennett Jenson, Georgetown University, Washington, D.C.

DAMD-17-88-H-8004	Immunological Studies of Anti-AIDS Drugs in ARC/AIDS. E. M. Hersh, University of Arizona, Tucson, AZ.
DAMD-17-88-Z-8018	Calcium Channels (NYAS-Conference). New York Academy of Science, New York, NY.
DAMD-17-88-Z-8029	Gordon Conferences on Microbial Pathogenesis. P. P. Sparling, University of Rhode Island, Kingston, RI.
DAMD-17-88-H-8002	QSAR Chemical Database and Information Science. E. Stephen, Technassociates, Inc., Rockville, MD.
DAMD-17-88-H-8003	Evaluation of Candidate Anti-AIDS Drugs in Vitro. W. M. Shannon, Southern Research Institute, Birmingham, AL.
DAMD-17-88-C-8200	Enzyme Immunoassay for Anatoxin-A. R. A. Amos, Bio-Metric Systems, Inc., Eden Prairie, MN.
DAMD-17-88-C-8148	Characterization of P. brevis Polyether Neurotoxin Binding Component in Excitable Membranes. D. G. Baden, University of Miami, Miami, FL.
DAMD-17-88-C-8198	Algal Toxins: Production, Detection and Therapy. J. A. Benson, Neushul Mariculture, Inc.
DAMD-17-88-Z-8024	Investigation of Crimean-Congo Hemorrhagic Fever and Hemorrhagic Fever with Renal Syndrome. A. Antoniadis, Aristotelian University of Thessaloniki, Thessaloniki, Greece.
DAMD-17-89-C-9007	Rattlesnake Neurotoxin Structure, Mechanism of Action, Immunology and Molecular Biology. I. I. Kaiser, University of Wyoming, Laramie, WY.
DAMD-17-89-Z-8019	FASEB Conference: Trichothecene, Blue-Green Algal and Marine Toxins: Mechanisms, Detection and Therapy. A. E. Rogers, Federation of American Societies of Experimental Biology, Bethesda, MD.
DAMD-17-89-Z-8031	9th World Congress on Animal, Plant and Microbial Toxins. C. L. Ownby, Oklahoma State University, Stillwater, OK.

DAMD-17-89-Z-9002	Regulation of Potassium Transport Across Biological Membranes: A Symposium. L. Reuss, University of Texas, Galveston, TX.
DAMD-17-88-Z-9009	Investigations of Hemorrhagic Fever with Renal Syndrome (HFRS) in Yugoslavia. A. Gligic, Institute of Immunology and Virology, Vojvode Stepe, Yugoslavia.
DAMD-17-89-Z-9010	Study of Nephropathia Epidemica in Sweden. B. Niklasson, National Bacteriological Laboratory, Stockholm, Sweden.
DAMD-17-89-Z-9019	Structure-Function Relationship of Hydrophiidae Postsynaptic Neurotoxins. A. T. Tu, Colorado State University, Fort Collins, CO.
PO-89-PP-3904	SAR and CIR Analysis of Possible Rift Valley Fever Virus Breeding Sites in Kenya. P. Sebesta, National Aeronautics and Space Administration, Moffett Field, CA.

APPENDIX C

PRESENTATIONS
(Abstracts)

FY 88

Anderson, G. W., Jr., and C. J. Peters. 1987. Variation in virulence for rats among Rift Valley fever virus strains of different geographical origin. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Anderson, G. W., Jr., and J. F. Smith. 1987. Rift Valley fever virus (RVFV) maturation at the plasma membrane of rat hepatocytes as revealed by immunoelectron microscopy. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Anderson, G. W., Jr., M. V. Slayter, and C. J. Peters. 1988. Pathogenesis of a phleboviral infection (Punta Toro virus) in golden Syrian hamsters. Presented at the Annual Meeting of the American Society for Virology, Austin, TX, June.

Anderson, G. W., Jr., J. F. Smith, and C. J. Peters. 1988. A model to evaluate vaccines, anti-viral drugs, and innate mechanisms of resistance to Rift Valley fever virus (RVFV). Presented at the Army Science Conference, Westpoint, NY, June.

