SECOND ASIA - PACIFIC CONGRESS OF MEDICAL VIROLOGY

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Virus Diseases: The Global Challenge to Health for All

November 17-22, 1991
Ambassador Hotel, Bangkok
THAILAND

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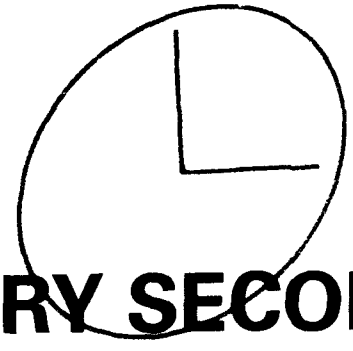

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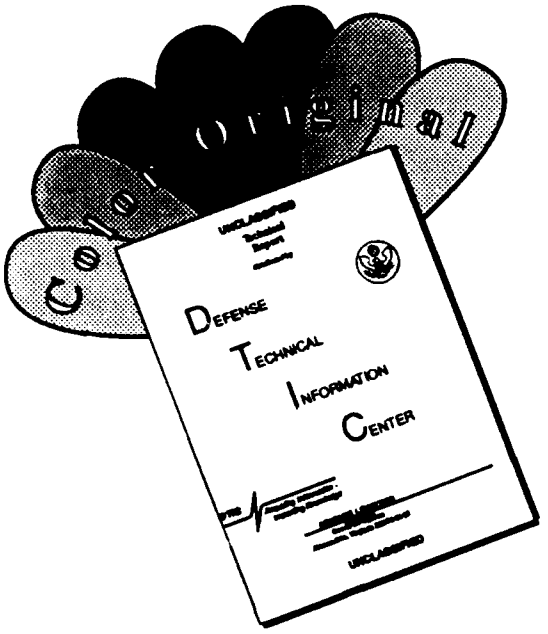

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ABSTRACTS

Virus Diseases : The Global Challenge to Health for All

November 17-22, 1991
Ambassador Hotel, Bangkok
THAILAND

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MESSAGE

Dear Colleagues,

It is our pleasure to invite you to the Second Asia-Pacific Congress of Medical Virology, which will be held in Bangkok, Thailand between November 17-22, 1991.

The Congress has been called in response to requests of the International Committee of the First Asia-Pacific Congress of Medical Virology held in Singapore in 1988. There has been a continuing need for a comprehensive regional organization that will bring together all those interested in medical virology i.e., virologists, microbiologists, immunologists, epidemiologists, infectious diseases specialists, clinicians, medical technologists, public health workers and other scientists. It is hoped that this Congress will demonstrate to the academical, political and industrial world how extensive and serious the unsolved problems of viral infections in developing and developed countries are. The need to bring the benefit of modern research with high technology to the populations of world in the quickest, safest and simplest manner is a certain theme.

Your are cordially invited to come to exotic Thailand, enjoy our famous cuisine, shopping, etc. in a warm and friendly atmosphere which will be long remembered.

We therefore look forward to your favourable response and we are certain that with your active participation and support, a major breakthrough will be achieved in the fulfillment of our common goals.

The Organizing Committee

The Second Asia-Pacific Congress of Medical Virology

Bangkok, Thailand.

MESSAGE

Thailand holds a special significance for the Asia-Pacific Congress of Medical Virology. It was in Bangkok in 1986 that the idea of holding a regional scientific meeting for virologists in the Western Pacific and Southeast Asian countries was conceived. Two years later, in 1988, the First Asia-Pacific Congress of Medical Virology was held in Singapore. The Singapore Congress proved to be an instant success with virologists coming not only from regional countries but also from the international community.

The Singapore Congress also saw the inauguration of the Asia-Pacific Congress of Medical Virology Board and the Pacific Basin Respiratory Virus Research Group, as well as the formal association of the Congress with the Asian Group for Rapid Viral Diagnosis. At the Singapore Congress, the Board decided that the Congress would be held every three years with Thailand hosting the Second Congress (1991) and China hosting the Third Congress (1994).

I am very pleased to see that the Second Asia-Pacific Congress of Medical Virology is being held as scheduled in Bangkok, Thailand. The Bangkok Congress holds promise that the Congress will become a regular major scientific meeting for virologists in the Asia-Pacific region. I wish to congratulate Professor Prasert Thongcharoen, Dr. Chantapong Wasi, and other members of the National Organizing Committee for organizing a first-rate Congress and hope that all participants will have a stimulating and enjoyable meeting.

Professor Chan Yow Cheong
President,
First Asia-Pacific Congress of Medical Virology

MESSAGE

With great pleasure I welcome you to the Second Asia-Pacific Congress of Medical Virology in Bangkok, Thailand from 17 to 22 November, 1991. This is yet another expression of our fervent desire and support for the continuing attention of the international community in helping to solve some of the problems which underscore our fight against viral infections. We are fortunate that eminent scholars from different parts of the world have confirmed their participation and will contribute significantly to this conference.

With your active collaboration we hope to achieve the objectives of the Congress.

P. Thongcharoen M.D.

Professor Prasert Thongcharoen
President,
Second Asia-Pacific Congress of Medical Virology

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MAHIDOL UNIVERSITY

Mahidol University has its origin in 1887. Siriraj Hospital was estabished then by His Majesty King Chulalongkorn in memory of his son, His Royal Highness Prince Siriraj. In 1899 a medi al school was founded and was later developed into the University o Medical Sciences in 1943.

In 1969 His Majesty King Bhumibol Adulyadej ga e the Universtiy the name "MAHIDOL" to honor his father, His Royal Highness Prince Mahidol of Songkhla, who had played a very important role in the development of medical education and public health in Thailand.

Mahidol Universtiy is located in three areas *i.e.*

BANGKOK NOI, BANGKOK : These facilities, situated on the west bank of the Chao Phya River, Thonburi side, cover an area of about 29 acres.

PHAYA THAI, BANGKOK : These facilities cover an area of 198 acres.

SALAYA, NAKHON PATHOM : This facilities covers an area of approximately 500 acres.

SUNDAY 17 NOVEMBER 1991

PRE-CONGRESS WORKSHOP

Session No. 1 — WORKSHOP I

Date : Sunday 17 November 1991 — Time 10:00-12:00
Room : Room B

WORKSHOP I Molecular Virology
Chairpersons : ***Skorn Mongkolsuk***, THAILAND
Chin Yih Ou, USA

1. Polymerase Chain Reaction (PCR) : A Powerful Diagnostic Tool for Diseases Control
   Suvicha Chitpatima, THAILAND
2. Recent Advances in Automated DNA Sequencing and Data Analysis
   Richard H. Lussier, USA
3. Chemiluminescence Detection of Digoxigenin - Labeled Nucleic Acids : Optimization of the Detection Protocol
   Hans J. Hoeltke, GERMANY

Session No. 2 — WORKSHOP II

Date : Sunday 17 November 1991 — Time 13:00-15:00
Room : Room B

WORKSHOP II Flow Cytometry
Chairpersons : ***Alan D. King***, USA
H. Kyle Webster, USA

1. Flow Cytometry in Virology Research
   Bruch A. Bach, USA
2. Clinical Application of Flow Cytometry
   Jeff Harvey, USA
3. Use of Flow Cytometer in Thailand - FACS Users Club
   Kovit Pattanapanyasat, THAILAND

SUNDAY 17 NOVEMBER 1991

OPENING CEREMONY AND WELCOME RECEPTION

Convention Hall and Garden Bar **Time 15:30-20:30**

15:30 Multivision Slides Show

15:45 Guests and Delegates are requested to be seated

16:00 Introductory Reports
Professor Dr. Prasert Thongcharoen
Chairman of the Organizing Committee

Inauguration Remarks
Dr. Pirote Ningsanonda
Minister of Public Health

Welcoming Address
Professor Dr. Pradit Chareonthaitawee
Dean of Faculty of Medicine Siriraj Hospital

Presentation of Recognition Awards

17:00 **KEYNOTE LECTURE**
Virus Vaccines : Achievements and Challenges
Professor Dr. Joseph L. Melnick, USA

17:45 Opening of the Exhibition

18:30 Welcome Reception at the Garden Bar, on the second floor

Session No. 3 **KEYNOTE LECTURE**

Date : Sunday 17 November 1991 **Time 17:00-17:45**
Room : Room A & B

KEYNOTE LECTURE
Virus Vaccines : Achievements and Challenges
Professor Dr. Joseph L. Melnick, USA

WS I-1

THE POLYMERASE CHAIN REACTION: A POWERFUL DIAGNOSTIC TOOL FOR DISEASE CONTROL

S. Tim Chitpatima

Department of Biochemistry, Pramongkutklao College of Medicine, 315 Rajvithee Road, Payathai, Bangkok 10400, Thailand.

In recent years, significant advances in the knowledge of DNA and its make up have led to the development of a powerful technique call Polymerase Chain Reaction (PCR) (1-3). The term PCR can be defined as "A primer-mediated enzymatic amplification of specific genomic or cloned sequences". Since the advent of PCR, laboratories around the globe have been exploiting this technology to bridge limitations or to overcome common problems normally encountered in molecular biology techniques. In addition, this technology has been employed successfully in diagnostic and basic scientific research and development. Nowaday, PCR technology is fast becoming a commonplace among molecular biology laboratories.

PCR can be considered as a second generation molecular biology technique with several inherent advantages. The technique is fast and relatively easy to perform. The virtue of the technique lies in its sensitivity and specificity. The true potentials of this technology is realized in early detection of pathogens and genetic abnormalities. More recently, with the advances in knowledge of genetic basis of disease pathogenesis, PCR has been utilized to study genetic predisposition and diseases association. Genetic markers of diseases have been extensively studied and identified. Regulative gene expression and disease association has been elucidated in several systems in mammals. The recent utility of antisense RNA and DNA in regulating gene expression has been realized. PCR can be used as quantitative tools to measure the extent of gene expression as a function of regulative agents both *in vivo* and *in vitro*.

One important consideration for performing routine PCR is the issue of quality control. This is based on the fact that this powerful technique can offer both advantages and disadvantages. The advantages have been discussed above. The disadvantages involved fallacious laboratory practices, not the inherent problem of the technology. While PCR is the most sensitive detection techniques, careless handling of PCR protocols could lead to spurious results. Exogenous sources of contamination can easily been introduced into samples which otherwise would not be amplifiable. This could lead to difficulties in interpretation of data. Minute contamination of sample and reagents by target DNA will be amplified and scored as positive. Several precautionary steps must be taken to insure fidelity of the assay.

The most commonly practiced PCR protocols involved amplification of target DNA or cDNA sequences and analysis of PCR product by electrophoresis. The product is normally further identified by hybridization with probes complementary to region inside of both primers, A validated detection technique. Other alternative PCR protocol involved enhancing sensitivity and specificity by use of successive amplification, the so called "booster or Nested PCR". While this practice has good utilities in enhancing sensitivity, the practice itself is not yet practical for routine diagnosis use, especially when performed by novice or careless laboratory technicians. The procedure calls for amplification of targat DNA for a few rounds, followed by transfering part of the reaction to another tube containing "booster or internal primers". Thus this opens a chance for cross contamination of internal primers". Thus this opens a chance for cross contamination of post-PCR product from the first amplification to pre-PCR mixture of the second amplification. In addition, this procedure is a few times the cost of normal amplification in that at the frequently used and accepted protocol calls for a two-step amplification of 4 sets of primers in succession. Nested PCR's true potentials are then realized on research scale in obtaining low abundant and rare target DNA for further analyses in research fields and not in routine diagnostic research work. It full potentials should be realized in the future with acquired maturity of technical personnel.

Addtional utilities for PCR includes environmental monitoring for presence of pathogens and genetic abnormalities. PCR potentials in diagnosis of prenatal and perinatal genetic abnormalities such as Phenylketonuria, Duchene's Muscular Distrophy, Sickle Cell Anemia, Thalassemia, etc. have been realized in clinical diagnosis. Within next decades, it would not be surprising if PCR has become so common that it has a place in all research and diagnostic laboratories benches.

WS I-2

RECENT ADVANCES IN AUTOMATED DNA SEQUENCING AND DATA ANALYSIS

Richard H. Lussier

Applied Biosystems Japan, Inc.

3-3-6 Minami-suna, Koto-ku, Tokyo 136 Japan

DNA sequencing is one of the most commonly used techniques in modern molecular biology. It has traditionally employed radioactive precursors as a method of labeling and ultimately detecting reaction products in conjunction with ultra-thin denaturing polyacrylamide gels and X-ray sensitive films.

In 1986, Applied Biosystems made the first automated real-time gel reader, utilizing fluorescence labeling and the Sanger chain termination method, available to researchers throughout the world. The system takes advantage of muticolor labeling and detection of sequencing reaction products. By using four unique fluorophores, one to label each of the A,G,C, and T reaction products, a "one lane one template" detection system was devised. Sanger sequencing reaction products from one template labeled with these fluors is applied to a single lane of a conventional denaturing polyacrylamide gel inside the instrument. The products are then automatically electrophoresed, detected, and finally specialized software reads the sequence of the DNA based on the order of appearance of the colored fragments at the multicolor detector. Thus, the instrument automates the electrophoresis, fragment detection, base calling, and data entry steps associated with DNA sequencing. Improvements in software and reaction conditions throughout the years (including the unique "cycle sequencing" method) have resulted in the ability to routinely read 450 to 500 bases per double or single stranded template.

In an effort to automate the entire process of DNA sequencing, instrumentation has also been developed to perform the actual sequencing reaction. The technology employed in this instrument reduces the amount of template and enzyme necessary to generate sequencing products while eliminating tedious manual manipulations such as pipeting and liquid handling. Human intervention in the process is reduced to a minimal amount. In turn, an increase in reliability and reproducibility of DNA sequencing experiments are realized.

Finally, a system which utilizes an array of microprocessors developed by the TRW corporation in conjunction with computer hardware and software has been developed to automate some of the data analysis typically performed on newly derived DNA sequence. This device, the Fast Data Finder (FDF), reduces the time required to search a DNA or protein data base to seconds rather than hours or days typically required with conventional hardware and software. A unique programming language of FDF allows the design of custom "queries" of a data base to explore relationships previously unattainable. This instrument is also capable of rapid and extremely accurate assembly of "cosmid size" DNA sequencing projects.

Each system shall be described.

WS I-3

CHEMILUMINESCENCE DETECTION OF DIGOXIGENIN-LABELED NUCLEIC ACIDS. OPTIMIZATION OF THE DETECTION PROTOCOL

H.J. Holtke

Boehringer Mannheim GmbH, Biochemical Research Center, Nonnenwald 2, D-8122 Penzberg, Germany.

A fast and simple protocol for the chemiluminescent detection of digoxigenin (DIG) labeled nucleic acids with antidigoxigenin antibody Fab- fragments coupled to alkaline phosphatase and AMPPD as substrate is described. The washing and blocking procedure was optimized to yield low background even on highly charged nylon membranes. The sensitivity of the system is equal or better than of radioactive mehods. Exposure to X-ray of Polaroid films for up to 30 minutes is sufficient for highly sensitive detection of DIG-labeled nucleic acids down to 30 fg homologous DNA. Human single-copy genes are detected in Southern-blots of as low as 0.3 ug total placenta DNA. Blots can be reprobed multiple times very easily without loss of signal intensity.

The advantages of the DIG-system, high sensitivity, absence of background and ease of reprobing, are illustrated by applications for single-copy gene detection in genomic blots of human DNA, Northern-hybridizations to rare mRNA, detection of *E. coli* genes on blots of genomic digests after pulse field gel electrophoresis as well as for nonradioactive DNA sequencing blots with DIG-labeled primers.

WS II-1

FLOW CYTOMETRY IN VIROLOGY RESEARCH

Bruch A. Bach
Worldwide Clinical Research Director Becton Dickinson Immunocytometry Systems
2350 Qume Drive San Jose, California, USA.

WS II-2

CLINICAL APPLICATION OF FLOW CYTOMETRY

Jeff Harvey
Becton Dickinson Immunocytometry Systems 2350 Qume Drive San Jose, California, USA

WS II-3

USE OF FLOW CYTOMETER IN THAILAND

K. Pattanapanyasat
The Thalassemia Center, Division of Hematology, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Interests in performing flow cytometry in Thailand began in 1983 when Armed Forces Research Institute of Medical Sciences (AFRIMS) introduced the first FACS analyser into the country. Most of the studies that were carried out during the early years were connected with the enumeration and characterization of blood cells in malaria patients. It took almost another 5 years before the second system was installed in Siriraj Hospital Medical School. In the years that have followed, the demand for the instrument remained high. By 1990, AFRIMS obtained a more advanced flow cytometer equipped with high power and sorting facilities. It is from this system that many experiments, incuding a novel technique for simultaneous determination of malaria parasite DNA and red blood cell antigens, as well as parasite antigens, was described. At about the same time, the proliferation and public awareness of AIDS has prompted the Ministry of Public Health to purchase 16 systems. This is probably the largest single order of such an instrument in this region. These 16 systems are now being used to study the levels of CD 4 + cells in HIV patients throughout the country. There are now a total of 23 sets of low cytometers in Thailand. Four are located in the North, 3 in the Northeast and the South, and 13 are in Central Thailand with 11 of these in Bangkok.

During a training course on fow cytometry held in April, 1991 by the Ministry of Public Health, a FACS Users Club was set up after a proposal from the participants of that course. The purpose of the Club is to provide the users with information of the latest development in this field and related subjects. It also help the members solving some troubleshootings of the instrument. The club now involves the participation of more than 50 members.

In this workshop, I shall discuss some of the achievements done by us and others working in this field. The discussion will also cover several other on-going projects as well as the story of FACS Users Club in Thailand.

KL

VIRUS VACCINES: ACHIEVEMENTS AND CHALLENGES

Joseph L. Melnick
Baylor College of Medicine, Houston, Texas.

Through the development and use of vaccines, many virus diseases have been brought under control. The current conventional vaccines consist either of infectious, attenuated viruses or of noninfectious killed viruses or subviralantigens. Thus far, it is the live vaccines that have yielded the greatest successes in controlling and even eradicating diseases.

Historical landmarks in the development of vaccines for virus diseases of humans span almost two centuries, beginning with Jenner's demonstra-tion in 1798 that a mild disease, produced by design with one virus, could protect against more virulent disease caused by a related agent. A momentous recent achievement was the licensing in 1982 of a hepatitis B vaccine consisting of subviral particles prepared from plasma of chronic virus carriers, and subsequently from yeast expressing a recombinant gene of hepatitis B virus. Other potential vaccines are under investigation. Their development is based on genetic manipulation, nucleic acid sequencing, and monoclonal antibody availability.

Dramatic results of widespread vaccination are being seen in the decreases, particularly in the industrialized countries in the temperate zones, of cases of four childhood viraldiseases: poliomyelitis, measles, rubella, and mumps. Two viral vaccines are included in the World Health Organization Expanded Programme on Immunization, and as a result millions of infants are being protected from the ravages of poliomyelitis and measles. However, challenges remain.

The type of overall immunity required should determine the optimal vaccine to be developed and used. Thus a vaccine (such as IPV), which prevents a systemic infection and protects the individual, is not as effective for the community as one (such as OPV) which prevents infections involving mucosal surfaces. Proper use of vaccines is crucial in eliminating disease from a community or a region, and for maintaining freedom from recurrences. The conditions for breaking the chain of viral transmission and eradicating disease by optimal use of polio, measles, and hepatitis B vaccines will be discussed.

Session No. 4 PLENARY LECTURE 1

Date : Monday 18 November 1991 Time 09:00-09:45
Room : Room A & B

PLENARY LECTURE 1
Chairpersons : *Michiaki Takahashi*, JAPAN
John W. Boslego, USA
Emerging Virus Diseases
Frank Fenner, AUSTRALIA

Session No. 5 SYSPOSIUM 1

Date : Monday 18 November 1991 Time 10:15-11:45
Room : Room A

SYMPOSIUM 1 New Agents of Viral Hepatitis
Chairpersons : ***Bruce Innis***, USA
Termchai Chainuvatti, THAILAND

1. Hepatitis E Virus (HEV) : Epidemiology, Characterization and Animal Models
   John Ticehurst, USA
2. Molecular Characterization and Emerging Epidemiology of the Hepatitis E Virus (HEV)
   Gregory R. Reyes, USA
3. Hepatitis C Virus : A New Member of the *Flaviviridae*
   Micheal Beach, USA
4. Prospects for Hepatitis C Vaccines
   Colin R. Howard, UK

Session No. 6 SYSPOSIUM 2

Date : Monday 18 November 1991 Time 10:15-11:45
Room : Room B

SYMPOSIUM 2 Herpesviruses Update
Chairpersons : ***Koichi Yamanishi***, JAPAN
Prasong Pruksananonda, THAILAND

1. Human Herpes Virus 6 (HHV-6) Infection
   Koichi Yamanishi, JAPAN
2. The Natural History of Early Lymphotropic Herpesvirus Infection in Hong Kong
   R.B. Heath, UK
3. Human Herpes Virus 6 (HHV 6) Infection and Exanthem Subitum in Thailand
   Kruawan Balachandra, THAILAND
4. Diagnosis of Herpes Simplex Virus Encephalitis by Detection of Class-Specific Antibodies and DNA in CSF
   Eric Uren, AUSTRALIA
5. Endonuclease Cleavage Analysis of HSV-2 Isolated in Chiangmai, Thailand
   Wasana Sirirungsi, THAILAND

Session No. 7 **SPECIAL SESSION I**

Date : Monday 18 November 1991 **Time 12:45-13:45**
Room : Room A

SPECIAL SESSION I Hepatitis A and Hepatitis B Vaccines
Chairpersons : ***Prayura Kunasol,*** THAILAND
Bruce Innis, USA

1. Hepatitis A Vaccine
   Assad Safary, BELGIUM
2. Long Term Follow up of the Efficacy of rDNA Hepatitis B Vaccine in High Risk Neonates
   Yong Poovorawan, THAILAND
3. Control of Hepatitis B Infection in Asia with Universal Infant Immunization as Part of the EPI
   Mark Kane, WHO, GENEVA
4. Current Status of Global Control of Hepatitis B Through Vaccination
   James Maynard, PATH, USA

Session No. 8 **SYMPOSIUM 3**

Date : Monday 18 November 1991 **Time 14:00-15:15**
Room : Room A

SYMPOSIUM 3 Molecular Biology, Creative Uses of New Technology to Solve Difficult Problems
Chairpersons : ***John Aaskov,*** AUSTRALIA
Suharyono Wuryadi, INDONESIA

1. Pathogenic Study of Transgenic Mice Carrying Poliovirus Receptor Gene
   Takeshi Kurata, JAPAN
2. Detection of Viruses in Water Samples Using Amplification of Viral RNA Through Polymerase Chain Reaction
   N. Jothikumar, INDIA
3. Rapid Identification of Dengue Viruses by Polymerase Chain Reaction
   K. Morita, Akira Igarashi, JAPAN
4. *n Situ* DNA Amplification : Method for Detecting DNA Viruses at Cellular and Subcellular Resolution
   Wasunt Chantratita, THAILAND
5. A Novel Variant of Human Interferon Alpha 1 Gene
   Hou Yunde, CHINA

Session No. 9 SYMPOSIUM 4

Date : Monday 18 November 1991 Time 14:00-15:15
Room : Room B

SYMPOSIUM 4 Respiratory Viruses
Chairpersons : ***Claude M. Hannoun***, FRANCE
Nancy Cox, USA

1. Rapid Diagnostic Methods and Influenza Monitoring in France
   Claude M. Hannoun, FRANCE
2. Molecular Epidemiology of Influenza
   Christoph Scholtissek, GERMANY
3. Evolutionary Differentiation of the H3 Hemagglutinin Genes of Human, Swine and Avian Influenza A Viruses
   Kuniaki Nerome, JAPAN
4. A Large-Scale Expression of the Influenza Virus Hemagglutinin in Silkworms by a Baculovirus Vector
   Kuniaki Nerome, JAPAN
5. Pacific Basin Surveillance on Respiratory Viruses
   Helen Regnery, USA

Session No 10 WORKSHOP 1

Date : Monday 18 November 1991 Time 15:30-17:00
Room : Room A

WORKSHOP 1 Viral Hepatitis
Chairpersons : ***John Ticehurst***, USA
Yong Poovorawan, THAILAND

1. Active Hepatitis A Vaccination : Immunogenicity and Reactogenicity of an Accelerated Immunization Schedule
   Hans L. Bock, GERMANY
2. Development, Safety and Immunogenicity of New Inactivated Hepatitis A Vaccines : Effect of Adjuvants
   Reinhard Gluck, SWITZERLAND
3. Inhibition of Duck HBV DNA Replication Using Combination Antiviral Chemotherapy
   Stephen A. Locarnini, AUSTRALIA
4. Hepatitis Type E Studies in China
   Yutu Jiang, CHINA
5. Further Work on Enteric Non-A, Non-B Hepatitis
   Hema Gupta, INDIA
6. Incidence of Hepatitis B Virus Infections in 3-5 Year Old Children in Sichuan, China
   Li-Hua Liu, CHINA
7. The Effect of *Mycobacterium phlei* Substance in the Treatment of Chronic Hepatitis B and Chronic Hepatitis B Virus Carriers
   X.Z. Pu, CHINA
8. Lymphoblastoid Interferon With or Without Steroid Pretreatment in Chronic Hepatitis B Infection
   Thelma E. Tupasi, PHILIPPINES

Session No. 11 **WORKSHOP 2**

Date : Monday 18 November 1991 Time 15:30-17:00
Room : Room B

WORKSHOP 2 Respiratory Viruses
Chairpersons : ***Kennedy F. Shortridge,*** HONG KONG
Kuniaki Nerome, JAPAN

1. Co-operative Respiratory Virus Research in the Pacific Basin-Rationale and Potential
   Kennedy F. Shortridge, HONG KONG
2. Emergence of A New Influenza Virus in Horses in China
   Yuanji Guo,, Robert G. Webster, CHINA
3. Influenza Surveillance System and Virus Antigenic Analysis in Guangdong Province, China
   Gui Zhang Shen, CHINA
4. Influenza Surveillance in Pune, India, 1990
   Lalitha B. Rao, INDIA
5. Influenza Outbreak in a Subdistrict of Jakarta, Indonesia
   C. Maûroef, INDONESIA
6. Epidemiology of Viruses Associated with Acute Respiratory Tract Infection in Thai Children
   Subharee Suwanjutha, THAILAND
7. The Virological Surveillance and Molecular Epidemiology of Respiratory Virus Infections
   Sirima Pattamadilok, THAILAND
8. Analysis of Antigenic Drift of Influenza Isolates with Polymerase Chain Reaction and Nucleic Acid Sequencing
   Wu-Tse Liu, TAIWAN

17:15-18:15 **GROUP MEETING** **Room B**
Pacific Basin for Respiratory Virus Research Group

18:15-19:00 **ROUND TABLE DISCUSSION** **Room A**
Vaccination Strategies against Influenza

PL1

EMERGING VIRUS DISEASES

Frank Fenner
John Curtin School of Medical Research, Canberra, Australia.

Emerging virus diseases are not really a new phenomenon; they have been occurring ever since humans ventured into new environments. However, they have been brought to popular attention by the recent emergence of the human immunodeficiency viruses and their spread around the world. Perhaps the only "new" viruses involved in such diseases are the reassortant viruses that periodically cause pandemic influenza, and even these are produced by recombination of genes from pre-existing strains of human and animal influenza viruses.

Most instances of emerging virus diseases occur as a result of the movement of human populations into new ecological situations, the most widespread of which are the rapidly growing cities of the developing world. Other factors that have resulted in the appearance or recognition of new human virus diseases are the movement of farmers into forest fringes and the availability of better methods of virus diagnosis. The talk will trace the history of some of the more dramatic emerging virus diseases: pandemic influenza, AIDS, the hemorrhagic fevers, and human monkeypox.

HEPATITIS E VIRUS (HEV): EPIDEMIOLOGY, CHARACTERIZATION, AND ANIMAL MODELS

J. Ticehurst, *CF Longer, JD Caudill, LVS Asher, LN Binn, CH Hoke*

Walter Reed Army Inst Res/TA Mlele, Amer Registry Path, Washington DC; LL Rhodes, S Denny, JW Leduc, US Army Res Inst Infect Dis, Frederick MD; TJ Popkin, RHPurcell, Nati Inst Allergy Infect Dis/JP Bryan, LJ Legtera, Uniformed Svcs Univ Health Sci, Bethesda, MD; K Krawczynski, DW Brandley, Ctre Disease Control, Atlanta GA; C-C Huang, D Nyugen, GR Reyes, Genelabs Inc, Redwood City CA; RW Jansen, SM Lemon, Univ NC, Chapel Hill NC; USA. J Dilawari, Postgraduate Medical Inst, Chandigarh, India; M Iqbal, I Malik, Pakistan-US Lab Seroepidemiology, Rawalpindi, Pakistan; BL Innis, Armed Forces Res Inst Med Sci, Bankok, Thailand.

HEV is recognized as the etiologic agent of enterically-transmitted non-A, non-B hepatitis. It has infected people in Asia (including India since 1956 or earlier), Africa, and Mexico. A peculiarity of HEV infections in that levels of antibody to HEV (anti-HEV) are nearly always highest during the acute phase. Thus, sera from experimentally-infected primates were essential for identifying HEV; animals are still the only source of laboratory propagated virus bacuse HEV has not been isolated in cell culture. Although HEV is not well-characterized, its positive-sense RNA genome*, morphology, and physical features are somewhat distinct but most closely resemble those of caliciviruses. Our recent studies with HEV have attempted to identify additional infections, to determine the pattern of HEV excretion from patients, to identify and further characterize animal models, and to understand immunity. Immune electron microscopy (IEM) was used for detecting both anti-HEV and particulate HEV antigen. Immunofluorescence (IF) microscopy was used for detecting HEV antigen (HEVAg)in liver and anti-HEV by IF-blocking. Antibody was also detected by a prototype enzyme immunoassay* (EIA) and, in addition, HEV by an affinity capture-polymerase chain reaction (AC-PCR) method. For evidence of hepatitis, we measured serum levels of alanine aminotransferase (ALT) and observed liver tissue by light microscopy for histopathologic changes. We identified HEV infections in Lebanon during 1982 (in a US Marine), Nepal during 1987, Pakistan during 1987 and 1988, and India during 1989. In a study of an outbreak in Pakistan, we used IEM to detect HEV in feces from 1 of 12 individuals who developed jaundice 5 to 30 days later and from 9 of 36 patients within 7 days of the onset of icteric hepatitis; all 11 feces from day 8 to 15 were negative for HEV. We serially transmitted HEV strain Mexico '86 in cynomolgus and owl monkeys. Disease in cynomolgus monkeys was similar to that which was previously reported; these animals have been our best source of virus (in bile and feces) and HEVAg-positive liver. Such HEV particles were 29 nm in diameter, morphologically preserved in lyophilized feces, and resistant to extraction with trichlorotrifluoroethane. During the 2nd passage of HEV, 2 cynomolgus monkeys had peak levels of ALT on Day 31 after inoculation. Four more cynomolgus monkeys were given the same inoculum and then had bile and liver tissue collected between Days 12 and 22; the peak of viral replication (as determined by IF microscopy of liver and by IEM and AC-PCR of bile) appeared to occur prior to the development of anti-HEV and the most severe hepatitis. Owl monkeys were infected with HEV and developed high, sustained levels of anti-HEV, but only 3 of 6 monkeys developed hepatitis during the 1st passage. A chimpanzee had been infected with HEV Pakistan '85R and developed hepatitis; 3 years later, it was challenged with HEV Mexico '86 and did not develop hepatitis but responded with greatly increased levels of antibody that peaked by Day 11. Our results indicated that HEV may be a disease threat in the Middle East, as it continues to be in southern Asia. The virus appears to be resistant to an organic solvent. Cynomolgus monkeys still appear to be the best mode of human hepatitis E but HEV can infect owl monkeys and induce high levels of antibody without necessarily inducing hepatitis. A chimpanzee (like cynomolgus monkeys and a tamarin that were previously described) was not susceptible to reinfection with a 2nd strain of HEV and appeared to have an anamnestic response to the challenge virus, thus offering hope that an HEV vaccine could induce protective immunity.

*See abstract, this Congress by GR Reyes

S1-2

MOLECULAR CHARACTERIZATION AND EMERGING EPIDEMIOLOGY OF THE HEPATITIS E VIRUS (HEV)

Gregory R. Reyes,
Molecular Virology Laboratory, GeneLabs Incorporated, 505 Penobscot Drive. Redwood City, California 94063, USA.

Epidemic hepatitis, transmitted by the waterborne or fecal/ oral route, has been recognized as a major problem in developing countries. Although for the most part self-limited, the disease has been documented to cause significant morbidity and mortality in pregnant women. A recently characterized virus has been identified as the etiologic agent of what was formerly reported as enterically-transmitted non-A, non-B hepatitis. The discovery of this agent came about by the application of new cloning and screening methodologies that led to the elucidation of the 27-34 nm nonenveloped agent as a single-stranded, positive-sense, polyadenylated virus of approximately 7.5 kb in length. Two major open reading frames were recognized together with signature motifs for nonstructural genetic elements as well as those that presumably encode the viral structural gene (s). Recombinant expression of limited subgenomic regions of the virus has led to the development of prototype diagnostic tests for acute and past infection. These tests, currently under evaluation, will help to clarify the critical parameters of the host-virus interaction. In particular, the cyclical nature of virus outbreaks and whether they result from more virulent virus strains or perhaps waning host immunity will have profound implications for future efforts at virus control and eradication. An update will be presented on our current understanding of the virus genomic organization and expression as well as on our efforts to develop a sensitive and specific diagnostic test based on the expression of HEV recombinant proteins.

S1-3

HEPATITIS C VIRUS: A NEW MEMBER OF THE FLAVIVIRIDAE

M. Beach
Hepatitis Branch, Centers for Disease Control, Atlanta, GA, USA 30333.

The recent cloning and characterization of the primary agent responsible for parenterally-transmitted non-A, non-B hepatitis, now known as hepatitis C virus (HCV), has resulted in an explosion of information on the virus. The virus contains a single-stranded, positive-sense RNA genome approximately 9.4 kb in length. A single long open reading frame, which extends through almost the entire genome, could encode a polypeptide over 3000 amino acids in length. Comparison of the nucleotide and polyprotein sequences, and hydrophobicity plots with other viruses, indicates that HCV is related to members of the family Flaviviridae (flaviviruses and pestiviruses). The 5'-untranslated region exhibits sequence homology with the pestiviruses and contains multiple upstrean AUG codons which may be involved in translational regulation. The 5'-end encodes a putative core region and at least two glycoproteins which may be envelope proteins. Consensus sequences can be found in the 3'-end that suggest the presence of a serine protease, RNA helicase, and RNA-dependent RNA polymerase. HCV displays a high degree of sequence diversity throughout its genome with a hypervariable region found in the sequence encoding the putative envelope protein. This may indicate that HCV is capable of rapid mutation in order to evade immune detection by the host as found with other RNA viruses.

S1-4

PROSPECTS FOR HEPATITIS C VACCINES

C.R. Howard
The Royal Veterinary College, Royal College Street, London NW1 OTU.

The hepatitis C virus (HCV) genome consists of a l0kb singie-stranded, positive sense RNA organised similar to that of the pestivirus genus of the virus family Flaviviridae. Pestivirus infections of cattle and sheep show a number of similarities to HCV infections of humans. In the case of BVDV, persistent infections are frequent, it being estimated that 70% or more of cattle are chronically infected. The virus exists as two biotypes, persistence occurring as a result of infection *in utero* with the non-cytopathic type. Morbidity results when such animals are superinfected later in life with the second, cytopathic type. Two points particularly relevant to HCV are the requirement for an exact antigenic match for the superinfecting, cytopathic virus and the protection that is afforded by passive immunization. This suggests a sub-unit hepatitis C vaccine may be possible. On the negative side, however, HCV shares with pestiviruses the ability to undergo antigenic change in the structural proteins.

The development of HCV vaccines will require the answers to a number of questions, the most important being the protectivve value of anti-HCV antibodies and the identification of protective antigens. Antibody to non-structural antigens may be important, as shown for the flaviviruses yellow fever and dengue. Care also needs to be exercised that anti-HCV antibodies do not enhance rather than prevent virus growth on subsequent exposure to HCV.

Comparative analysis of HCV isolates and pestiviruses shows the presence of hypervariable hydrophilic domains in the E1 region that may represent potential immunogens. Hydrophilic domains in the E2 region of HCV appear more constant and we have identified 4 regions reactive for anti-HCV antibodies. Despite the lack of sequence homology to BVDV however, some cross-reactivity has been observed with anti-BVDV antisera. Expression of E2 proteins containing these regions is possible and most likely to represent first generation HCV vaccines.

S2-1

HUMAN HERPES VIRUS 6 (HHV-6) INFECTION

Koichi Yamanishi
Department of Virology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

A novel human herpesvirus was isolated from patients with lymphoproliferative disorders in 1986, and thereafter viruses with the similar characteristics were also isolated mainly from AIDS patients. This virus is now named HHV-6. In 1988, we reported that HHV-6 causes exanthem subitum (or roseola infantum). This virus has a high affinity for T cell with CD4 marker on the cell surface. In this symposium, the cellular tropism, the latent infection *in vitro* and *in vivo*, immunity in patients with exanthem subitum and the mode of transmission of HHV-6 will be discussed.

S2-2

THE NATURAL HISTORY OF EARLY LYMPHOTROPIC HERPESVIRUS INFECTION IN HONG KONG

R.B. Heath, *J. Tamm, M.H.Ng*
Queen Mary Hospital, Hong Kong and H.O. Kangro. St Bartholomew's Hospital, London.

The age at which young children in Hong Kong first become infected with CMV, EBV and HHV6 viruses was determined by testing sequential blood samples, taken during the first two years of their life, for the presence of antibodies to these viruses. The blood samples were taken at birth (cord blood) and thereafter at 4 monthly intervals. Antibodies to CMV and HHV6 were assayed by an indirect radioimmunoassay and those to EBV:VCA by immunofluorescence.

It was found that during the first 2 years of their life, 25.9% of the children had been infected with all 3 viruses, 44.4% with 2, 27.8% with 1 and only 1 out of 79 had escaped infection with all 3 viruses. Ninety-four percent of the children had been infected with HHV6, 61.1% with EBV and 38.8% with CMV.

The patterns of the rates of infection with these viruses were very different. CMV infection in particular, was unusual in that nearly all of the infections had occurred before the age of 4 months. The possible reasons for the different rates of infection will be discussed.

S2-3

HUMAN HERPESVIRUS 6 (HHV 6) INFECTION AND EXANTHEM SUBITUM IN THAILAND

***K. Balachandra*[1], *P. Bowonkiratikachorn*[2], *B. Poovijit*[3], *A. Thattiyaphong*[2], *C. Jayavasu*[1], *C. Wasi*[4], *M. Takahashi*[5], *K. Yamanishi*[5].**

[1]Department of Medical Science, [2]Charoenkrung Pracharuk hospital, [3]Children hospital, [4]Department of Virology, Faculty of Medicine, Siriraj Hospital, Mahidol University, [5]Research Institute for Microbial Diseases, Osaka University.