Arikawa, J., and C. Schmaljohn. 1987. Functional characterization of Hantaan virus antigenic determinants on the envelope glycoproteins defined by monoclonal antibodies. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Arikawa, J., and C. Schmaljohn. 1988. Functional characterization of antigenic sites on the G1 and G2 glycoproteins of Hantaan virus and their conservation among Hantaviruses. Presented at the 7th Annual Meeting of the American Society for Virology, Austin, TX, June.

Barerra Oro, J. G., R. H. Kenyon, J. Meegan, K. McKee, C. MacDonald, F. Cole, H. W. Lupton, and C. J. Peters. 1987. The immune response to Candid 1 (C #1) live, attenuated Junin virus (JV) vaccine against Argentine hemorrhagic fever. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Barrera Oro, J. G., H. W. Lupton, P. B. Jahrling, J. Meegan, R. H. Kenyon, and C. J. Peters. 1988. Cross-protection against Machupo virus with Candid #1 live-attenuated Junin virus vaccine. 1. The post vaccination prechallenge immune response. Presented at the 2nd International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar del Plata, Argentina, March.

Bartkus, J. M., and S. H. Leppla. 1988. Trausciptional regulation of anthrax toxin production. Presented at the 88th Annual Meeting of the American Society for Microbiology, Miami Beach, FL, May.

Battles, J., and J. M. Dalrymple. 1987. Nucleic acid sequence changes associated with reduced mouse virulence in monoclonal antibody escape mutants of Rift Valley fever virus. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Bhatnagar, R., and A. M. Friedlander. 1988. Calcium is required for anthrax lethal toxin activity. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, FLA, May.

Bhatnagar, R., Y. Singh, S. H. Leppla, and A. M. Friedlander. 1988. Calcium influx induced in cells by anthrax lethal toxin enhances inositol triphosphate formation. Presented at the International Conference on Biomembranes in Health and Disease, Lucknow, India

Chen, S.-Y., H. A. Thompson, and J. C. Williams. 1988. Cloning and characterization of the *Coxiella burnetii* origin of DNA replication.Presented at the Annual Meeting of the American Society of Rickettsiology and Rickettsial Diseases, Santa Fe, NM, April.

Chirigos, M. A. 1988. Maleic anhydride divinyl ether copolymer (MVE-2): the chemical stalking horse of biological response modifiers. Presented at the 3rd Chemical Congress of North America, Toronto, Canada, June.

Creasia, D. A., and M. L. Nealley. 1988. Acute inhalation toxicity of saxitoxin in the mouse. Presented at the 9th World Congress on Animal, Plant and Microbial Toxins, International Society on Toxinology, Stillwater, OK, July-August. To be published in *Toxicon.*

Creasia, D. A., G. A. Saviolakis, and K. A. Bostian. 1988. Effect of inhaled insulin: effect of adjuvant. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology, Las Vegas, NV, May.

Crosland, R. D. 1988. Development of drug therapies for snake venom intoxication. Presented at the International Society for Toxinology, Stillwater, Oklahoma, August.

Cosgriff, T. M., C. M. Hsiang, J. W. Huggins, M. Y. Guang, J. I. Smith, Z. O. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, P. H. Gibbs, X. E. Gui, G. W. Yuan, and T. M. Zhang. 1988. Predictors of fatal outcome in the severe form of hemorrhagic fever with renal syndrome (HFRS). Presented at the First International Symposium on Hataviruses and Crimean Congo Hemorrhagic Fever Viruses, Porto Carras, Halkidiki, Greece, September.

Cosgriff, T. M., D. B. Parrish, R. M. Lewis, P. B. Jahrling, J. W. Huggins, and C. J. Peters. 1987. The original hemorrhagic fever revisited: hemostatic derangement in rhesus monkeys infected with yellow fever virus. Presented at the 29th Annual Meeting of the American Society for Hematology, Washington, D. C., December.

Dalrymple, J. M., L. T. Kakach, and M. S. Collett. 1988. Mapping protective determinants of Rift Valley fever virus using recombinant vaccinia viruses. Presented at the Sixth Cold Spring Harbor Conference on Modern Approaches to Vaccines, Cold Spring Harbor, New York, September.