HHV-6 has been proved to be the causative agent of exanthem subitum by serology and virus isolation in Thailand. Thirty-one cases (62%) from fifty patients suspected clinically as exanthem subitum were serodiagnosed as HHV-6 infection, and sixteen strains of HHV-6 (52%) from 31 patients whose antibody titers had converted to positive during convalescent phase could be isolated from them during acute phase of disease. The disease occurred in infants from three months to one year of age and most frequently at age 5-6 months. Antibody converted to only HHV-6 in 23 cases (46%) of 50 patients, and seroconversion was also observed to both HHV-6 and dengue virus in 7 patients (14%), and to both HHV-6 and Coxsackie B virus in 1 case (2%). In the 23 patients in whom seroconversion was observed only to HHV-6, all had fever and rash which mostly appeared after subsidence of fever. Lymphadenopathy and relative lymphocytosis were prominently recognized, association with diarrhea, vomiting, running nose, cough and hepatomegaly, and furthermore convulsion during febrile phase was seen in some cases. All patients recovered completely within a week.

S2-4

DIAGNOSIS OF HERPES SIMPLEX VIRUS ENCEPHALITIS BY DETECTION OF CLASS-SPECIFIC ANTIBODIES AND DNA IN CSF

E.Uren, *J. Montanaro*
Virus Laboratories, Department of Microbiology/ Infectious Diseases, Royal Children's Hospital, Melbourne, Australia.

The value of polymerase chain reaction (PCR) in diagnosing herpes simplex encephalitis (HSVE) was assessed by examining CSF samples from HSVE patients diagnosed previously by demonstrating HSV antibodies in the CSF. HSV-specific IgM and IgA antibodies were detected by enzyme immunoassay (EIA) capture technique and HSV-IgG antibodies were detected by EIA using antigen coated wells. PCR was performed using primers which flanked the conserved region within the glycoprotein D gene of HSV type 1 and 2.

Of 25 CSF samples from 12 HSVE cases which had previously been tested by EIA, 20 were available for PCR tests. Five of 8 CSF samples taken from 6 patients within 4 days of onset of illness gave a positive PCR reaction whereas only 2/12 samples taken after this period were positive. In contrast, HSV-specific IgA, IgM or IgG antibodies were detected in CSF within 4 days of onset in only 3/11 cases but were regularly detected in CSF submitted later in the course of the illness. The combined testing by PCR and EIA gave the greatest chance of diagnosing HSVE, with HSV-DNA detection being the more sensitive in the early stage of illness and the class specific antibody method being the more appropriate for specimens received in the latter stages.

S2-5

ENDONUCLEASE CLEAVAGE ANALYSIS OF HSV 2 ISOLATED IN CHIANG MAI, THAILAND

***W. Sirirungsi*[1], *T. Iwamasa*[2]**

[1]Department of Clinical Microbiology, Faculty of Associated Medical Science, Chiang Mai University, Thailand.

[2]Department of Pathology, Ryukyu University, School of Medicine, Japan.

Herpes simplex viruses (HSV) type 2 isolated from Chiang Mai, Okinawa strains and standard HSV-2 (SAV strain) were characterized using the 4 restriction endonucleases (RE) *BamHI, KpnI, BglII* and *EcoRI*. By observation of presence or absence of cleavage sites and variation in mobility of DNA fragment at terminal and joint regions of HSV-2 genomes, 42 Chiang Mai isolates and 2 Okinawa isolates were analyzed. *Bam HI* RE profiles showing differences between the DNAs of Chiang Mai isolates as 5/21 gain site in G region, 7/21 non-coin-cidence of site in P and Q, 6/21 gain site between B and C, 1/21 lost site in G and 2/21 lost site in D region. This preliminary results revealed that RE cleavage patterns of Chiang Mai isolates showing variations and some of them differed from those obtained with Okinawa or SAV strains.

SS I-1

HEPATITIS A VACCINE

A. Safary
SmithKline Beecham Biologicals, Rixensart, Belgium.

Hepatitis A is endemic in all parts of the world, and its occurrence is influenced greatly by the level of sanitation or hygiene in the environment. At present, serum immune globulin offers the only means of protection, but it is impractical when repeated exposure is anticipated. An effective vaccine is essential as increasing numbers of adults are becoming susceptible to infection with HAV.

SmithKline Beecham Biologicals has developed an inactivated hepatitis A vaccine, derived from the HM175 viral strain. The safety and immunogenicity of this vaccine were established through *in vitro* and *in vivo* testing before being administered to human volunteers. Clinical trials using SmithKline Beecham Biologicals hepatitis A vaccine started in April 1988. To date, 31 studies had been initiated, by 42 investigators in 17 countries. Several thousand volunteers, and over twenty thousand children in a field trial, have received the vaccine.

No clinical signs of hepatitis A infection have been observed and serum liver enzyme levels have shown no significant changes. Reactions after vaccination are generally mild, rarely lasting more than 24 hours; the most common reaction is soreness at the site of injection.

The immunogenicity of the vaccine was measured using an in-house ELISA technique and commercially available RIA. Some serum samples have been tested for neutralizing antibodies. The vaccine is highly immunogenic, inducing over 99% seroconversion in volunteers after two doses. Antibody response is shown to be related to the vaccine dose level and the number of doses given. Antibodies persist for at least one year following vaccination.

Studies carried out on the simultaneous administration of hepatitis B vaccine (Engerix -B) with the inactivated hepatitis A vaccine have shown no interference between the two vaccines with regard to antibody response. Passive-active immunization studies resulted in subjects acquiring anti-HAV antibodies more rapidly, but the antibody response to the vaccine plus immunoglobulin was lower than when the vaccine was administered alone.

SS I-2

LONG TERM FOLLOW-UP OF THE EFFICACY OF rDNA HEPATITIS B VACCINE IN HIGH RISK NEONATES

Y. Poovorawan, *S. Sanpavat, W. Pongpunlert, S. Chitinand, S. Chumdermpadetsuk, P. Sentrakul, R. Sakulramrung, A. Safary*
Departments of Pediatrics, Ob & Gyn, Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok. SmithKline Biologicals, Belgium.

The immunogenicity and protective efficacy of a yeast derived hepatitis B vaccine was studied in normal newborns and neonates at risk (n = 497). Doses of 10 mcg were given following two different vaccination schedules : 0, 1, 2, and 12 months; 0, 1, 6 months. The effect of concurrent HBIG administration at birth was also evaluated.

The vaccine was highly immunogenic in all groups of neonates. No significant adverse effect was observed. The protective efficacy of vaccine was assessed alone or in combination with HBIG in neonates born to HBsAg/HBeAg +ve mothers. Two of 59 (3.4%) newborns receiving vaccine (0,1,2 and 12 months) alone became chronically infected with hepatitis B virus while only 1 of 65 (1.5%) infants administered vaccine plus HBIG became a carrier. The results for vaccine protocol 0,1 and 6 the infected rate at one year was 3 of 59 (5.1%) in vaccine alone group and 1 or 60 (1.7%)in infants received vaccine plus HBIG. The protective efficacy of vaccine alone or in combination with HBIG was more than 90%. After following the children of vaccine alone (0,1, 2, + 12) at 4 years and vaccine + HBIG at 3 year, no additional infection was seen. The yeast derived vaccine is therefore effective in preventing perinatal transmission of hepatitis B virus. It can reduce carrier stage in a given population where hepatitis B is prevalent.

SS I-3

CONTROL OF HEPATITIS B INFECTION IN ASIA WITH UNIVERSAL INFANT IMMUNIZATION AS PART OF THE EPI

Dr.M. Kane
WHO, Geneva, Switzerland.

The Expanded Programme on Immunization (EPI), the Technical Advisory Group on Viral hepatitis, the WHO Regionall Office for the Western Pacific (WPRO), and most National Health Authorities in Asia have recommended University infant Immunization with HB vaccine as part of the EPI. HB immunization has been successfuly introduced by most Asian and Pacific Basin countries, and the remaining countries are seeking resources to begin immunization. The Asian Region is the most successful in the world in adopting HB vaccination. Several critical issues remain as problems to public health officials. These include whether to begin or continue maternal HBsAg screening, how to improve immunization coverage at birth follow-ing home deliveries, how to obtain HB vaccine to use, and whether countries should manufacture HB vaccine. Countries successfully using HB vaccine in infants also want to know which population groups would benefit most from additional use of the vaccine. WPRO and WHO, Geneva, hae been actively involved in helping countries plan and implement HB immunization programmes. and we are now involved in the issues of vaccine price, transfer of technology, combination vaccines, and future ùone shot' vaccines that will greatly simplify the delivery of HB and other vaccines.

SS I-4

CURRENT STATUS OF GLOBAL CONTROL OF HEPATITIS B THROUGH VACCINATION

James Maynard

International Task Force on Hepatitis B Immunization, Seattle, Washington, USA.

Enormous progress has been made in the last several years in the ongoing conquest of hepatitis B through immunization. With recent decisions in several countries of low HBV endemicity to recommend immunization of all infants with HB vaccine, a single global immunization strategy may now be proposed which provides for such immunization as an integral part of EPI. Data from several countries now indicate that there are no barriers to effective integration of HB vaccination into EPI. In the Asian Pacific region, over 20 countries have started such EPI integrated programs. A key to further expansion of EPI integrated HB immunization is the anticipated early availability of a polyvalent DPT/HB vaccine.

S3-1

PATHOGENETIC STUDY OF TRANSGENIC MICE CARRYING POLIOVIRUS RECEPTOR GENE

T. Kurata[1], S. Koike[2], C. Taya[2], H. Yonekawa[2], A. Nomoto[2]
[1]Department of Pathology, N.I.H., [2]Department of Microbiology, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Poliovirus-sensitive transgenic mice were produced by introducing the human gene encoding cellular receptors for poliovirus into the mouse genome. Expression of the receptor mRNAs and receptor antigens in tissues of transgenic mice was examined by using RNA blot hybridization and polymerase chain reaction, and immunofluorescence, respectively. Poliovirus receptor genes and antigens were expressed in many tissues of the transgenic mice just as in tissues of humans and monkeys. The transgenic mice were susceptible to all three poliovirus serotypes. The mice inoculated with type 1 poliovirus (Mahoney strain) showed typical paralytic signs of bilateral hind legs, finally resulting in death. Immunohistochemical studies revealed the presence of poliovirus antigens in the motor neurons of the anterior horn of the spinal cord and medulla oblongata. The virus caused similar signs and pathological findings by any routes of infections, for example intracerebral, intraperitoneal, intravenous and oral administration. Even higher titer of the virus solution failed to produce any clinical signs, lesions and viral antigens in non-transgenic mice. These results indicate that poliovirus requires receptor for active infection and this transgenic mouse model may become an excellent new animal model to study molecular mechanism of pathogenesis of poliovirus and to assess oral poliovirus vaccines.

S3-2

DETECTION OF VIRUSES IN WATER SAMPLES USING AMPLIFICATION OF VIRAL RNA THROUGH POLYMERASE CHAIN REACTION

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Enterovirus, the most common waterborne viruses in contaminated water, is responsible for outbreaks of illness particularly amongst children in developing countries. Special measures need to be devised for viral elimination in the treatment process of water supply systems as the normal chlorination only eliminates bacterial pathogens. The need for rapid and efficient detection of viruses is an essential prerequisite in this process. Conventional cell culture methods for the detection of viruses require several weeks besides their specificity. The advent of recombinant DNA techniques makes it possible to develop gene probes for detection of viruses through dot-blot hybridization. However, the sensitivity of this method is restricted beyond 10^3 virus particles. The introduction of polymerase chain reaction (PCR) could provide a breakthrough in situations of low titres in environmental samples where theoretically even a single-copy gene can be amplified and seen as distinct bands on agarose gels.

Present study describes concentration of viruses from large volumes of water samples to a microlitre quantity (around 10,000 fold concentration) facilitating subsequent detection. The detection for enterovirus included various steps, viz extraction of single stranded RNA, treatment with ribonuclease inhibitor, cDNA synthesis, amplification of gene by PCR, extraction of PCR products and finally running on agarose gel for visualization of specific band. PCR was very sensitive and ultimately makes the detection of enterovirus easier, shorter, and more adoptable for water quality surveillance programmes in developing countries.

S3-3

RAPID IDENTIFICATION OF DENGUE VIRUSES BY POLYMERASE CHAIN REACTION

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Dengue virus infection is highly prevalent in many tropical countries. In the southeast Asia appearance of severe disease manifestation of dengue hemorrhagic fever (DHF) and shock syndrome (DSS) has made dengue virus infection a leading cause of morbidity and mortality among children under 14 years old.

Since dengue vaccine is still under development, we considered that rapid identification of the disease agent would provide useful information to the clinicians for proper treatment. Such information would also be valuable for the epidemiologists to issue appropriate preventive measures. In our laboratory, studies on the nucleotide sequence of dengue virus genomes have provided basic information to work out a procedure for the detection and typing dengue virus genome by polymerase chain reaction following cDNA synthesis by reverse transcriptase (RT-PCR). Four sets of primer pairs were prepared so that different size of target sequence could be amplified for each type of dengue virus genome, and the amplified sequences were detected as ethidium bromide stained bands after agarose gel electrophoresis. The result of the standard RT-PCR was almost the same as a simplified RT-PCR in which all ingredients were mixed in a reaction tube from the beginning. The results were almost the same whether the template was phenol-extracted and ethanol precipitated RNA or nonionic detergent solubilized infected fluid. The method gave consisting results for all strains of dengue viruses so far tested.

S3-4

IN SITU DNA AMPLIFICATION: METHOD FOR DETECTING DNA VIRUSES AT CELLULAR AND SUBCELLULAR RESOLUTION

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In situ DNA amplification, the combined useful techniques of *in vitro* amplification of DNA target, the polymerase chain reaction (PCR) and *in situ* hybridization has recently established in our laboratory. Herpes simplex virus (HSV) infected Vero cells and HeLa cells containing Human papilloma virus (HPV) genomes were used as models to develope the technique. The cells were fixed on the glass slide and viral DNAs were amplified using conventional PCR procedure and specific oligonucleotide primers in the present of 10% glycerol. The 25 cycles of amplification were performed in a programable, fan force circulating air oven using either two-or three-temperature protocol. In addition, one-step procedure for nonradiolabeled, amplified products without using probes also developed by including biotin-14-dATP during amplification. A signalling group (alkaline phosphatase) covalently attached to streptavidin was used to detect biotinylated products located inside the cells. We obtained strong signals from both types of cells. The system is specific and sensitive, does not require sophisticated molecular biology expertise or radioisotope.

S3-5

A NOVEL VARIANT OF HUMAN INTERFERON ALPHA 1 GENE

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A 520-base pair human interferon-alpha gene was isolated by using PCR method twice from chromosome DNA of a Chinese (Han Nationality) fetal liver. The nucleotide sequences were determined. These two separately amplified DNA fragments shared the completely identical nucleotide sequence but possessed C and G at position 410 and 541, respectively, which differ from those in interferon alpha 1 and interferon alpha D previously described. Therefore the deduced amino acid sequence would have an Ala at position 114 and a Val at position 158. At all other sites it has the same amino acids as those in interferon alpha 1 and D. We recommend the interferon alpha D gene, interferon alpha 1 gene and interferon alpha 1/158V gene found in our laboratory, being named IFN-a1a, IFN-a1b and IFN-a1c, respectively.

S4-1

RAPID DIAGNOSTIC METHOD AND INFLUENZA MONITORING IN FRANCE

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Influenza pandemics extending to the whole planet can be devastating. Milder episodes cause yearly thousands of deaths and heavy economic losses. Our ideas on flu epidemiology depend on data on severe cases seen in hospitals which provide information on virulent variants, but not on the real circulation of the agent in the bioenvironment. "True" cases of influenza are seen by first line practitioners and typing of the virus is a first clue on the actual risk. Our experience after several winters shows that early information in this field is still more useful than we initially thought. Detection has to be made at the level of primary cases, in families, but also in the open population, in schools, in military recruits, in nursing homes, with special attention to children since they are the first to be attacked, and they play an important role in later virus dissemination.

GROG system has been elaborated in France along these principles. GROGs are early warning networks for detection and surveillance of influenza outbreaks, designed since 1984 to complement WHO influenza surveillance system. They collect specific and non-specific data, transformed into indices and graphs. The first GROG has been experimented in Paris and its area during three years, and after evaluation, was then extended to all regions in France. It uses more than 20 specific and non-specific indicators computed from data collected by nearly 1 000 sentinels (virologists, GPs, pediatricians, pharmacists, Social Security centres, army medical services,..) under the responsability of WHO Influenza National Reference Center (Paris and Lyon) and coordinated by two GPs. Kits are provided to sentinels : swab, transport medium, patient history card, self-addressed envelope, instructions. Samples are sent immediately to the laboratory; it has been checked that transport does not reduce sensitivity of isolation. They are processed according to an ELISA-immunocapture (EDIC) method; the results are available within four hours. The test is very specific, but less sensitive than culture. At the same time, the sample is inoculated into MDCK cultures which are observed for 2 weeks by EDIC or for hemagglutinin. This protocol is efficient for A, B and C types and RSV; it can be adapted to other agents.

Information on virus isolations and evolution of indices is treated on mondays to be sent to authorities, participants of the network and to other destinations where it can be useful, including, under convenient formats, to the medical profession (daily medical journals) and to the public. Results of influenza monitoring are useful to practitioners, to vaccine and drug manufacturers and distributors, to other profes-sionals involved in medicine or security, and finally to the public. In addition, the availability of as many isolates of fresh viruses as possible coming from different regions is essential for the evaluation of the epidemiological situation.

In western countries, it is estimated that no more than 10% high risk patients were vaccinated during the last winters. In France, after 20 years of efforts of monitoring and information, this rate has increased from 20% in 1970 to 70% in 1990.

S4-2

MOLECULAR EPIDEMIOLOGY OF INFLUENZA

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The nucleoprotein (NP) of influenza A viruses plays a major role in determination of species specificity. A phylogenetic analysis of the NPs revealed that there exist two main branches, one comprising all human and a few swine isolates, the other all avian and the other mammalian isolates including some other swine viruses. The human NPs are under a strong selection pressure, while the avian NPs are not. Since swine isolates were found in both branches of the phylogenetic tree, the genes of the "classical" swine viruses and of more recent "swine-like" isolates have been studied in more detail. The role of pigs in the creation of human pandemic strains will be discussed in more detail.

S4-3

EVOLUTIONARY DIFFERENTIATION OF THE H3 HEMAGGLUTININ GENES OF HUMAN, SWINE AND AVIAN INFLUENZA A VIRUSES

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A total of 55 H3 influenza viruses isolated from pigs and domestic fowls during the period 1976 to 1982 were characterized by HI tests with antisera. Swine H3N2 viruses were divided into two antigenic variant groups, including (i) early strains and (ii) recent strains. It was also found that all the avian viruses only reacted with antisera to early strains of human H3N2 virus, whereas they possessed a hemagglutinin (HA) antigen distinguishable from that of the earliest human and swine viruses. To define the evolutionary mechanisms and differentiation of the above viruses, nucleotide sequences of 12 HA genes determined in the present study and17 published HA gene sequences were analyzed in more detail from phylogenetic points of view. We found that the earliest swine strain isolated in Japan in 1969 is less related to the other swine viruses isolated between 1976 and 1982 than all of the avian H3 viruses examined. It was also evident that the number of nucleotide and amino acid changes in swine HA genes of H3 virus was not proportional to the amount of time for virus isolations. The phylogenetic tree constructed by neighbor-joining method allowed to differentiate three host-specific lineages constituting of human, swine and avian strains. Undoubtedly, four swine isolates belonged to human lineage, suggesting that epidemic strains in humans might be transmitted to swine populations. In phylogenetic analysis of the swine viruses isolated in China and Hong Kong during the six-year period between 1977 and 1982, all without exception were shown to be their evolutionary separation from avian lineage. The rate of synonymous and nonsynonymous substitutions for the HA gene of human and swine viruses was estimated to be 0.01291-0.01274 and 0.0027 per site per year, respectively, indicating that they evolve with an identical rate at both sites. Avian influenza virus was further characterized by their evolutionary pattern different from that of human and swine viruses

S4-4

A LARGE-SCALE EXPRESSION OF THE INFLUENZA VIRUS HEMAGGLUTININ IN SILKWORMS BY A BACULOVIRUS VECTOR BACULOVIRUS

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In order to develop more effective and safe vaccine through the increase of antigen content, we have studied the establishment for the suitable and efficient model expression system in silkworms. In this system the baculovirus *Bombix mori* nuclear polyhedrosis virus (BmNPV) was employed, and a cDNA was synthesized from viral RNA of A/sw/Ehime/1/80(H1N2) with use of a synthe- tic universal primer. A cDNA encoding for the hemagglutinin(HA) has been inserted into the *Bgl II site* of the pBM050 polyhedrin vector. The resultant recombinant vBMHA1-B4 obtained through homologous packaging has been used in a series of expression and evaluation of the immune responses. The cultured silkworm cells produced immune reactive HA after pBM050 virus infection, showing that the infected cell surfaces was strongly stained with immunofluorescent labelling antibody specific for H1N1 virus. The results of HA titration of the culture fluids collected daily showed that its peak titer reached 64 on the third day. In contrast to this, higher HA titer was detected in silkworm, and the final HA titer was found to be extremely high (32,768). Monoclonal antibodies to HA of A/NJ/8/76 (H1N1) demonstrated that HA expressed in silkworm shows immunoreaction patterns identical to those of a parental virus. This HA could be highly purified by a combination of the fixed chicken red blood cell and DEAE-cellulofine A 800. The present study revealed that the level of antibody induced by HA derived from silkworm is approx. 4-fold higher than those of fertile hen's egg prepared influenza vaccine.

S4-5

PACIFIC BASIN SURVEILLANCE ON RESPIRATORY VIRUSES

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WS1-1

ACTIVE HEPATITIS A VACCINATION : IMMUNOGENICITY AND REACTOGENICITY OF AN ACCELERATED IMMUNIZATION SCHEDULE

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Hepatitis A remains a frequent course of morbidity and occasionally mortality in central Europe. In addition both lack of natural immunity and tourism to high endemic countries increase. Therefore, SmithKline Beecham Biologicals has developed formaldehyde-inactivated candidate hepatitis A vaccines derived from the HM175 viral strain. In this prospective study different vaccination schedules and hepatitis A candidate vaccine were tested for possible accelerated immunization. Immunogenicity was measured using a sensitive ELISA technique (cut-off > 20 IU/ml).

RESULTS : SEROCONVERSION (%) & GMT's (mIU/ml)

Vacc. Sched./Ag-Content		Day 14	Day 28	Mon.2	Mon.6
0-14 d/	%	43.9	100.0	100.0	100.0
720 El-U	GMTs	259.3	596.0	417.6	350.0
n = 48					
0-28 d/		46.2	78.6	94.9	95.0
720 El-U		118.3	250.6	458.8	388.5
n = 49					
0-14-28 d/		36.8	97.4	97.4	100.0
720 El-U		144.6	506.6	676.2	522.2
n = 45					
0-14-28 d/		22.5	94.4	97.4	97.7
360 El-U		176.0	516.0	446.3	394.0
n = 46					

The study proved that given by an abbreviated vaccination schedule (days 0-14) the vaccine of an antigen content of 720 El-U induces earlier high seroconversion rates and GMT's without increase of side effects, thus being an appropriate schedule for accelerated immunization in lack of time.

WS1-2

DEVELOPMENT, SAFETY AND IMMUNOGENICITY OF NEW INACTIVATED HEPATITIS A VACCINES : EFFECT OF ADJUVANTS

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The RG-SB-strain of Hepatitis A virus (HAV) was purified after growth on MRC-5 human diploid cells. The virus was inactivated by treatment with formalin 0.05% at 37°C for 10 days. Vaccine 1 was prepared by incorporating inactivated virus into immunostimulating reconstituted influenza virosomes while vaccine 2 consisted of inactivated virus adsorbed to $Al(OH)_3$. Both vaccines contained 150 ng of HAV antigen per 0.5 ml dose. Seronegative adult volunteers received 2 intramuscular injections on day O and a booster dose on day 7 in the deltoid region. No systemic reactions or alterations in blood chemistries were noted. Most subjects who received the alum-adsorbed vaccine presented with a local reaction characterized by pain and indurations at the injection site. There was no local reactions in the group that received the liposome preparation. With both preparations, all subjects were seropositive by 21 days post vaccination. The geometric mean titer (range) for the alum-adsorbed and liposome vaccine formulations on day 21 was 251 mIU/ml (39-750) and 357 mIU/ml (29-940) respectively.

Due to the fear of neuropathogenic damage of alum, this immunoenhancing form of hepatitis A vaccine represents a step ahead in future vaccine technology.

WS1-3

INHIBITION OF DUCK HBV DNA REPLICATION USING COMBINATION ANTIVIRAL CHEMOTHERAPY

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Hepatitis B virus (HBV) supercoiled DNA (SC DNA) is both the main transcriptional template for hepadnaviral replication and the replicative species most resistant to therapy with conventional antiviral agents such as interferon and nucleoside analogues (e.g. ganciclovir). In this study we investigated the effects of the prokaryo- tic DNA gyrase inhibitor nalidixic acid, alone and in combination with ganciclovir, on replication of duck HBV *in vivo*. Congenitally infected 5 weeks old ducklings were treated for 4 weeks with nalidixic acid and ganciclovir. Sera obtained at weekly intervals before, during and after treatment were tested for DHBV DNA by dot blot hybridization. Liver specimens for analysis by Southern hybridization were obtained at the beginning and end of treatment and also 4 weeks later. Within the first week of treatment serum DHBV DNA decreased to levels which were undetectable by conventional dot blot hybridization and remained so for the duration of therapy. Analysis of liver tissue revealed a 20-40 fold decrease in total viral DNA levels compared to placebo-treated ducks and, more importantly, a significant and reproducible decrease in viral SC DNA was also observed. These results demonstrate that the prokaryotic DNA inhibitor nalidixic acid can inhibit SC DNA generation and processing, and in combination with conventional antiviral agents such as ganciclovir, can reduce the generation and stability of all the recognized DNA replicative intermediates of DHBV. The use of combinations of antiviral agents targeted to different parts of the viral life cycle should offer improved therapeutic responses, as well as reducing the extent and frequency of the rebound phenomenon.

WS1-4

HEPATITIS TYPE E STUDIES IN CHINA

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Epidemics of hepatitis E (HE) have been documented to occur in many Asian countries including China. Several HE epidemics had occurred in Xinjiang, Northwest and in Liaoning, Northeast China in 1986-1987.

Epidemiologic studies of HE epidemics in Hotian in 1986 and Khashi in 1987 in Xinjiang showed that the epidemics in Hotian and in Baheqi township in Khashi were water-borne and HE in Baren, Khashi was food-borne.

Incidence and case fatality rates between men and women were not significantly different, whereas the deaths in pregnant women were significantly higher than those in men and non- pregnant women. The other risk factors were age above 10 years, peasants, and contact with hepatitis patients. Mean incubation period was 34.9 $\pm$10.1 days. Experimental infection of Rhesus monkeys with the virus-like particles (VLP) from stool specimens of a patient at incubation period and serial passages in the monkeys were carried out in 1987. Five out of seven and 4/4 infected animals with stool and liver biopsy material respectively developed illnesses within 20 - 49 days after inoculation. Clinical manifestations included fever, loss of appetite and elevation of ALT. VLPs, 27-34 nm in diameter were found in the stools of the inoculated monkeys 2-3 days before the rise of ALT. Sera of the animals were negative for IgM anti-HAV, IgM anti-HBc and HBsAg.

Isolation of HEV in human embryonic lung 2BS cells was made by inoculation of the stool of the above-mentioned woman patient. Infected cell cultures were passaged for 4-8 times and slight changes began in the 4th generation cultures, 27 days after inoculation. In the 7th passage, CPE changes consisted of widening of the cells, decrease in transparency and slight cell deterioration without separation from the container wall. VLPs were found in the cell suspension of the 5th passage cultures. Two of three monkeys inoculated with a mixed culture fluid developed illness like those inoculated with patient's stools.

WS1-5

FURTHER WORK ON ENTERIC NON-A, NON-B/HEV

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We had earlier reported an enzyme immunoassay and 27-34 nm virus like particles (VLPs) to be antigen/viral related with hepatitis E virus (HEV). HEV has been successfully transmitted with more than five passages to *Maccaca mulatta* monkeys by oral, I.V. and portal routes.

To develop a dependable antibody based serological assay, homogenous HEV VLPs were procured from gall bladder and virus were pelleted by sucrose cushion. The nuclear material of HEV was characterised and found to contain RNA. cDNA library was made. 21 clones with inserts ranging from 2.9 to 1.5 kb were picked up. Most of the above clones were found to react specifically with serum samples from patients with HEV but not with serum samples from patients with HAV, HBV and amoebic infection.

WS1-6

INCIDENCE OF HEPATITIS B VIRUS INFECTIONS IN 3-5 YEARS OLD CHILDREN IN SICHUAN, CHINA

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To determine the incidence of infection with hepatitis B virus among the Chinese children of 3-5 years old who were in a day nursery of Zhigong city, Sichuan. 1,167 children were tested for HBV markers by RIA. This trials were started in 1985. 41.13% (480/1,167) were infected with HB [HBsAg (+) and/or Anti-HBs (+) and/or Anti-HBc (+)]. Of these only 12.68% were HBsAg (+). The frequency of HBsAg (+) was higher in boys (15.0%, 87/579) than in girls (10.37%, 61/588) ($P < 0.01$), and the percentage of HBsAg (+) was much higher in children (50.0%, 19/38) whose mothers with HBsAg (+) than in children (9.97%, 37/371) whose mothers with HBsAg (-) ($P < 0.001$) 762 (65.3%) children were randomly followed up for one year. Of them, 58.8% (448/762) were initially (in 1985) susceptible subjects. By the time of 1986, 12.95% (58/448) developed HBV infection, 3.79% (17/448) had HBsAg (+) and 9.15% (41/448) with other HBV markers. The new incidence of infections was associated with the frequency of HBV markers in the classes of nursery. The new infected children (15.6%, 44/282) in the classes with HBV markers > 10% were higher than that of (8.43%, 14/166) in the classes with HBV markers < 10% ($P < 0.05$). Conclusions : 1). It was showed a high HBsAg prevalence was 12.68%. New (annual) infection incidence among 3-5 years old in Zhigong city of China was 12.95%; 2) An increased risk of HBV infection was related to two factors: (1) HBsAg (+) mother was a high risk factor in this region and (2) the infected children with HBV may transmit to others through child to child with close contact in the nursery; 3) There were 409 children who were matched 409 mothers, 13.69% (56/409) of them were HBsAg (+) in whom 33.9% (19/56) were related to their HBsAg (+) mothers (maternal transmission) and the other 66.1% (37/56) probably from horizontal transmission. It is clear that preschool-age of children living in the high HBsAg prevalence regions should be vaccinated also.

WS I-7

THE EFFECT OF MYCOBACTERIUM PHLEI SUBSTANCE IN THE TREATMENT OF CHRONIC HEPATITIS B AND CHRONIC HEPATITIS B VIRUS CARRIERS

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This article reports a double-blind placebo- controlled study on the effect of *Mycobacterium phlei* substance (MPS) in animal experiments as well as in the treatment of chronic hepatitis B (HB) and chronic hepatitis B virus (HBV) carriers. MPS might enhance both the nonspecific immune function as indicated by raising the activity of macrophages and the specific humoral immunity as shown by increasing the number of antibody-produc- ing spleen cells of mice. MPS showed rather good effect in eliminating duck HBV-DNA. Chronic patients with positive HBsAg, HBeAg and anti-HBe and chronic HBV carriers, in a total number of 40, were divided into 3 groups, i.e., 10 in the MPS group, 10 in the placebo-control group, 20 in the MPS plus HB vaccine group. Results of the treatment showed that the negative conversion of HBeAg, HBV-DNA and DNA-polymerase in the MPS group was superior to that in the control group. The negative conversion of HBeAg, HBV-DNA and DNA-polymerase in the MPS plus HB vaccine group was even more superior to that in the MPS group ($P < 0.05$), and furthermore some patients produced anti-HBe.

CO-OPERATIVE RESPIRATORY VIRUS RESEARCH IN THE PACIFIC BASIN - RATIONALE AND POTENTIAL

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Institution of control measures in the face of a new influenza pandemic has been difficult because the subtype, place and time of emergence of the virus were unknown quantities.

Since the 1968 pandemic, caused by Hong Kong (H3N2) virus, two of these uncertainties are now more clear:-

1. Southern China has been recognized as a hypothetical influenza epicentre and countries around the Pacific Basin places for the emergence of many important interpandemic strains.

2. The time of peak influenza activity in the epicentre is in the summer.

This new appreciation of pandemic influenza and that 20 years had passed since the Hong Kong virus appeared, provided the main impetus for interested parties to form the Pacific Basin Respiratory Virus Research Group at the First Asia-Pacific Congress of Medical Virology in 1988 with the object of developing co-operative research in the Pacific Basin to improve basic understanding of respiratory viruses and methods for their control.

Integral with this activity is better understanding of the epidemiology of respiratory syncytial virus, an important cause of morbidity in certain age groups, and other respiratory viruses and the need to distinguish them quickly from influenza virus in respiratory virus outbreaks.

As a contribution towards these goals, an improved respiratory virus reporting system is being established. Further, a course on the rapid diagnosis of these infections has been held in southern China. Consolidation of these efforts and the undertaking of new ones are being planned. This approach has application in the Basin and beyond.

WS2-2

EMERGENCE OF A NEW INFLUENZA A VIRUS IN HORSES IN CHINA

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Two severe outbreaks of influenza-like disease occurred in the Jilin and Heilongjian Provinces of Northeast China in the Springs of 1989 and 1990, respectively. Aetiologic, seroepidemiologic studies, as well as the horses of experimental infection with isolates indicated that the two outbreaks in horses associated with great losses due to H3N8 subtype of influenza A virus were confirmed. The antigenic and genetic and phylogenetic analyses indicated that the new viruses isolated from sick horses were not derived from equine-2 viruses circulating in foreigh horses, but were closely related to avian influenza virus. However, the new isolates failed to replicate in Beijing ducks, but did replicate and cause disease in mice on initial inoculation and on subsequent passaging cause 100% mortality. So it seems that new equine-2 virus perhaps was derived from some avian virus, not derived from duck virus directly.

WS2-3

INFLUENZA SURVEILLANCE SYSTEM AND VIRUS ANTIGENIC ANALYSIS IN GUANGDONG PROVINCE, CHINA

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Influenza surveillance has been carrying out in Guangdong Province since 1971. Institute of Epidemic Control in Guangdong Health and Epidemic Prevention Stations the centre of provincial surveillance system. Some major cities were selected as sentinels and the laboratories in the stations in these cities were connected as a network. They worked according to the unified procedures. Surveillance contents included : epidemiology data collection, virus isolation and identification, virus antigenic antibody level investigation in general population. Antigenic analysis of influenza A virus in Guangdong Province and antigenic drift of influenza A both subtype H3N2 and H1N1 in the past 20 years were presented. Authors had a discussion on the character of influenza epidemiology in Guangdong Province, and point out the problem that new variants of influenza H3N2 virus in China were first found in Guangdong Province.

WS2-4

INFLUENZA SURVEILLANCE IN PUNE, INDIA, 1990

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Acute respiratory diseases (ARD) cause enormous morbidity and mortality particularly in infants and children in developing countries. Of the several respiratory viruses which cause ARD, influenza is important as it causes frequent epidemics and periodic pandemics. As part of human influenza surveillance several outbreaks of influenza have been investigated by the National Institute of Virology, Pune from 1976 onwards according to the methods described by the WHO. The present study reports influenza surveillance conducted in Pune during the year 1990. During this study a total of 633 cases of ARD were investigated. Most of them were children. Throat/nasal swab specimens collected from these cases yielded 36 influenza virus isolates: 14 A (H1N1) and 22 type B. The A(H1N1) isolates were identified as similar to A/Singapore/6/86(H1N1) strain and all the type B isolates as similar to the new variant; B/Yamagata/16/88 strain. Type A(H3N2) strain did not circulate in 1990. It was the predominant strain in 1989. Thirty-five of the 36 isolates were from the specimens collected from ARD cases during the rainy season (June, July, August and September). Twenty two of the isolates were from July. These results confirmed our earlier observation on the regular occurrence of influenza outbreaks in rainy months in Pune which has a tropical monsoon climate.

WS2-5

INFLUENZA OUTBREAK IN A SUBDISTRICT OF JAKARTA, INDONESIA

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A dengue surveillance program was established in November 1990 at the local health clinic in a West Jakarta subdistrict. Duri Utara is nearly 36 hectares in size, with a population of approximately 18,000 comprised of 3800 families in 2500 houses. Serum samples were collected for dengue virus isolation and serology, while throat swabs were collected for influenza virus isolation. Beginning the fourth week of January 1991, there was an increase in the weekly average number of fever cases, from 8 to 18. Patients presented with primary symptoms of fever, cough, and/or nasal discharge. The number of cases peaked in the fourth week of February, with a total of 111 patients in February and 104 patients in March. During this time, there was no increase in the number of dengue virus infections, but influenza virus was isolated from 16 patients. Prior to and after this period, there were no influenza isolates. Isolates were found throughout the subdistrict, with the majority (69%) from children 0-3 years of age. Three types of influenza virus, type A subtype H1, Type A subtype H3, and type B, were circulating simultaneously in the community. The influenza diversity described here for this small area and relatively short period, is in contrast to previous descriptions of mixed infections which involved large, city-wide epidemics and rarely involved both types A and B.

WS2-6

EPIDEMIOLOGY OF VIRUSES ASSOCIATED WITH ACUTE RESPIRATORY TRACT INFECTION IN THAI CHILDREN

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A combined Hospital and community based study for the viral etiologic agents of acute respiratory tract infection (ARI) in children under 5 years of age were conducted at Ramathibodi Hospital and Din-Daeng community during January 1986 and December 1987.

Virologic studies included nasopharyngeal aspirate for rapid viral diagnosis and isolation, as well as paired serum for serology. The 739 children from hospital and 674 children from community were included. The results showed that 47% of URI and 45% LRI in hospital based study were positive for viruses whereas 4.5% of mild URI, 32.5% of moderate to severe URI and 39% of LRI in community based study were positive. RSV was the most common virus found in both hospital based and community based populations which account for 42% and 30% of virus-positive cases respectively. The monthly distribution of virus-positive cases were the same in both groups. The incidence of infections due to RSV peaked in July and August, while that of infections due to parainfluenza viruses peaked in February and March, influenza viruses and adenoviruses were isolated throughout the years.

Virologic studies performed by our group in the year 1988 to 1990 revealed the same seasonal pattern especially for RSV.

National ARI control program as well as vaccine development are centainly needed.