Darwish, M. A., W. H. Ennis, I. H. Allen, S. El Said, F. M. Feinsod, and C. J. Peters. 1987. Neutralizing antibodies to RVF virus in sheep from Aswan, Egypt. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Dixon, R. S., W. Gibcsn, L. S. Holt, S. L. Clements, J. G. Miller, and L. Hoffman. 1987. Laboratory animal care and use in a high-hazard (BSL-4) environment. Presented at the 38th Annual Meeting of the American Association for Laboratory Animal Science, Denver, CO, November.

Driscoll, D., and K. Wilson. 1988. High-containment isolation, American style. Presented at the Intenational Society of Infection Control, London, England, September.

Franz, D. R., D. A. Creasia, and R. D. LeClaire. 1988. Effects of *Ptychodiscus brevis* (red tide) toxin on respiratory function in the awake guinea pig. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology, Las Vegas, NV, May.

Franz, D. R., R. D. LeClaire, D. Hernandez, and K. B. Moody. 1988. Effects of saxitoxin on respiratory function in the awake guinea pig. Presented at the 9th World Congress on Animal, Plant and Microbial Toxins, International Society on Toxinology, Stillwater, OK, July-August. To be published in Toxicon.

Franz, D. R., R. D. LeClaire, W. B. Lawrence, and D. L. Bunner. 1988. The role of hypoxia in the pathogenesis of cyanoginosin intoxication. Presented at the 9th World Congress on Animal, Plant and Microbial Toxins, International Society on Toxinology, Stillwater, OK, July-August. To be published in Toxicon.

Friedlander, A. M., S. H. Leppla, and E. M. Cora. 1988. Molecular interactions of anthrax toxin components with target cell receptors. Presented at the Army Science Conference, Westpoint, NY, June.

Gabrielson, B. J., M. A. Ussery, P. G. Canonico, G. R. Pettit, E. M. Schubert, and W. M. Shannon. 1988. An antiviral structure/activity study of the phenanthridone alkaloids, pancrastatin, narciclasine and analogs. Presented at the American Chemical Society's North American Meeting, Totonto, Canada, June.

Gabrielsen, B., M. A. Ussery, P. G. Canonico, G. R. Pettit, E. M. Schubert, and R. W. Sidwell. 1988. Anti-RNA-viral activities of phenanthridones related to narciclasine (I, R=H, R'=OH). Presented at the 2nd International Conference on Antiviral Research, Williamsburg, VA, April.

Gad, A., F. M. Feinsod, A. Shokry, B. A. Soliman, G. O. Nelson, S. El Said, P. H. Gibbs, and A. J. Saah. 1987. Exposure variables in human *Wucheria bancrofti* filariasis in the Nile Delta. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygiene, Los Angeles, CA, November-December.

Genovesi, E. V., A. J. Johnson, and C. J. Peters. 1988. Pathogenesis of lymphocyte choriomeningitis virus (LCMV) infections of inbred hamsters. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, FLA, May.

Graham, R. R. 1988. Current trends in veterinary laboratory operations. Presented at the Brooke Army Medical Center Annual Meeting for Veterinary Officers, Fort Sam Houston, TX, May.

Gravely, S. M., D. M. Waag, and J. C. Williams. 1988. Time- and dose-dependent effects of the *Coxiella burnetii* phase I whole cell vaccine on splenocyte and thymocyte populations. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, FLA, May.

Hasty, S., B. Lidgerding, C. French, and J. Dalrymple. 1987. Hantavirus propagation and plaque assay on cells derived from the CV-1 African green monkey kidney cell line. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Hewetson, J. F., S. E. Hall, J. I. Smith, and J. E. Beheler. 1988. Comparison of an ELISA and sodium channel assay for detection of paralytic shellfish poisoning in clams and clinical specimens from intoxicated individuals. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology, Las Vegas, NV, May.

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Hodgson, L. N. Pesik, and J. Smith. 1988. Analysis of the structural and nonstructural proteins of Crimean-Congo hemorrhagic fever virus. Presented at the 7th Annual Meeting of the American Society for Virology, Austin, TX, June.

Hoover, T. A., and J. C. Williams. 1988. Cloning and expression of the *Coxiella burnetii* pyrB gene. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, FLA, May.

Huggins, J. W., C. M. Hsaing, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T., M. Zhang, P. H. Gibbs, and D. D. Oland. 1988. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the Army Science Conference, Westpoint, NY, June.

Huggins, J. W., C. M. Hsaing, T. M. Cosgriff, M. Y. Guang, J. I. Smith, Z. A. Wu, J. W. LeDuc, Z. M. Zheng, J. M. Meegan, C. N. Wang, X. E. Gui, K. W. Yuan, T., M. Zhang, P. H. Gibbs, and D. D. Oland. 1988. Intravenous ribavirin therapy of hemorrhagic fever with renal syndrome (HFRS). Presented at the Second International Conference on Antiviral Research, Williamsburg, VA, April.

Jahrling, P. B., R. W. Trotter, J. G. Barrera Oro, H. W. Lupton, T. M. Cosgriff, and C. J. Peters. 1988. Cross-protection against Machupo with Candid #1 live-attenuated Junin virus vaccine. Presented at the 7th Annual Meeting of the American Society for Virology, Austin, TX, June.

Jahrling, P. B., R. W. Trotter, J. G. Barrera Oro, H. W. Lupton, T. M. Cosgriff, and C. J. Peters. 1988. Cross-protection against Machupo with Candid #1 Junin virus vaccine. III. Postchallenge clinical findings. Presented at the 2nd International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar del Plata, Argentina, March.

Kende, M. 1988. Antiviral potential of biological response modifiers (BRM). Presented at the Second International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Carribbean Region, Mar Del Plata, Argentina, March.

Kende, M., and P. G. Canonico. 1988. Antiviral potential of biological response modifiers (BRM). Presented at the Second Symposium on Prevention and Tatment of Viral Infection, Bechyne, Czechoslovakia, June.

Kende, M., P. G. Canonico, M. Contos, and A. Gabizon. 1988. Liposomes in the therapy of Rift Valley fever virus infection. Presented at the UCLA Symposium, entitled "Liposomes in the Therapy of Infectious Diseases and Cancer," Lake Tahoe, CA, February.

Kende, M., P. G. Canonico, and T. R. Tice. 1988. Drug targeting strategies for the treatment of experimental Rift Valley fever virus (RVFV) infection. Presented at the International Conference on Pharmaceutical Sciences and Clinical Pharmacology, Jerusalem, Israel, May-June.

Kende, M., W. L. Rill, M. J. Contos, and P. G. Canonico. 1988. Efficacy of a novel orally active immunomodulator, S-26308 against arbovirus infections. Presented at the 2nd International Conference on Antiviral Research, Williamsburg, VA, April.

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Kenyon, R. H., J. Barrera Oro, C. MacDonald, J. Meegan, and C. J. Peters. 1988. Lymphocyte transformation assays for Junin virus (Argentine hemorrhagic fever). Presented at the Second International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar Del Platas, Argentina, March.

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Kirsi, J. J., and P. G. Canonico. 1988. The comparative antiviral action of ribavirin against selected Bunyaviruses. Presented at the 2nd International Conference on Antiviral Research, Williamsburg, VA, April.

Knauert, F, K., and B. A. Parrish. 1987. Detection of Rift Valley fever virus (RVFV) RNA in immune complexes. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Knudson, G. B., S. F. Little, and B. E. Ivins. 1988. Immunization against anthrax with a transposon Tn*916*-generated aro mutant of *Bacillus anthracis*. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, FL, May.

Ksiazek, T. G., and J. Smith. 1987. A quantitative 24 hour assay for Rift Valley fever virus neutralizing antibody. Presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

Latham, P. S., S. B. Sepelak, D. Y. Pifat, and J. F. Smith. 1987. Hepatocytes and Kupffer cells express age-related susceptibility to murine hepatitis caused by a bunyavirus. Presented at the Annual Meeting of the Association for the Study of Liver Disease, November.