WS2-7

THE VIROLOGICAL SURVEILLANCE AND MOLECULAR EPIDEMIOLOGY OF RESPIRATORY VIRUS INFECTIONS

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Despite the numerous reports, the significance of virus isolations in association with acute respiratory illness in Southeast Asia (ARI) is not well understood. Epidemiological knowledge of ARI due to viruses depends on the ability to identify viruses being isolated. Thus, a combination of different cell lines, addition to immunofluorescent test, would be possible to prove the viral infection in a large number of cases. The present study was designed to define the viral etiology with the virus types, multiple infections and clinical features. A total of 134 nasopharyngeal specimens were collected during August-December 1989. The virus infections were documented in 31 cases (23.1%), 13 (41.6%) of which were due to mixed infections. They were RS, influenza, Adeno, small round and herpes viruses. Even though a total of 242 specimens were also subjected to the virological surveillance, the proportion of multiple infections with the above viruses was not small. To investigate the background in which influenza viruses circulate in the winter of 1990, they were characterized antigenically and genetically. The antigenic analysis determined that H3N2 viruses from Thailand are closely related to those circulating in 1990-91 season in Japan. In contrast, two B virus variants cocirculated in the same season, including B/Yamagata/16/88-like strain and its variant. The nucleotide sequences of the HA gene of the latter virus (B/Bangkok/163/90) had 98.5% homology with those of B/Yamagata/16/88. It was evident from a phylogenetic tree constructed by N-J method that B/Bangkok/163/90 is divided off from branch containing B/Yamagata/16/88.

WS2-8

ANALYSIS OF ANTIGENIC DRIFT OF INFLUENZA ISOLATES WITH POLYMERASE CHAIN REACTION AND NUCLEIC ACID SEQUENCING

*[1]S.R. Chern[1], S.T. Hu[1], S.H. We[2], C.S. Tsai[2], **W.T. Liu[1]***
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In order to understand the variation of hemagglutinin (HA) gene of influenza A (H1N1) in Taiwan area, several isolates collected at VGH-Taipei were selected for study. The DNA copies of the HA1 region of the *HA gene* obtained by reverse transcription were further amplified by polymerase chain reaction (RT/PCR). The *products from* PCR were then analyzed in restriction endonuclease digestion. The sequence of the nucleic acid was followed by Sanger's chain termination method. Compare the nucleotide sequence and deduced amino acid sequence with the reference reports, we found that our A/Taiwan/86 (H1N1) new isolates in 1990 had antigenic drift at amino acid 77 from serine to proline and some minor amino acid substitutions. The RT/PCR method was also used to evaluate the 1991 isolates and used as a reference method for influenza virus identification.

Session No. 12 PLENARY LECTURE 2

Date : Tuesday 19 November 1991 Time 9:00-9:45
Room : Room A & B

PLENARY LECTURE 2
Chairpersons : *Teera Ramasoota*, THAILAND
Philip A. Pizzo, USA
Prevention of HIV Transmission in Healthcare Setting
Bruce G. Weniger, THAILAND

Session No. 13 SYMPOSIUM 5

Date : Tuesday 19 November 1991 Time 10:15-11:45
Room : Room A & B

SYMPOSIUM 5 Pediatric AIDS
Chairpersons : *John S. McKenzie*, AUSTRALIA
Soawanee Chumdermpadetsuk, THAILAND

1. HIV Infections in Women
   Suporn Koetsawang, THAILAND
2. Recent Advances in Laboratory Methods for the Diagnosis and Management of HIV Infections
   Chin Yih Ou, USA
3. HIV Infection in Children : The Experience in Thailand
   Somsak Lolekha, THAILAND
4. Clinical Problems and evolving treatment principles for children with AIDS.
   Philip A. Pizzo, USA

Session No. 14 SPECIAL SESSION II

Date : Tuesday 19 November 1991 Time 12:45-13:45
Room : Room A

SPECIAL SESSION II New Tests
Chairpersons : *Chan Yow Cheong*, SINGAPORE
Ai Ee Ling, SINGAPORE

1. Two Minute Diagnosis of Viral Diseases
   Carmel J. Hillyard, AUSTRALIA
2. A Modified Western Blot Format for Serological Discrimination of HIV-1 and HIV-2
   Lily Chan, SINGAPORE
3. HCV Rapid Test Comparative Studies with New HCV ELISA Assays
   D. Chien, A. Chu, USA
4. Use of Dengue Blot in Dengue Diagnosis : The Malaysian Experience
   Randel Fang, MALAYSIA

Session No. 15 SPECIAL SESSION II A

Date : Tuesday 19 November 1991 Time 12:45-13:45
Room : Room B

SPECIAL SESSION II A New Drugs and Vaccine
Chairpersons : ***Arnold Monto***, USA
Urabala Boonyaprakob, THAILAND

1. Current Research in Combination Therapy in HIV infections
   D.H. King, UK
2. Development of Attenuated Respiratory Virus Vaccines : Progress toward the Use of Live Cold-adapted Influenza Virus Reassortant Vaccine in Man
   Hunein F. Maassab, USA

Session No. 16 SYMPOSIUM 6

Date : Tuesday 19 November 1991 Time 14:00-15:15
Room : Room A

SYMPOSIUM 6 Viral Hemorrhagic Fever
Chairpersons : ***Ho Wang Lee***, KOREA
Yutu Jiang, CHINA

1. Update on Epidemiology of Hemorrhagic Fever with Renal Syndrome and Vaccine
   Ho Wang Lee, KOREA
2. Development of A Recombinant Vaccine for Hemorrhagic Fever with Renal Syndrome
   Joel M. Dalrymple, USA
3. Yellow Fever : Risk Assessment for Asia
   Thomas P. Monath, USA
4. Xinjiang Hemorrhagic Fever in China
   Fu-Xi Qiu, CHINA

Session No. 17 SYMPOSIUM 7

Date : Tuesday 19 November 1991 Time 14:00-15:15
Room : Room B

SYMPOSIUM 7 Chlamydial Infections
Chairpersons : ***Micheal Ward***, UK
Chuinrudee Jayavasu, THAILAND

1. Chlamydia - Their Cell Biology and Clinical Significance
   Micheal Ward, UK
2. The Prevalence of *C. pneumoniae* and *C. trachomatis* Antibody in Thai Children
   Pongpun Nunthapisud, THAILAND
3. Association of Chlamydial and Gonococcal Infections with Ectopic Pregnancy
   Utidsak Hariratanakul, THAILAND
4. Diagnosis and Typing of Chlamydial Infections by the Polymerase Chain Reaction
   Suphat Pecharatana, THAILAND
5. Chlamydial Vaccines - Dream or Reality ?
   Micheal Ward, UK

Session No. 18 WORKSHOP 3

Date : Tuesday 19 November 1991 Time 15:30-17:00
Room : Room A

WORKSHOP 3 Improved Vaccines for Expanded Program on Immunization

Chairpersons : *Surin Pinichpongse*, THAILAND
Yuri Ghendon, WHO, GENEVA

1. A Novel Type of Combined Measles-Mumps-Rubella Vaccine (HDCV)
   Reinhard Gluck, SWITZERLAND
2. Measles Immunization at 6 Months
   Christopher J. Clements, WHO, GENEVA
3. Laboratory Diagnosis of Poliomyelitis : The Important Key for Polio Eradication
   Yuri Ghendon, WHO, GENEVA
4. Antibody Response to Polio Vaccines : An Evaluation Using Two Strains of Poliovirus Type 1
   J.M. Deshpande, INDIA

Session No. 19 WORKSHOP 4

Date : Tuesday 19 November 1991 Time 15:30-17:30
Room : Room B

WORKSHOP 4 HIV Diagnosis

Chairpersons : *Jean-Claude Cherman*, FRANCE
Debhanom Muangman, THAILAND

1. Biological Variability of Various HIV Isolates
   Jean-Claude Cherman, FRANCE
2. Expression of HIV-1 Autologous P55- and P55/GP120-V3 Core Particles : A New Approach in HIV Vaccine Development
   Hans J. Wolf, GERMANY
3. Clinical and Laboratory Observations on the Specificity for HIV Antigens in the Abbott ELISA Test
   David C. Shanson, UK
4. Defined HIV-1 Indeterminate Western Blot Profiles as Laboratory Markers of Seroconversion and Late Stage Infection
   Jean C. Downie, AUSTRALIA
5. Proteins of Human Retroviruses and their Use in Diagnosis of Retroviral Infection
   M.G. Sarngadharan, USA
6. *n Vitro* AZT Sensitivity of HIV-1 Isolated from Patients Before, During and After Therapy
   K.H. McGavin, AUSTRALIA
7. A Follow up Study of HIV Carriers in Japan
   Takashi Kurimura, JAPAN
8. Increase in HIV Seropositivity among Male Patients Attending STD Clinics and Female Prostitutes at Poona, India
   Kalyan Banerjee, INDIA
9. The Legislative Response to a New Virus : A Global Survey of Legislation Relating to Human Immunodeficiency Virus
   D.C. Jayasuriya, SRI LANKA

THE HIV CHALLENGE : PROTECTING HEALTH CARE WORKERS AND PATIENTS FROM EACH OTHER

Bruce G. Weniger
The HIV/AIDS Collaboration
Nonthaburi 11000, Thailand.

Through 30 June 1991, out of a total of 143,404 adult AIDS cases reported in the United State, 6,782 (4.7%) were health care workers (HCWs). Of these, 94% (6,385) had risk factors for HIV infection such as homosexual contact, injecting drug use, heterosexual contact with an infected or high-risk person, or receipt of blood products. Only 3 (.04%) were infected by occupational exposure. In 6% (394) the transmission mode could not be classified.

HCWs and patients can be infected during medical care by the transfer of infected blood via percutaneous inoculation and by mucous membrane exposure. Prospective surveillance from 15 August 1983 through 30 June 1990 of 1,372 initially-HIV-seronegative HCWs with documented exposures to HIV-infected blood and at least one year of followup revealed percutaneous exposure resulted in HIV transmission to 0.33% (4/1207) and mucous membrance exposure to 0% (0/165).

Evidence from genetic sequencing of HIV-1 strains from an American dentist who practiced with AIDS and from five of his patients strongly suggest HIV transmission from the dentist to the patients, but the circumstances how transmission occurred remain unclear.

The Centers for Disease Control recommends that all HCWs practice "universal precautions" in which blood and body-fluids from all patients-not only those known to be HIV-positive-are handled carefully. These precautions include using gloves when handling these materials and wearing masks during procedures likely to cause splashes. CDC also recommends that HIV-infected HCWs inform patients and/or refrain from performing invasive procedures likely to inoculate their blood into patients. As of late 1991 this recommendation is being debated and the details of its eventual application and implementation remain unclear.

S5-1

HIV INFECTIONS IN WOMEN

Suporn Koetsawang
Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok, Thailand.

S5-2

RECENT ADVANCES IN LABORATORY METHODS FOR THE DIAGNOSIS AND MANAGEMENT OF HIV INFECTIONS

Chin-Yih Ou

Division of HIV/AIDS, National Center of Infectious Diseases, Centers for Disease Control, Atlanta, Georgia, USA.

The diagnosis of HIV infection has been based primarily on the detection of antibodies to the virus in the blood of infected persons. The detection of viral antigens and genetic material is often extremely difficult due to the low titer of viruses and low copy number of proviral sequences present in infected persons. HIV detection in infants born to seropositive mothers can be complicated by the presence of maternal antibodies. This causes serious delays in the identification of infants truly infected with HIV and initiation of antiviral therapy. However, recent technological advancements including site-directed serology, antigen detection, polymerase chain reaction (PCR)), IgA antibody detection and antibody-producing cell detection have drastically improved the identification of several million fold, allows the direct detection and genetic manipulation of the viral sequences from infected patients. A population-based HIV genetic survey thus becomes feasible. The interplay of these new technological advancements in the diagnosis and management of HIV infection and their use in monitoring HIV transmission will be discussed.

S5-3

HIV INFECTION IN CHILDREN: THE EXPERIENCE IN THAILAND

S. Lolekha, *V. Sirisanthana*
Department of Pediatrics, Mahidol University and Chiang Mai University, Thailand.

The first case of pediatrics AIDS in Thailand was reported in November 1988. Up until December of 1990, there were 65 cases of HIV infection in children less than 15 years of age reported to the Department of Epidemiology, Ministry of Public Health. Four main routes of transmission were heterosexual transmission(adolescent prostitutes), vertical transmission, IV drug users, and transfusion of blood or blood products. The number of children born to the known HIV positive mothers increase rapidly during the last three years. There were 6 cases in 1988, 47 in 1989, 221 in 1990 and 169 cases in the first 9 months of 1991.

From January 1989 to August 1991, 28 cases of symptomatic perinatally acquired HIV infection were admitted at Chiang Mai University hospital. All of their parents were infected by heterosexual route (nine mothers were prostitutes and 16 fathers gave history of visiting prostitutes). The average age to the fathers and mothers were 29 and 24 years respectively. Most of the parents were healthy. The age when the first sign or symptom appeared ranged from 1 to 9 months (median 3 months). The median age at the time of diagnosis was made was 5 months. The common presenting symptoms were fever, cough, dyspnea, diarrhea, failure to thrive and delayed development. Other common finding were hepatomegaly (100%), abnormal chest roentgenogram (81%), splenomegaly (73%), generalized lymphadenopathy (65%). Secondary infectious diseases included pneumonia, septicemia, otitis media, meningitis, tuberculous lymphadenitis, cryptosporidium gastroenteritis, Penicilium marneffei infection and disseminated herpes simplex infection. The major cause of death was septicemia. WHO criteria for provisional diagnosis of pediatric AIDS is more practical in developing countries.

S5-4

HIV INFECTIONS IN CHILDREN : THE CLINICAL ISSUES

Philip A. Pizzo

Pediatric Branch, Infectious Disease Section, National Cancer Institute; Professor of Uniformed Services University of the Health Sciences, USA.

Although children constitute only 2% of the AIDS population of the USA, the incidence of HIV- infected infants is rising rapidly and in tandem with the increasing numbers of HIV-infected women. On a global level, children comprise 5-25% of the cases of AIDS, underscoring the importance of this disease in the world's pediatric population. AIDS in children is characterized by a more accelerated course and by differences in clinical presentation. Of particular note is the impact of HIV infection on neurodevelopmental function in infants and children. In this presentation, the unique epidemiologic, diagnostic and clinical features that characterize AIDS in children will be discussed. Important advances in antiretroviral therapy and in the management of infectious and noninfectious complications will also be presented.

SS II-1

TWO MINUTES DIAGNOSIS OF VIRAL DISEASES

C. Hillyard, P. Bundesen, M. Gerometta, B Kemp, S. Martin, D. Rylatt

AGEN Biomedical Ltd, Brisbane, St. Vincent's, Institute of Medical Research, Melbourne, Australia.

A novel, rapid, whole blood assay technology has been developed. The test is a one step pro- cedure performed on whole blood samples, with a visible result in two minutes. It was developed in response to the need for a rapid, accurate diagnostic test, which could be performed in field conditions, casualty departments, physician's offices and veterinary practices. Two viral assays have been developed to date, for HIV-1 antibody and hepatitis B surface antigen (HBsAg). The HIV reagent consists of a Fab fragment of an erythrocyte binding antibody coupled to a peptide from the transmembrane gp41 of HIV-1. This reagent binds circulating antibodies, causing agglutination of the patient's own red cells. The reagent has a specificity of 99.7% and sensitivity of 100% in Australian trials of 5197 normal donors and 401 patients with confirmed HIV infection. In Thailand, a trial of 1000 blood donors, 500 intravenous drug abusers and 300 patients from an STD clinic, yielded a sensitivity of 100% and specificity of 99.5%. The test has exceptional sensitivity to early infection and, in an independent trial of 6 homosexual patients, detected anti-HIV antibodies in 33% of samples collected in the first week of seroconversion illness, compared with the next most sensitive assay, which detected 17%.

The hepatitis B reagent is a bispecific antibody, consisting of two coupled Fab molecules, one with specificity for erythrocytes and the other for HBsAg. This reagent, in initial trials of samples from 1002 patients and normal donors has a specificity of 99.5% and sensitivity of 95.1%.

This assay technology is applicable to many viral infections, where a rapid diagnosis is of importance and can detect either circulating viral antigen or antiviral antibodies.

SS II-2

A MODIFIED WESTERN BLOT FORMAT FOR SEROLOGICAL DISCRIMINATION OF HIV-1 AND HIV-2

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Diagnostic Biotechnology, Singapore

It is ofter not possible to distinguish Indeterminate HIV-1 Western blot specimens from true HIV-2 seropositive specimens due to the extensive immunological cross reactivity of these 2 viruses. A modified western blot format, incorporating the well characterized native HIV-1 viral antigen profile, a specific HIV-2 peptied, with a band for internal control for sample addition, was developed to characterize such samples. The latter band minimized the risk of false negative due to operational errors. The specificity and sensitivity of this modified Western blot system was assessed with a panel of blood donor sera, sera from viral infections other then HIV-1 and HIV-2 gag reactive sera and HIV-1 and HIV-2 seropositive sera. Gag reactive and HIV-1 seropositive sera had characteristic viral lysate profile bands on the immunoblots but did not react with the specific HIV-2 band. All HIV-2 sera tested showed characteristic indeterminate Western blot patterns but also reacted with the specific HIV-2 band. Our results show that this modified format greatly improved the ability to characterize samples that are Western blot indeterminate from truly HIV-2 infected sera.

SS II-3

HCV RAPID TEST COMPARATIVE STUDIES WITH NEW HCV ELISA ASSAYS

D. Chien[1], A. Chu[2], J. Tam[2], C. Yeung[2], J. McFarland, A. Tabrizi[1], M. Houghton[1], M. Nelles[3], S. Lee[3], G. Kuo[1]

[1]Chiron Corporation, Emmeryville, AC. [2]EY Laboratories, Inc., San Mateo, CA, USA [3]Ortho Diagnostics Systems, Raritan, N.J.

We present here a new HCVCHEK rapid test for the detection of antibodies to Hepatitis C virus. The test performance was compared with the Ortho second generation HCV ELISA and a new C2.5 ELISA which is based on a single recombinant HCV antigen (C25). The HCVCHEK is a ten-minutes membrane format assay. The membrane was coated with HCV core, NS3 & NS4 regions antigens. The conjugate used in the assay is a Protein A-colloidal Gold reagent. The sample size is about 40 microliter per assay. In this study, the rapid test has demonstrated the good assay specificity and sensitivity. In the voluntary blood donor random selected fresh specimens (N=600), the reaction rate is about 0.5%. The assay sensitivity from seroconversion panel samples and paid donor samples are comparable to the second generation ELISA and 2.5 ELISA. The assay requires to use fresh sample and needs to avoid specimen freeze and thawing cycles. From these studies, we conclude that the HCVCHEK is a user friendly test. It should be very useful to serve the rapid test market needs.

SS II-4

USE OF DENGUE BLOT IN DENGUE DIAGNOSIS : THE MALAYSIAN EXPERIENCE

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Dengue fever/Dengue haemorrhagic Fever (DF/DHF) has been a public health problem in Malaysia with an endemic level of about 7 per 100,000 population per year. In 1990, Malaysia experienced its most severe outbreak of DF/DHF with a record total of 5,590 cases referred to the Div.Virology, Institute for Medical Research (IMR). Of these, 1880 were confirmed serologically to be DF/DHF. The conventional serological procedure, the haemagglutination inhibition (HI) test, for the diagnosis of DF/DHF is cumbersome and causes delay in diagnosis. Another problem associated with the HI test has been that it has often been difficult to obtain a second convalescent serum sample for an accurate diagnosis. This has raised an urgent need to establish a "rapid" test for diagnosis of DF/DHF. As such the authors recently carried out an evaluation of a newly available commercial rapid test namely the Dengue Blot Assay (Diagnostic Biotechnology Singapore Pte. Ltd.). The test is intended for use in the laboratory confirmation of dengue virus infection. The evaluation was to determine if the test could be utilized as a routine laboratory test and establish its sensitivity and specificity. Over 400 samples were tested against the Dengue Blot Assay. Results were checked against an in-house Dengue IgM ELISA and the HI assay. Preliminary results indicate that the sensitivity and specificity of the Dengue Blot is satisfactory. Our results also indicate that the Dengue Blot has a useful role to play in a routine laboratory especially since it provides rapid results on single serum samples thereby reducing the workload in a busy diagnostic laboratory. A flow chart for the laboratory diagnosis of DF/DHF incorporating the Dengue Blot, IgM ELISA and HI is proposed.

SS IIA-1

CURRENT RESEARCH IN COMBINATION THERAPY IN HIV INFECTION

D.H. King
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Zidovudine (AZT) has been clearly shown to increase the quality and length of life in patients with ARC and AIDS. More recently it has demonstrated efficacy in mildly symptomatic and asymptomatic patients. However the disease does still ultimately progress and this combined with a reported decrease in the *in vitro* sensitivity of HIV isolates to zidovudine (the clinical significance of which is still unclear), suggests a need to investigate the possibility of combining zidovudine with other antivirals:

Benefit has been demonstrated in combination with acyclovir, most probably due to a reduced incidence of herpes infection.

Interferon alpha appears to act on the late stages of virus replication, probably viral assembly and release. Combination of interferon alpha annd zidovudine has demonstrated *in vitro* synergistic inhibition of HIV replication and early clinical trials show promise.

Two dideoxynucleoside analogues, ddI and ddC in combination with zidovudine have both demonstrated synergistic inhibition of HIV replication in cell cultures of both lymphocytic and monocytic origin.

An update on and rationale for a research programme evaluating the safety and efficacy of combination therapy involving the above agents will be given. It is hoped that combination of anti-retroviral agents will prolong further the duration of the antiviral state and the period of immune restoration whilst at the same time keeping toxicity to a minimum.

S12-2

DEVELOPMENT OF ATTENUATED LIVE RESPIRATORY VIRUS VACCINES : PROGRESS TOWARD THE USE OF LIVE COLD-ADAPTED INFLUENZA VIRUS REASSORTANT VACCINE IN MAN

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The University of Michigan, School of Public Health Department of Epidemiology
109 Observatory, Ann Arbor, MI 48109.

Adaptation of respiratory viruses to growth at suboptimal temperature (25°, cold-adaptation) has been found to be a reliable technique for development of attenuated viruses as candidates for live virus vaccines for use in man. The extensive experience acquired in our laboratory in the past years in the development of cold-adapted influenza virus vaccines and the limited feasibility data accumulated with other human respiratory viruses has allowed us to state that cold-adaptation using human isolates is a reliable technique to attenuate respiratory viruses such as respiratory syncytial virus.

Data will be provided on the progress achieved toward development, characterization and testing in man of cold-adapted live influenza virus vaccine of type A and of type B influenza viruses. The technique followed is based on development of attenuated "Donor" strain of type A influenza virus (A/Ann Arbor/6/60/H2N2) and a "*Donor*" strain of influenza B virus (B/Ann Arbor/1/66). Through genetic reassortment and using the attenuated "Donor" strain, it is possible to update the antigenicity of the vaccine line of influenza virus to confirm to the new antigenic prototype of the circulating epidemic strains of influenza virus.

The reassortment technique has provided the rapidity and reproducibility necessary in the development of cold reassortment influenza virus vaccines with a 6/2 gene profile where the six internal genes are derived from the "*Donor*" strain and the two genes coding for the two surface glycoproteins derived from the wild type circulating strain.

In general, the attenuated cold-adapted reassortant vaccines of type A and type B influenza viruses have been documented to have the following characteristics:

1) administration by the natural route (intranasally).
2) proper level of attenuation and immunogenicity.
3) non-transmissibility in the different age groups.
4) availability of a marker system (ca,ts) to monitor vaccine shedding in the field.
5) genetic stability
6) production in an acceptable substrate.

Molecular basis of the attenuation of these cold reassortant (CR) influenza virus vaccines will also be discussed.

S6-1

UPDATE ON EPIDEMIOLOGY OF HEMORRHAGIC FEVER WITH RENAL SYNDROME AND VACCINE

H.W. Lee[1], L.J. Baek[1], C.N. Ahn[2]

[1] WHO Collaborating Centre for Virus Reference and Research (HFRS), The Institute for Viral Diseases, Korea University, Seoul, Korea, [2]Mogam Biotechnology Research Institute, Kyunggi-do, Korea.

World-wide, about 200,000 people are hospitalized with hemorrhagic fever with renal syndrome (HFRS), caused by Hantaan virus, occurs in Asia and eastern parts of Europe, a moderate form, caused by Seoul virus, occurs in Asia, and a mild form, caused by Puumala virus, occurs in Europe, Hantaan virus occurs in Asia and in eastern parts of Europe, Seoul-like viruses occur worldwide, Puumala virus occurs in Europe, and Prospect Hill and Leaky viruses (other hantaviru- ses) have been isolated in the U.S.A. The reservoirs of hantaviruses are rodents and other small mammals. Serologic studies of 42 hantaviruses isolated from HFRS patients and from animals indicated that there are 6 or 7 serotypes. Vaccine inactivated with formalin was prepared from the suckling mice brains and the antigenic potency of the vaccine was determined by ELISA. Mice immunized with the vaccine were protected when challenged with live Hantaan virus. The antibody responses of 456 vaccinees given inactivated Hantaan virus vaccine against were then studied. SC injection of the vaccine was better than IM injection for production of antibodies in humans. Optimal immunogenic dose of the vaccine given SC to humans were 5,120 ELISA antigen units. Of 456 vaccinees, immunization of 336 with two doses of vaccine at one month interval resulted in 99% seroconversion by IFAT. The vaccine was safe and only minimum side effect were observed. In the 1990s, it is highly possible to identify HFRS and HFRS-like illnesses caused by hantaviruses in parts of the world where HFRS is not known because of the availability of serodiagnostic tests. The efficacy of this vaccine against HFRS in the endemic areas of HFRS remains to be determined.

S6-2

DEVELOPMENT OF A RECOMBINANT VACCINE FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME

J.M. Dalrymple, *C.S. Schmaljohn, F.J. Mallnoski, S.E. Hasty, Y-K. Chu*

US Army Medical Research Institute of Infectious Diseases, Frederick, Maryland, USA.

A recombinant vaccinia virus, expressing genes from both the M and S RNA segments of Hantaan virus, strain 76-118, has been propagated under conditions suitable for the production of human vaccines and was proposed as a candidate vaccine to prevent hemorrhagic fever with renal syndrome (HFRS). The vaccine candidate expressed both envelope glycoproteins and the nucleocapsid protein in infected cell cultures and induced antibody to all three Hantaan viral structural proteins following infection of experimental animals. Hamsters immunized with this recombinant vaccinia candidate vaccine resisted infection with a homologous Hantaan virus challenge. Protected animals did not exhibit a high-titered immune response or display Hantaan virus antigen in their lungs and kidneys following challenge as did unvaccinated control animals. It is not yet known if this vaccine will protect against the numerous antigenic variants within the *Hantavirus* genus, which vary considerably in their ability to be neutralized with immune sera to Hantaan virus. Numerous virus isolates from around the world have been compared serologically as well as by nucleotide sequence analysis in an effort to estimate future vaccine efficacy. Isolates from the Bashkiria region of the USSR were of specific interest because of proposed vaccine trials in that area and a preliminary analysis of selected nucleotide sequences of these isolates suggest that they are not identical to the prototype Hantaan virus but rather share similarities with those of Puumala virus. Studies to add additional Puumala antigens to the recombinant HFRS vaccine are in progress.

S6-3

YELLOW FEVER : RISK ASSESSMENT FOR ASIA

T.P. Monath
Virology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA.

In the first decade of this century, there was widespread apprehension that yellow fever (YF) might be introduced into Asia upon completion of the Panama Canal, and the establishment of "rapid" communication with the American tropics. Similar fears persist today, but are linked to the expansion of air travel. The assessment of risk centers on three questions: 1. How receptive is Asia to the introduction and spread of YF virus? 2. What is the likelihood that such an introduction would occur? and 3. How prepared are national and international health agencies to recognize and control a yellow fever outbreak? Some empirical data, largely derived from laboratory experiments, are available to define the question of receptivity. Human immunity to dengue viruses-highly prevalent in Asiamay be an important obstacle to the introduction and spread of yellow fever. This view is supported by cross-challenge experiments in nonhuman primates. Heterologous cross-protection, which reduces the titer of yellow fever viremia, would be most effective in the presence of a biologically incompetent population of *Aedes aegypti*, and, indeed, Asian strains of *Ae.aegypti* are relatively inefficient vectors. These factors may limit the risk of spread, but are unlikely to provide an absolute barrier, as will be illustrated by data from a recent urban YF epidemic in Africa. The assessment of risk of introduction centers on the present distribution of yellow feve. in Africa and South America. During most of the era of air travel, YF has been largely confined to remote, inland, rural regions, with little likelihood of escape. However, recent epidemics of urban YF in Africa and the reinvasion of coastal areas and urban centers in South America by *Ae. aegypti* increase the potential for dissemination of YF virus by air travelers. Implications for surveillance and control are discussed in the light of the current status of diagnostic tests and vaccine supply.

S6-4

XINJIANG HEMORRHAGIC FEVER IN CHINA (REVIEW)

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Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing, China.

Outbreaks of an infectious disease with fever and hemorrhages, designated as Xinjiang hemorrhagic fever, occurred in Southern Xinjiang, China since 1965. This article reviews the results of epidemiologic and etiologic studies. Epidemiologic investigations included general and geographic epidemiology, case studies, home visits, animal reservoir and arthropod vector. Etiologic studies included isolation of virus from patients, vertebrate animals and arthropods by inoculation of samples into newborn mice, and identification by biologic and physicochemical properties and serologic reactions. The results showed that this disease possessed endemicity and natural midii and was widely distributed in desert pasturelands throughout the Talimu River Valley. Wild animals were mainly rabbits and rats. Herding domestic animals were sheep, cow, horse and camel. The index of *Hyalomma asiaticum* on sheep was the highest. The occurrence of this illness was mainly sporadic. It showed a spring-summer seasonality with the peak in May. Majority of patients were herders or persons who had contac' with pastureland. Most cases were 15-45 years of age. The case fatality was 23.6%. Between 1966 and 1980, 32 virus strains were isolated from sera of patients, 2 from sheep, 7 from *Hyalomma asiaticum*, 1 from *Euchoreutes naso* Sclater; in 1982, 1 from urine of a patient. These 43 virus strains were proved to be identical in antigenicity. Identification showed that they were very closely related to and might be variant strains of Crimean-Congo hemorrhagic fever virus. *Hyalomma asiaticum* played the role of both the vector and the main reservoir host, and sheep served as a minor reservoir host.

S7-1

CHLAMYDIA-THEIR CELL BIOLOGY AND ITS CLINICAL SIGNIFICANCE

Micheal E Ward
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Chlamydia were for many thought to be viruses due to their obligate intracellular parasitism, sensitivity to interferon and resistance to many common antibacterial drugs. To this day it is virology laboratories which usually undertake the diagnosis of chlamydial infections, hence their appropriate inclusion in a virology congress as "honorary viruses". Like many viruses, chlamydial infection of cells results in either productive (lytic) or non-productive (cryptic, persistent) infections. Cryptic chlamydial infection is characterized by only intermittent, transient shedding of infectious elementary bodies. Thus, cell culture is inappropriate for the demonstration of cryptic chlamydial infection. Their clinical significance is only beginning to be appreciated due to the advent of new, non viability dependent assays of chlamydial infection. Products of cell mediated immunity, including IFN, can induce cryptic infection leading to the chronic excretion of chlamydial heat shock protein implicated in the immunopathology of scarring damage to the eyes (trachoma) or fallopian tubes (pelvic inflammatory disease, PID). This presentation will review evidence for the cinical significance of cryptic chlamydial infection in the pathogenesis of severe disease.

S7-2

THE PREVALENCE OF *C. PNEUMONIAE* AND *C. TRACHOMATIS* ANTIBODY IN THAI CHILDREN

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Chlamydia pneumoniae is a new species of *Chlamydia*. The organism causes acute respiratory tract infection especially pneumonia, bronchitis, sinusitis. The previous seroepidemiological studies have found the prevalence of *C. pneumoniae* antibody to be 40 to 70% in adults in many areas of the world. The antibody prevalence was low (1-2%) in young children in Seattle and Denmark, but was relatively high in Taiwan suggesting that *C. pneumoniae* might be a more significant pathogen in young children in tropical countries. The preliminary study in Thailand determined the prevalence of *C. pneumoniae* antibody was compared to the *C. trachomatis* antibody in children. The studied population were school children (group 1), children with pneumonia (group 2) and new born (group 3). The sera were tested for the antibodies by the method of microimmunofluorescence test. In children group 1,2,3 the antibody against *C. pneumoniae* and *C. trachomatis* were detected in 37%, 37%, 62% and 4%, 36%, 31% respectively. There was an increase in prevalence of antibody against *C. pneumoniae* with increasing age, but not of the *C. trachomatis* antibody.

S7-3

THE ASSOCIATION OF CHLAMYDIAL AND GONOCOCCAL INFECTIONS WITH ECTOPIC PREGNANCY

***U. Haririattanakul*[1], *U. Kositanont*[2], *C. Thirapakawong*[1], *C. Wasi*[2], *V. Gherunpong*[2]**

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A case-control study was designed to determine the association of past exposure to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with ectopic pregnancy. Sera from 50 women with ectopic pregnancy and 50 age-macthed women with intrauterine pregnancy were tested by enzyme linked immunosorbent assay (ELISA) using *C. trachomatis* L1 and *N. gonorrhoeae* pili as antigens. In the ectopic pregnancy group 80% had IgG antibodies to *C. trachomatis* compared with 44% in the controls. The prevalence of gonococcal IgG antibody was 74% in the ectopic pregnancy group and 48% in the controls. There were no statistical differences of prevalences of IgM antibodies between ectopic pregnancy group and controls to *C. trachomatis* (28% VS 22%) and to *N. gonorrhoeae* (38% VS 42%). Cultures for *C. trachomatis* and *N. gonorrhoeae* of endocervical swabs form the two groups were negative. The results demonstrate that pastinfection with *C. trachomatis* and *N. gonorrhoeae* are associated with ectopic pregnancy.

S7-4

DIAGNOSIS AND TYPING OF CHLAMYDLAL INFECTIONS BY THE POLYMERASE CHAIN REACTION

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The laboratory diagnosis of chlamydial infections by tissue culture is time consuming, of variable sensitivity and misses cryptic chlamydial infections characterized by the intermittent shedding of viable elementary bodies. PCR, using primers directed against the plasmids or genes encoding the ribosomal or outer envelope proteins, potentially offers high sensitivity and specificity for the diagnosis of chlamydial infections. Unfortunately published methods suffer from the following problems : cumbersome DNA extractions; lack of *Chlamydia genus specificity, no positive confirmation of the result*; inability to distinguish between the three chlamydial species; a requirement for DNA purification and restriction digests for typing purposes, the necessity for confirmatory probes or digests. We describe simpler approaches to these problems. In a study of endemic trachoma in a W. African village, full length copies of the MOMP gene of *C. trachomatis* were amplified using species specific primers. Serovar specific primers and fresh Taq polymerase were used in a nested PCR permitting serotyping and identification of double infections. Naturally occurring variants could be sequenced. Secondly, in a study of genital and respiratory infections, primers directed against conserved (sense) and species specific regions (antisense) on the gene encoding the 60 KDal chlamydial cysteine rich protein. When *C. trachomatis* was detected, the MOMP gene was amplified and nestd PCR used to identify the responsible serovar. PCR based techniques achieve the sensitive and specific diagnosis of chlamydial infections and offer unsurpassed opportunities for investigating the molecular epidemiology of chlamydial infections.

S7-5

CHLAMYDIAL VACCINES-DREAM OR REALITY ?

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Chlamydial infections are a major cause of blindness and of infertility or ectopic pregnancy. Limited natural immunity to chlamydial infections appear to be produced following exposure to repeated infections. Thus active trachoma is essentially a childhood disease in endemic areas. Moreover chlamydial infections are less common in prostitutes than other marker organisms of sexual exposure. Early trachoma vaccines, using crude whole organisms provided some evidence of immunity but also enhanced disease severity in some individuals. Current strategy is to use defined subcomponent vaccines notably the major outer membrane protein (MOMP). Antibody to selected MOMP B cell epitopes neutralizes chlamydial infection *in vitro* and *in vivo.* This presentation describes 1) definition of discontinuous of MOMP B cell epitopes of man and mouse to single amino acid resolution; 2) delineation of Th epitopes on MOMP recognized by the primary immune response in humans; 3) vaccination experiments with recombinant MOMP in the highly relevant mouse model of chlamydial salpingitis and infertility. Mucosal immunization achieved significant reduction in chlamydial colonization but will probably be difficult to sustain. Parenteral immunization may be required for reduction in the severity of complicated infection. It is suggested that a vaccine to protect host cells in a sustained manner from initial chlamydial infection may still be a dream. A vaccine to modify the scarring sequelae of chlamydial infection is probably realistic.

WS3-1

A NOVEL TYPE OF COMBINED MEASLES-MUMPS-RUBELLA VACCINE (HDCV)

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Persons with a history of anaphylactic reaction following ingestion of eggs or a receipt of neomycin are at risk when vaccinated with today's combined measles-mumps-rubella vaccines. For this reason a new highly attenuated combined human diploid cell vaccine (HDCV) against measles, mumps and rubella has been developed which is free of any avian proteins, animal protein extracts or antibiotics. The new, lyophilized vaccine was prepared from the following 4 components:

1) **The Edmonston-Zagreb (EZ 19)** measles virus, an Edmonston Enders strain virus which was further attenuated and adapted on human diploid cells by D. Ikic.

2) **The Rubini mumps** virus has been attenuated on human diploid cells (HDC) by R. Gluck. This Rubini mumps vaccine strain represents a recently developed new component. The virus has been isolated from an 8 years old boy with an acute parotitis. Two passages on WI-38 cells (HDC), 13 passages on fertilized specific pathogen-free hen-eggs and 13 further passages on MRC-5 cells (HDC) yielded the new highly attenuated mumps vaccine strain.

3) **The Wistar RA-27/3** is the rubella vaccine strain adapted to human diploid cells by S.A. Plotkin.

4) The vaccine has been stabilized by a new stabilizer containing no substances of animal origin. This guarantees stability of all components for at least 7 days at 37°C.

The efficacy of the new vaccine has been evaluated in more than 1000 seronegative children aged 9 to 24 months. Seroconversion rates, determined 4 to 6 weeks after vaccination were 95% to 100%. There were no reports of any serious side effects received from the physicians participating in the clinical studies.

W3-2

MEASLES IMMUNIZATION AT 6 MONTHS

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Expanded Programme on Immunization, World Health Organization, Geneva, Switzerland.

Despite global coverage with measles vaccine reaching 78%,measles virus continues to circulate. Up to one third of measles cases occur in children before the recommended age of immunization. In order to control measles, there has been a search for measles vaccines which can be given effectively at early ages.

The first vaccine to be recommended by WHO for administration at six months has been the Edmonston Zagreb vaccine manufactured in Zagreb. Now other manufacturers of this vaccine strain are showing comparable field trial results. In addition, other strains are being explored with a view to use at early ages.

More research will be required to ascertain the most appropriate strain, age and route of administration, dose and titer of vaccine for use at early ages.

WS3-3

LABORATORY DIAGNOSIS OF POLIOMYELITIS: THE IMPORTANT KEY FOR POLIO ERADICATION

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World Health Organization, Geneva, Switzerland.

In 1988 the 41st World Health Assembly Commited WHO initiative by the year 2000. Appropriate laboratory service are an important part of the WHO initiative on the global eradication of poliomyelitis. However most methods of laboratory diagnosis of poliomyelitis currently in use were developed more than 30 years ago, and some of them should by improved.

First of all, rapid methods for direct detection of polioviruses in clinical specimens without cultivation of virus are badly needed. Data obtained recently indicates that it is possible with PCR to detect picornaviruses directly in stool sample without viral cultiva- tion. Such methods may detect viral genomes in specimen where the virus was inactivated during incorrect ransportation of the sample or due to another reasons.

Characterization of poliovirus isolates from patients as wild or vaccine-like, and, if wild, indigenous or imported is very important in the analysis of sporadic cases or outbreaks of poliomyelitis, especially in countries in the last stage of eradication of poliomyelitis and also for analysis of vaccine-associated cases of poliomyelitis. Special test is needed which can characterise vaccine-like isolates as a virus which is really responsible for acute flaccid paralisis.

To make a decistion about eradication of poliomyelitis, we should be sure that there are no wild poliovirus cir culating in the population and no virus can be isolated from healthy people or from environmental samples. Environmental surveillance will, therfore, be crucial to the success of the eradication programme and the absence of circulation of wild polioviruses will be one of the criteria to declare countries free from polioviruses. New sensitive methods that allow to detect wild polioviruses in environmental specimens in the pre sence of vaccine viruses is urgently needed.

WS3-4

ANTIBODY RESPONSE TO POLIOVACCINES : A EVALUATION USING TWO STRAINS OF POLIOVIRUS TYPE 1

J.M. Deshpande, *K.H. Dave*
Enterovirus Research Centre, Parel, Bombay, India.

Epidemiological data on paralytic poliomyelitis in Bombay and other cities in India reveal that approximately 20 percent of the total number of patients had received full course of oral poliovaccine [OPV] immunization. Although many factors have been suggested to explain vaccine failure in children in tropical countries, consideration of antigenic relationship between the vaccine strains and infecting poliovirus gained importance after the poliomyelitis epidemic in Finland in 1984.

Majority of vaccine failure cases in Bombay were due to poliovirus type 1 [PV1] infections. We have, therefore, evaluated the serum antibody response in children immunized with either OPV [3] or IPV[2] using PV1 Sabin vaccine strain and a local neurovirulent isolate. Neutralization index [NI] of each serum against the two strains of PV1 were compared in 56 OPV immunized and 36 IPV immunized children. Significant difference in NI against the two strains of PV1 was detected in children immunized with either vaccine. In both cases antibody response was superior against Sabin vaccine strain. It was also found that 35 per cent of children in OPV and 44 per cent in IPV groups had developed poor antibody response only against the wild virus. It is thought that such children may not be protected if infecting wild-virus dose is high. Such situation is expected to prevail in areas of high endemic poliomyelitis. It is suggested that seroconversion studies may be performed using local wild poliovirus serotypes instead of standard strains in countries where vaccine failure rates are substantially high.

WS4-1

BIOLOGY VARIABILITY OF VARIOUS HIV ISOLATES

Jean-Claude Cherman
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EXPRESSION OF HIV-1 AUTOLOGOUS P55- AND P55/GP120-V3 CORE PARTICLES: A NEW APPROACH IN HIV VACCINE DEVELOPMENT

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Max V. Pettenkofer Institut, LMU, Munchen; [1]Batanisches Institut LMU, Munchen, FRG

In the past, considerable efforts have been directed towards developing a safe and effective vaccine against HIV infection. To avoid adverse side effects like e.g. induction of enhancing antibodies or immune suppression as described for gp160/120, a candidate vaccine should exclude such critical epitopes and restrict to immunologically well defined antigenic regions. Due to its particulate nature and its early appearence in infection, the virus core protein p55-GAG is an appropriate carrier molecule for defined, immunologgically relevant epitopes derived from other reading frames e.g. env. p55-core expression was achieved by recombinant vaccinia and baculo viruses. Shedding of 90-110 nm core particles into culture medium was observed in both expression systems and proven by ultrathin-section electron microscopy and sedimen- tation analysis. Addition of the protease coding sequence resulted in an efficient processing of the p55-precursor molecule in the Baculo-system only. Significant protease mediated processing of the GAG precursor in the Vaccinia-system was only achievable by addition of the entire POL coding sequenze. In contrast to other retroviruses (MMLC), HIV-1 protease mediated processing is not dependent on the myristylation of p55-GAG.

As protease mediated processing does not complete the maturation process of the p55-precursor particles, but, in contrast, inhibits particle formation, further investigations focused on the appication of premature p55-GAG-particles as carrier proteins. To extend their immunological spectrum, a consensus sequence of the HIV-major neutralizing epitope V3 of gp120, designed in our lab, was inserted into different regions of the p55 carrier molecule. Broad reactivity of the corresponding V3-consensus peptide was proven in ELISA with patient sera (> 90%). Moreove V3-IIIB specific cytotoxic T-cells of the balb-C mouse killed target cells treated with V3-consensus peptides. The expression of the p55-V3 chimeric proteins in the Vaccinia expression system was proven western blot and immuno fluorescence analysis using (i) monoclonal antibodies directed to p55 and the inserted V3 region and (ii) polyclonal monospecific anti-V3 peptide sera. Particulate conformation of chimeric particles produced in mamalian and insect cells was show by ultrathin section electron microscopy. Immunological data using purified particles or recombinant vaccinia viruses as immunogens wil be presented.

WS4-3

CLINICAL AND LABORATORY OBSERVATIONS OF THE SPECIFICITY FOR HIV ANTIGENS IN THE ABBOTT ELISA TEST

D.C. Shanson

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Serum HIV antigen tests have been used to detect early HIV infection in patients who have not yet developed HIV antibodies as well as to assist with monitoring anti-HIV drug therapy. The Abbot ELISA HIV antigen test has been used at St Stephen's and Westminster Hospitals since 1988 to report positive results in over 1000 patients who developed clear clinical and other serological evidence of HIV infection. However, 3 male patients with positive HIV antigen test results lacked long-term clinical and serological evidence of HIV infection. One patient who lacked evidence of HIV infection gave consistently positive HIV antigen test results over a period of a few months. 'False-positive' results arising from non-specific reactions were concluded to have occurred in this patient. In a separate study experiments using the Western Blot method demonstrated that the Abbott test detects not only p24 HIV antigen but also other proteins apart from the p24 HIV antigen. The Abbott test should not be used alone to diagnose HIV infection and should not be used specifically to detect p24 antigenaemia in patients known to be infected with HIV. In practice the test is most useful for monitoring HIV antigenaemia due to various HIV antigens in patients with known HIV infection who are receiving retroviral drug therapy.

WS4-4

DEFINED HIV-1 INDETERMINATE WESTERN BLOT PROFILES AS LABORATORY MARKERS OF SEROCONVERSION AND LATE STAGE INFECTION

J. Downie, C.B. Chew, A. Cunningham
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The aim of this study was to determine whether there was a predictive relationship between the Western blot band profile and the clinical status of the patient with particular reference to seroconversion and late stage infection.

METHODS:

Western blot (Diagnostic Biotechnology) profiles of 92 sera which were categorized as Indeterminate Group 4 (reactive to envelope proteins gp160/120 and to not more than two other viral proteins, Australian Guidelines)from indivi- duals known to be either seroconverters (32) or patients with ARC/AIDS (35) were analysed for their exact band specificity for envelope (ENV, gp 160/120), polymerase (POL, p68, p34) or core (GAG, p24) proteins.

RESULTS:

It was found that of the 32 known seroconverters, 3 (9%) were initially reactive to ENV proteins only. The remaining 29 (91%) were initially reactive to ENV/GAG proteins but not to POL proteins. Of the 25 seroconverters tested for antigen (p24), 22 (88%) were negative. Of the 35 late stage infection patients, 8 (23%) showed moderate to strong reaction of ENV proteins only, 9 (26%) to ENV/GAG proteins and 19 (54%) to ENV/POL proteins. Of the 26 late stage patients tested for antigen, 25 (96%) were positive. Antigen was detected in 60% of those reacting to ENV only, 100% reacting to ENV/GAG proteins and 84% reacting to ENV/POL proteins.

CONCLUSIONS:

Band analysis of Western blot indeterminate (Group 4) profiles of 67 individuals showed that seroconverters were more likely (91%) to be reactive to gp160/120 and p24 while late stage infection patients showed strong reactivity of gp 160/120 and p34 (54%). The majority (88%) of seroconverters were antigen (p24) negative while 96% of late stage infection patients were positive for p24 antigen.

WS4-5

PROTEINS OF HUMAN RETROVIRUSES AND THEIR USE IN DIGNOSIS OF RETROVIRAL INFECTION

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Department of Cell Biology, Advanced Bioscience Laboratories, Kensington, MD, USA.

Human retroviruses have been identified as the primary etiological agents for a number of human diseases. Thus HTLV-I causes adult T-cell leukemia and tropical spastic paraparesis and HIV-1 causes AIDS. The major routes of transmission for these viruses are sexual,through mothers' milk (for HTLV-I) and through blood. Transmission through clinical use of blood can be prevented by adequate testing for antibodies to these viruses. Antigens of HTLV-I and (HTLV-II) and HIV-1 have been identified and characterized. The major antigens are products of the structural genes *gag,pol and env* and these are all immunoreactive with sera of infected individuals. Therefore the first generation tests used extracts of purified virus as the antigen in a variety of testing formats. In these tests the sensitivity and specificity were highly dependent on the quality of the antigen preparation. A systematic study of relative immunoreactivity of specific HIV-1 proteins to sera of virus infected subjects identified envelope protein (gp160) as the most reactive, followed by the reverse transcriptase (p66/p51) and the gag protein p24. In an ELISA test gp160 exhibited 100% specificity and 100% sensitivity on a variety of panels that included 400 Western blot characterized sera, 1000 unselected blood donor sera, 40 sera known to interfere in HIV-1 and HTLV-I ELISA tests and sequential pre- and post-seroconversion sera samples from 4 individuals.

WS4-6

IN VITRO AZT SENSITIVITY OF HIV-1 ISOLATED FROM PATIENTS BEFORE, DURING AND AFTER THERAPY

K.H. McGavin, *S.A. Land, R.E. Edwards, D. Hugo, R. Lucas, C.J. Birch, S.A. Locarnini*

Virology Department, Fairfield Hospital, Victoria, Australia.

Treatment of HIV infected individuals with AZT commenced at Fairfield Hospital in March 1987. Since then the *in vitro* AZT sensitivities of 372 isolates, collected from 237 patients before, during and, in a number of cases, after treatment with the drug have been obtained. A miniaturized peripheral blood mononuclear cell culture system was used in conjunction with a reverse transcriptase microassay to enable the testing of this large number of isolates. The study has shown that:

- pretherapy isolates display a range in their sensitivity to the drug,
- a majority of patients develop resistant virus after long term treatment with AZT,
- the rate of development of resistance in asymptomatic individuals is slower than in patients with disease symptoms and,
- in some cases, cessation of therapy leads to the emergence of drug sensitive virus.

WS4-7

A FOLLOW-UP STUDY OF HIV CARRIERS IN JAPAN

T. Kurimura, O. Yamada, A.M. Choudhury
Department of Pathology, Research Institute for Microbial Diseases,
Osaka University, Japan.

The profile of the epidemics of HIV infections in Japan is different from other countries. Early in 1980's, AIDS patients were found mainly among hemophiliacs. They were infected with the virus *via transfusion of contaminated blood products*. In 1983, first AIDS case of male homo/ bisexuals was found and an AIDS case in heterosexual contact in 1986.

We have been performing a follow-up study of Japanese HIV carriers mainly consisted of hemophiliacs since 1987 employing virus isolation, detection of viral DNA/RNA by PCR and antibody/ antigen testing.

For first 3 years of our study, virus isolation rate using peripheral blood mononuclear cells (PBMC) was around 20% while it increased to ca.30% since 1990. This phenomenon predicts the increase of AIDS patients among the subjects in near future since virus isolation rate reflects the virus load in the blood. Virus isolation correlates with the presence of viral RNA in plasma or viral RNA in PBMC. It was demonstrated that loss of anti-p17 is also a useful marker which correlates with the progression of clinical status.

WS4-8

INCREASE IN HIV SEROPOSITIVITY AMONG MALE PATIENTS ATTENDING STD CLINICS AND FEMALE PROSTITUTES AT POONA, INDIA

K. Banerjee, *A.D. Divekar, J.J. Rodrigues, S. Kulkarni, M. Thakar, N.K. Athalye*

National Institute of Virology, Post Box No. 11, Pune, India.

Serum samples were collected from male patients attending STD clinics at Poona. Serum samples from prostitutes were collected from brothels and free clinics for assorted ailments. The sera were tested in ELISA and the positives confirmed by Western Blot (WB). Of 4868 persons attending STD clinics,1895 cases were of Chancroid, 633 cases of acute granuloma inguinale, 179 cases of primary syphilis, 379 cases of secondary syphilis, 164 cases of LGV, 100 cases of venereal warts, 101 cases of Herpes progenitalis, 130 cases of nonspecific urethritis, 64 cases of granuloma inguinale, 594 cases gave history of exposure to prostitutes but had no other symptoms. The percentage of ELISA positivity ranged from 3 to 5 percent in different conditions except in secondary syphilis which was 17.68, and in granu loma inguinale it was 12.5%. The WB positivity was slightly lower. The percentage of seropositivity was logit transformed [$P = {}^{m}/_{n}$: m = Nos. of +ve samples; n = total Nos. of samples. $Y = l_n\ (m + 0.5)/(n-m + 0.5)$] and was plotted quarterly from 1-7-1987 to 31-3-1991, the curve followed the equation $Y = 5.177 + 0.191\ X$. There was no change in the rate of increase of seropositivity in different years. Similarly the seropositivity in prostitutes increased from 4.69 percent in the first quarter of 1989 to 31 percent in the first quarter of 1991. The curve plotted with percentage of seropositivity (Y) and each quarter of the year (X) had the regression equation $Y = -2.843 + 0.235\ X$. Here again there was no significant change in the rate of increase in seropositivity in the different years.

The data are being used for making prediction models for HIV cases in the Pune area.

WS4-9

THE LEGISLATIVE RESPONSE TO A NEW VIRUS: A GLOBAL SURVEY OF LEGISLATION RELATING TO HUMAN IMMUNODEFICIENCY VIRUS

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In the wake of the AIDS pandemic, almost all developed, and several developing countries have enacted AIDS-related laws and regulations. The law-making process which commenced in 1983 has not only gathered monentum over the years, but there has also been a significant evolution in the scope and content of the laws and regulations. Different legal and judicial systems have responded in different ways to the pandemic and to the underlying causative agent. There are differences in the way in which infection with HIV is classified. Notification procedures and measures to be taken by persons who are HIV seropositive or who engage in high risk activities also differ from one law to another. Some of the legal requirements, especially those pertaining to screening of high-risk groups and foreign travel, have generated much controversy, while the requirement to screen blood before transfusions, for instance, has had a salutary effect. Countries which have already enacted HIV- or AIDS-related laws or those which are planning to do so in the future must formulate and apply eartain criteria to ensure that legal provisions conform to principles of human rights and wı serve the intended purposes.

Session No. 20 **PLENARY LECTURE 3**

Date : Wednesday 20 November 1991 Time 09:00-09:45
Room : Room A & B

PLENARY LECTURE 3
Chairpersons : ***Frank Fenner,*** AUSTRALIA
Suttipant Sarasombath, THAILAND
Neurologic Complication of Measles
Dianne Griffin, USA

Session No. 21 **SYMPOSIUM 8**

Date : Wednesday 20 November 1991 Time 10:15-12:15
Room : Room A

SYMPOSIUM 8 Rabies : Diagnosis, Pathogenesis, Prevention and Control
Chairpersons : ***Mukda Trishnanonda,*** THAILAND
Thelma E. Tupasi, PHILIPPINES

1. Identification of the Geographic Distribution of Rabies Virus Variants by Molecular Techniques
   Jean S. Smith, USA
2. Neuronal Cell Cultures as a Model for the Study of Rabies Virus Pathogenicity
   Pinhas Fuchs, ISRAEL
3. Pathogenesis of Human Rabies
   Thiravat Hemachudha, THAILAND
4. Ante- and Postmortem Diagnosis of Rabies by PCR Using Nested Primer
   Nuanthip Kamolvarin, THAILAND
5. Advances in Rabies Vaccine Technology
   Reinhard Gluck, SWITZERLAND
6. Antibody Responses to PCEC Rabies Vaccine Following 0.1 ml Immunization Schedules
   Vara Meesomboon, THAILAND
7. An Alternative Economical Schedules for Rabies Postexposure Vaccination : The Thai Red Cross Regimen
   Prapan Phanuphak, THAILAND
8. 5 Years Experience with PCEC Rabies Vaccine - What about Side Effects and Treatment Failures ?
   Ihor Harabacz, GERMANY

Session No. 22 **SYMPOSIUM 9**

Date : Wednesday 20 November 1991 Time 10:15-12:15
Room : Room B

SYMPOSIUM 9 Gastroenteritis Viruses
Chairpersons : *an H. Holmes*, AUSTRALIA
Wandee Varavithya, THAILAND

1. Serotypic and Host-Specific Differences in Rotavirus Proteins
   Ian H. Holmes, AUSTRALIA
2. Serotyping of Human and Bovine Rotaviruses by Polymerase Chain Reaction
   Koki Taniguchi, JAPAN
3. Changing Pattern of Rotavirus Infection Associating with Infant Development
   M.H. Ng, HONG KONG
4. Epidemiology of Group A Rotavirus Strains in Bangladesh from 1987-1990
   Leanne E. Unicomb, BANGLADESH
5. An Ongoing Study of Human Rotavirus Epidemiology in Thailand
   Pantipa Sinarachatanant, THAILAND
6. Detection and Serotyping of Astrovirus from Viral Gastroenteritis in Kunming,China
   Ji-Chang Liu, CHINA

PL-3

NEUROLOGICAL COMPLICATIONS OF MEASLES

D.E. Griffin

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Measles remains a worldwide problem due to problems with vaccine delivery and efficacy. Secondary infections, diarrhea and pneumonia, cause most of the mortality but neurological disease causes most of the longterm morbidity. Three types of neurologic disease occur. (1) The most common (1/1000 cases) is postinfectious encephalomyelitis, a perivenular disease that occurs at the time of the acute disease. This is an autoimmune process and myelin basic protein is an important target antigen for immunologic attack. Virus has not been detected in the central nervous system (CNS). (2) Inclusion body encephalitis is caused by CNS infection with measles in immunocompromised individuals that have not contained the infection with anappropriate immune response. The original measles infection may not elicit a rash due to the lack of an immune response. Neurologic disease appears within a few weeks of measles virus infection and progresses to death within 2-3 months. (3) The most uncommon (1/10 cases) neurologic complication of measles is subacute sclerosing panencephalitis. This disease occurs 7-12 years after the original virus infection and is associated with CNS inflammation and high titers of antibody to measles virus in serum and CSF. A defective form of measles virus is found in neural cells. The disease is usually relentlessly progressive with death a few years after symptoms begin.

S8-1

IDENTIFICATION OF THE GEOGRAPHIC DISTRIBUTION OF RABIES VIRUS VARIANTS BY MOLECULAR TECHNIQUES

J. Smith, *L. Orciari, P. Yager, C. Warner, D. Seidel*
Rabies Laboratory, Viral and Rickettsial Zoonoses Branch, DVRD, CID, Centers for Disease Control, Atlanta, GA, USA.

Distinctive characteristics of the ribonucleoprotein of rabies virus isolates from different species and from different areas of the world can be identified by antigenic and genetic analysis. Antigenic typing is useful for broad surveys of rabies variants, and consistent differences among isolates taken from different species or from different geographic areas imply independent cycles of virus transmission and separate enzootics. The epidemiologic and evolutionary relationships between groups of isolates can be estimated from cDNA sequence homology. We have used panels of monoclonal antibodies for antigenic typing and dideoxy sequencing of polymerase chain reaction-amplified cDNA for genetic typing of rabies isolates from dog rabies enzootic areas of Latin America, Africa, and Asia. Isolates from different enzootic areas could be differentiated by their distinctive antigenic or genetic charac- teristics, and isolates collected from the same enzootic area could be identified by similarities in their antigenic profile and nucleotide sequence. These methods were used to investigate the source of infection for unexplained human and animal rabies deaths in the United States and to show that their infection occurred in dog rabies enzootic areas outside of the United States.

S8-2

NEURONAL CELL CULTURES AS A MODEL FOR THE STUDY OF RABIES VIRUS PATHOGENICITY

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P. Fuchs
Department of Virology, Israel Institute for Biological Research, Ness-Ziona, Israel.

The use of *in vitro* model systems to replace animal studies is nowadays more important than ever. Neuronal cell cultures infected with rabies virus provide valuable information on the tropism of the virus and the ultrastructural and neurochemical changes in the infected cells.

Primary dispersed and organotypic cultures of selected brain areas and spinal cords were prepared from mouse fetuses and neonates. Following synapse formation and myelination (2nd week in culture) cells were infected with the CVS-21 strain of rabies virus (about 10^4 mouse LD_{50}/culture). Cultures were maintained for additional 1-7 days before processing for viral replication and ultrastructural studies. Virus replication was determined by the fluorescent foci assay on BHK-21 cells.

We found that the peak of viral replication was at day 4 post-infection. Fluorescent and electron microscopy revealed that during the first 3 days post-infection the virus multiplied specifically in neurons. However, only 10-20% of the neurons were infected, indicating the possibility that only certain types of neurons were susceptible to the virus. From day 4 post-infection onwards, viral antigens were detected also in glial cells and macrophages. The damage induced by the virus to the cells and the myelin sheath was minimal even 1 week following infection.

Our results are comparable to those published for *in vivo* studies indicating that neuronal cell cultures may serve as a reliable model for the characterization of cell : virus interaction in rabies and other encephalitic viruses.

S8-3

PATHOGENESIS OF HUMAN RABIES

Thiravat Hemachudha
Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand.

Rabies is regarded as an universally fatal disease. Human rabies may present as encephalitis or paralysis. Encephaiitic rabies patients usually have a rapidly progressive course. It has been considered that there are defects in immune responsiveness resulting to rapid dissemination of virus. Further, strain and virus localization may determine the clinical presentations.

In order to determine whether there is "immune paralysis" in human rabies, we evaluate immune activation, cytotoxic killing process, and antibody responses to N and G antigens. Examination of serum cytokines showed that levels of sIL-2 r were comparable in groups of fatal rabies and nonfatal encephalitis due to other viruses. However, fewer paralytic rabies patients had elevated sIL-2r. sCD 8 levels were elevated in only a few rabies and nonrabies patients. IL-6 was detectable in only sera of encephalitic rabies patients and in none of the patients with paralysis.

Analysis of antibody responses in rabies patients showed that only 20% of them had neutralizing antibodies. In these antibody-positive rabies patients, levels of both antibodies, particularly of anti-N antibody, were lower than in vaccinated group.

Regarding cytotoxic system, although number of NK cells in the peripheral blood was significantly diminished (no demonstrable NK cells in the brain), their functions were well preserved. These suggest that inadequate response to N protein rather than defects in immune activation and killing mechanism may play role in rapid virus dissemination.

Study of rabies viral antigen distribution and strain characterization failed to show any correlation with specific clinical manifestations. Rabies viral antigen is usually demonstrable in the spinal cord and brainstem if the survival period is shorter than 7 days, regardless of clinical types. Cellular reactivity to rabies virus, as determined by *in vitro* lymphocyte proliferation test, appears to correlate well with clinical types. Further, cellular reactivity to myelin basic protein may also influence the clinical course. MBP-positive patients tend to die faster.

We conclude that delayed appearance of neutralizing antibody in the early phase and process of immune destruction in the late phase may explain the high virulence of rabies in man.

S8-4

ANTE - AND POSTMORTEM DIAGNOSIS OF RABIES BY PCR USING NESTED PRIMERS

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Diagnosis of rabies may be achieved by isolation of virus (mouse inoculation test, tissue culture infection technique), demonstration of antigen or antibody in tissue and biological fluids (immunoassay). Unfortunately, none of these tests is simple, sensitive and reliable. Antibody may appear in the serum (rarely in the CSF) in only 20% of patients with rabies regardless of survival time. This is due to the defect in immune recognition of nucleocapsid antigen, which is crucial for elicitation of neutralizing antibody. Fluorescent antibody test on brain impression smear also carries a false negative rate of 0.2-0.5%. MIT requires at least 21 days for the result.

We have successfully developed methods to extract and amplify rabies virus RNA from brain and CSFs. Our techniques involve 2 step PCR after reverse transcription, first with outer primers and then with inner primers nested within the first pair. The product of 524 base pairs of N gene is visualized by agarose gel electrophoresis and ethidium bromide staining, thus avoiding hybridization to confirm its specificity. Preliminary results with 200 specimens of rabid dog and human brains as well as CSFs showed an excellent correlation with MIT. This test can also be applied to brain specimens that were left over at room temperature for 6-8 hours. Total time required for the whole process is less than 24 hours.

This technique is promising and can be applicable for routine use at a large rabies diagnostic centre.

S8-5

ADVANCES IN RABIES VACCINE TECHNOLOGY

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Optimal pre- and post-exposure treatment of rabies should involve a limited number of immunizations that induce a long lasting immune response of both T and B cells against a number of different rabies virus strains with virtually no side-effects. According to WHO requirements the treatment should be inexpensive.

Advances in biotechnology such as the culture of MRC-5 human diploid cells on micro-carriers in large scale bioreactors or new, highly effective down-stream processes (purification-techniques) of rabies viruses economically produced in embryonated duck eggs have made possible the production of potent and safe rabies vaccines at industrial scale and at low cost.

S8-6

ANTIBODY RESPONSE TO PCEC RABIES VACCINE FOLLOWING 0.1 ml IMMUNIZATION SCHEDULES

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The tissue culture vaccine will remain too expensive for routine national use in Thailand. Thus, immunogenicity of more economical regimens were evaluated in 146 healthy adult male volunteers who had no previous antirabies immunization. Neutralizing Antibody (N Ab) to Purified Chick Embryo Cell (PCEC) Rabies Vaccine of Behringwerke, Germany, Lot No. 88011, potency 5 IU/ml was studied. Volunteers were randomly allocated to 5 different multi-site regimens. In group 2, 35 volunteers were given the standard intramuscular (IM) regimen (1 ml inoculation on days 0, 3, 7, 14, 30 and 90 at the deltoid). Volunteers in the other 3 experimental groups were given 0.1 ml intradermally (ID). In group 1, 57 subjects were given 22211 regimen (2 sites inoculations on days 0, 3, and 7 and 1 site inoculation on days 30 and 90). In group 3, 20 subjects received 42101 regimen. In group 4, 15 subjects received 22111 regimen. The fifth group with 19 subjects received 222011 with 0.1 ml of vaccine given subcutaneously (SC). On days 14 and 30, all volunteers except one vaccinee of the SC group had titers of > 0.5 IU/ml. The geometric mean titers (GMTs) on days 14, 30, 90, 180 and 365 of group 1 were 7.94, 4.29, 2.26, 3.63 and 1.79 IU/ml respectively. The other 2 ID regimens were shown to have similar N Ab response to the first group. The standard in regimen gave higher GMTs than all other regimens on every occasion tested. The GMTs on days 14, 30, 90, 180, and 365 were 8.16, 12.92, 4.93, 8.19 and 4.75 IU/ml, respectively.

However, no significant advantage could be observed. The SC regimen seem to have the lowest GMTs especially on days 14 and 30 with no significant difference. The vaccinees with N Ab > 0.5 IU/ml on days 90, 180 and 365 were found to be 88.24-98.18%, 71.43-100 % for the ID regimens, 100% for the IM regimen and 72.22-84.21% for the SC regimen.

This investigation suggested that the multi-site 0.1 ml ID regimens are quite satisfactory compared to the standard 1 ml IM regimen. However, these regimens should be used by skillful staffs.

S8-7

AN ALTERNATIVE ECONOMICAL SCHEDULES FOR RABIES POSTEXPOSURE VACCINATION : THE THAI RED CROSS REGIMEN

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Intradermal (ID) vaccination is the only practical approach in postexposure rabies vaccination in developing countries if brain tissue derived vaccine is to be totally replaced by tissue culture vaccine. The Rabies Clinic of the Thai Red Cross Society (TRCS) which treats 20,000 or 1/5 of Thailand's postexposure rabies cases annually has been using purified Vero cell rabies vaccine (PVRV) since 1987. The reconstitu- ted vaccine contains 0.5 ml per vial dose. The TRCS's ID regimen of PVRV consists of 2 ID injection of 0.1 ml (1/5 vial) each of PVRV on days 0, 3, 7, followed by 0.1 ml ID injection on days 28 and 90, so called the 2-2-2-0-1-1 or 2-site ID regimen. Although the 2-site ID PVRV regimen is less immunogenic than the 4-site ID regimen (4-4-4-0-1-1) but it is as immunogenic as the full-dose intramuscular (IM) regimen while only one-third of the vaccine is needed. In addition to the neutralizing antibody response, the ID PVRV regimen can also result in 1 week earlier specific cell-mediated immune response than the IM regimen. The antibody response to TRCS's ID PVRV regimen is not significantly suppressed by the concurrent administration of equine rabies immune globulin and will last as long as 1 year. In addition, if either one or both of the 2 ID injections were misplaced subcutaneo- usly, the vigorous antibody response was still maintained. And finally, the efficacy and safety of this ID regimen have been well established including the efficacy and safety records in pregnant women. As a result of these findings, TRCS 2-site ID regimen has already been accepted by WHO Expert Committee on Rabies as the economical alternative for postexposure rabies vaccination which will appear in the 1991 WHO Revised Guidelines on Rabies Prevention. This will be most practical for any primary or secondary healthcare center which see at least 1 patient with rabies exposure per week since we have demonstrated that the potency of reconstituted PVRV would not significantly decline after 1 week of storage.

S8-8

5 YEARS EXPERIENCE WITH PCEC RABIES-VACCINE WHAT ABOUT SIDE EFFECTS AND TREATMENT FAILURES?

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Drug Surveillance, Behringwerke AG, Marburg, Germany.

All data on suspected side effects after tissue culture rabies vaccination reported spontaneously to Drug Surveillance of Behringwerke AG, Marburg, Germany are analyzed and an adverse reaction profile is presented with special emphasis on allergy, neurological events, use during pregnancy and treatment failures.

From this date,it can be concluded that there is no difference between PCEC vaccine and other tissue culture rabies vaccines in terms of efficacy and safety.

S9-1

SEROTYPIC AND HOST-SPECIFIC DIFFERENCES IN ROTAVIRUS PROTEINS

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The number of recognized serotypes of group A rotaviruses is increasing steadily. Serotype depends on both of the virus surface proteins, the glycoprotein VP7 and the projecting protein VP4. At least 13 glycoprotein (G) serotypes have been established, and there are probably at least as many VP4 (P) serotypes although they are technically more difficult to define.

Host specificity is considered to depend on VP4, but in cases where particular VP7 serotypes occur in a range of animal hosts (eg. G3) the amino acid sequence of VP7 also varies slightly between hosts. We and others have identified antigenic epitope regions within VP7 by sequence analysis of variants resistant to neutralizing monoclonal antibodies. It is suspected that these regions may be close to a host cell receptor-binding region but in the absence of eg. x-ray crystallographic structural data this is currently impossible to prove.

We have recently been studying a non-antibody inhibitor found in the serum of BALB/c mice which neutralizes rotaviruses with certain G serotypes. The specificity appears to depend on VP7, not VP4. Within serotype G3, some rotavirus strains are sensitive and others resistant to the inhibitor. Sensitive and resistant strains differ in only a few amino acids in or near the antigenic epitope regions of VP7. We believe that the inhibitor resembles a mouse cell receptor for rotavirus and may help us to identify regions in VP7 involved in receptor-binding.

S9-2

SEROTYPING OF HUMAN AND BOVINE ROTAVIRUSES BY POLYMERASE CHAIN REACTION

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In human rotavirus (HRV),seven serotypes have been identified. The serotype specificity is mainly associated with an outer capsid protein VP7. Enzyme Immunoassay using anti-VP7 serotype-specific monoclonal antibodies (EIA-typing) has widely been employed for serotyping HRV. In contrast, nucleotide sequence data of VP7 genes of numerous rotavirus strains made it possible to apply polymerase chain reaction for serotyping HRV (PCR-typing).

Stool specimens were collected from infants with diarrhea in Sapporo between Oct. 1990 and Feb. 1991. Rotaviral RNA was prepared by using NP-40 and proteinase K. After full length (1,062 bases), DNA of VP7 gene was amplified (Ist PCR), DNA fragments with different sizes depending on the serotype were amplified by 2nd PCR using serotype-specific primers.

By EIA-typing, 95 of 115 samples (82.6%) could be serotyped : 83 were serotype 1, 5 were serotype 2 and 7 were serotype 3. In contrast, PCR-typing determined the serotype of 108 out of 115 samples (93.9%). PCR-typing results were in excellent agreement with EIA-typing results. The serotype of the samples which had been kept for 14 months at room temperature could be determined by the PCR. Thus, PCR-typing of HRV was found to be more sensitive than EIA although it is more expensive and not suitable for a large-scale survey. Furthermore, PCR-typing could be applied for serotyping bovine rotaviruses including serotypes 6, 8 and 10, whose prevalence in Thailand was recently elucidated. The presence of four different kinds of type has also been reported for VP4, the other outer protein of HRV. Typing of VP4 by PCR is now being studied.

S9-3

CHANGING PATTERN OF ROTAVIRUS INFECTION ASSOCIATING WITH INFANT DEVELOPMENT

M.H. Ng, *B-J Zheng*
Department of Microbiology, University of Hong Kong, Queen Mary Hospital, Pokfulam Rd, Hong Kong.

Rotavirus infection occurred constantly during the first 2 years of infancy, but less than 5.5% of which resulted in diarrhoea. The ratio of overt infection to asymptomatic infection (overt infection rate) varies between serotypes. The prevalent serotype 1 virus was the most virulent, with an overt infection rate of 11.8%, while serotype 3 and those isolated from neonates were the least virulent, with overt infection rate of 2.6% and less than 1.3%, respectively. The outcome of the infection also changed with age. Neonatal infection was characterized by asymptomatic excretion of the virus in stool. Overt infection mainly occurred before 12 months of age, accompanied by virus excretion and a vigorous antibody response. The large majority of the infection after neonatal period was asymptomatic evidenced by antibody response, but apparently without accompanying virus excretion. The changing pattern of infection was correlated with maternal antibodies and the development of immunity by the infants against successive episodes of infection.

S9-4

EPIDEMIOLOGY OF GROUP A ROTAVIRUS STARINS IN BANGLADESH FROM 1987-1990

L.E. Unicomb, *Fu Bingnan, A. Ali, N.N. Banu, J.G. Gomes, Z. Rahim, S.R. Tzipori*

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh.

A study of group A rotavirus was conducted to ascertain the prevailing strains in different parts of Bangladesh. A total of 4335 rectal swab specimens and 6527 stool specimens were collected from patients of all age groups from 2 rural locations (Matlab and Teknaf) and from the capital city of Bangladesh (Dhaka). Group A rotavirus (RV) was detected by ELISA from 756 rectal swabs (17.4%) and 1070 stool samples (16.4%). Of the 1826 RV positive specimens, 1714 (93.9%) were tested for serotype by oligonucleotide probe hybridization. A serotype could be determined from 64.7% of stool RVs, whereas only 36.1% of RV positive rectal swabs could be serotyped (X^2 = 136.17, $p < 0.001$).

Serotypes 1-4 were found in Dhaka and Matlab throughout the years studied, whereas only serotype 1 strains exhibiting the same electropherotype were found during a RV epidemic in 1989 in the remote area of Teknaf.

Our results suggest that rectal swab specimens are adequate for the detection of RV but are not appropriate where RV serotyping is required.

S9-5

AN ONGOING STUDY OF HUMAN ROTAVIRUS EPIDEMIOLOGY IN THAILAND

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A continuous study on rotavirus infections among pediatric diarrheas visited hospitals in Bangkok, Lampang, Hat Yai, and Nakhon Ratchasima was made by polyacrylamide gel electrophoresis and silver staining method. Rotavirus was endemic in all studied provinces and caused annual epidemic. The epidemic started earlier in Bangkok than that in other provinces, i.e, observed during August and February, while it occurred during November and May in Lampang and Hat Yai. The magnitude of epidemic was different in different seasons and different areas of study; the epidemic was initiated by a 1-2°C drop of temperature from 28-29°C, be maintained at low relative humidity (<75%) and suspended in warmer climate.

The incidence during the non-epidemic months was high (20%) in Bangkok and low (<10%) in other provinces. Genomic variability of rotaviruses detected in each geographical area per season ranged from 9 to 48 electropherotypes, however, only one or a few electropherotypes predominated. Within the same epidemic season, the same or different predominated electropherotypes observed in different studied areas.

A long term study in Bangkok during 1982-1990 and in the provinces during 1987-1990 showed the epidemics of serotype 1 rotaviruses with serotype 2 co-existed throughout the study period. However, serotype 2 rotaviruses predominated in Bangkok and Lampang during 1987-1988 and 1989-1990. Serotype 4 predominated in Bangkok during 1982-1983 and appeared at low frequencies in Bangkok, Hat Yai and Nakhon Ratchasima during 1988-1989. Serotype 3 was detected, at low frequencies, in Bangkok, Hat Yai and Lampang only during 1989-1990. A serotype 3 subgroup I and a virus with unusual short RNA migration pattern were observed in this study.

S9-6

DETECTION AND SEROTYPING OF ASTROVIRUS FROM VIRAL GASTROENTERITIS IN KUNMING, CHINA

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Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing, China.

This article reports the detection of astrovirus from viral gastroenteritis in Kunming area in the past several years, and the serotyping of astrovirus for the first time in China. Electron microscopy (EM) was applied for the detection of virus particles in fecal samples of patients with this illness. Antibody was assayed by complement fixation test (CFT), immune electron microscopy (IEM), solid-phase immune electron microscopy (SPIEM) and enzyme-linked immunosorbent assay (ELISA). A kind of virus particle was found by EM in 1985. It appeared round or hexagonal, 32-38 nm in diameter, without envelope, having a core of about 32 nm in diameter with honey -comb like capsomeres. It has an outer layer of about 38 nm in diameter with radially arranged capsomeres and irregular surface. IEM showed positive results. SPIEM confirmed positive adsorption. Serologic assay by CFT, IEM, SPIEM and ELISA revealed a greater than 4-fold rise in titer of the antibody against this virus in paired sera. Results of ELISA with Group A rotavirus and Group B rotavirus were negative. Six strains of morphologically similar virus found in 1983 showed cross serologic reaction with this virus. Sera of 125 healthy adults were examined for this antibody by CFT, of which 15 were positive. Whereas, results of CFT on sera of 57 non-gastroenteritis infants were all negative.

This virus was different from rotavirus, adenovirus, coronavirus, calicivirus, enteroviruses, Norwalk agent, etc. in size and morphology. Finally, it was identified as astrovirus by morphologic characterization, enzyme immunoassay and cultivation, and was serotyped as Type 3 astrovirus.