Leatherman, D. L., and J. L. Middlebrook. 1988. Effect of emetine upon the association of T-2 toxin with CHO cells. Presented at the 9th World Congress on Animal, Plant and Microbial Toxins, International Society on Toxinology, Stillwater, OK, July-August.

LeClaire, R. O., W. B. Lawrence, K. A. Bostian, and K. A. Mereish. 1988. Acute toxicity of microcystin-LR in the rat. Presented at the Annual Meeting of the Society for Toxinology, Dallas, TX, February.

LeClaire, R. D., K. B., D. R. Franz, D. L. Bunner. 1988. Hemodynamic changes induced by cyanoginosin-LR in the rat and the effect of pharmacological intervention. Presented at the 9th World Congress on Animal, Plant and Microbial Toxins, International Society on Toxinology, Stillwater, OK, July-August. To be published in Toxicon.

LeDuc, J. W. 1987. Hantaan virus infections: old lessons and new developments in Korean hemorrhagic fever. Presented at the Third Annual F. Edward Hebert School of Medicine Conference on Military Medicine, Uniformed Servoces University of Health Sciences, Bethesda, MD, October.

LeDuc, J. W. 1988. Recent advances in the epidemiology and diagnosis of Hantaviruses, cause of hemorrhagic fever with renal syndrome. Presented at the Biennial Army Medical Entomology Training Course, San Antonio, TX, February.

LeDuc, J. W. 1988. Recent advances in the epidemiology and diagnosis of hantaviral infections. Working Conference and Symposium, sponsored by the U.S.-Japan Viral Diseases Panel of the U.S.-Japan Cooperative Medical Sciences Program, Tokyo, Japan, July.

Linthicum, K. J., C. L. Bailey, F. G. Davies, A. Kairo, and T. Logan. 1988. Observations on the horizontal distribution of ground pool Aedes pupae within a flood dambo in Kenya. Presented at the Annual Meeting of the American Mosquito Control Association, Denver, CO, February.

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Linthicum, K. J., T. M. Logan, C. L. Bailey, D. M. Watts, and D. J. Dohm. 1987. Experimental infection of Dugbe virus (family Bunyaviridae, genus *Nairovirus*) in five North American and one African ixodid ticks. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Liu, C. T. 1988. Body surface areas and K values in strain 13 guinea pigs with different weights and ages. Presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology and Medicine, Las Vegas, NV, May.

Logan, T. M. 1988. Crimean-Congo hemorrhagic fever. Presented at the Biennial Army Medical Entomology Training Course, San Antonio, TX, February.

Logan, T. M., K. J. Linthicum, C. L. Bailey, D. M. Watts, and J. Moulton. 1987. Transstadial transmission of Crimean-Congo hemorrhagic fever virus in orally infected *Hyalomma truncatum* with observations on virus replication in *Rhipicephalus appendiculatus*. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Logan, T. M., K. J. Linthicum, C. L. Bailey, D. M. Watts, and J. Moulton. 1988. Experimental transmission of Crimean-Congo hemorrhagic fever virus (family Bunyaviridae, genus *Nairovirus*) by *Hyalomma truncatum* Koch. Presented at the First International Symposium on hantaviruses and Crimean-Congo hemorrhagic fever viruses, Porto Carras, Halkidiki, Greece, September.

Loridan, C., and J. Middlebrook. 1988. Cloning and sequencing of the cDNA coding for myotoxin A. Presented at the 9th World Congress on Animal, Plant and Microbial Toxins, International Society on Toxinology, Stillwater, OK, July-August. To be published in Toxicon.

Lupton, H. W., P. B. Jahrling, J. G. Barrera Oro, and C. J. Peters. 1988. Cross-protection against Machupo virus with Candid #1 live-attenuated Junin virus vaccine. II. Post-challenge virological and immunological findings. Presented at the 2nd International Conference on the Impact of Viral Diseases on the Development of Latin American Countries and the Caribbean Region, Mar del Plata, Argentina, March.