Session No. 23 **PLENARY LECTURE 4**

Date : Thursday 21 November 1991 Time 09:00-09:45
Room : Room A & B

PLENARY LECTURE 4
Chairpersons : ***Joseph L. Melnick***, USA
Petcharin Srivatanakul, THAILAND
Viruses in Human Cancers
Harald zur Hausen, GERMANY

Session No. 24 **SYMPOSIUM 10**

Date : Thursday 21 November 1991 Time 10:15-11:45
Room : Room A

SYMPOSIUM 10 EBV Associated Cancer
Chairpersons : ***Hans J. Wolf***, GERMANY
Chalobon Yoosook, THAILAND

1. Epidemiology of EBV Associated Cancer
   D.M. Parkin, FRANCE
2. EBV Biology : A Review on Pathogenicity and Prevention of Disease
   Hans J. Wolf, GERMANY
3. Use of Recombinant Epstein-Barr Virus Membrane Antigen in an ELISA Format to Measure IgA Antiboby Responses : A Screening Test for Determining Individuals at High Risk for Nasopharyngeal Carcinoma
   Paul J. Durda, USA
4. The Development of Epstein-Barr Virus Vaccines
   Andrew J. Morgan, UK

Session No. 25 **SYMPOSIUM 11**

Date : Thursday 21 November 1991 Time 10:15-11:45
Room : Room B

SYMPOSIUM 11 Arboviruses I : Diagnosis and Pathophysiology
Chairpersons : ***Thomas P. Monath***, USA
Suchitra Nimmanitaya, THAILAND

1. Validity of the Diagnosis of DHF by Physician
   Sompon Tassniyom, THAILAND
2. Risk Factors in Dengue Virus Infections
   Soe Thein, John Aaskov, MYANMAR

3. Detection and Typing of Dengue Viruses in the Culture Fluid by Reverse Transcriptase Polymerase Chain Reaction
Niwat Maneekarn, THAILAND
4. Lack of an Augmenting Effect of IFN gamma on Dengue Virus Replication in Human Peripheral Blood Monocytes
Nopporn Sittisombut, THAILAND
5. Hematopoietic Regulatory Functions of Dengue Virus Infected Stromal Cells in Cultures of Human Marrow Cells
Vincent F. La Russa, USA
6. Reverse Transcriptase Inhibits Taq Polymerase Amplification
L.N Sellner, ***Robert J. Coelen***, AUSTRALIA
7. Detection of Japanese Encephalitis Virus Infection in Mosquitoes and Cell Culture by Polymerase Chain Reaction (PCR)
Deepak A. Gadkari, INDIA

Session No. 26 **SPECIAL SESSION III**

Date : **Thursday 21 November 1991** **Time 12:45-13:45**
Room : **Room B**

SPECIAL SESSION III Japanese Encephalitis Vaccine
Chairpersons : ***Nadhirat Sangkawibha***, THAILAND
Kalyan Banerjee, INDIA

1. Japanese Encephalitis Vaccine Development in Japan
Akira Igarashi, JAPAN
2. Experience with the Improved JE Vaccine
A. Oya, JAPAN

Session No. 27 **WORKSHOP 5**

Date : **Thursday 21 November 1991** **Time 14:00-15:15**
Room : **Room A**

WORKSHOP 5 Virus Associated Cancers
Chairpersons : ***Harald zur Hausen***, GERMANY
D.M. Parkin, FRANCE

1. Association between Hepatitis B Virus, Hepatitis C Virus and Risk for Hepatocellular Carcinoma in Thailand
Petcharin Srivatanakul, THAILAND
2. Juvenile Laryngeal Papillomatosis in Thailand
Tochinobu Fujiyoshi, JAPAN
3. Human Papillomavirus in Cervical Carcinoma in Thailand
Sukhon Sukvirach, THAILAND
4. Human Interferon Alpha 1b Has High Therapeutic Efficacy in Threatment of Papillomavirus Infections of the Cervix
Zhiwei Qian, CHINA
5. Synthetic Peptide LA-I of Herpes Simplex Virus Type-2 (HSV-2) as a Diagnostic Marker for Cervical Intraepithelial Neoplasia
A. Parashari, M.M. Gupta, INDIA

Session No. 28 WORKSHOP 6

Date : Thursday 21 November 1991 Time 14:00-15:15
Room : Room B

WORKSHOP 6 Arboviruses II : Basic Virology
Chairpersons : *Joel M. Dalrymple*, USA
Mah-Lee Ng, SINGAPORE

1. Genetic Analysis of the Envelope Proteins of Dengue Isolates TH-36 (Den-5) and TH-Sman (Den-6)
   Stephen Yuen Wing Shiu, HONG KONG
2. A Model for the Antigenic Structure of the Dengue Virus Glycoprotein, NS1
   Paul R. Young, AUSTRALIA
3. RNA Polymerase of the Flavivirus Kunjin - Molecular and Morphological Analyses
   Edwin G. Westaway, AUSTRALIA
4. Probable Presence of RNA in Kunjin Virus-induced Vesicles
   Mah-Lee Ng, SINGAPORE
5. Analysis of Dengue Virus Proteins Expressed by Baculovirus Recombinants
   Xinyong Qu, ***Frank J. Austin***, NEW ZEALAND
6. Epitope Mapping to Define the Function of Dengue Virus Proteins
   John Aaskov, AUSTRALIA

Session No. 29 WORKSHOP 7

Date : Thursday 21 November 1991 Time 15:30-17:00
Room : Room A

WORKSHOP 7 Improved Techniques in Viral Diagnosis
Chairpersons : *Monica Grandien*, SWEDEN
Savanat Tharavanij, THAILAND

1. Quality Control in Clinical Virology
   Monica Grandien, SWEDEN
2. Serological Diagnosis of Epstein-Barr Virus Infection Using Three Novel Recombinant ELISAs
   Hans J. Wolf, GERMANY
3. Antibody Responses to Influenza Virion Proteins by Western Blot Analysis
   Diwen Qui, AUSTRALIA
4. *n Situ* Enzyme Immunoassay for Antiviral Susceptibility Testing of Respiratory Syncytial Virus
   Jung-Oak Kang, KOREA
5. Rapid Identification of Respiratory Viruses in Neonates
   Dennis Maurer, AUSTRALIA
6. Studies on Rapid Clinical Diagnosis of Influenza Virus Type A and B
   Tao Sanju, CHINA
7. Rapid Diagnosis of Respiratory Viral Infections - Do We Still Need Confirmatory Tests ?
   Monica Grandien, SWEDEN
8. Detection of Rubella IgG and IgM Antibodies in Whole Blood on Whatman Paper; Comparison to Serum Samples
   Vanna Punnarugsa, THAILAND

Session No. 30 **WORKSHOP 8**

Date : Thursday 21 November 1991 **Time 15:30-17:00**
Room : Room B

WORKSHOP 8 Arboviruses III : Epidemiology
Chairpersons : ***Tissa Vitarana,*** SRI LANKA
Soe Thien, MYANMAR

1. The Molecular Epidemiology of Ross River Virus Isolates From Australia and the South Pacific Region
   Micheal D. Lindsay, AUSTRALIA
2. Arbovirus Activity in Northern Australia Over the 1990/91 Wet Season
   Annette K. Broom, AUSTRALIA
3. *Epidemiological Trends of Japanese Encephalitis*
   Akira Igarashi, JAPAN
4. Japanese Encephalitis Virus Infections in Indonesians
   Suharyono Wuryadi, INDONESIA
5. Dengue Virus Surveillance at a Jakarta Hospital, 1988-1990
   Ratna Irsiana Tan, INDONESIA
6. Spread of Dengue in Villages of Western India
   Kalyan Banerjee, INDIA
7. Problems in Dengue Control - A Case Study
   S. Poovaneswari, Sai-Kit Ken Lam, MALAYSIA
8. Dengue Hemorrhagic Fever : A Continuing Problem in Sri Lanka
   Tissa Vitarana, SRI LANKA
9. Dengue Type 1 Epidemic with Hemorrhagic Manifestations in Fiji 1989-90
   A.H. Fagbami, FIJI

18:30-22:00 **BANQUET and LOY-KRATONG** **Sala Thai**

PL-4

VIRUSES IN HUMAN CANCERS

H. zur Hausen
Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Viruses may contribute to the development of human tumors by different mechanisms: indirectly by inducing immunosuppression or by modifying the host cell genome without persistence of viral DNA; directly by inducing oncoproteins or by altering the expression of host cell proteins at the site of viral DNA integration. Human cancers associated with papillomavirus, hepatitis B virus, Epstein-Barr virus, and human T cell leukemia lymphoma virus infections are responsible for approximately 15 percent of the worldwide cancer incidence. Cancer of the cervix and hepatocelular carcinoma account for about 80 percent of virus-linked cancers. Because experimental and epidemiologic data imply a causative role for viruses, particularly in cervical and liver cancer, viruses must be thought of as the second most important risk factor for cancer development in humans, exceeded only by tobacco consumption.

S10-1

EPIDEMIOLOGY OF EBV ASSOCIATED CANCER

D.M. Parkin
International Agency for Research on Cancer, Lyon, France.

Infection with Epstein-Barr Virus (EBV)) is ubiquitous, and is involved in acute, chronic and malignant diseases. Infection early in life, with persistence of a large load of viral genetic material, has been associated with the later development of several malignant disorders, most notably Burkitt's Lymphoma (BL), Nasopharyngeal Carcinoma (NPC) and Hodgkin's Disease. Although EBV fulfills some of the epidemiological criteria of causality for these cancers, in none is the relationship very direct. Thus, in BL, the essential step in the malignant process is a chromosomal translocation, and EBV infection (in conjunction with holoendemic malaria in Africa) may act simply by causing hyper-proliferation of B cells, during which the genetic change occurs. In NPC, EBV is present at higher levels than in controls, but other factors important in tumour development are NPC-susceptibility gene(s) and exposure to dietary and/or occupational carcinogens.

S10-2

EBV BIOLOGY : A REVIEW ON PATHOGENICITY AND PREVENTION OF DISEASE

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EBV is a vvery common virus in humans. More than 90% of the adult population worldwide is infected. The virus ensures its survival by infecting cells which ar .. most rarely producer progeny virus, Differentiation of cells is an additional prerequisite for viral replication. The mechanisms involved are complex and involve viral and cellular components. In vitro immortalization of certain cells by EBV is a rapid and efficient event, however the outgrowth of neoplasias in the immuncompetent host is very rare. Although mechanisms are still not completely understood, suppression of synthesis of immunogenic viral genes and of cellular genes involved in recognition of altered cells (HLA, adhesion) seem to be important.

The regionally widely differing incidence of major EBV associated neoplasias (Nasopharyngeal carcinoma in Southern Chinese, African type of Burkitts Lymphoma) suggests the exploitation of this association for diagnostic and prophylactic purposes. Both approaches have been pursured.

Antigens of relevance for diagnosis of EBV related acute and neoplastic diseases have been developed, tested and are being introduced for routine use.

Two independent approaches have been followed for the development of a vaccine. A vaccinia based life recombinant product expressing the major envelope glycoprotein gp 250/350 of EBV is already under evaluation in humans. Another product, where the same EBV antigen is produced as an antigen by secretion into the supernatant of transfected chinese hamster ovary cells, is under evaluation for use in human trials.

S10-3

USE OF RECOMBINANT EPSTEIN-BARR VIRUS MEMBRANE ANTIGEN IN AN ELISA FORMAT TO MEASURE IgA ANTIBODY RESPONSES: A SCREENING TOOL FOR DETERMINING INDIVIDUALS AT HIGH RISK FOR NASOPHARYNGEAL CARCINOMA

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Objectives:
Determine if a soluble truncated form of the gp350/250 membrane antigen (rMA) of Epstein-Barr Virus (EBV) can be used in an ELISA format to quantify human IgA responses to EBV. Determine if the ELISA is competitive with indirect immunofluorescence assays for IgA quantitation.
Methods:
rMA was purified from supernatant fluids from clones of the recombinant cell line GH3 19(J. Virol 1987; 61: 1796) by lectin affinity and ion exchange chromatography. Purified rMA was adsorbed to polystyrene 96-well microtiter plates. Various sera, previously characterized for IgA antibodies to EBV by immunofluorescence (IF), were then examined for reactivity with the solid phase rMA in an ELISA formatted to be specific for human IgA antibody responses.
Results:
Of 27 samples positive by IF for IgA responses to EBV VCA, 25 scored positive in the rMA based ELISA. Of 25 IgA IF negative samples (titers of < 10), 14 had low level reactivity (titers of 20 or 40) in the ELISA. With a serum panel from patients with WHO type 1 or type 2 NPC, 12 of 13 WHO type 2 sera were scored positive by ELISA, as were 7 of 15 sera from WHO type 1 patients.
Conclusions:
A recombinant soluble form of the EBV membrane antigen gp350/250 can be used in an ELISA format to screen for the presence of IgA antibodies to EBV. This simple and objective assay, which appears to be 4 to 8-fold more sensitive than IF depending on the cutoff used to determine positivity, may be capable of replacing the more cumbersome and less objective IF presently being used to screen for individuals having high titer IgA antibodies to EBV who are at high risk for having and/or developing nasopharyngeal carcinoma.

S10-4

THE DEVELOPMENT OF EPSTEIN-BARR VIRUS VACCINES

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The role of Epstein-Barr virus (EBV) in the causation of certain important human cancers has come under increasingly intense scrutiny in recent years. EBV is the direct cause of infectious mononucleosis and is implicated along with cofactors in endemic Burkitt's lymphoma, lymphoma in immunodepressed patients including AIDS sufferers, and undifferentiated nasopharyngeal carcinoma. Recent evidence suggests a link between EBV and certain Hodgkin's lymphomas. The association of Epstein-Barr virus with BL and NPC led to the proposal for the development of anEBV vaccine for use in man, since approximately 80,000 new cases of the latter disease per year are being reported. A subunit vaccine has been developed based on the EBV envelope glycoprotein, gp 340, and will be the subject of a Phase I human trial in the near future. Gp 340 produced in a bovine papillomavirus expression vector system and isolated from tissue culture supernatants has been successfully used to protect cottontop tamarins against experimentally induced EBV lymphoma. The cottontop tamarin has been used to evaluate various prototype vaccines. Inoculation of the tamarin with a large standard dose of EBV invariably leads to the development of malignant lymphoma within 14 to 21 days, ultimately leading to death. The tumours induced in cottontop tamarins by EBV are monoclonal or oligoclonal and have been identified as true malignant lymphomas using the most stringent criteria. They express the EBV latent antigens but no late antigens, although these are expressed almost immediately on growing tumour cells *in vitro*. A cDNA library has been constructed from a tumour biopsy and a search is being made for those EBV genes expressed. A major transcript from the BamH1A region not previously associated with latency is expressed in EBV-induced tamarin tumours.

Gp340 has been successfully used in conjunction with a muramyl dipeptide (MDP) adjuvant or incorporated into Quil A immunostimulating complexes to vaccinate tamarins. Both adjuvants may be formally accepted for human use in the near future and therefore could be used in the coming human trial. Alternative adjuvants are also being investigated. Recombinant viruses expressing gp340 have been investigated in the tamarin model and protection against EBV-induced lymphoma was achieved using a vaccinia recombinant laboratory strain but not a vaccine strain. Varicella and adenovirus vaccine strain recombinants are also under investigation. Where protective immune responses in the tamarin were obtained with a vaccinia recombinant, antibodies to gp340 were absent indicating that T cell immunity is important in the protective response. Subsequently it has been shown that human seropositives carry $CD4^+$ T cells specific for gp340 and that gp340 can induce T cell mediated inhibition of EBV-induced B cell transformation *in vitro*. T cell responses to gp340 vaccination in man may be critical and will be monitored in the coming human trial.

In future vaccine design it will be necessary to know the location and type of immunological information in the EBV gp340 envelope glycoprotein. The gp340 gene has been cut in twelve fragments spanning the whole gene. Each of the fragments have been expressed in quantity as B-galactosidase fusion proteins. Antibodies from the sera of NPC, BL patients and normal seropositives have been shown to react with particular fragments. Experiments to locate B and T cell epitopes using these fragments and a panel of synthetic peptides are being carried out.

S11-1

VALIDITY OF THE DIAGNOSIS OF DHF BY PHYSICIAN

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In Thailand, reported cases of DHF (dengue hemorrhagic fever) has been increasing during the past 30 years. Median incidences for each ten year period, started from 1958, were 10, 23 and 54 per 100,000 population respectively. The largest outbreak in 1987 resulted in the incidence of 325 cases/100,000 population. It has been speculated that this may be due to over diagnosis. We conducted a study during Sept 20-Oct 10, 1989 at out-patient department of a provincial hospital. When a patient was diagnosed as DHF, their blood was collected for viral isolation and antibody test. Two weeks later these patients were followed-up for clinical assessment and blood drawing for convalescent serum. Twenty-six cases were studied. Nineteen (73%) cases were males. Fifteen (58%) cases were between 5-9 years of age. Duration of fever before coming to hospital was 3 days in 19 (73%) cases. Dengue viruses were isolated from 10 patients (38.5%). Dengue type 1,2 and 3 were detected in 4, 4 and 2 cases respectively. From ELISA test for dengue antibodies, 13 (52%) cases showed rising antibody to dengue virus. Seven (28%) of these showed primary infection and the other six patients showed secondary infection. Clinical course of all dengue infection cases were mild. All of them were not admitted except one with diagnosis of UTI. All other non-dengue infection cases were recovered without admission. The validity of DHF diagnosis from this study was about 50%. Because of a small sample size and possible selection bias, more studies are needed.

S11-2

RISK FACTORS IN DENGUE VIRUS INFECTIONS

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Severe dengue infections occur most commonly in patients experiencing anamnestic infections with dengue serotypes not previously encountered. Onset of shock in dengue haemorrhagic fever patients is preceded by activation of the serum complement system. IgM antibody does not activate the classical complement pathway when it combines with soluble antigens. IgG1 and IgG3 antibodies are very efficient activators of the complement system when they combine with either soluble or particulate antigens. We have compared serum levels of IgG1, IgG2, IgG3 and IgG4 antibodies against dengue 1, 2, 3 and 4 in acute and convalescent sera from dengue fever, dengue haemorrhagic fever and dengue shock syndrome patients using an indirect enzyme linked immunosorbent assay (ELISA).

Our data suggest that severe disease may correlate better with the absence of high levels of non-complement fixing antibody than with high levels of IgG1 and IgG3.

S11-3

DETECTION AND TYPING OF DENGUE VIRUSES IN THE CULTURE FLUID BY REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

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Simple Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for the detection of dengue virus genome in the culture fluid was developed in such a way that dengue virus genome could be reverse transcribed and amplified in a single reaction mixture without previous RNA extraction. Four pairs of type-specific oligonucleotide primers were used as mixed primers in order to detect and type dengue virus genome simultaneously. Sixty-six serum specimens obtained from patients hospitalized with the clinical diagnosis of dengue hemorrhagic fever in Bangkok and Chiang Mai were inoculated into C6/36 cell cultures. After 6-7 days of incubation, the culture fluids were tested for the presence of dengue viruses (DV) by RT-PCR in parallel with further subculture and detection of dengue virus infected cells by peroxidase-antiperoxidase (PAP) staining procedure, using DV type-specific monoclonal antibodies as primary reagents. It was found that 32 dengue virus isolates were obtained from 66 serum specimens tested (48.5%), of these, 5 were DV1, 24 were DV2 and both DV1 and DV2 were detected in 3 samples. The results obtained by RT-PCR were in good agreement with PAP detection except that 3 isolates of DV1 that failed to be detected by PAP staining could be identified by RT-PCR.

S11-4

LACK OF AN AUGMENTING EFFECT OF IFN GAMMA ON DENGUE VIRUS REPLICATION IN HUMAN PERIPHERAL BLOOD MONOCYTES

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Gamma interferon (IFN) can augment dengue virus replication in a human monocytic cell line, U937 (Kontny et al. J. Virol. 62:9228, 1988). The augmenting effect was seen when U937 was infected with dengue virus which preincubated with low level of antidengue antibody, but not with free virus. In order to determine this effect of IFN on human peripheral blood monocytes (PBMn), we purified PBMn from Thai adults by using Ficoll-Hypaque centrifugation, adhesion to gelatin/plasma-coated flask and elution with EDTA. Pretreatment of 80-90% pure PBMn with purified human leukocyte IFN or recombinant IFN for 24 hours before dengue virus infection at the MOI of 5 resulted in a dose-dependent reduction of virus titer in the supernatant at 24 hours after the infection. The decrease in virus replication was seen after infecting PBMn with free virus and with virus which was preincubated with an optimal amount of an anti-flavivirus monoclonal antibody, 4G2. When infected PBMn was stained for dengue virus antigen, there was a parallel reduction of the number of dengue virus-infected cells among IFN-treated monocytes. Thus, in contrast to its effect in U937, IFN does not augment dengue virus replication in human PBMn. This finding does not support the idea that IFN might contribute to the pathogenesis of dengue hemorrhagic fever through an increased uptake and subsequent replication of dengue virus in monocytes.

S11-5

HEMATOPOIETIC REGULATORY FUNCTIONS OF DENGUE VIRUS INFECTED STROMAL CELLS IN CULTURES OF HUMAN MARROW

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Recent studies from our laboratory as well as others have suggested that both the stromal cells which make up the micro-environment in long term marrow cultures (LTBMC) (Exp Hematol 19:479; 1991) and hematopoietic progenitors from unpurified marrow cell preparations (Blood 74:1235, 1989) may be infected by Dengue (DEN) virus. In this study 3 to 4 week old irradiated (9.5 Gy) adherent stromal cells developed in LTBMC were assessed for their ability to support hematopoiesis following DEN type 2 infection. Our objectives were: 1) To determine if virus infection of stromal cells affects the proliferation of hematopoietic progenitors CFU-MIX, CFU-GM, BFU-E, and high proliferative potential (HPP) CFU-GM, (HPP) BFU-E in LTBMC, 2). To determine if DEN 2 infection of stromal cells alters their hematopoietic accessory functions, and 3). To determine if CD34+ marrow cell isolates can be infected directly by DEN 2 virus and whether infection alters the proliferation of clonogenic hematopoietic progenitors in methylcellulose cultures. There was a marked reduction in the numbers of hematopoietic progenitors which initially seed on the DEN 2 infected adherent stromal cells as measured 1 week after recharge with highly purified CD34+ cells. This suggests that DEN 2 virus infection of stromal cells impairs the critical cell to cell interactions which are necessary for the binding of primitive CD34+ hematopoietic cells on the the stroma which sustains hematopoiesis in LTBMC. The cumulative production of all the hematopoietic progenitors in LTBMC is significantly lower in DEN 2 infected cultures. Accessory functions of virus infected stromal cells were altered when measured in feeder co-cultures with CD34+ cell isolates. Colony formation by all clonogenic progenitor cells was reduced when compared to uninfected stromal feeder layers. Addition of giant cell tumor conditioned media to co-cultures containing infected stromal cell feeder layers restored colony formation. Incubation of CD34+ cell isolates with DEN 2 only 2 hrs results in a significant loss in colony formation. However, we were not able to detect virus replication in these cells by conventional plaque assay. These results suggest that DEN 2 virus can alter the proliferation of hematopoietic progenitors and the hematopoietic regulatory functions of stromal cells.

S11-6

REVERSE TRANSCRIPTASE INHIBITS TAQ POLYMERASE AMPLIFICATION

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Detection of viral RNA in host tissue by polymerase chain reaction (PCR) requires the prior reverse transcription of the viral RNA. When simultaneously processing large numbers of samples, it is advantageous to reduce the number of manual manipulations. One way to achieve this, when attempting to detect viral RNA is to add all reagents required for both reverse transcription and amplification to one tube, and to design a single, non-interrupted thermal cycling program. Whilst some compromises, particularly with respect to Mg2+ ion concentration and pH, must be made the sensitivity of the assay should not suffer (cf 2 separate reaction system). Since, theoretically at least, a single cDNA molecule should suffice for amplification, conditions may be biased to favour the Taq polymerase. Whilst attempting to set up a one-tube system with Taq polymerase (Taq: Biotech International) and avian myeloblastosis virus (AMV) reerse transcriptase (RT), we noticed a substantial decrease in the sensitivity of detection of RNA. Investigation of this phenomenon has revkealed direct interference of RT with Taq, Evidence supporting this conclusion includes the following observations; (1) increasing the ratio of Taq to RT improves sensitivity; (2) adding non-homologous RNA improves sensitivity; (3) RT that has been heat inactivated prior to Taq addition does not exert this effect; (4) the effect is not sequence restricted; (5) The Mg2+ ions are not sequestered by RT. In addition, the effect is not limited to AMV RT, Moloney murine leukaemia virus RT also affected Taq activity.

S11-7

DETECTION OF JAPANESE ENCEPHALITIS VIRUS INFECTION IN MOSQUITOES AND CELL CULTURE BY POLYMERASE CHAIN REACTION (PCR)

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Japanese encephalitis virus, a member of the family flaviviridae,is an important human pathogen in South and East Asia. *Culex vishnui* group of mosquitoes are the major vectors of JEV. Isolation of JEV from human cases is rare and time consuming. The rapid diagnosis therefore mainly relies on the detection of virus specific IgM antibodies from serum or CSF. The PCR technology has given a new boost to rapid diagnosis and here we report detection of JEV specific nucleotide sequences using PCR. Mosquitoes were infected intra-thoracically with JEV and stored at 12,24,48,72 and 96 hours post infection. Five infected mosquitoes were ground in 1 ml volume. 50 ul of this suspension was used for RNA extraction which was finally suspended in 40 ul. One ul of this was used for reverse transcription reaction using random primers. The entire 20 ul RT reaction was then used for PCR. Two primer pairs were used to give amplified products of 1700 bp and 765 bp. JEV RNA was detected in infected mosquitoes as early as 48 hours post inoculation. Authenticity of the amplified product was confirmed by using internal(nested) primers and by cloning the product in M13 vector followed by partial sequencing. Four different strains of JEV grown in cell culture could be detected by PCR but two strains of West Nile virus failed to give positive signal using the same assay conditions. Cell culture grown JEV was diluted serially, RNA was extracted and the ability to detect JEV RNA was compared to plaque assay. PCR was more sensitive than plaque assay. Attempts to detect JEV from mosquitoes fed on viremic chick and from clinically diagnosed JE cases are in progress.

SS III-1

JAPANESE ENCEPHALITIS VACCINE DEVELOPMENT IN JAPAN

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Japanese encephalitis (JE) is an acute viral disease with high fatality and grave sequelae. Its causative agent, JE virus, is a member of flavivirus, and is transmitted by *Culex tritaeni-orhynchus* and related mosquitoes which breed in watered rice fields. Swine and wild birds are amplifier vertebrates while humans are dead-end host with high rate of inapparent infection. JE is present not only in Japan but also in several countries in Asia monsoon area where rice cultivation in watered paddy fields and swine raising are common. There is no specific treatment for JE except supportive care, while the disease is preventable by effective human vaccination.

In Japan, formalin-inactivated Nakayama strain JE vaccine was developed from infected mouse brain homogenate. The first national standard in 1956 was revised several times by improved production processes, and the current vaccine is almost pure virion free from contaminating brain materials. The vaccine potency is determined by neutralizing antibody production in mice, and its efficacy was demonstrated by field studies in Taiwan and recently in Thailand. Studies on the neutralizing antibody level in the vaccinees in JE-nonendemic areas showed that the protective level of neutralizing antibodies were obtained by the primary vaccination of 2 shots with 1 week interval followed by a booster shot in the next year, and was maintained by booster immunization in every 3-4 years. JE vaccine is one of the safest human vaccines without serious side effects.

SS III-2

EXPERIENCE WITH THE IMPROVED JE VACCINE

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WS5-1

ASSOCIATION BETWEEN HEPATITIS B VIRUS, HEPATITIS C VIRUS AND RISK FOR HEPATOCELLULAR CARCINOMA IN THAILAND

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Liver cancer is the most frequent malignant neoplasm of males in Thailand, and third in females. Among cases notified with a histological diagnosis, hepatocellular carcinoma (HCC) accounted for 61% of cases in males and 45% in females.

A hospital-based case-control study was designed to investigate association between Hepatitis B virus (HBV), Hepatitis C virus (HCV) and risk for HCC in residents of northeast Thailand. 65 cases from three hospitals, with matched controls, were included. Infection with Hepatitis B virus was the major risk factor. Twenty-seven (41.5%) of HCC cases were positive HBsAg compared to 5(8.4%) of controls-the odds ratio on matched analysis was 12.0. The presence of any marker of HBV infection (HBsAg or anti-HBs and/or anti-HBc) was associated with an odds ratio of 5.3. With a 10% prevalence of carriers, the percentage of cases in the Thai population which can be attributed to HBsAg carriage is 58%.

Several recent reports have suggested that in areas where prevalence of hepatitis B infection is lowthen hepatitis C virus may be responsible for a substantial proportion of cases of HCC. There was a low prevalence of antibody to HCV - only 7 positive tests in 63 case-control pairs, and the odds ratio associated with the presence of anti-HCV was close to unity. It is clear, however, that HCV plays a minor role, if any, in Thailand, and the prevalence of anti-HCV is low.

WS5-2

JUVENILE LARYNGEAL PAPILLOMATOSIS IN THAILAND

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Juvenile laryngeal Papillomatosis (JLP) is a histologically benign tumor, but causes a serious problem of recurrent airway obstruction. JLP is the most common tumor of larynx in children though the incidence is so low as the prevalence (per 100,000 children aged at 0-14 years), 0.7 in Denmark, 0.6 in USA and 0.1 in Japan. On the other hand, the prevalence in Thailand was 2.8 that is 5-10 times more than those in other countries. In order to elucidate the etiology of the JLP,we investigated human papillomavirus (HPV) DNA in tissues from 25 Thai patients with JLP by dot blot hybridization and polymerase chain reaction (PCR). HPV11 DNA was positive in 21 cases of 25 JLP patients (84%) and HPV 6 DNA was positive in one case (4%). Southern blot study after digestion with restriction enzyme Bam HI revealed one band sized 8.0 Kb indicating that HPV DNA exist as plasmid state instead of integrated form. It was estimated that more than 60 copies of HPV genome in each cell with double PCR using HPV primers and *B*-globin primers. We isolated HPV DNA from DNA of JLP patient with gemonic cloning using EMBL3 as vector. Further restriction mapping and partial DNA sequencing results revealed that the isolated HPV DNA was same as known HPV 11. Thus the high prevalence of JLP in Thailand can not be explained by virulency of these HPV strains, and there may be some other cofactors involved in the high prevalence of JLP in Thailand.

E6 and L2 gene regions of the isolated HPV DNA from JLP were subcloned with expression vector pEX1 and pEX3 and recombinant E6 and L2 proteins were obtained. Antibodies reacted with this recombinant L2 protein were detected in a serum of JLP patient.

WS5-3

HUMAN PAPILLOMAVIRUS IN CERVICAL CARCINOMA IN THAILAND

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Uterine cervical cancer is the second most common cancer in Thai female. Association between cervical cancer and sexual activity has been reported for a long time. In recent years, specific type of human papillomaviruses appeared to be one necessary factor although other factors such as oral contraceptive use, smoking and immunosuppression are also important.

Identification of HPV type and distribution in Thai patients can give informative data for epidemiological study about HPV and cervical cancer. In this study, cervical tissue from 69 Thai patients with invasive carcinoma were tested for HPV type, using Southern blot hybridization with isotope-labeled HPV DNA probes (HPV type 11,16 and 18) under non stringent condition. HPV DNA was found in 31 of 69 (44.93%) cervical carcinoma biopsies; HPV type 16 in 20 (28.98%); HPV type 18 in 2 (2.90%); HPV type 35 in 3 (2.4%); HPV type 16 and 35 in 3 (4.35%) and HPV 16 related type in 4 (3.2%). Further study will be performed to investigate for other possible HPV type.

WS5-4

HUMAN INTERFERON ALPHA 1b HAS HIGH THERAPEUTIC EFFICACY IN TREATMENT OF PAPILLOMAVIRUS INFECTIONS OF CERVIX

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Chronic cervicitis was shown to be related to papillomavirus type 16 (HPV-16), herpes simplex virus type 2 (HSV-2) and cytomegalovirus (CMV) infections as demonstrated by DNA hybridization technique and virus isolation method from samples taken from erosive and normal cervices. After one course of treatment with recombinant interferon alpha 1b(rIFN- a1b). 93.8% of cases showed clinical improvement and 60% marked improvement. The HPV- 16 and HSV detection rates were also significantly dropped down after rIFN- a1b treatment as compared with that before treatment. A Chinese herb drug, *Astragalus membranaceus*, was shown to be synergic to interferon therapy. Results showed rIFN-a1b has high therapeutic efficacy in treatment of papillomavirus infections of cervix.

WS5-5

SYNTHETIC PEPTIDE LA-I OF HERPES SIMPLEX VIRUS TYPE-2 (HSV-2) AS A DIAGNOSTIC MARKER FOR CERVICAL INTRAEPITHELIAL NEOPLASIA

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A school of thoughts believes that sub-clinical HSV-2 infection is an associated co-risk factor in cervical carcinogenesis. Advances in molecular technology made it possible to use monospecific antibodies to detect tumour associated HSV-2 proteins in cancer cells and tissues. LA-I a synthetic polypeptide corresponds to 13 residues at the aminoterminus of the herpes simplex type-2 protein ICP 10, that is encoded by the transforming viral DNA sequences (Bgl II C fragment). Antibody to LA-I is shown to have diagnostic potential for identifying cervical intraepithelial neoplasia and invasive cancer patients. Antibody to LA-I was raised in rabbits using KLH as hapten. Formalin fixed tissue sections of 55 patients were deparaffinized and stained with LA-I antibody by ABC peroxidase method. The antibody to LA-I specifically stains anatypical epithelium from 47% dysplasia cases, 60% invasive cancer and 23% chronic cervicitis cases. It rarely reacts with normal cervical epithelium. Results indicate that LA-I may be one of the marker for cervical carcinogenesis.

WS6-1

GENETIC ANALYSIS OF THE ENVELOPE PROTEINS OF DENGUE ISOLATES TH-36 (DEN-5) AND TH-SMAN (DEN-6)

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Complementary DNAs (cDNAs) were synthesized from the envelope genes of two isolates of dengue viruses (TH-36 and TH-Sman, tentatively classified as dengue-5 and dengue-6 respectively) and amplified by polymerase chain reaction (PCR) using sense and antisense primers designed from conserved dengue sequences. The amplified cDNA clones were sequenced in both directions by double-stranded dideoxy sequencing. Alignment with published dengue sequences enabled us to assign these viruses accurately to classified serotypes. Amino acid changes, detected between these two isolates and strains of their respective serotypes, may account for the significant antigenic differences observed during previous serological typing of these viruses.

WS6-2

A MODEL FOR THE ANTIGENIC STRUCTURE OF THE DENGUE VIRUS GLYCOPROTEIN, NS1

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An extensive panel of monoclonal antibodies specific for the dengue 2 virus non-structural glycoprotein NS1 have been used to define the spatial distribution of epitopes on both the membrane-bound (mNS1) and secreted (sNS1) dimer forms of this protein.

We have previously reported the binding of a subset of these monoclonals with a nested set of peptides corresponding to the entire length of the protein (Falconar and Young). These experiments identified five distinct epitopes at the amino acid sequence level and in the current study, we have used these monoclonals as site-specific probes. Classic competition ELISA's and the pattern of reactivity on immunoblots of partial enzyme digests (generated with trypsin, chymotrypsin and *S. aureus* V8 protease) with these monoclonals in comparison with the remaining panel has enabled us to build a basic model for the antigenic structure of NS1.

WS6-3

RNA POLYMERASE OF THE FLAVIVIRUS KUNJINMOLECULAR AND MORPHOLOGICAL ANALYSES

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We showed previously that the flavivirus RNA-dependent RNA polymerase synthesizes genomic- size RNA asymmetrically and semi-conservatively in a replicative intermediate using a fully double-stranded recycling template, the replicative form (RF). Subcellular fractions of Kunjin virus-infected cells were prepared and analysed by density gradient centrifugation for intracellular content of viral RNA polymerase, RNA and proteins. These viral products in cytoplasm at 24 hours post infection were located in "heavy membrane" fractions which also contained virions and three types of virus-induced morphologically distinct membranes. After treatment with 0.5% NP40 detergent, these fractions were again sedimented and analysed. Electron microscopy of detergent-treated fractions showed that all membranes were solubilized. Viral RNA polymerase activity sedimented and was retained in regions of this second gradient containing RF, no structural proteins, and enrichment of several specific nonstructural proteins. This is the first report which characterize the RNA polymerase in terms of membrane location and associated ultrastructures, together with co-sedimenting viral RNA and proteins.

WS6-4

PROBABLE PRESENCE OF RNA IN KUNJIN VIRUS-INDUCED VESICLES

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The observation of the 60-80 nm smooth membrane vesicles have been noted for several years to be flavivirus-induced. Usually at early infection,these vesicles contained a thread-like enclosure. However the nature of this enclosure is not known. In this study, we used various microscopy and enzyme digestion techniques to decipher the composition. The use of immunofluorescence and acridine orange staining indicated the presence of RNA in clusters at the perinuclear region. The clusters were arranged similarly to the vesicles observed under the electron microscope. Autoradiography, is now being performed with ^{3}H-uridine to further confirm the presence of RNA in these vesicles. Besides visual observation, enzyme digestion procedures were carried out. Two enzymes, ribonuclease type III-A and T_1 were used. From the results, the composition of the thread-like enclosures appeared to be made up of a mixture of double and single stranded RNA. The finding would correlate to our postulation that these vesicles are vehicle for the progeny RNA of the flaviviruses.

WS6-5

ANALYSIS OF DENGUE VIRUS PROTEINS EXPRESSED BY BACULOVIRUS RECOMINANTS

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[1]HRC Virus Research Unit, University of Otago, P. O Box 56, Dunedin, New Zealand.

Dengue virus type 2 cDNA sequences encoding the nonstructural proteins NS1 and 2a, and the envelope structural protein(E) with signal sequences of different lengths were transfected into the baculovirus *Autographa californica*. NSI and E proteins were expressed most efficiently in *Spodoptera frugiperda* (Sf9) cells by recombinants which contained 123 bp of the envelope gene as a signal sequence for NS1 and 165 bp of premembrane gene as a signal sequence for E. The proteins were glycosylated and transported to the surface of the Sf9 cells, and were similar in molecular size and antigenicity to the authentic proteins of dengue virus-infected *Aedes albopictus* cells. NS1 was expressed as a dimer which is the native form of the authentic protein and Sf9 cells which were expressing E protein exhibited the phenomenon of haemadsorption which is a characteristic of authentic E protein. When injected into mice the expressed proteins stimulated the production of dengue virus specific antibodies, and immunized mice were protected against challenge with a virulent dengue virus strain. Ultimately it is hoped that these proteins may be incorporated into a multivalent subunit dengue virus vaccine, but in the more immediate future they have potential as antigens for assessing the immune response to individual virus proteins by dengue patients.

WS6-6

EPITOPE MAPPING TO DEFINE THE FUNCTION OF DENGUE VIRUS PROTEINS

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A multifaceted approach is being used to identify the function and functional regions of the envelope (E) and capsid (C) proteins of dengue 2 virus. The PEPSCAN system has been used to identify linear epitopes in the E and C proteins of dengue 2 virus. Monoclonal antibodies of specific antibody classes directed against some of these epitopes and against whole dengue virus have then been used to
(a) localize these proteins within infected cells and
(b) attempt to identify functional determinants on these two proteins.

Peptides corresponding to selected known epitopes have been evaluated for their ability to agglutinate gander erythrocytes or to interfere with infection of cells and/or agglutination of erythrocytes. Several regions of the dengue 2 E protein likely to be involved in haemagglutination have been identified and preliminary data confirms the report (Tadano et al; Makino et al; 1989, J. Gen Virol, *70*, 1409, 1417) that part of the dengue capsid protein may appear in the nucleus of dengue infected cells.

WS7-1

QUALITY CONTROL IN CLINICAL VIROLOGY

Monica Grandien
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In virology, unreliable results may lead to serious consequences for patients and communities. Quality assurance is a necessity and should embrace a combination of efforts leading to high quality results:

1. *internal quality control* is undertaken by the laboratory staff and includes a continuous monitoring of working practices, equipment and reagents used. It has an immediate influence on the results and strengthens day-to-day consistency of the diagnostic tests.

2. *external quality assessment* is a system for objective checking of laboratory results by an external body. It is performed by a regular dis- tribution of specimens of known but undisclosed content for testing by the laboratory. By surveys of results comparability between laboratories is established. Ideally, the activity is carried out on a national basis by a national or reference laboratory.

3. *quality control of commercial kits* for viral diagnosis (or of commercially available reagents) is of fundamental importance, Besides test results giving the accuracy of the test (sensitivity, specificity and predictive values) the validation should also include reproducibility and robustness of the test as well as risks for contamination of the close surroundings.

4. *quality assurance by duplicate testing* During periods when new techniques are introduced into a laboratory routine and confirmation of results are difficult to obtain by assaying other parameters,examination of duplicate specimens at a centre experienced with the technique can be used to assure correct diagnosis. The system is often used for quality control of immunofluorescence diagnosis and should cover the entire introduction period in an unexperienced laboratory.

The national reference laboratory should be responsible for making these quality control functions available, preferably in their own laboratory.

WS7-2

SEROLOGICAL DIAGNOSIS OF EPSTEIN-BARR VIRUS INFECTION USING THREE NOVEL RECOMBINANT ELISAs

H. Wolf[1], P. Wutzler[2], I. Farber[2] W. Hinderer[3], H.-H.Sonneborn[3]
[1]Max von Pettenkofer Insitute, University of Munich, FRG; [2]Medical Academy, Erfurt, FRG; [3]BIOTEST AG, Dreieich, FRG.

A novel indirect EBV ELISA system (BIOTEST anti-EBV recombinant) has been evaluated with respect to its usefulness in routine diagnosis of EBV primary infection. The assay system is composed of three different microtest plate ELISAs and utilizes the antibody capture principle. Three highly purified recombinant EBV antigens are immobilized on the solid phase. The early antigens p138 (BALF2, truncated) and p544 (BMRF1, whole sequence) are coated as a mixture for testing IgM (assay 1) and IgG (assay 2). In addition, the EBNA-1 antigen p72 (BKRF1, carboxy-half) is used for detecting IgG antibodies (assay 3).

Three panels of sera were examined in direct comparison with standard immunofluorescence (IF): Specimens of a) 114 infectious mononucleosis (IM) patients, b) 60 patients with acute CMV infection, toxoplasmosis or rheumatic disease, respectively, and c) 185 apparent healthy blood donors as a control group.

Among the IM patients 113 were clearly recognized as having acute primary infection (sensitivity 99.1% compared to VCA-IgM). Three apparent false-positive results were obtained with patients of other diseases and one (IgM-positive) within the control group (specificity 98.4%).

In conclusion, the data suggest that the recombinant ELISA system can be advantageously used for standardized rapid diagnosis of acute EBV primary infection. Compared to the classical IF methods the novel assay system offers the following advantages: Easy performance, objective reading of results, strongly reduced interference with reheumatoid antibodies, and no interference with antinuclear antibodies.

WS7-3

ANTIBODY RESPONSES TO INFLUENZA VIRION PROTEINS BY WESTERN BLOT ANALYSIS

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[2]Department of Microbiology, The University of Melbourne, Victoria, 3052, Australia.

An immunoblotting procedure was developed to detect responses in mice and humans to influenza virion antigens. The technique was capable of detecting 1-3 ug of the HA on NC strips at a 1/5000 dilution of a mouse serum with an initial HI titre of 20. The effects of the use of the blocking agent Tween 20 upon virion proteins after electrophoretic transfer were also studied. The commonly used concentration of 0.05% (v/v) Tween 20 greatly affected the detection of the M protein when included in blocking and incubation buffers, due either to elution of the protein from the NC membrane or to denaturation. By contrast, Tween 20 caused no detectable loss to the HA and NA/NP proteins. Responses to the proteins of influenza A Mem_H-Bel_N and B/AA/1/86 were detectable by immunoblotting after separation by PAGE under reducing and non-reducing conditions. Under reducing conditions, the antibody response to HA2 was much stronger than to that of HA1 in both human convalescent sera and mouse sera prepared after i.n. inoculation. However, under non-reducing conditions, largest responses to HA proteins were obtained compared with other influenza viral proteins. Antibody responses in mice to influenza viral antigens were different for IgG subclasses and the distribution of each IgG subclass was influenced by the route of inoculation. These studies indicate that considerable heterogeneity exists in the responses of the various immunoglobulin subclasses of influenza virion proteins.

WS7-4

IN SITU ENZYME IMMUNOASSAY FOR ANTIVIRAL SUSCEPTIBILITY TESTING OF RESPIRATORY SYNCYTIAL VIRUS

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[2]Department of Microbiology and Infectious Diseases, University of Calgary Health Sciences Centre, Calgary, Canada.

An enzyme immunoassay (EIA), performed directly on fixed infected monolayers of HEp-2 cells in microtiter plates, was compared with the conventional plaque reduction assay (PRA) method for the determination of antiviral activity of ribavirin against respiratory syncytial virus. A 50% reduction in virus replication was observed at 3.4 and 5.9 mg/L of the drug by EIA and PRA, respectively. EIA is simple to perform and reproducible and has objective end points. Moreover, EIA has advantages over PRA in that results are available sooner and a much wider range of inoculum size can be used without affecting susceptibility data. EIA is suitable for the rapid and accurate susceptibility testing of a large number of clinical isolates.

WS7-5

RAPID IDENTIFICATION OF RESPIRATORY VIRUSES IN NEONATES

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Direct fluorescent antibody (DFA) and culture amplified direct fluorescent antibody (CA-DFA) were compared with standard tissue culture isolation for the identification of respiratory syncytial virus, parainfluenza type 1, parainflu-enza type 2 and parainfluenza type 3 viruses. The three methods were used to examine 286 nasopharyngeal aspirates from children. One of the above viruses was identified from 28% of these specimens.

DFA identified 84%, CA-DFA 76% (72 hours incubation) and tissue culture 41%. DFA and CA-DFA together identified viruses in 57% of the specimens which were tissue culture negative. Tissue culture identified viruses in 4% of the specimens which were DFA and CA-DFA negative. DFA is the method of choice for the rapid identification of these viruses. The combination of DFA and CA-DFA results in the identification of 96% of these viruses in three days.

WS7-6

STUDIES ON RAPID CLINICAL DIAGNOSIS OF INFLUENZA VIRUS TYPE A AND B

Tao Sanju[1], *Yong Dongrong*[1], *Wang Huanqin*[1], *Guo di*[1] *Zheng Qijing*[2], *Yun Zhibing*[2]

[1]Institute of Virology, Chinese Academy of Preventive Medicine, [2]Department of Virology, Beijing Friendship Hospital.

Rapid clinical diagnosis of influenza was carried out using monoclonal antibodies to type A and B influenza virus by indirect immunofluorescence(IF) method. 85 nasopharyngeal swab specimens were collected from outpatients suspected of influenza. Using conventional embryonated eggs isolation method as a control, 3 of 85 specimens were positive during the first passage of virus isolation, while 8 of 85 specimens were positive for the second passage. It needs 6-7 days at least. However, the antigen of influenza virus in MDCK cells inoculated with the specimens can be detected as early as 24 hours after inoculation, and 7 of 85 specimens were positive. The sensitivity and specificity was 87.5% (7/8) and 100% respectively, as compared with the second passage isolation in eggs. Another, the antigen of influenza virus in nasopharyngeal cells was detected directly. 5 of 85 specimens were positive with sensitivity and specificity of 62.5% (5/8) and 100% respectively. It needs only 3-4 hours. Therefore, IF method for rapid diagnosis of influenza was superior to conventional method. It is not only rapid, but also capable of direct detection of the type of influenza virus.

WS7-7

RAPID DIAGNOSIS OF RESPIRATORY VIRAL INFECTIONS DO WE STILL NEED CONFIRMATORY TESTS

Monica Grandien

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By rapid diagnosis the precise etiological agent of a respiratory infection is identified in time to influence managment and treatment of patients and their contacts. Efficient techniques and high quality of reagents are prerequisites for reliable results.

RS, influenza A and B, parainfluenza 1, 2, 3 and adenoviruses, as well as measles before the rash, can be routinely diagnosed by immunofluorescence or ELISA techniques directly in clinical specimens (nasopharyngeal secretions). Introduction of monoclonal antibodies for diagnosis has increased the accuracy of both methods.

A high degree of accuracy can be reached by use of standardised procedures, quality control of reagents and the exchange of specimens with a center experienced in immunofluorescence diagnosis. Appearance and distribution of viral antigens in the infected cells confirm positive immunofluorescence results. In ELISA objective results are recorded in OD values to be compared with OD values obtained by measuring negative and positive controls. Since the two techniques are used for detection of viral antigens no infectious virus is needed making handling and transportation of specimens less crucial.

The specificity of the antigen detection methods varies with the quality of reagents used. The sensitivity also varies with the type of virus infection. For diagnosis of some viruses by immunofluorescence the sensitivity equals virus isolation, e.g. RS virus diagnosis in children. Other viral infections may be more easily diagnosed by use of ELISA. In adenovirus infections results of the antigen detection methods correlate to acute infection while a positive virus isolation may be caused by an adenovirus carrier state.

In adults the IgG antibody response is often used to diagnose or confirm viral respiratory infections. In infants the presence of maternal antibodies or other immaturity interferes with development of detectable antibody titers. So far the IgM response is not routinely measured in respiratory infections because of technical difficulties, the relatively late appearance of IgM and its unreliable appearance in reinfections.

The needs for confirmatory tests in rapid viral diagnosis are based on all the given parameters. Results obtained from quality control of rapid diagnosis in several developing countries exemplifies the needs.

WS7-8

DETECTION ON RUBELLA IgG AND IgM ANTIBODIES IN WHOLE BLOOD ON WHATMAN PAPER; COMPARISON TO SERUM SAMPLES

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We compared detection of rubella HI antibody and rubella specific IgM in dried whole blood spotted on Whatman filter paper and serum samples, both obtained by venipuncture from same subject. Of 1,000 paired samples for HI antibody study, 807 dried blood samples had HI titers identical to corresponding serum samples and 193 showed 1 dilution difference. Storage of dried blood at room temperature for 28 days did not affect the HI antibodies. Study for specific IgM by solid phase immunosorbent hemagglutination inhibition test (SPIHIT) on healthy subjects and rubella patients, the results of presence (positive or negative) of specific IgM from both sources of sample were correspondent when the dried blood were stored at room temperature from 5 hours to 38 days. This study demonstrated that the use of Whatman paper as transport medium for blood samples in determination of rubella immunity and the diagnosis of rubella infection is possible.

WS8-1

THE MOLECULAR EPIDEMIOLOGY OF ROSS RIVER VIRUS ISOLATES FROM AUSTRALIA AND THE SOUTH PACIFIC REGION

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Ross River virus (RRV), the mosquito-borne aetiological agent of epidemic polyarthritis, occurs throughout coastal Australia and has also been found in Fiji, Western Samoa, and the Cook Islands. In most areas, levels of disease in humans are low. Occasionally under certain environmental conditions, epidemics of major proportions occur, such as the 1988-89 epidemic in the south-west of Western Australia which involved over 600 reported human cases. It is not known whether epidemics are initiated by viruses impor- ted into the area or by locally circulating strains. In this paper we report the findings of a comparison of the genomes of 67 isolates of RRV using RNase T_1 oligonucleotide mapping to address this question. This procedure allows the comparison of strains which have no more than 10% sequence divergence. Analysis using the UPGMA method revealed that the isolates were distributed in three clusters (topotypes) which linked at a similarity coefficient of less than 68%. During the south-west epidemic two topotypes were found to cocirculate. Furthermore, one human isolate (from 1989) had complete identity with a mosquito isolate obtained in 1987 from the same geographic region. Although it appears that human movement may be responsible for the introduction of strains of RRV to various areas, our results show that epidemics are dependent on the presence of suitable mosquito vector and vertebrate host populations, rather than the introduction of an epidemic virus strain.

WS8-2

ARBOVIRUS ACTIVITY IN NORTHERN AUSTRALIA OVER THE 1990/91 WET SEASON

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[2]Health Department of Western Australia.

Several medically important arboviruses are known to be circulating in mosquito-vertebrate cycles in northern Australia. Murray Valley encephalitis (MVE) virus is recognized as the main aetiological agent of Australian encephalitis (AE), although a few cases are caused by the closely related Kunjin (KUN) virus. Ross River virus (RRV) and Sindbis virus are two alphaviruses that have been isolated from mosquitoes in northern Australia and RRV is known to cause a polyarticular disease in humans often associated with fever and a rash.

The 1990/91 wet season has been exceptional in NorthernAustra lia with a near record wet in Darwin, and the Kimberley region of Western Australia has experienced the heaviest wet season since 1981. Increased rainfall has resulted in large scale flooding of some areas with a subsequent increase in mosquito numbers and a concomitant increase in virus activity. Considerable MVE activity has been detected in the North-West during the period March to May 1991. This was demonstrated by a large number of seroconversions from sentinel chicken flocks sited at Broome, Derby, Kununurra, Wyndham and Kalumburu. Although several MVE isolates have been obtained from mosquitoes trapped at Broome in April 1991 there have been no cases of AE reported from the area. However there has been a significant increase in the number of RRV cases reported from Broome. Additional evidence of virus activity was obtained from an ongoing surveillance study of the Billiluna Community, 150 km south of Halls Creek in Western Australia where six children have recently seroconverted to MVE.

Five cases of AE, two from Western Australia, two from the Northern Territory and one from Queensland have been diagnosed in 1991. Four of the cases have been caused by MVE virus and one by KUN virus. The significantly increased incidence of MVE virus activity in Northern Australia this year is of concern should increased rainfall or flooding occur in central Australia. This may then facilitate eventual virus movement to south-eastern areas of Australia resulting in a serious desease threat to the region.

WS8-3

EPIDEMIOLOGICAL TRENDS OF JAPANESE ENCEPHALITIS

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Japanese encephalitis (JE) has been a serious public health problem in several countries in Asian or Asia where rice cultivation in watered paddy fields and swine raising are common. These agricultural characteristics are favorable for the transmission of JE virus by offering ample breeding sites for vector mosquitoes and large numbers of amplifier hosts. In Japan, the number of JE patients decreased dramatically after 1966 due to reduced vector density and mass-vaccination. The former was the result of wide spraying pesticides for the control of insect rice pests. Field-collected vector mosquitoes in recent Japan were found highly resistant to pesticides particularly organophosphates. Continued low endemicity of JE in Japan was attributed to sustained vaccination. The number of JE cases showed remarkable decline after 1981 in the Republic of Korea. This decrease was also the result of mass-vaccination. In contrast, outbreaks of JE or JE-like disease have been reported in Vietnam and Thailand after 1960, and in India and Nepal after 1978, respectively. Such increased JE cases in several countries could probably be related with socioeconomic development through population explosion requiring increased food production. Since food production in Asian or Asia has been depending on the paddy fields and swine population, and both of them could have offered suitable condition for the transmission of JE virus by supplying ample vector breeding sites and amplifier hosts.

WS8-4

JAPANESE ENCEPHALITIS VIRUS INFECTIONS IN INDONESIANS

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Ministry of Health, Jakarta, Indonesia; Sanglah Hospital, Denpasar, Indonesia; U.S. Naval Medical Research Unit No.2, Jakarta, Indonesia.

Japanese encephalitis virus (JEV) has been isolated previously from pigs and mosquitoes in Indonesia, but the existence of human clinical disease has not been documented. A report of a JEV infection in an Australian child who visited Bali, prompted establishing a hospital based surveillance of pediatric encephalitis cases in Denpasar on the Island of Bali, Indonesia. There were 7 encephalitis cases between October 1990 and December 1990, and 10 cases between March and May 1991. There were no cases between December 1990 and March 1991. The patients, 11 males and 7 females, ranged in age from 3 months to 11 years. Acute and convalescent sera and cerebrospinal fluid (CSF) were collected. All samples were assayed by ELISA for anti-dengue virus/anti-JEV IgM/IgG antibodies. Isolation of virus from all samples was also attempted. Four patients (24%) had acute JEV infections, based upon a diagnostic rising antibody titer in serum and CSF, however virus was not isolated. These patients ranged in age from 2 to 11 years, with three males. Symptoms included fever, convulsions, stiffness of the back and/or paralysis. All children recovered, but some children had slurred speech and some developed paralysis six months after infection. The results represent the first IgM confirmed cases of clinical JEV infection diagnosed in Indonesia.

WS8-5

DENGUE VIRUS SURVEILLANCE AT A JAKARTA HOSPITAL, 1988-1990

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A hospital-based surveillance program was conducted in West Jakarta to monitor the incidence of dengue virus infection and the prevailing dengue serotype. Sera from suspected dengue cases were collected for virus isolation using C6/36 mosquito cell culture and/or by mosquito inoculation. In 1988, a city-wide dengue epidemic occurred with 1431 cases of suspected dengue infection presenting to this hospital. This was followed by two interepidemic years, 1989 and 1990 with 187 and 616 cases, respectively. A total of 401 (17.9%) dengue viruses were isolated during the period. The predominate isolate in 1988 and 1989 was dengue serotype 3 (53.8% and 70%, respectively), but in 1990 the predominate isolate was serotype 1 (40%). Isolates were found throughout the year, with peak rates occurring at different times each year. Dengue virus was isolated primarily (32.7%) from patients between 5-9 years of age. There were more isolates from primary dengue infections than from secondary infections (63.8% vs 36.2%). There was no correlation between serotype and severity of infection. These findings indicate that in an urban area with endemic dengue virus, virologic surveillance may be of minimal epidemiologic value, since all four dengue serotypes were found throughout the year, and that during the epidemic the frequency of isolation for all serotypes increased.

WS8-6

SPREAD OF DENGUE IN VILLAGES OF WESTERN INDIA

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There has been a gradual but relentless spread of *Aedes aegypti* in the towns of Maharashtra. Recently this mosquito has been found breeding in villages of Maharashtra as well in Gujarat States. Epidemics of dengue fever have occurred in several villages of these two States in Western India. Virus type 1,2,3 have been isolated from human cases as well as from mosquitoes. Seroconversion to dengue virus has been demonstrated in a large number of cases.

There has been no case with haemorrhagic manifestations though in Jalore, Rajasthan, in 1985 a few cases have been seen with haemorrhagic manifestations.

The spread of the vectors of dengue in the rural areas of the country together with the occurrence of epidemics in the villages as well as in large towns and cities pose a serious public health problem.

WS8-7

PROBLEMS IN DENGUE CONTROL-A CASE STUDY

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The current dengue 2 outbreak in Malaysia is the most severe since dengue became a notifiable disease in 1973. Many areas have been badly affected, including an area referred to as TTDI which is only a few kilometers outside of Kuala Lumpur.

TTDI is a residential area of 18 square kilo- meters with an upper middle income population living in some 3,600 houses made up of bungalows, double-storey link houses and single storey houses. The first case of dengue was reported towards the end of November 1990 and in December, there were 3 confirmed hospitalized cases. The numbers increased in January and February and started to decline slowly over the next few months.

Control measures consisting of perifocal fogging as well as fogging by ULV were initiated and the *Aedes* indices were constantly monitored. Teams from the Health Department carried out mass source reduction for the whole area and health education in the form of exhibition to publicize the danger of dengue was also mounted. The residents themselves took an active role in promoting health education and community participation.

Despite identifying the outbreak early and the initiation of control measures, it took several weeks to bring down the number of dengue cases and it has not been possible to eradicate the disease. Factors which contributed to the prolonged outbreak and better control strategies for future outbreaks will be presented.

WS8-8

DENGUE HAEMORRHAGIC FEVER-A CONTINUING PROBLEM IN SRI LANKA

Tissa Vitarana, Nalini Jayasekera, Nalini Withane
Medical Research Institute, Sri Lanka

Up to 1988 while Dengue transmission due to all 4 types was endemic, few cases of Dengue Haemorrhagic Fever (DHF) were seen (less than 10 cases per year in the preceding 20 years). Since 1989 the pattern has changed and DHF has progressively increased and appears to have become endemic. In 1989 there were 203 clinical cases with 20 deaths (10%) and 87 laboratory confirmed. In 1989 the cases were mainly from Colombo and the suburbs. In 1990 there was a marked increase with 1,121 cases, 61 deaths (5.4%) and 345 laboratory confirmed. There was a spread of DHF cases to the other towns throughout the country. In 1991 there were 642 cases up to August with 28 deaths (4.4%) and 185 laboratory confirmed. There has been more cases during the rainy season, May to August, each year.

Unlike in Cuba where there was an explosive outbreak in 1981, in Sri Lanka the pattern of DHF seems to be a high endemic one.

During this period Dengue types 2 and 3 have been isolated, but there does not appear to be a significant increase of Dengue Fever cases nor has there been an increase of vector mosquito *(Aedes aegypti* and *Aedes albopictus)* densities.

The DHF cases have most commonly manifested with bleeding in to the bowel rather than in to the skin. The tourniquet test positivity has also been low. 75% of cases have been in children under the age of 12 and they appear to have a better than average nutritional status. But significant number of adults too have been affected.

W8-9

DENGUE TYPE 1 EPIDEMIC WITH HEMORRHAGIC MANIFESTATIONS IN FIJI 1989-1990

A.H. Fagbami, J. Mataika, M. Shrestha, A. Adiao, D. Gubler, V. Vorndam, I. Gomez, G. Kuno

Fiji School of Medicine, Centers for Disease Control, Fort Collins USA and San Juan Laboratories, Puerto Rico.

Dengue has emerged as a leading cause of morbidity in the South Pacific region; since 1971 to date dengue epidemics have occurred in several Pacific island countries. Although there was clinical evidence of dengue activity in Fiji annually for the past 20 years, only two large epidemics were previously documented. In July 1989, cases of dengue and dengue-like illnesses appeared in hospitals and Health Centers in Suva, the capital city. Investigations were carried out to determine the viral etiology and epidemiological features of the outbreak.

Virus isolation studies were carried out by inoculation of C6/36 cell cultures and *Toxorhynchites* mosquitoes; specificity of isolates was determined by indirect immunofluorescence and complement fixation test.

A total of 3686 cases were recorded by the Ministry of Health; a vast majority of these had classical dengue. Forty-three per cent of patients hospitalised at the Colonial War Memorial Hospital had hemorrhagic manifestations. Thirty-six strains of Dengue type 1 virus were isolated from sera of patients.

Dengue is now a major public health problem in the South-West Pacific; all the 4 serotypes are circulating in the region. Although few epidemics have been recognized in Fiji, more are expected to occur. It is therefore necessary to improve upon the present level of surveillance and vector control in order to reduce dengue mortality and morbidity.

Session No. 31 **PLENARY LECTURE 5**

Date : Friday 22 November 1991 **Time 09:00-09:45**
Room : Room A & B

OPEN FORUM
Chairpersons : ***Ken Lam,*** MALAYSIA
Sricharoen Migasena, THAILAND
Vaccine for Children in the Next Decade
Children Initiative Vaccine
Expert Viewpoints
Audience Opinion

Session No. 32 **SYMPOSIUM 12**

Date : Friday 22 November 1991 **Time 10:15-11:45**
Room : Room A & B

SYMPOSIUM 12 Vaccine Development
Chairpersons : ***Chev Kidson,*** THAILAND
Stitaya Sirisinha, THAILAND
1. Strategies in Vaccine Development
Pornchai Matangkasombut, THAILAND
2. The Brightening Prospect for AIDS Vaccines
Marc P. Girard, FRANCE
3. Live Attenuated Dengue Viruses Vaccine
Natth Bhamarapravati, THAILAND
4. Development in Vaccine Research
Gary B. Calandra, USA

Session No. 33 **SPECIAL SESSION IV**

Date : Friday 22 November 1991 **Time 12:45-13:45**
Room : Room A

SPECIAL SESSION IV Varicella Vaccine
Chairpersons : ***Sombodhi Bukkavesa,*** THAILAND
Wu-Tse Liu, TAIWAN
1. Current Status and Prospect of a Live Varicella Vaccine
Michiaki Takahashi, JAPAN
2. Use of Varicella Vaccine in Healthy Children
Max Just, SWITZERLAND

3. The First Varicella Vaccinees in the Philippines with Live Attenuated OKA Strain Vaccine (1987) and a Two-Year Follow Up
Fe del Mundo, PHILIPPINES

Session No. 34 **SYMPOSIUM 13**

Date : Friday 22 November 1991 **Time 14:00-15:15**
Room : Room A & B

SYMPOSIUM 13 Antiviral Drugs and Interferon
Chairpersons : ***Prasert Thongcharoen.*** THAILAND
Hou Yunde, CHINA

1. Management of Herpes Zoster Infections
Stewart Glover, UK
2. Treatment of Hepatitis C with Interferon
Hiroshi Yasuhashi, JAPAN
3. Development and Use of Oral Low Dose Interferon Therapy in Animals and Man
Allen L. Richards, J.M. Cummmins, USA

15:15-15:45 **CLOSING CEREMONY** **Room A & B**

PL-5

VACCINE FOR CHILDREN IN THE NEXT DECADE CHILDREN INITIATIVE VACCINE EXPERT VIEWPOINTS AUDIENCE OPINION

S12-1

STRATEGIES IN VACCINE DEVELOPMENT

Pornchai Matangkasombut
Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

S12-2

THE BRIGHTENING PROSPECT FOR AIDS VACCINES

Marc Girard
Department Virology, Institut Pasteur, Paris, France.

A very substantial progress has been made during the last year and a half in the field of AIDS vaccines. First, it was demonstrated that whole inactivated virus vaccines were able to provide protection against experimental infection of rhesus macaques with simian immunodeficiency virus (SIV). Second, protection of chimpanzees agaist experimental infection with human immunodeficiency virus (HIV-1)was achieved by immunization of the animals with either the virus envelope glycoprotein gp120 alone, or with a variety of antigens, among which the envelope glycoprotein gp160 and a synthetic peptide with the sequence of the HIV-1 principal neutralization determinant. The latter, the V3 loop of gp120, is a highly variable, type-specific determinant. Antibodies to the V3 loop neutralize virus infectivity at a late stage of virus penetration by blocking fusion between the viral envelope and the membrane of the target cell. Protection of chimpanzees against HIV infection has also been reported using only pg160 as a vaccine. The presence of V3-specific neutralizing antibodies seems to correlate in all cases with protection against virus challenge. Third, it was recently reported that a humanized monoclonal antibody to the V3 loop was able to provide passive protection of chimpanzeen against virus challenge, thus confirming that the basis for protection against HIV infection lies in neutralizing antibodies. Several questions still however remain to be addressed, among which: can vaccination provide protection against challenge with cell-associated virus? against challenge by the mucosal route? How long will such protection last? How can we cope with virus variability: An answer to these questions is a prerequisite to the development of an efficacious HIV vaccine for humans. Still, the recent advances in the field foster the hope that it will eventually be possible to develop a successful vaccine and provide an optimistic outlook for the future.

S 12-3

LIVE ATTENUATED DENGUE VIRUSES VACCINE

Natth Bhamarapravati

S12-4

DEVELOPMENT IN VACCINE RESEARCH

Gary Calandra
Merck Sharp & Dohme Research Laboratories

Changes in needs and opportunities for prevention of infectious diseases require not only development of new vaccines but evaluation of possible improvement in present vaccines, of new schedule regimens, and new combinations. Examples of two vaccines in these catagories are attenuated measles, mumps and rubella (M-M-R^{R} II) and inactivated hepatitis A vaccine (iHAV). The Merck measles, mumps and rubella vaccines have been used worldwide in more than 300 million children.

However, the occurrence of measles out-breaks, related to inappropriate vaccine delivery, has led to new schedules of two doses of measles vaccine or M-M-R^{R}II. The change in maternal measles antibody from that due to natural measles to that due to vaccine has led to re-evaluation of earlier (6,9, or 12 month) initiation of the first dose of measles vaccine.

Future studies will involve higher titers of the Moraten strain. The iHAV in clinical development is a highly purified vaccine which resultsin development of neutralizing antibodies within two to four weeks of the first doe and can be used in a two-dose regimen of 0,24 weeks. The trend toward universal immunization with hepatitis B vaccine could provide the momentum and opportunity for a pediatric combined hepatitis A and B vaccine. Hepatitis A could also be added to the proposed pediatric combination of DPT, PRP-OMPC for *Haemophilus influenzae* (PedvaxHIB^{R}) and hepatitis B vaccine (H-B-VaxII^{R}).

SS IV-1

CURRENT STATUS AND PROSPECT OF A LIVE VARICELLA VACCINE

M. Takahashi
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A live varicella vaccine (Oka strain) has been developed and was first licensed for high risk children in European countries in 1984. In Japan and Korea, it has been widely used for normal as well as for high risk children since 1986 and 1988, numbers of immunized children amounting to approximately 900,000 and 800,000 respectively. No or very few adverse reaction has been observed after vaccination, with good protective effect. As for occurrence of zoster after vaccination, it has been demonstrated in Japan and U.S. that skin infection, i.e. appearance of rash is closely correlated with later development of zoster in acute leukemic children. Since no rash usually appear after vaccination in normal children, it is expected that immunization with varicella vaccine will considerabely reduce incidence of zoster in future.

SS IV-2

USE OF VARICELLA VACCINE IN HEALTHY CHILDREN

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Chicken-pox is often considered a benign disease. However, certain infrequent but severe complications such as encephalitis, Reye's syndrome and bacterial superinfection have been observed, which have raised the issue of the vaccination of healthy children. Indeed, cost-benefit studies have demonstrated the advantages of administering the varicella vaccine to all children. Moreover, vaccination may help to protect against the later development of zoster.

The Oka strain vaccine, developed in Japan, has been used for the mass vaccination of children against varicella in Japan and South Korea since 1986. It has been shown to be safe and immunogenic and that even if there is some excretion of the vaccine viral strain, there is no reversion to virulence. Further studies have been carried out in the U.S., where a product license for this vaccine is currently pending. The Oka vaccine is manufactured in Europe by SmithKline Beecham Biologicals, and is licensed for use in immunocompromized subjects. It has been shown to prevent approximately 90% of all varicella infections and to protect against the serious complications of the disease in the immunocompromized patient. In addition, it has been studied in clinical trials which have enrolled over 1000 healthy children; trials are underway to confirm the safety and immunogenicity of production lots of the varicella vaccine in healthy infants. In one of these, the reactogenicity of the varicella vaccine was compared to that of a placebo in a total of 35 infants between the ages of 12 and 18 months. The vaccine was shown to be no more reactogenic that the placebo. All initially seronegative children had antibodies following vaccination.

General vaccination with the varicella vaccine will be the most feasible if it is combined with the measles-mumps-rubella vaccine. Potential problems, such as the stability of the varicella component and the competition between viral strains, now need to be addressed.

SS IV-3

THE FIRST VARICELLA VACCINEES IN THE PHILIPPINES WITH LIVE ATTENUATED OKA STRAIN VACCINE (1987) AND A TWO-YEAR FOLLOW UP

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In 1987 the Children's Medical Center Philippines (CMCP) obtained fifty doses of live attenuated varicella virus vaccine, OKA-BIKEN strain, from the University of Osaka, Japan, Virology Department through M. Takahashi, MD.

In the first year, 50 well children 1 to 19 years old from Metro Manila were the participants in this study. The following year additional doses of the vaccine increased the total vaccines to 121 children. Of this number 107 were completely followed up.

Immediate clinical reactions were minimal, consisting mainly of local injection site reactions like pain (12%), redness (15%) and swelling (4%), fever in 12% and rashes in 5%.

A limited amount of skin test antigen was included. Twenty-five children had both pre- and post-vaccination. Results of these tests will be described in the paper.

Of the 107 children followed up, 49 (46%) has been exposed to natural varicella since immunization and 16 cases (33%) of varicella were observed. All cases were mild with less than 30 vesicles in 63%. No Herpes Zoster was observed in this study.

The health status of the vaccinees was generally good following vaccination. Altogether clinical observations at CMCP of 107 children in a 3-year follow-up show that the varicella used is safe and effective.

S13-1

MANAGEMENT OF HERPES ZOSTER INFECTIONS

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LYMPHOBLASTOID INTERFERON WITH OR WITHOUT STEROID PRETREATMENT IN CHRONIC HEPATITIS B INFECTION

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A double blind randomized trial of lymphoblastoid interferon with or without steroid pretreatment was undertaken in 26 Filipino adults positive for HBsAg, HBeAg and HBV-DNA polymerase. Only 2 of these patients were symptomatic. Another 14 patients who did not receive treatment were followed up as controls. After a minimum of 12 months follow-up, all the controls remained positive for HBsAg and HBeAg. Of those treated with lymphoblastoid interferon, inhibition of DNA polymerase activity was noted in 4/13 pretreated with steroid and in 2/13 pretreated with placebo. Seroconversion to anti-HBe was observed in only 2 of 13 pretreated with steroid, 1 of whom also lost HBsAg compared to 1 of 13 pretreated with placebo. Only those patients with symptoms and/or elevated AST benefitted from interferon therapy and no obvious difference was observed with steroid pretreatment in this small population studied.

S13-3

TREATMENT OF HEPATITIS C WITH INTERFERON

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Nagasaki, Japan.

Hepatitis C virus (HCV) is the predominant agent of both transfusion-associated and sporadic non-A non-B hepatitis, and hepatitis C frequently progresses to chronic hepatitis which may eventuate in cirrhosis and hepatocellular carcinoma. Interferon (IFN) is the only therapeutic agent which may prevent progression. Our studies on treatment of 134 hepatitis C patients with IFN indicated that most patients decrease serum alanine aminotransferase (ALT) levels during treatment. In order to evaluate the state of HCV after treatment with IFN, HCV-RNA by using PCR and serological markers of HCV were measured in 30 patients with chronic hepatitis C who had been treated with 6MU of IFN per day for 8 weeks. In 37% of patients, HCV-RNA disappeared from serum for at least 12 months and anti-C100-3 decreased progressively during this time. Seventeen percent of patients were positive for HCV-RNA in spite of the improvement of ALT levels and decline of anti- C100-3. In 46% of patients HCV-RNA disappeared transiently or remained persistently positive. The result indicates their IFN-mediated improvement of ALT and decrease of anti-C100-3 were not always related to disappearance of HCV-RNA from serum. On the other hand, sustained disappearance of HCV-RNA from serum was demonstrated in the patients who did not have post-treatment ALT relapse. This indicated that IFN can eradicate HCV frequently and produce a cure state of chronic hepatitis C.

S13-4

DEVELOPMENT AND USE OF ORAL LOW DOSE INTERFERON THERAPY IN ANIMALS AND MAN

A.L. Richards, *J.M. Cummmins*

POSTER PRESENTATION

MONDAY 18 NOVEMBER 1991

Hepatitis - P1

1.1. Prevalence of Hepatitis B Virus Markers in Some Population Groups in Macau
M. Fernanda Ferreira, HONG KONG

1.2. Efficacy of Recombinant and Plasma-Derived Hepatitis B Vaccines in the Prevention of Perinatal Transmission
Li-Hua Liu, CHINA

1.3. Long Term Follow-up of Recombinant and Plasma-Derived Hepatitis B Vaccines in Infants Born to HBsAg-Carrier Mothers
Li-Hua Liu, CHINA

1.4. Epidemiologic Characteristics of HBV Infection in Human Population
Xin-Shang Zhang, CHINA

1.5. Prevalence of HAV and HBV Infections in Children and Adolescence in Thailand
Chantapong Wasi, THAILAND

1.6. Liver Morphology in Filipino Adults Who are Chronic HBV Carriers
Normando C. Gonzaga, PHILIPPINES

1.7. The Construction and Application of Hybridoma Cell Line Anti-HBs Monoclonal Antibody Secretion
Shao Huixun, CHINA

1.8. ThePrevalenceofHepatitisB Virus (HBV) Infection in Institution for Healthy Children in Japan
K. Baba, Akira Yamada, JAPAN

1.9. A Rapid Enzyme Immunoassay Using Recombinant Expressed Antigen and Monoclonal Antibody Conjugate to Detect Anti-HBc Antibodies
Jong Seong Ahn, KOREA

1.10. HBs Ag Containing Pre-S1 and Pre-S2 Regions Expressed in Yeast
S ung-Jin Kim, KOREA

1.11. Detection of 27-32 nm Virus-Like Particles in Stools of Non-A, Non-B Hepatitis Patients in Thailand by Immunoelectron Microscopy
Surang Saguanwongse, THAILAND

1.12. Hepatitis C Virus Infection in Blood Donors
A.M. Beardsley, Chye Keat Cheah, AUSTRALIA

1.13. Hepatitis C Virus in Queensland Aborigines
Dennis Ma·rer, AUSTRALIA

1.14. The Prevalence of Anti-HCV among Risk Groups in Thailand
Yong Poovorawan, THAILAND

1.15. Prevalence of Anti-HCV in Selected Population Detected by Various Commercial Reagents
Suda Louisirirotchanakul, THAILAND

1.16. Evaluation of a Post Transfusion Non-A, Non-B Hepatitis ELISA and Immunoblot test
Y.H. Chew, SINGAPORE

1.17. Hepatitis B Immunization of Infants in the State of Hawaii - A Model for Universal Immunization for Infants in the United States
Arwind R. Diwan, USA

1.18. Immunogenicity of Hepatitis B Vaccine in Low Birth Weight Neonates
Pagakrong Lumbiganon, THAILAND

1.19. Immune Response to Hepatitis B Vaccine in Premature Neonates
Somying Chawareewong, THAILAND

1.20. Low Cost EIAs for Hepatitis B Virus Surface Antigen (HBsAg) and Anti-HBsAg Detection
L. Mendis, SRI LANKA

Herpesvirus - P2

2.1. The Effect of Family Members in Childhood on Seroprevalence to Herpes Simplex Virus
Masanori Toba, JAPAN

2.2. Subtyping of Herpes Simplex Virus Type 1 Isolated in Taiwan by Restriction Endonuclease Cleavage Pattern
Chuan-Liang Kao, TAIWAN

2.3. Rapid Identification of Herpes Simplex Virus
Dennis Maurer, AUSTRALIA

2.4. The Virological Study of Female Herpes Genitalis
Sontana Siritantikorn, THAILAND

2.5. Characterization of Human Cytomegalovirus (HCMV) Antigen Recognized by HCMV Specific Monoclonal Antibodies
C. Park, KOREA

2.6. Rapid Detection of Human Cytomegalovirus (AD-169) Antigens in Cell Culture with Peroxidase-Conjugated Monoclonal Antibody
E. Hwang, KOREA

2.7. Detection of Human Cytomegalovirus DNA by Polymerase Chain Reaction (PCR)
Xiong Qi Hua, CHINA

2.8. Prevalence of Antibody to Human Herpesvirus-6 among Normal Thai Population
Ruengpung Sutthent, THAILAND

2.9. A Dot Immunobinding Assay in Comparison with the Gel Diffusion Test for the Detection of Equine Herpes Virus Type-1 (EHV-1) Antigen from Field Samples
Y.P. Grover, INDIA

2.10. Impairment of Herpes Simplex Virus Type 2 Encapsidation during Growth at Suboptimal pH Environment
Parvapan Bhattarakosol, THAILAND

Respiratory Viruses - P3

3.1. The Application of Polymerase Chain Reaction to the Detection of Influenza Virus in Throat Swab
Akira Yamada, JAPAN

3.2. Morphogenesis of Respiratory Syncytial Virus in a HEp-2 Cell Line
Miyako Matsumoto, JAPAN

3.3. The Morphological Identification of Surface Glycoproteins and Nucleoprotein in Respiratory Syncytial Virus-Infected Cells by Electron Microscopy Techniques with Monoclonal Antibodies
Miyako Matsumoto, JAPAN

3.4. Respiratory Viruses in Children Admitted with Lower Respiratory Tract Infection, 1989-1990
Pilaipan Puthavathana, THAILAND

3.5. Adenovirus Surveillance in Japan, 1981-1989 ' Yearly Trend and Association with Respiratory Illness
Shizuko Yamadera, JAPAN

3.6. Efficient Protection Against Influenza Virus Infection by Recombinant Vaccinia Virus Expressing Influenza HA Gene from Strong Promoter
Shigeyuki Itamura, JAPAN

3.7. A549 Cell Line and Adenovirus Isolation in Routine Virus Diagnosis
Aishah Ibrahim, AUSTRALIA

Molecular Biology - P4

4.1. The FLV^R Gene of Mice Alters the infectability of Murine Embryo Fibroblasts
Robert J. Coelen, AUSTRALIA

4.2. Cloning and Nucleotide Sequence Analysis of the Measles Virus Genome in an Autopsied Brain Tissue of a Subacute Sclerosing Panencephalitis (SSPE) Patient
Michiko Watanabe, JAPAN

PI-1

PREVALENCE OF HEPATITIS B VIRUS MARKERS IN SOME POPULATION GROUPS IN MACAU

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A seroepidemiological survey was conducted to determine the prevalence of HBV markers in Chinese Macau residents (estimated Chinese population 440,000).