Maiztegui, J. I., F. Feinsod, A. M. Briggiler, C. J. Peters, D. A. Enria, P. Gibbs, A. M. Ambrosio, E. Tiano, M. R. Feuillade, G. Gamboa, O. Conti, D. Vallejos, C. MacDonald, and J. Barerra Oro. 1987. Inoculation of Argentine volunteers with a live-attenuated Junin virus vaccine. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, November-December.

Malinoski, F. J., S. Hasty, J. Dalrymple, and P. C. Canonico. 1988. Prophylactic ribavirin treatment of dengue type 1 infection in rhesus monkeys. Presented at the 2nd International Conference on Antiviral Research, Williamsburg, VA, April.

Mangiafico, J. A., J. L. Sanchez, L. T. Figueriedo, J. W. LeDuc, and C. J. Peters. 1987. Isolation from man of a newly recognized subtype of Cache Valley virus. Presented at the Annual Meeting of the American Society for Tropical Medicine and Hygeine, Los Angeles, CA, December.

McCaul, T. F., C. E. Snyder, Jr., and J. C. Williams. 1988, Immunocytochemical detection of an immunogenic protein of *Coxiella burnetii* by monoclonal antibodies. Presented at the 9th European Congress on Electron Microscopy, York, U.K., September.

McCaul, T. F., H. A. Thompson, and J. C. Williams. 1988. Ultrastructural studies of sporulation of *Coxiella burnetii.* Presented at the Annual Meeting of the American Society of Rickettsiology and Rickettsial Diseases, Santa Fe, NM, April.

McCaul, T. F., and J. C. Williams. 1988. Immunocytochemical detection of antigenic determinants of *Coxiella burnetii.* Presented at the 88th Annual Meeting of the American Society for Microbiology, Miami Beach, FL, May.

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GLOSSARY

AcNPV	*Autographica californica* nuclear polyhedrosis virus
AIDS	Acquired immune deficiency syndrome
AHF	Argentine hemorrhagic fever virus
AVHRR	Advanced very high resolution radiometer
BARD	United States-Israel Bi-National Agricultural Research and Development Fund
BCG	*Bacillus globigii*
BW	Biological warfare
CCHF	Crimean-Congo hemorrhagic fever virus
cDNA	Complementary DNA
CHIK	Chikungunya virus
CHO	Chinese hamster ovary cells
CME	Chloroform-methanol extract
CMR	Chloroform-methanol-extracted residue
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EF	Edema factor
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
G1, G2	Glycoproteins
GC-MS	Gas chromatography-mass spectroscopy
HFRS	Hemorrhagic fever with renal syndrome

HIV	Human immunodeficiency virus
HT-2	HydroxyT-2 toxin
HPBL	Human peripheral blood lymphocytes
HPLC	High-pressure liquid chromatography
IC	Intracoelomic
IFA	Indirect fluorescent antibody
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IND	Investigational New Drug
Il	Interleukin
ISC	Inmune suppressive complex
i.p.	Intraperitoneal
i.v.	Intravenous
Kb	Kilobase
Kd	Kilodalton
KHF	Korean hemorrhagic fever
LCV	Large cell variant
LCMV	Lymphocytic choriomeningitis virus
LD_{50}	Median lethal dose
LF	Lethal factor
LPS	Lipopolysaccharides
LT	Lymphocyte transformation
MAB	Monoclonal antibodies

mRNA	Messenger RNA
NAP	Neutrophil activating protein
NC	Nucleocapsid protein
NCI	National Cancer Institute
NIH	National Institutes of Health
NMR	Nuclear magnetic resonance
NOAA	National Oceanic and Atmospheric Administration
P2, P3	Biological containment levels 2 and 3
PA	Protective antigen
PbTx-2 and 3	Brevetoxins 2 and 3
PFU	Plaque-forming unit
PHA	Phytohemagglutinin
PLA_2	Phospholipase 2
PRN	Plaque-reduction neutralization
QAE	QAE chromatography
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SCID	Severe-combined immunodeficient mouse
SCV	Small cell variant
SIV	Simian immunodeficiency virus

TCF	Lymphocyte chemotactic factor
$TCID_{50}$	Median tissue culture infectious dose
VEE	Venezuelan equine encephalomyelitis virus
WCI	Whole cells, phase I

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