A total of 8,259 blood samples were obtained from: high risk groups identified as male and female prisoners (890), male drug addicts (89), female prostitutes (274), health workers (110), voluntary blood donors-Ist donation (1,401), pregnant women (3,762), territorial service men (675) and children from 1 to 14 years (1,058).

Hepatitis B virus markers, assayed using ELISA test kits (Behring), were: HBsAg, antiHBc, antiHBs, HBeAg, antiHBe and, in some cases, IgM-HBc.

The results were evaluated by sex and age in each group, for each marker. They are the following.

- more than 80% of the residents with 45 years old have HBV markers and 70% are immune.
- 30% of the children under 5 years old have, at least, one of HBV markers.
- more than 65% of the residents with 20 years old have, at least, one of the HBV markers.
- 13% of the pregnant women are HBsAg carriers at the moment of delivery and 33% of them are HBeAg+.
- the carrier rate in the total population is 11.6% at the time of the survey.

On the basis of these data, a prevention programme has been settled consisting of immunization of all newborn, beginning on January 1989; an expanded vaccination programme for school children and health workers will start on 1991/92.

P1-2

EFFICACY OF RECOMBINANT AND PLASMA-DERIVED HEPATITIS B VACCINES IN THE PREVENTION OF PERINATAL TRANSMISSION

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In order to evaluate the vaccine efficacy on recombinant and plasma hepatitis B vaccines in neonates of HBV carrier mothers, from July 1983 to June 1990, 46,443 pregnant women in four maternity hospitals in Sichuan Province were tested by RPHA. Of those, 5.68% (2636/46443) were HBsAg (+). HBeAg was detected by ID in 30.8% (813/2636) HBsAg (+) women. 350 infants born to HBsAg and HBeAg carrier mothers were randomly assigned into 12 groups. 28 in the control group with placebo, 51 in the HBIG plus 30 mcg plasma vaccine, 124 in the plasma vaccine groups and 147 in the five yeast recombinant vaccine groups. These infants were vaccinated with the 0, 1, 6 or 0, 1, 2, 6 months schedule and have completed at least 9 months of follow-up. The sera of infants were tested for HBsAg, Anti-HBs and Anti-HBc by RIA (Abbott). The results showed that HBsAg carrier rate in placebo group was 89.3%, the protective rate in each immunization group as followd, (1) Chinese plasma-derived vaccine 30 mcg x 4 plus HBIG was 93.4%, 30 mcg x 4 plasma-derived vaccine alone was 87.6%, 15 mcg x 3 vaccine was 56%, 10 mcg x 4 vaccine was 56.2%. (2) American (MSD) plasma-derived vaccine 20 mcg x 3 was 76.4%, 10 mcg x 3 was 62.7%. (3) The different type of recombinant vaccines (a) MSD rDNA 5 mcg x 3 was 87.1%, 10 mcg x 3 was 82.32%. (b) Amgen rDNA 10 mcg x 3 was 80%, 5 mcg x 3 was 72.0%. (c) S.K rDNA 20 mcg x 3 was 68.9%.

Anti-HBs seroconversion rate in early phase (1-9 months) was the same as protective rate. The antibody value of 5 mcg rDNA MSD vaccine was a little lower than that of 30 mcg plus HBIG, 30 mcg and 10 mcg Amgan rDNA vaccine groups, and was similar to that of 10 mcg MSD plasma vaccine. No side-effect was observed in all groups.

Conclusions: (1) The five vaccine groups (30 mcg Chinese plasma vaccine plus HBIG, 30 mcg group, 5 or 10 mcg rDNA MSD, 10 mcg rDNA Amgen) had a good efficacy for prevention of perinatal transmission. (2) The efficacy of 5 mcg rDNA MSD vaccine was similar to that of 30 mcg Chinese plasma vaccine and was a little higher than that of 20 mcg MSD plasma. However there was no significant difference among these three groups. (3) It is noted that the efficacy of 15 mcg Chinese plasma was obviously lower than that of 30 mcg group ($P < 0.03$).

P1-3

LONG-TERM FOLLOW-UP OF RECOMBINANT AND PLASMA-DERIVED HEPATITIS B VACCINES IN INFANTS BORN TO HBsAG-CARRIER MOTHERS

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In an attempt to evaluate the long-term immunogenicity and efficacy of recombinant and plasma-derived HB vaccines in preventing HBV infection. We have observed 206 infants for 2-6 years. They were born to HBsAg(+) and HBeAg(+) mothers and were successful in immunization of HB vaccine during the perinatal period. We cellected the sera at 24, 36, 48, 60, and 72 months respectively. They were tested for HBsAg, Anti-HBs, Anti-HBc by RIA (Abbott). The results showed as below:

The Observative Time(month) and New Infective Number

GROUP	cNo.of Subject	now infective number/observative number					
		24	36	48	60	72(M)	TOTAL
1. 30 mcg plasma plus HBIG (China)	48	0/20	0/15	0/18	5/25	0/25	5(1)*
2. 30 mcg plasma (China)	24	0/18	0/20	0/16	0/14	0/16	0
3. 15 mcg plasma (China)	17	1/14	1/12	0/12			2(1)*
4. 10 mcg plasma (China)	14	0/11	0/9	0/6			0
5. 10 mcg plasma (MSD)	18	1/14	1/17	1/10			3(2)*
6. 5 mcg rDNA (MSD)	23	0/15	0/12	2/9	0/3		2(1)*
7. 10 mcg rDNA (Amgen)	23	0/18	0/13	0/13			0
8. 20 mcg rDNA (S.K)	39	1/28	1/30	1/7			3(1)*

( )* : The mumber of HBsAg, Anti-HBs and Anti-HBc (+) among the new infective infants.

It showed that the groups whith used high does of vaccine or produced the high valus anti-HBs (30 mcg plus HBIG, 10 mcg rDNA Amgen vaccine) usually were not found new HBV infection. Althrough new infection was occured, the time of HBsAg produced was later than other groups.

Conclusion: 1. The results suggested that continuity of immunogenicity and protective efficacy were related to the valus of Anti-HBs and the does and quility of vaccine. 2. As 6 infants had had effective protective value of Anti-HBs, but HBsAg and Anti-HBc were also (+), whether there is a mutant HBV is needed to further researth. 3. Althrough the lower does rDNA vaccine reached to the same protective rate and efficacy as 30 mcg plasma-derivde vaccine. However the Anti-HBs persistent time is not so long as high does plasma-derived vaccine. 4. According to our observation, additional booster doses of vaccine should be given on time to maintain the satisfactory protective efficacy.

P1-4

EPIDEMIOLOGIC CHARACTERISTICS OF HBV INFECTION IN HUMAN POPULATION

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1. Cohort effect of HBV infection: Cross-sectional studies on HBV infection were carried out in a village of Beijing rural area in 1982 and 1988. Birth cohort method was applied in the analysis of the changes of HBV infectious markers (HBsAg, anti-HBs, anti-HBc, by RIA) in 424 individuals who sustained the study both in 1982 and 1988. The results showed that the cohort effect was very obvious in HBV infection of human population. The birth cohort curve of HBsAg in 1-5 age group was a straight line and was parallel with the abscissa, but the curves of anti-HBs and anti-HBc in same age group went up, indicating the important influence of age on the outcome of HBV infection.

2. Familial clustering of HBsAg persistent carriers: In the same study, 29 of the 36 HBsAg-positive individuals discovered in 1982 were re-examined in 1988 and 24 (82.8%) of them remained HBsAg-positive twenty-three belonged to 78 families with a total of 316 persons were analyzed for the patterns of family distribution by means of binomial theorem. The results demonstrated that the distribution of HBsAg persistent carriers did not fit well with the binomial distribution, indicating their familial clustering.

3. The density of HBV infection was found to be significantly correlated with the HBsAg positivity rate: Cross-sectional study on HBV infection was carried out in 5 units of People's Armed Forces in 1983. The positivity rates of HBsAg were significantly different in the 5 units. After one and half years follow-up, it was found that the densities of HBV infection were still significantly different and it was correlated with the positivity rates of HBsAg. Fitting with the regression curve, the equation of the density of HBV infection was $Y=10^{(1.082+0.0806x)}$, and the coefficient of correlation was $R^2=0.7973$.

P1-5

PREVALENCE OF HAV AND HBV INFECTIONS IN CHILDREN AND ADOLESCENCE IN THAILAND

Chantapong Wasi

P1-6

LIVER MORPHOLOGY IN FILIPINO ADULTS WHO ARE CHRONIC HBV CARRIERS

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Tropical Disease Foundation , Makati Medical Center, Makati, Metro Manila, Philippines.

A descriptive study of the morphology of the liver before and 1 year after lymphoblastoid interferon therapy in 18 Filipino adults with chronic HBV infection was undertaken. HBsAg and HBcAg localization was done with peroxidase-antiperoxidase (PAP) and confirmed by immune-electron microscopy with immune gold labelling. Initial liver biopsy of the 18 patients disclosed chronic active hepatitis (CAH) in 8, chronic persistent hepatitis (CPH) in 9 and minimal change in 1. PAP done in 18 patients revealed cytoplasmic HBsAg in all, membraneous HBsAg in 13, nuclear HBcAg in 17 and cytoplasmic HBcAg in 12. Three of 18 patients had seroconversion to anti-HBe and the histopathology showed a change from mild to severe CAH in 1, from severe CAH tc CPH in 1 and persistent CAH in the other. One of the remaining 5 patients with CAH changed to CPH with no change in the 4. Three of the 9 CPH had worsening to CAH. Evidence of chronic liver disease and presence of HBV liver cell markers have thus been demonstrated in these patients including 16 asymptomatic patients. This study also showed that excepting in two patients, interferon therapy failed to improve the histologic characteristics of these patients.

P1-7

THE CONSTRUCTION AND APPLICATION OF HYBRIDOMA CELL LINE ANTI-HBs MONOCLONAL ANTIBODY SECRETION

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A spleen cell of BALB/C mice is immunized by HBsAg with myeloma cell (SP2/0) fusion, creating two strains of hybridoma cells. One secretes anti-HBs a subtype (IC 11), and another secretes anti-HBs d subtype (IC 8). Both are IgG subgenus.

The titers of antibody of the two cell strain are 2^{13} and 2^{14} by PHA (passive haemagglutination) respectively, neither the hybridoma cell's ability to secrete anti-HBs affected after they have been repeatedly frozen and thawed five times, nor after twenty generations.

The supernatant and ascites secreted by the two strains of hybridoma cells are thermally stable. The titer of the monoclonal antibody (McAb) at 37°C and 56°C, does not change after 60 minutes. McAb secreted by the two hybridoma strains can not be neutralized completely, the affinity of McAb is stronger than PcAb's

The ascites of mice are fractionated by 50% saturated ammonium sulphate, then eluted by DEAE- cellulose, yielding the purified McAb.

Human 0-group erythrocytes sensitized with the McAb pair were detected HBsAg using R-PHA (reverse passive haemagglutination), having a sensititivity of : < 20 ng/ml. Using coated solid phase polystyrene plate produced from the McAb pair, labelled PcAb by HRP (horse radish peroxidase), the ELISA diagnostic kit can prepared. This kit is used to detect HBsAg, with a sensitivity of : < 2 ng/ml.

P1-8

THE PREVALENCE OF HEPATITIS B VIRUS (HBV) INFECTION IN INSTITUTION FOR THE HEALTHY CHILDREN IN JAPAN

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The prevalence of hepatitis B virus infection and antigen and antibody positivity in 641 children and 319 staff members at institutions for healthy children in Osaka Prefecture was studied, and the results were compared with those in 1163 medical workers and 191 individuals not engaged in medical service.

The prevalence of HBV positivity among these groups decreased in the following order : staff members (24.8%), medical workers (18.9%), institutionalized children (14.2%), and individuals not engaged in medical service (7.9%). The prevalence of HVB infection among institutionalized children tended to increase with the duration of institutionalization rather than with their age. Among staff members, on the other hand, the prevalence increased with increase of both age and duration of employment.

Among institutionalized children, 1.9% were HBV carriers, of whom 66.7% were positive for HBe antigen, a finding suggesting the importance of this group as a source of infection.

These results suggest that a vaccination program, such as that provided for medical workers, is needed for children and staff members at institutions for healthy children.

P1-9

A RAPID ENZYME IMMUNOASSAY USING RECOMBINANT EXPRESSED ANTIGEN AND MONOCLONAL ANTIBODY CONJUGATE TO DETECT ANTI-HBc ANTIBODIES

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OBJECTIVE :

Anti-HBc antibodies can be the only serologi cal marker of acute Hepatitis B Virus (HBV) infection in the absence of HBsAg and anti-HBs. The use of human polyclonal anti-HBc as conjugate implies difficulties in the assay system concerning availability, risk of infection and batch variation. To avoid these problems we investigated the competitive enzyme immunoassay (EIA) with use of a monoclonal anti-HBc antibody.

METHODS :

The competitive reaction occurs in one step between the specific anti-HBc monoclonal antibody conjugate and anti-HBc antibody in serum toward the microwell strip coated with yeast-derived recombinant Hepatitis B core antigen (HBcAg). The incubation time is 4 hours at 37°C, followed a washing cycle and 30 min. of color development.

RESULTS :

1067 determinations on serum or plasma were performed. Results were compared with commerci- alized RIA kit. Sensitivity of polyclonal human anti-HBc conjugate ranged from 91.8% to 99.2%. Whereas monoclonal antibody conjugate showed a sensitivity of 99.5%, when compared to Sorin RIA kit. Polyclonal conjugate varied the specificity from 83.8% to 95.1%. As far as specificity is concerned value of 99.6% was calculated after testing 1067 paired sera and plasma drawn from healthy blood donors using monoclonal antibody conjugate.

CONCLUSION :

Competitive EIA using monoclonal anti-HBc conjugate allows a short incubation time, highly sensitive and specific method for the detection of anti-HBc.

P1-10

HBsAg CONTAINING PRE-S1 AND PRE-S2 REGIONS EXPRESSED IN YEAST

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It has been postulated that the inclusion of pre-S1 and pre-S2 epitopes into Hepatitis B vaccines may circumvent the non-responsiveness to S-protein and induce pre-S protective antibodies. In order to develop a more immunogenic HB vaccine, the large surface protein (L) of HBV, containing pre-S1 and pre-S2 sequences, was expressed in *S. cerevisiae* by placing the coding region under the control of a yeast hybrid phosphoglycerate kinase promoter. Cel chromatography and sucrose gradient analyses showed that the L-protein assembled into particles whose size and density were similar to those of plasma derived HBsAg. The particles were purified from yeast cell lysate by subsequent processes: PEG precipitation, binding to colloidal silicate and selective elution, ion exchange chromatography, isopycnic ultracentrifugation, and sucrose density gradient ultracentrifugation. The immunogenicity of the L-protein particles absorbed onto aluminium hydroxide was evaluated in Balb/C mice. A strong S-protein response comparable to plasma derived HBsAg was obtained; high titer of antibody reacting with peptide 126-140 (pre-S2) was also obtained. Our results demonstrate that HBsAg containing pre-S1 and pre-S2 regions produced in yeast is highly immunogenic and induces a pre-S specific immune response.

P1-11

DETECTION OF 27-32 NM VIRUS-LIKE PARTICLES IN STOOLS OF NON-A, NON-B HEPATITIS PATIENTS IN THAILAND BY IMMUNOELECTRON MICROSCOPY

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The objective is to detect non-A, non-B hepatitis virus in stools of viral hepatitis patients by IEM technique. From two hospitals, Vachira Hospital (Bangkok) and Prapokklao Hospital (Chantaburi), 142 sporadic cases of viral hepatitis were tested for HAV and HBV infections. Thirty-nine and 58 cases were serologically found to be associated with HAV and HBV infections respectively. The remaining 45 cases were unrelated to infection of HAV or HBV. In nine cases of these, 27-32 nm virus-like particles in stools by immune electron microscopy were detected, by using a reference serum of enterically transmitted non-A, non-B hepatitis. (Fausta 3/87). This finding implies that enterically transmitted non-A, non-B virus is prevalent also in Thailand.

P1-12

HEPATITIS C VIRUS INFECTION IN BLOOD DONORS

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With the availability of an assay to detect antibodies to hepatitis C virus (HCV), Australian Blood Banks now screen donations as a major contribution towards the reduction of posttransfusion hepatitis. The rate of anti-HCV- positive donations is currently around 0.4%. Twenty-four positive donors with elevated alanine aminotransferase (ALT) were referred to the Infectious Diseases Clinic for further investigation. Liver biopsy samples were examined and showed that all of the donors had ongoing liver disease; half of them were diagnosed with chronic persistent hepatitis while the remainder showed chronic active hepatitis. Polymerase chain reaction (PCR) to detect HCV RNA was positive in 21/24 patients using primers from the 5' non-coding region of the virus genome whereas only a proportion of these patients were positive using primers from the NS3/ NS4 region. No correlation was noted between histological diagnosis, ALT levels and PCR positivity. The proportion of anti-HCV-positive individuals with viraemia was much higher in this than in previous studies and it is possible that this is a result of studying patients who have raised ALT. A similar study to examine the incidence of viraemia in anti-HCV-positive blood donors with normal ALT values is currently ongoing.

P1-13

HEPATITIS C VIRUS IN QUEENSLAND ABORIGINES

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A number of aboriginal groups in Queensland have been studied previously for the incidence of Hepatitis A and B. No studies have been reported about the incidence of Hepatitis C.

The sera were tested with a commercial second generation EIA kit according to the manufacturers instruction.

Five separate communities were screened and showed an incidence of antibodies to Hepatitis C ranging from 12% to 86% (Yarrabah 12%, Cherburg 22%, Mornington Island 27%, Edwards River 81% and Kowanyama 86%).

The incidence of Hepatitis C showed a marked variation between the different communities. There was no correlation between the incidence of exposure to Hepatitis C and the exposure to Hepatitis A or B.

P1-14

THE PREVALENCE OF ANTI-HCV AMONG RISK GROUPS IN THAILAND

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The prevalence of anti-HCV is high in patients with multiple blood transfusions, IV drug abusers, chronic liver diseases with and without HBsAg. High risk Thai adults, children and blood donors were studied for anti-HCV, using ELISA (Abbot kit) to determine the prevalence among the risk groups. The results are shown in Table.

Diagnosis	No.tested	+ve(%)
Children		
Thalassemia with multiple transfusion	98	14 (14)
Infantile cholestasis	22	0 (0)
Miscellaneous chronic liver diseases	16	0 (0)
Adults		
HBV healthy carriers	73	0 (0)
Health care workers	53	0 (0)
Non-A Non-B post-transfusion hepatitis	8	5 (62.5)
Chronic hepatitis(Histologically proved)		
HBsAg +ve	18	0 (0)
HBsAg -ve	24	10 (42)
Cirrhosis		
HBsAg +ve	3	0 (0)
HBsAg -ve	13	3 (23)
Hepatocellular carcinoma		
HBsAg +ve	16	0 (0)
HBsAg -ve	12	0 (0)
IVDU	50	26 (52)
Prostitutes	150	4 (3.8)
Blood donors	101	2 (2)

In contrast to other reports, in the chronic liver disease adult group markers of dual infection with hepatitis B and hepatitis C viruseswere not found. When hepatitis B was not the cause of chronic liver disease or in cases of post-transfusion non-A non-B hepatitis, it is recommended that anti-HCV should be studied to make a proper etiologic diagnosis.

P1-15

PREVALENCE OF ANTI-HCV IN SELECTED POPULATION DETECTED BY VARIOUS COMMERCIAL REAGENTS : A PRELIMINARY STUDY

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New generation of anti-HCV detection, using both structural and non-structural (NS) antigen, from 4 commercial enzyme immunoassay (EIA) kits i.e., Abbott (USA), Diagnostic Biotechnology, DB (Singapore), Innotest (Belgium) and UBI (USA) were evaluated. Sera, collected in 1990 and 1991, from 4 groups of population i.e., 100 Thalassemic patients (50 children and 50 adults), 30 intravenous drug users (IVDU), 30 medical students and 11 repeatably reactive sera by a recombinant HCV C100-3, were studied.

It was found that 11 anti-HCV positive sera were positive by all of these three HCV-EIA. Of 160 tested sera, 50 samples gave positive reactions for anti-HCV by all four EIAs kits; 4 by three EIAs (Abbott and UBI together with either DB or Innotest), 5 by two EIAs (Abbott together with either DB or UBI) and 10 by one (either Innotest, DB or UBI). The discripant sera were confirmed using Immunoblot (Inno-Lia, Belgium) based on NS 4, NS5 and core antigen. It could be found false positive and false negative result in these new assays. The positive sera gave antibody to either both NS and core, or core only. Overall, the prevalence of anti-HCV varied 44%-48% in Thalassmic children, 12-22% in Thalassemic adults, 83-93% in IVDU and 0-2% in medical students. The reactivity of anti-HCV detected by using both structural and non-structural antigen assay were positive more than the previous report using non-structural antigen, HCV C100-3 (93% VS 67%) in IVDU group. Thus, these new generation HCV-EIA commercial assay seem to be more sensitive but the specificity of the test need to be verified.

P1-16

EVALUATION OF A POST TRANSFUSION NON-A, NON-B HEPATITIS ELISA AND IMMUNOBLOT TEST

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The DB HCV ELISA and Immunoblot tests incorporated antigens from the structural and nonstructural regions of the HCV genome. The specificity of the ELISA test was evaluated with blood donor sera and sera from viral infections other than hepatitis. The donor sera were previously tested with another HCV ELISA (Abbott) kit. All repeatedly reactive samples were further tested with more specific supplementary tests like the Ortho RIBA IV and DB HCV Blot test. Specificity was estimated to be 99.7%. A panel of sera from patients with chronic liver disease (n = 30) were also evaluated against the DB HCV ELISA and Immunoblot test. These sera were concurrently tested with Abbott second generation ELISA as well as UBI peptide ELISA. 3 sera were reactive with Abbott HCV EIA but non reactive with both the UBI and DB HCV ELISA. The 3 sera were similarly not reactive with any of the nonstructural and structural proteins on the DB Immunoblots. Further tests using RIBA IV are being performed. In summary, the DB HCV ELISA has a specificity of greater than 99.7% and a sensitivity of detection similar to other available second generation HCV ELISA products.

P1-17

HEPATITIS B IMMUNIZATION OF INFANTS IN THE STATE OF HAWAII - A MODEL FOR UNIVERSAL IMMUNIZATION FOR INFANTS IN THE UNITED STATES

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Hepatitis B Surface Antigen (HBsAg) carrier rates vary among the multi-ethnic residents of the State of Hawaii. A low dose trial 2.5 mg of yeast recombinant HB vaccine (HBV) (Recombivax-HB) was undertaken in 300 infants using four schedules of immunization. Antibody responses were measured at various times following 3 doses of HBV. Results showed that Recombivax-HB was highly immunogenic using all 4 schedules. Based upon results of these studies the State of Hawaii will be undertaking immunization programs for all infants in the State. It is anticipated that a similar strategy will be recommended for all infants in the United States of America (USA) by the American Committee of Immunization Practices (ACIP) and the immunization committee of the American Academy of Pediatrics. These recommendations will help all states in the USA to start their Hepatitis B immunization programs for the infants similar to the program undertaken by the State of Hawaii. It is expected that universal immunization of infants in the USA with HBV will reduce the cost of HBV globally; so that Hepatitis B virus endemic countries in S.E. Asia and the Pacific will undertake the routine immunization of all infants with HBV. Over the years this will reduce the transmission of Hepatitis B virus, the number of new HBsAg carriers, the cases of Hepatitis B and primary hepatitis cellular carcinoma associated with Hepatitis B.

P1-18

IMMUNOGENICITY OF HEPATITIS B VACCINE IN LOW BIRTH WEIGHT NEONATES

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Low birth weight was usually the exclusion criterion of most of the hepatitis B vaccine studies. We conducted a comparative study evaluating the immunogenicity of hepatitis B vaccine on normal and low birth weight(<2500 grams) neonates. Fifty one low birth weight neonates and 51 controls matched by sex and date of delivery were available for study. Hepatitis B vaccine (Hevac B Pasteur(R)) were given to all neonates at birth, 1,2 and 12 months. Blood specimens were drawn for the detection of HBsAg, anti HBs and anti HBc at birth, 4,9 and 13 months using micro ELISA technique. The geometric mean titers of anti HBsAb were found to be 165.9, 123.0 and 1479.1 at 4,9 and 13 months respectively for low birth weight neonates while they were 165.9, 120.2 and 1698.2 respectively for normal birth weight neonates. The seroconversion rates for the low birth weight group were 68.3, 70.7 and 91.7 at 4,9 and 13 months. The corresponding rates for the normal birth weight group were 65.1, 72.5 and 89.2 respectively. It was concluded that the immunogenicity of hepatitis B vaccine in low birth weight neonates was as good as in normal birth weight neonates.

P1-19

IMMUNE RESPONSE TO HEPATITIS B VACCINE IN PREMATURE NEONATES

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The immunogenecity of a recombinant yeast-derived hepatitis B vaccine in 25 premature infants who were born to HBsAg negative mothers was studied. The gestational ages were 28-37 weeks and birth weights were 1300-2000 grams. Serconversion occurred in 22 infants, the anti-HBs titers varied between 50 and 13,470 IU/L (Geometric mean titer = 542 IU/L). Seroconversion rate = 88%. The response did not vary with gestational age, birth weight or illness status.

PI-20

LOW COST EIAs FOR HEPATITIS B VIRUS SURFACE ANTIGEN (HBsAg) AND ANTI-HBsAg DETECTION

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The aim was to develop EIAs for detecting HBsAg and anti-HBsAg whose cost was more suitable for third world country budgets in comparison with the cost of commercial kits.

High titer anti-HBsAg was raised in sheep immunized with Engerix-B vaccine. Sheep immunoglobulins (Ig) were purified by NH_4SO_4 precipitation and sephadex G-200 and DEAE cellulose column chromatography. Purified Ig was concentrated by ultrafiltraion and labelled with biotin. Hepatitis B virus (HBV) from the serum of carriers was purified using a sepharose 6B column and a blue-sepharose CL-6B affinity column. Virus was concentrated by ultrafiltration. Immune plates (Nunc) were used in both EIAs. In the HBsAg EIA, catching antibody was goat anti-HBsAg (Dako) detecting antibody, biotin-labelled sheep anti-HBsAg described above; avidin-peroxidase conjugate (Sigma); and substrate TMB. 53 positive and 48 negative sera were tested. Compared to RIA (Abbotts)the EIA developed was 90.6% sensitive and 100% specific. The sensitivity of a commercial EIA (Biotest) compared to RIA was 96%. In the EIA for anti-HBsAg, plates were coated with purified virus, the conjugate was rabbit anti-human-peroxidase (Dako); and substrate TMB. 75 sera containing anti-HBsAg by RIA (Abbott) and 25 negative sera were tested. 100% of 25 high positives (> 150 mIU); 84% of 25 moderate positives (50-80 mIU); and 12% of 25 low positives (10-20 mIU) were positive. All 25 negative sera were negative. The overall sensitivity was 65% and specificity 100%. The sensitivity of both assays needs to be improved, in the case of the HBsAg EIA perhaps with the use of monoclonals which we are developing.

P1-21

SEROEPIDEMIOLOGY OF HBV, HCV AND HDV INFECTION IN MONGOLIAN POPULATION

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Hepatocellular carcinoma (HCC) and chronic hepatitis (CH) are still remaining a major public health problem in Mongolia as in the most Asain coutries. Prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) infection was determined by testing of HBsAg, anti HCV, anti HDV antibodies (HCVAb, HDVAb), 48,3% of the HCC patients, 60.7% of the CH patients and 6.8% of the healthy population were positive for HBsAg. Anti-HCV were detected in 62.1% of HCC patients, in 46.4% of CH patients and in 16,3% of healthy population. In 36 (87.8%) of the 44 HBsAg positive serum samples were detected HDVAb subtype was only.

In the HBsAg negative HCC patients 93,3% were found for HCVAb.

In the HBsAg positive HCC patients 50% were also positive for HCVAb.

P2-1

THE EFFECT OF FAMILY MEMBERS IN CHILDHOOD ON SEROPREVALENCE TO HERPES SIMPLEX VIRUS

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We found that positive rate of antibody to herpes simplex virus (HSV) in Japanese young adults (20 to 29 years of age) had decreased to about 40% in 1984, compared to those in 1960 (82% Yoshino et al.) and in 1969 (51%; Hondo). To elucidate factors affecting this phenomenon, serum samples and data regarding family members in childhood were collected from college students aged 18 to 19 years and analyzed the relationship between number of inhabiting members of the household in childhood and seropositivity to HSV.

Sera of new students of a women's college in Yokohama City were collected yearly from 1985 to 1989 and stored frozen at -50°C until use. Neutralizing antibody to HSV was assayed with micro-method by using RK-13 cells and HF, a standard type 1 strain of HSV, in serum samples diluted 2-fold serially from 1:5. Information concerning family members at age 6, 12, and 18 were obtained by conducting self-recording questionnaires at the time of bleeding.

The following results were obtained:

1. Although positive rates of antibody to HSV in new students of each year showed some fluctuation between 32 to 38%, no year-dependent decreasing tendency from 1985 to 1989 was observed.
2. Numbers of total family members and cohabiting grandparents in childhood were larger in seropositives than in seronegatives, but those of parents and siblings were not different between two groups.
3. Antibody positive rates among students cohabiting in infancy with 0, 1, 2, and more than 2, grandparents were 31.8, 40.0, 40.4, and 42.9%, respectively, and the difference observed was statistically significant (Chi-square test, $p < 0.05$).

These results show the possible effects of total family size and number of grandparents upon the chance of primary infection with HSV in childhood.

P2-2

SUBTYPING OF HERPES SIMPLEX VIRUS TYPE 1 ISOLATED IN TAIWAN BY RESTRICTION ENDONUCLEASE CLEAVAGE PATTERN

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In order to know the genomic polymorphism of herpes simplex virus type 1 (HSV-1) isolated in Taiwan, 134 strains of HSV-1 were collected at National Taiwan University Hospital for study during the period from January 1981 to December 1990. Restriction endonucleases (BamHI, SalI and KpnI) were used for digesting viral DNA extracted from infected Vero cells. The RE digestion patterns of these strains were compared to the result obtained in standard strain F. The criteria of subtyping of HSV-1 described by Dr. Sakaoka was used in this study. There were 6 subtypes (A-F) occurred in these strains. The distribution of subtype A to F were 35%, 3%, 6%, 6%, 47% and 3% respectively. The distribution of HSV-1 subtypes shows: 1) no difference in years when they were isolated. 2) no difference in ages of patients who were infected with each subtype. 3) no difference in patients with or without vesicular lesion. 4) no dramatic difference between Taipei and outside of Taipei.

P2-3

RAPID IDENTIFICATION OF HERPES SIMPLEX

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Direct fluorescent antibody (DFA) and culture amplified direct fluorescent antibody (CA-DFA) were compared to tissue for the identification of *Herpes simplex* virus.

In the CA-DFA procedure MRC5, HEp2 and Vero cell lines were compared. DFA identified and typed 44% whereas CA-DFA using MRC5 cells only identified and typed 86% of *HErpes simplex*. HEp2 cells identified 4% of *Herpes simplex* which were not identified in MRC5 cells but MRC5 identified 18% which were missed by Hep2 cells. Vero cells demonstrated poor adhesion to the chamber slides. Culture amplified direct fluorescence is the method of choice for the rapid identification of *Herpes simplex* with the best results being obtained using both MRC5 and HEp2 cell. Inoculation of duplicate sets for incubation overnight and 72 hours results in a further increase in sensitivity.

P2-4

THE VIROLOGICAL STUDY ON FEMALE HERPES GENITALIS

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From 1980-1991 herpes simplex virus (HSV) isolation from clinical specimens of 1,118 female patients attending at female sexually transmitted disease (STD) clinic, Siriraj Hospital were studied. These women were clinically diagnosed herpes genitalis. The specimens were collected from cervix and genital lesions. HSV was isolated in cell cultures, Vero and human embryonic lung fibroblast (HEL). HSV isolates were identified by immunofluorescence test using rabbit polyclonal antibodies to HSV-1 and HSV-2. Of these patients, 655 (59%) showed positive isolation. The isolation of HSV from cervix of patients with primary episode could be detected more than patients with recurrent episode (46% vs 20%) while the isolation rates from genital lesions of these two groups showed no significant difference. Of these cases, 66% had recurrent episode within six months; 7% and 5% after one year and two years. Of the 115 HSV-isolates further investigated by direct immunofluorescence test using mouse monoclonal antibodies specific to HSV-1 or HSV-2, 97.4% were HSV-2 and 2.6% were HSV-1.

HSV isolation in cell cuture is quite a convenient and easy technique in virology laboratories which have cell culture facilities. High frequency of HSV isolation from patients attending at STD clinic could be detected in 24 to 48 hours. The earliest cytopathic effect could be observed in 8 hours. This technique is more sensitive than direct specimens examination using the immunofluorescence technique.

P2-5

CHARACTERIZATION OF HUMAN CYTOMEGALOVIRUS (HCMV) ANTIGEN RECOGNIZED BY HCMV SPECIFIC MONOCLONAL ANTIBODIES

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To characterize human cytomegalovirus antigens recognized by HCMV specific monoclonal antibodies (MCMVA 51, 93) several tasks were performed as follows : immunoprecipitation, N-linked glycosylation blockade with tunicamycin, Western blotting, biochemical blockade of protein synthesis using antiviral agents, virion purification and dot blot immunoassay, immunofluorescence (IF)staining to delineate morphologic characteristics.

IF staining of HCMV infected cells with mono- clonal antibody MCMVA 51 showed yellowish green cytoplasmic mass which was revealed to have molecular weight of over 220kd by immunoprecipitation. The binding epitope was glycosylated. MCMVA 51 cross-reacted with 38kd antigen of human pancreatic islet cell which was supposed to be an autoantigen in type-1 diabetes mellitus.

On the contrary, IF staining with MCMVA 93 showed diffuse yellowish green stains confined to the nucleus and the corresponding antigen was identified to be a 150kd protein which did not contain N-linked glycosylation on their binding epitope.

With the above result we could assume that the corresponding antigen of MCMVA 93 may be a regulatory protein not to be unveiled yet and that of MCMVA 51 must be closely linked to the pathogenetic factor of type-1 diabetes mellitus. For the further evaluation of corresponding antigens of MCMVA 93 and 51, molecular cloning should be performed.

P2-6

RAPID DETECTION OF HUMAN CYTOMEGALOVIRUS (AD-169) ANTIGENS IN CELL CULTURE WITH PEROXIDASE-CONJUGATED MONOCLONAL ANTIBODY

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A direct immunoperoxidase staining method was performed for the detection of human cytomegalovirus (HCMV) in the cell culture as rapid and accurate as possible using HCMV-specific monoclonal antibodies previously characterized by our laboratory.

Onto the monolayer of human fetal lung fibroblast (HFLF) cultured on the round cover glass in the 24-well plate, HCMV in a concentration of 500 pfu/ 0.2 ml, 50 pfu/ 0.2 ml, and 5 pfu/0.2 ml was inoculated. From 24 hrs after inoculation, the cover glass was retrieved from the well everyday for 7 days and stained with peroxidase-conjugated monoclonal antibodies.

In the heavily inoculated specimens (500 pfu/ 0.2 ml) MCMVA-93 could detect HCMV antigens within 24 hrs but MCMVA-57 within 48 hrs after inocula-tion. In the lightly inoculated specimens (5 pfu/ 0.2 ml) HCMV antigens could be detectable within 120 hrs with MCMVA-93 and at 96 hrs with MCMVA-57 before the appearance of the cytopathic effect.

The dot-like, dark brownish tints appeared in the cytoplasm of the HCMV-infected cells stained with MCMVA-57 at 48 hrs after inoculation. In the case of the infected cells stained with MCMVA-93, the brownish tint appeared at the perinuclear area at 24 hrs and in the nucleus at 72 hrs.

P2-7

DETECTION OF HUMAN CYTOMEGALOVIRUS DNA BY POLYMERASE CHAIN REACTION (PCR)

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The PCR technique has been applied to detect DNA of human cytomegalovirus in HEL cell infected with AD169 strain. Sequence of the fourth Exon of the immediate early (IE) gene was amplified by using the 19A and 19T.

The PCR was performed in a mixed solution with Tris-HCl buffer including $MgCl_2$, dNTPs, DTT, Gelatin, DMSO, BSA, and CMV DNA. To each reaction 1-2 units Taq Polymerase and 25 pMol of the primers were added. For each cycle, the reactants were heated at 95°C for 5 mins and cooled at 45°C. The PCR consisted of 30 cycles. After reaction, the products will be extended at 65°C for 5 mins, the samples were kept at 4°C for electrophoresis.

The amplification products were accomplished by agarose gel and were stained with ethidium bromide, on which the bands appeared clear. Further the Southern blots and hybridization test were followed in order to identify the correct amplification products. The results showed that the products were related to DNA of human cytomegalovirus.

P2-8

PREVALENCE OF ANTIBODY TO HUMAN HERPESVIRUS 6 AMONG NORMAL THAI POPULATION

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Human herpesvirus 6 (HHV-6) is a human herpesvirus isolated from lymphoid cells. It is recently recognized as the causative agent of exanthem subitum. HHV-6 infection seems to be ubiquitous like other members of herpes group. To investigate the prevalence of HHV-6 infection among the general population in Thailand, 325 sera obtained from individuals in various age groups were tested for antibody to HHV-6. Human T lymphocyte cell line HSB2 infected with HHV-6 strain isolated in USA were used in indirect immunofluorescence test. Sera were tested at a dilution 1:10.

The seropositive rate is low in the newborn group (48.1%), which is similar to that of adult with age more than 30 years. The seropositivity in infants age group 3-6 months is 77.8%, then the antibody positive rate increases to 85% in the age group 7-12 months, and maintains at this levelin the adult with ages less than 30 years. The seropositive rate declines to 43.3%, 36.7%, and 40.9% in the age group 31-40, 41-50, and 51-60 years respectively. This result is agreed to that reported by Balachandra that Thai children has low seropositive rate about 23.1% to 15.4% from birth until 3 months and start to increase at age of 6 months. This study confirms that there is high prevalence of antibody to HHV-6 in Thailand and the infection is acquired early in life.

P2-9

A DOT IMMUNOBINDING ASSAY IN COMPARISON WITH THE GEL DIFFUSION TEST FOR THE DETECTION OF EQUINE HERPES VIRUS TYPE-1 (EHV-1) ANTIGEN FROM FIELD SAMPLES

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A rapid and simple dot immunobinding assay (DIA) is described for detection and identification of EHV-1 antigen in field samples from the cases of abortion, still birth, perinatal foal mortality and paralysis. The assay employs the use of nitrocellulose membrane and the antigen is adsorbed onto it as a dot. The antigen is identified as a coloured dot with a procedure based on the principle of enzyme immunoassay (EIA). In all, 61 samples were tested by DIA and the utility of the test was compared with conventional agar gel immuno-diffusion (AGID) test. With DIA 44 (out of the 61) samples were positive and 17 samples were negative, while with AGID only 22 samples were positive and 39 samples were negative. It was seen that DIA was rapid, more sensitive and specific along with the obvious advantage of being reagent-conservative, inexpensive and simple.

P2-10

IMPAIRMENT OF HERPES SIMPLEX VIRUS TYPE 2 ENCAPSIDATION DURING GROWTH AT SUBOPTIMAL pH ENVIRONMENT

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Herpes simplex virus type 2 (HSV-2) replication was inhibited during growth at suboptimal pH condition. At pH 6.5, the maximal viral yield decreased 10^2-10^3 folds compared to that recovered at pH 7.5 while viral DNA synthesis was unaffected. Electron microscopic observation of the infected cells maintained at these two pH conditions indicated that approximately equal amounts of immature virions were synthesized 6 hr after infection. However, at 18 hr the majority of viruses present in the nucleus of infected cells maintained at pH 6.5 were empty or partially cored capsids and some of these particles were enveloped and appeared in the cytoplasm whereas at pH 7.5 mature virions already appeared at the cytoplasmic membrane.

Analysis of the viral polypeptides by radio-immunoprecipitation indicated that the synthesis of p40, a family of polypeptides closely involved in viral DNA encapsidation, was significantly impaired in infected cells maintained at the lower pH condition.

P3-1

THE APPLICATION OF POLYMERASE CHAIN REACTION TO THE DETECTION OF INFLUENZA VIRUS IN THROAT SWAB

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An assay protocol based on exploiting the polymerase chain reaction (PCR) for the direct detection of influenza virus in throat swabs is described.

Two pairs of oligonucleotide primers were synthesized using an Applied Biosystems 381 A synthesizer. For H1 gene, the oligonucleotides corresponding to the nucleotide positions 385-404 and 815-796 (numbered according to the positive strand sequence of the HA gene of A/PR/8/34) were used. For H3 gene, the oligonucleotides corresponding to the nucleotide positions 179-198 and 756-737 (numbered according to the positive strand sequence of the HA gene of A/Aichi/68) were used.

By use of the mixture of H1 and H3 primers, it was possible to determine the subtype of influenza simultaneously. No visible band was detected after PCR of influenza B or A (H2N2) viruses with a pair of H1 or H3 primers. Dilution experiments showed that as little as 1.3-6 plaque-forming unit of virus was sufficient for detecting the HA gene by the PCR. All throat swab samples from which influenza viruses had been isolated by conventional methods were also positive by the PCR method.

P3-2

MORPHOGENESIS OF RESPIRATORY SYNCYTIAL VIRUS IN A HEP-2 CELL LINE

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The Respiratory Syncytial (RS) virus belonging to the family Paramyxoviridae are known to share, in addition to shape of virus particle and progress in virus assembly, a number of biological characteristics with animal and human parainfluenza viruses. Although they have been reported to mature in the formation of virions by budding from the membrane surface of infected cells. Recently, we observed another pathway of RS virus maturation different from other parainfluenza viruses. To define the development of RS virus in HEp-2 cells, RS virus (Long) -infected cells were fixed with glutaraldehyde on day 5 post infection and viewed in a Hitachi H7000 electron microscope with and without antibody in a combination of colloidal gold. As already reported, in the release of progeny virus from an infected cells, the plasma membrane plays important role through budding off process, whereas a number of virus particles seem to mature inside the cells. They are either round or filamentous, approx. 1000 nm in length, and it is of particular interest to observe that the majority of virus is surrounded by cytoplasmic vesicle. Following the arrangement of glycoprotein spikes on the intracytoplasmic membrane, ribonucleoprotein-like viral structural unit was found to accumulate underneath the above modified membrane. Immunoelectron microscope using colloidal gold also determined a number of ribonucleoprotein clusters in cytoplasm. The intracellularly matured viruses contain glycoprotein spikes that are closely arranged on their surfaces. Probably they are transported to outside the infected cells by transporting vesicles.

P3-3

THE MORPHOLOGICAL IDENTIFICATION OF SURFACE GLYCOPROTEINS AND NUCLEOPROTEIN IN RESPIRATORY SYNCYTIAL VIRUS-INFECTED CELLS BY AN ELECTRON MICROSCOPY TECHNIQUE WITH MONOCLONAL ANTIBODIES

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Respiratory Syncytial (RS) virus has been reported to mature by budding-off mechanism from the infected cell membrane in similar fashion to that of other parainfluenza viruses. However, the former virus does not contain hemagglutinating and neuraminidase activities that play a potential role at the first and last stage of virus infection. The present study was undertaken to follow synthesis and distribution to two glycoproteins G and F, and nucleoprotein in RS virus (Long) infected cells. The infected cells were treated with monoclonal antibodies in a combination of colloidal gold protein A complex followed by fixation with glutaraldehyde or paraformamide. A great number of vesicles with different sizes are distributed in a wide range of cytoplasm, and most of them contain colloidal golds associated with monoclonal antibody to G on their outer and inner surfaces. The colloidal gold associated with monoclonal antibodies demonstrated a lot of F antigens in the surface of empty granule. Coupled with the evidence that nucleoprotein is not detected in the above cell granules. This may reflected on the process of transportation of G and F proteins through the rough ER Golgi to the cytoplasmic membrane. In addition, the presence of a large number of small empty vesicles with 100 nm in diameter on the outer surface of the infected cell led us to conclude that the RS virus also mature through another pathway relating to transporting vesicles.

P3-4

RESPIRATORY VIRUSES IN CHILDREN ADMITTED WITH ACUTE LOWER RESPIRATORY TRACT INFECTIONS, 1989-1990

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From June 1989 to December 1990, this study investigated roles of respiratory viruses in 283 children aged between 1 month to 5 years who were admitted with acute lower respiratory tract infection at Siriraj Hospital, approximatey 88% were under 2 years of age.

Of 283 cases, 145 (51.2%) were positive for viral infection, which 9 (3.2%) of them had mixed infection. The most common virus detected was respiratory syncytial virus (33.9%), followed by adenovirus (5.7%), parainfluenza 1 (4.6%), parainfluenza 3 (3.9%) and influenza A (2.1%). Influenza A, H3N2 was found in 1989 and the H1N1 was found in 1990. Study on RSV subgroup showed that in 1989 subgroup A was predominate (75%) and in 1990 subgroup B was predominate (91%).

For seasonal incidence, RSV was found during August to October and parainfluenza 1 and 3 were found during Febuary to May.

In addition, incidence of *Chlamydia trachomatis* was determined in pneumonic cases aged under 6 months. of 65 cases, 6 (9.2%) were positive, all cases were under 3 months.

P3-5

ADENOVIRUS SURVEILLANCE IN JAPAN, 1981-1989 YEARLY TREND AND ASSOCIATION WITH RESPIRATORY ILLNESS

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National Epidemiological Surveillance of infectious Diseases in Japan gathers reports on infectious diseases through two information sources; case incidence reports from sentinel clinic/hospital and virus isolation reports from diagnostic laboratories including public health institutes and other institutions.

During 1981-1989, the yearly number of adenovirus isolations approximately ranged from 800 to 1,800, representing 10% to 23% of the total virus isolations in each year.

The most frequently reported adenovirus serotype was type 3 which fluctuated markedly from year to year, mainly contributing to a changing pattern of the total adenovirus reports. Reports of adenovirus type 4 which had been rare in Japan increased after the end of 1970s with a peak in 1984, and declined again thereafter. Isolations of types 1, 2, 5 and 6 were reported continuously at a low level, but type 7 was rarely isolated in Japan. In general, isolations of adenoviruses peaked in summer, but a fair number were reported throughout the year.

Upper respiratory illness was the major clinical manifestations associated with infections of types 1, 2, 5 and 6. Type 3 which was also highly associated with upper respiratory illness, often accompanied by eye diseases, e.g. PCF and EKC. While, for type 4 infections, frequency of upper respiratory illness was much lower and eye diseases proportionally increased. Most of other adenovirus isolations, types 8, 19 and 37 were predominantly related to eye diseases and recovered from eye specimen. However, type 11 was highly associated with urinary diseases and majority of isolations were from urine.

Adenoviruses were often associated with inflammation of the lower respiratory tract/pneumonia; the frequency represents more than 10% of yearly isolations in some types.

P3-6

EFFICIENT PROTECTION AGAINST INFLUENZA VIRUS INFECTION BY RECOMBINANT VACCINIA VIRUS EXPRESSING INFLUENZA HA GENE FROM STRONG PROMOTER

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Recombinant vaccinia viruses (RVV) have been successfully used as live vaccines to protect animals from many infectious pathogens. However, the use of attenuated strain as a safe vaccine vector and inactivation of vaccinia viral genes for insertion of a foreign gene into vaccinia viral genome reduce the immunogenicity of RVV. To overcome these disadvantages, we constructed vaccinia virus vector which can express a foreign gene from an efficient synthetic promoter, because larger amounts of antigen may be required to elicit higher immune responses. Synthetic promoter consists of the late promoter of cowpox virus A type inclusion body (ATI) and tandemly repeated sets of the early promoter of the 7.5 k promoter, and could express a foreign gene more efficiently at both early and late time of infection than the widely used 7.5 k promoter.

To analyze the effect of strong promoter on immunogenicity of RVV, we constructed two RVV, vR-7 and vSFB4 inf 1, which express the HA gene of influenza virus A/SW/Cambridge/39 (H1N1) from the 7.5 k promoter and the ATI-7.5 k hybrid promoter, respectively. Both RVV could express authentic influenza HA, however, HA protein was expressed at higher levels in vSFB 4 inf 1 than in vR-7. Immunization of mice with these RVV showed that vSFB 4 inf 1 elicited higher neutralizing antibody response and primed for an influenza virus-specific CTL response more efficiently than vR-7. Challenge infection of mice with influenza virus was also protected more efficiently by vSFB 4 inf 1 in a low dose than by vR-7 although all mice were protected by both immunization of high doses of RVV. These results demonstrate that the construction of a strong promoter is a good way to improve VV-based live vaccines.

P3-7

A549 CELL LINE AND ADENOVIRUS ISOLATION IN ROUTINE VIRUS DIAGNOSIS

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A549 cell line was evaluated in 1986 using a panel of known viruses, routine respiratory specimens, faecal samples, specimens from HIV patients and eye swabs. It was found to be useful for the primary isolation of clinically significant viruses such as Adenovirus (Ad), Herpes Simplex Virus (HSV) and Respiratory Syncy- tial Virus (RSV). A549 was chosen for the routine isolation of Adenovirus from eye swab specimens and more recently from faecal specimens from HIV infected patients.

Figures of Adenovirus isolates recovered from eye swab specimens since August 1986 further confirmed the increased sensitivity and relatively shorter incubation times when using A549 cells.

Adenovirus isolated from HIV infected patients consisted of mainly the uncommon types, such as Ad9, 26, 28, 30, 35, 44 and 46. An interesting feature was the recurrence of the same Adenovirus type in the same patient.

P4-1

THE FLV^R GENE OF MICE ALTERS THE INFECTABILITY OF MURINE EMBRYO FIBROBLASTS

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A number of genes have been identified in various strains of inbred mice, each of which confer resistance to a specific type of virus infection. The *Flv*^{r} gene confers resistance to flaviviruses. and is inherited as a single autosomal dominant trait. This system is used as a model to study genetic resistance to faviviruses. Mice which possess this gene (C3H.RV) support the replication of flaviviruses, but virus yields in their brain tissues are 100-fold lower and the spread of infection is slower when compared to congenic mice with the susceptibility allele of the *Flv* locus (C3H/HeJ). Various mechanisms have been proposed to explain the observed differences. They all relate to effects on the virus replication step. In one mechanism, the *Flv*^{r} gene is said to have an effect through the increased production of defective interfering (DI) particles, which in turn affects the amount of infectious virus produced. Our work suggests that the gene appears not to influence the production of DI particles, or the virus production in each infected cell, but possession of the gene decreases the infectability of the cell. Four strains of Murray Valley encephalitis (MVE) and one of West Nile virus (WN) were serially passaged in primary mouse embryo fibroblasts from C3H.RV and C3H/HeJ strains. Virus titers exhibi ted either a cycling of infectivity or complete elimination of infectious titer by the third passage. At no time could any DI particles be detected. Whilst immunofluorescence analysis showed C3H/HeJ MEF to be substantially and consistently more susceptible to infection than the C3H.RV MEF, the output of infectious virus per infected cell was similar for MEF from both donor types. Our working hypothesis therefore is that the *Flv*^{r} gene is early acting, operating at either the level of virus binding or uncoating, rather than at the level of RNA or whole virus replication.

P4-2

CLONING AND NUCLEOTIDE SEQUENCE ANALYSIS OF THE MEASLES VIRUS GENOME IN AN AUTOPSIED BRAIN TISSUE OF A SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE) PATIENT

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SSPE is a fatal disease caused by persistent infection of a defective mutant of measles virus in the central nervous system (CNS). Number of isolated causative virus is not enough to study its pathogenicity at present. Moreover, virus isolation has become more difficult, because SSPE patients can survive longer than before.

In the present study, we detected genomes of measles virus from an autopsied brain tissue of an SSPE patient using specific primers of hemagglutinin (H), fusion protein(F) and nucleoprotein (NP) genes of measles virus by polymerase chain reaction (PCR). The amplified complementary DNAs were purified and directly sequenced. Comparison of nucleotide sequences with measles virus (Edmonston (Ed) and Nagahata (Na)) and SSPE virus (Yamagata-I (YI) and Biken) revealed substitution A at nucleotide 1128 of NP gene of these strains for G of this new isolate. Furthermore, one nucleotide (G) insertion was found at 1599 of Ed strain. The same insertion was found in Na, YI and Biken strains. Nucleotide deletion that was in YI (position 1607) and Na (position 1644) strains was not found in Biken and this strain. The biological significance of these nucleotide substitutions is under study.

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Human Immunodeficiency Virus - P5

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M. Morita, JAPAN

5.2. Performance Comparison of Different HIV Antigens (Affinity Purified gp120+gp160 of Viral Lysate and Recombinant Proteins) When Used as the Capture Antigen in a HIVCHEK^{tm} Format
Carmen Woo, HONG KONG

5.3. Evaluation of Rapid HIV-1 Diagnostic Tests for Screening of Pre-Autopsy Specimens
Jean C. Downie, AUSTRALIA

5.4. Characterization of a HIV Isolate from Thailand
O. Yamada, JAPAN

5.5. Spread of HIV in Punjab State and Chandigarh in India
Sobha Sehgal, INDIA

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5.7. Immunodeficiency Virus-Like Particles Isolated from Peripheral Blood of a Chinese Patient
Xin-Shang Zhang, CHINA

5.8. Contribution of Disulfide Bonds in the Carboxyl Terminus of the Human Immunodeficiency Virus Type 1 gp120 Glycoprotein to CD4 Binding
Udy Olshevsky, USA

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Rutt Chuachoowong, THAILAND

5.10. Rapid Loop Dilution Technique : A Modification for Anti-HIV, HBsAg and Anti-HBs Screening Test by Agglutination
Suda Louisirirotchanakul, THAILAND

5.11. Evaluation of Rapid Screening Tests for Anti-HIV
Suda Louisirirotchanakul, THAILAND

5.12. Clinical Correlation of the Immunologic Markers in HIV Infected Persons
Sunee Sirivichayakul, THAILAND

5.13. Comparison of the Sensitivity of Various Anti-HIV Tests in Early Seroconverted Sera
Urai Chaisri, THAILAND

5.14. Seropositivity for HIV Antibody among Intravenous Drug Users in Manipur, North Eastern Part of India
Yengkokpam Ibotomba Singh, INDIA

5.15. Study of HIV Antigen in Thai Voluntary Blood Donors
Srivilai Tanprasert, THAILAND

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Marc Morillon, NEW CALEDONIA

Chlamydiae - P7

7.1. Serovars of Urogenital *Chlamydia trachomatis* Isolates in Japan
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7.2. Seroprevalence of *C. trachomatis* and *N. gonorrheae* in Infertility Couples and other selected groups.
Chantapong Wasi, THAILAND

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Zhao-Zhuang Luo, CHINA

8.2. Hantaan Virus Related Structure Found in Kidneys of Acute Phase HFRS Corpses
Tao Hung, CHINA

8.3. A Survey on Cold Blood Reserviors of Hemorrhagic Fever with Renal Syndrome
Dong You Yan, CHINA

8.4. Recent Study of Simple and Rapid Diagnosis of HFRS
Liang Fang, CHINA

8.5. Antigenic Potency Test of Hemorrhagic Fever with Renal Syndrome-Inactivated
Vaccine from Challenge of Vaccinated Animal with Hantaan Virus
Jin Won Song, KOREA

8.6. Studies on the Landscape Structure of Natural Nidii of Hemorrhagic Fever with Renal Syndrome (HFRS) in China
Hua-Xin Chen, CHINA

8.7. The Study of Hemagglutination Activity on Nuclear Structural Protein of HFRS Virus
Lingshu Wang, CHINA

8.8. Rift Valley Fever in Madagascar : A Recent Epizootic Outbreak in Central Highlands
Jacques Morvan, MADAGASCAR

8.9. Seroepidemiologic Study of Hantavirus Infection of Wild Birds and Bats in Korea
Luck-Ju Back, KOREA

8.10. High Density Particle Agglutination (HDPA) for Rapid Serodiagnosis of Hantavirus and JE Virus Infections
Tetsuo Tomiyama, JAPAN

P5-1

DETECTION OF ANTIBODY AGAINST REVERSE TRANSCRIPTASE OF HIV-1 BY A NEW METHOD: ITS EVALUATION AND APPLICATION

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Antibodies against reverse transcriptase (RT) have been detected by immunoprecipitation, Western blotting ELISA or neutralization assay. We recently noticed that antibodies against HIV-1 RT stabilized RT upon its heat-inactivation. We examined the specificity and clinical significance of this assay.

Sera were obtained from normal and anti-HIV-1 antibody-positive subjects. Virus lysates containing RT's were prepared from culture supernatants of human T cells infected with HIV-1, HIV-2 or SIV. Neutralization assays for RT's were done as follows: sera (2 ul) were mixed with virus lysate (20 ul) and incubated at 37°C for 30 min. Then residual RT activities were determined. Stabilization assays were done as follows: sera (2 ul) were mixed with virus lysate (20 ul) and incubated at 37°C 10 min and then at 56°C 20 min. In the presence of normal sera, more than 99% of RT activities were inactivated by heat treatment at 56°C. In the presence of seropositive sera, heat inactivation of the enzyme was inhibited. We examined about 150 seropositive sera by these two assays. Antibody titers against RT determined by neutralization assays and stabilization assays were compared statistically with clinical characteristics of patients, such as CD4/CD8 ratio of PBL, clinical stages according to CDC criteria, aberrant expression of BM-1 antigen and numbers of CD4 or CD8 lymphocytes. Antibodies against HIV-1 RT were much more frequently detected by the stabilization assay than by the neutralization assay. RT's HIV-1 and HIV-2 were able to distinguished by the stabilization assay. Stabilization assay is a new assay to detect specific antibodies against RT's of HIV-1 and HIV-2.

P5-2

PERFORMANCE COMPARISON OF DIFFERENT HIV ANTIGENS (AFFINITY PURIFIED gp120 + gp160 OF VIRAL LYSATE AND RECOMBINANT PROTEINS) WHEN USED AS THE CAPTURE ANTIGEN IN A HICHEK™ FORMAT.

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The purpose of this study is to demonstrate if there are performance differences between pENV 9, a HIV recombinant antigens currently used in the HIVCHEK^{tm} and purified gp120 & gp160 of viral lysate or other recombinant protein.

The assay system used for this study is HIVCHEK^{tm}, which is 3-5 minutes, simple and manual test. It is a membrane based filtration assay where the antigen is immobilized on the device membrane. The antigen captures the antibody in the HIV infected serum sample as the sample filters through the membrane. The antibody presence willbe indicated by a red dot on the membrane, which is the result of the interaction between the captured antibody on the membrane by the antigen and the Protein A-collidal gold.

400 serum samples (25% HIV antibody reactive) and BBI seroconversion panel were used for the comparison study. The gp120 & gp 160 were purified from virallysate by lectin affinity chromatographic and gel filtration techniques. And the other antigens were obtained from the noted sources; pENV 9 (DuPont), rgp 120 & rgp160 (Repligen) and the recombinant protein of clone 566 (Diagnostic Biotechnology Ltd, Singapore). Electrophoretic protein pattern for all antigens demonstrated the homogenity of each protein.

This study demonstrated that there are equivalent specificity and sensitivity of each antigens used in the HIVCHEK^{tm} format as comparing with the pENV 9 recombinant protein.

P5-3

EVALUATION OF RAPID HIV-1 DIAGNOSTIC TESTS FOR SCREENING OF PRE-AUTOPSY SPECIMENS

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OBJECTIVE:

The objective of this study was to compare the specificity and sensitivity of two rapid test kits in detecting HIV-1 antibody in postmortem serum specimens prior to autopsy.

METHODS:

Two rapid test methods: Genetic Systems GENIE HIV-1 and Abbott HIV1/HIV2 Test Pack were compared for sensitivity and specificity against a number of serum panels according to the manufacturers specifications for testing.

Serum panels included were: Positive Forensic (15), Negative Forensic (61), Positive IVDU (32), Negative IVDU (50), Indeterminate Western blot (27), Non-Specific ELISA screening (40) and confirmed Positive (49).

RESULTS:

Both Rapid Test Kits identified all the confirmed positive samples tested. However the Abbott Test Pack gave more non-specific reactions (6%, 9/151) than the Genetic Systems GENIE (0/151). The majority of these (8) were weak reactions which could be distinguished from a true positive reaction and were probably the result of non-specific cross-reactions with the p24 antigenic determinants contained in the test antigen. However the Test Pack was found to be more specific than the Abbott Combo HIV1/HIV2 Screening ELISA when compared against the same seven panels.

CONCLUSIONS:

Both Rapid Test kits were considered suitable for testing Forensic Serum specimens with the provision that all sera tested by these Rapid Test Kits be repeated with the standard screening ELISA on the next working day.

P5-4

CHARACTERIZATION OF AN HIV ISOLATE FROM AN HIV CARRIER IN THAILAND

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Genomic diversity and distinct biologic features of HIV variants are well-known. We isolated HIV-1 from peripheral blood mononuclear cell (PBMC) of an HIV carrier in Thailand, and examined its biological features and genomic difference comparing with Japanese HIV isolates.

Methods :

HIV was isolated by coculturing PBMC of an asymptomatic Thai carrier and Japanese healthy donors. Fresh isolate was examined for the growth in PBMC from healthy donors. Cytopathic effect was observed and p24 antigen level was determined by ELISA kit (Abbott). PCR with reverse transcription (RT) was carried out as described previously (J Virol Meth, 27, 203, 1990). Primer pairs corresponding to HIV-1 gag, env and LTR region were used (most of HIV-1 isolates from Japanese carriers are detectable by these primers).

Results and Discussion :

Five days after infection with Thai isolate, p24 level of culture fluid reached to more than 10 ng/ml and CPE was observed. Thus, this isolate belongs to rapid/high category. RNA of this isolate was extracted from culture fluid and PCR with RT was carried out. Specifically amplified products were detected with all of three primer pairs as in case of Japanese HIV isolates and LAV. However amplified product of this isolate in LTR region could not be digested with a restriction enzyme, Hae III, which usually cut the amplified product into two fragments. Although there are a few genomic differences shown by restriction profile, these primer pairs were suitable to detect RNA of this isolate.

P5-5

SPREAD OF HIV IN PUNJAB STATE AND CHANDIGARH IN INDIA

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HIV infection has established itself in India. It is expected to take a heavy toll in the coastal regions and border States (Madras, Bombay, Manipur, Nagaland). The total number of positive cases in India is 4,082 out of 580,824 screened, the actual number being much more. In Manipur more than 1000 I.V. drug users are positive. The State of Punjab bordering Pakistan and seat of heavy international traffic attracted little attention till the surveillence Centre at Chandigarh reported the first full blown case in May, 1987. Toll today, of the total 63 full blown cases recorded in India, 15 have been diagnosed in this region with 10 deaths. Besides, 57 seropositive cases have also been recorded including 30 foreign students. The source of infection could be traced to Africa(57%), USA(9.2%),Europe(3%) ,Bombay(3.1%), Gulf(9.2%), Multiple Countries(3.1%) and in 15.4% of cases, precise source could not be ascertained and they were all Punjabis. As many as 65% foreign students and 23.8% of Indian were also positive for HIV-2 by ELISA: a fact of importance in formulating future strategies of screening for HIV.

P5-6

HIV INFECTIONS IN THAILAND

Sombut Taprasertsuk

P5-7

IMMUNODEFICIENCY VIRUS-LIKE VIRUS PARTICLES ISOLATED FROM PERIPHERAL BLOOD OF A CHINESE PATIENT

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This article reports for the first time in China the isolation of a strain of immunodefici-ency virus-like virus particles from peripheral blood of a Chinese patient. This patient, a male of 23, born in Yunnan Province, had low-grade fever of unknown cause for more than 2 months with edema of face, pneumonitis of bilateral lower lobes, ulceration of buccal mucosa, maculopapular rash over perineal skin and enlargement of axillary and inguinal lymph nodes. He admitted a history of random sexual relations. The titer of fluorescent antibody against epidemic hemorrhagic fever virus was 1:1280. He was suspected of having systemic lupus erythematosus and epidemic hemorrhagic fever. Peripheral blood was taken 7 days before death. Virus isolation, electron microscopy (EM), indirect immunofluorescence (IIF) test, enzyme-linked immunosorbent assay (ELISA) and Western Blot (WB) test were carried out. Mononuclear cells were separated from the blood sample and co-cultivated with HuT78 cells in a CO_2 incubator at $37^{o}C$. Formation of syncytial cells was observed. EM of ultrathin sections revealed virus particles, appearing round or oval, 110-130 nm in diameter, having an eccentric core, with a transparent outer ring around some of them. Some particles were released by budding out from the surface of cells. The virus particle had a cone-shaped core with stripe-like structure. These morphologic characteristics were consistent with those of human immunodeficiency virus (HIV). IIF test and ELISA showed the presence of the antibody against HIV in the patients serum. However, WB test gave no hand. The nature of this virus particle remains to be further studied.

P5-8

CONTRIBUTION OF DISULFIDE BONDS IN THE CARBOXYL TERMINUS OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I GP120 GLYCOPROTEIN TO CD4 BINDING

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The carboxy half of the HIV-1 gp120 glycoprotein, which has been implicated in binding to the CD4 receptor, contains two disulfide bonds linking cysteine residues at 378-445 and 385-418. To examine the necessity of these disulfide bonds for the formation and/or maintenance of a gp120 glycoprotein competent for CD4 binding, we created mutants of a soluble form of gp120 in which combi- nation of these cysteine residues were altered. The mutant glycoproteins were examined for export from the expressing cell and for CD4 binding ability. The expression and export of mutant gp120 for which neither of these two bonds could form, were dramatically decreased. However, mutants for which either disulfide bond could form, were fully competent for CD4 binding. In some cases, the presence of one of the pair of linked cysteines exert more detrimentaleffect on export or CD4 binding than did alteration of both cysteines. Thus, the evaluation of the contribution of a particular disulfide bond to a phenotype should include studies in which both cysteines involved in the bond, are simultaneously altered.

P5-9

ANTI-HIV TESTING AT SIRIRAJ HOSPITAL : 1988-1991

Rutt Chuachoowong

P5-10

RAPID LOOP DILUTION TECHNIQUE : A MODIFICATION OF ANTI-HIV, HBs Ag AND ANTI-HBs SCREENING TEST BY AGGUTINATION

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Agglutination method is one of a widely used procedure in immunoassay for variety of infectious diseases. In this study, 800 samples collected from 200 IVDU, 200 pregnant women, 200 women attending family planning clinic, 100 workers, 50 females with STD and 50 hospitalized cases were examined for Hepatitis B surface antigen (HBs Ag) by RPHA (Serodia, Japan), anti-HBs by PHA (Serodia) and anti-HIV by GPA, (Serodia) using a rapid loop dilution method compared to conventional dilution method. It was found that 73, 257 and 55 samples were positive for HBs Ag (at titre of 20) anti-HBs (titre of 20), and anti-HIV (titre of 16) using coventional method whereas 74, 276 and 55 were positive for HBs Ag, anti-HBs and anti-HIV respectively using loop method (at titre of 13.5). Coventional dilution method could not detect anti-HBs in 19 samples which positive by ELISA, nevertheless rapid loop method could gave positive results. However, the non-specific reaction to unsensitized cell and non-reproducibility of rapid loop method were found more common for anti-HBs detection than the convventional method. The rapid loop method is simple, rapid and should be used in the agglutination method for HBs Ag, anti-HBs and anti-HIV.

P5-11

EVALUATION OF RAPID SCREENING TESTS FOR ANTI-HIV

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New rapid (Non-ELISA) tests are developed for anti-HIV-1 detection i.e., SeroCard (USA), SimpliRED (Australia) and SUDS (USA); or simultaneous anti-HIV-1 and anti-HIV-2 detection i.e., HIVCHEK (USA), Immunocomb (USA) and Rapid Clonatech (USA). The sensitivity and specificity of 6 new rapid tests, 3 based on indirect EIA (Immunocomb (IC), Rapid Clonatech, Serolard); 1 based on autologous agglutination assay (SimpliRED); 1 based on gold colloidal indirect assay (HIVCHEK); and 1 based on latex agglutination/ microfiltration EIA (SUDS) were studied. Panel sera which 85 positive anti-HIV-1 were collected from 50 symptomatic HIV infected cases, 50 IVDU, 50 pregnant women, 30 female prostitutes, 30 females with STD, 30 medical students and 50 workers. In addition, 20 sera from early seroconverters which anti-HIV-1 could be detected by ELISA and gelatin particle agglutination (GPA) but gave indeterminate result (using criteria from reagent kits) by immunoblot were also included. These follow-up patients showed positive result in the confirmatory test.

Of the 85 positive panel sera, all of these rapid assays could detect 100%. Those 20 from early seroconversion sera, 19 could be picked up by both Rapid Clonatech and SimpliRED, 18 by IC, SeroCard and SUDS while only 13 by HIVCHEK. Thus, the sensitivity of SimpliRED, Rapid Clonatech, IC, SeroCard, SUDS and HIVCHEK were 99%, 99%, 98%, 98%, 98%, and 92% respectively. All of these assays gave 100% of specificity except IC and SUDS which were 99% and 97%, respectively. The simultaneous anti-HIV-1 and anti-HIV-2 detection with separate bispot antigen gave cross reaction to HIV-2 antigen i.e., 6.8% by IC and 0.4% by Rapid Clonatech.

P5-12

CLINICAL CORRELATION OF THE IMMUNOLOGIC MARKERS IN HIV INFECTED PERSONS

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Three cellular and three serologic markers were evaluated in 61 Thai HIV-infected patients. These included CD4+ T cells, CD8+ T cells, total T cells, serum B2-microglobulin level, p24 antigen level and anti-p24. They were evaluated for their correlations with the clinical staging and as predictors for clinical progression. The decreased CD4+ T cells, increased B2-microglobulin level, decreased anti-p24 titer and increased p24 antigen level correlated well with the stages of HIV infection in Thai patients. The abnormalities were most marked in AIDS, followed successively by ARC and PGL or asymptomatic HIV infection. In our sequential 2 year follow-up, the absolute number and percentage of CD4+ T cells, the absolute number (but not the percentage) of CD8+ T cells, the B2-microglobulin level, p24 antigen level and anti-p24 at entry could be reliably used as prognostic markers for HIV progression. The combinations of p24 Ag with the measurement of CD4+ T cells substantially increased the prognostic value than either was used alone. Furthermore, the levels of B2-microglobulin and CD4/CD8 ratio of the progressors and non-progressors were significantly different on first test whereas the other markers would become discriminatory only after 1 or more years of follow-up. The progressors, however, could not be differentiated from the non-progressors based on age, sex or risk behaviors. The annual rate of clinical progresssion from asymptomatic to symptomatic HIV infection in our study was approximately 6.8%. The results obtained in our study of the natural course or HIV infection in Thai patients are essential baseline data for future therapeutic interventions or health policy planning of the country.

P5-13

COMPARISON OF THE SENSITIVITY OF VARIOUS ANTI-HIV TESTS IN EARLY SEROCONVERSION SERA

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Paired sera from 4 patients with proven HIV infection whose initial specimens obtained 14-51 days earlier were indeterminate and simultaneously retested with 7 screening anti-HIV test kits and immunoblot assay. This was aimed to evaluate the sensitivity of various new and old anti-HIV screening tests. The test kits evaluated were 4 ELISA test kits from Wellcome (Wellcozyme), Organon (Vironostika anti-HTLV-III), Pasteur (Rapid Elavia) and Diagnostic Biotechnology (DB, HIV-1 ELISA), 2 rapid tests based on microfiltration enzyme immunoassay procedure from Rapport (SUDS) and Disease Detection International (SeroCard), and 1 particle agglutination (PA) test (Serodia-HIV). Immunoblot strips from Diagnostic Biotechnology (HIV-1 Western blot) were used to confirm these serum specimens. Out of the 4 initial serum specimens tested, all were positive by PA, 2 by SUDS, Wellcome and Pasteur, 1 by SeroCard and DB, and none by Organon. When tested by immunoblot, 1 was negative (i.e., completely without any bands) whereas 3 were indeterminate (i.e. with very weak band for p18, 1 with weak band for p24, 1 with very weak band for gp 160). All repeat specimens obtained 14-51 days later (mean 32.5 ± 16 days) were positive by all screening tests and by immunoblot. Therefore, with these 4 early seroconversion sera, the sensitivity of the PA was 100%, of SUDS, Wellcome and Pasteur was 50% of SeroCard and DB was 25%, and Organon was 0%. Whereas none of these sera was considered positive by immunoblot.

P5-14

SEROPOSITIVITY FOR HIV ANTIBODY AMONG INTRAVENOUS DRUG USERS IN MANIPUR, NORTH EASTERN PART OF INDIA

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A study of intravenous drug users (I.V.D.U.) from 1986 to 1990 revealed the percentage of seropositivity for HIV antibody was 43.9 (male: 42.6 and female: 1.3). The report of seropositive I.V.D.U. in this area for the first time was India in a small number and the increase of positivity rate among such group within a short period of one year since the first detection led to the second largest seropositive category in India. Thus there is an immediate need of intervention to prevent further spread of HIV infection as most of the seropositives are in the age group of 15 to 30.

P5-15

STUDY OF HIV ANTIGEN IN THAI VOLUNTARY BLOOD DONORS

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The prevalence study for HIV antigen in 2,700 random sample of Thai voluntary blood donors, National Blood Center between October 1990-January 1991 found all were non-reactive. Another group of 10,165 blood donors among 17,750 collected between April-May 1990 who came back for repeated donation after 3-6 months revealed that 10 blood donors became positive for anti-HIV. One out of these 10 blood donors found positive for HIV antigen when all 10 specimens were retrospectively tested. The recipient of this blood donation was found positive for anti-HIV. Based on the total donations of 228,504 in 1990, the current estimation of undetected positive HIV donation is 1/10,000. This study documents HIV transmission by a "WINDOW" donations demonstrates the value of a serum/plasma repository in documentary HIV "WINDOW" transmission. The detection of HIV antigen is suggested to become a normal practice in routine laboratory in order to reduce the risk of blood transfusion-transmitted HIV infection.

P5-16

HIV AND HCV INFECTIONS AMONG THE THAIS

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HIV and HCV are blood borne viral infections. Thailand is already endemic for HIV, and it is worth investigating if HCV, a fellow blood borne viral infection is transmitted together with HIV in Thailand. The study was made in intravenous drug users (IVDU); pregnant women, service girls and patients with hepatocellular carcinoma (HCC). Anti-HIV, anti-HCV, were applied to sera from the study groups. High prevalences of infections by HIV and HCV were found in IVDU, while the prevalence of anti-HCV is much lower in other study groups mentioned. Almost half of IVDU were found positive for anti-HIV or anti-HCV, alone or together. HCV plays only a minor role in HCC. It is suspected that in this country HCV may be transmitted together with HIV, mostly by intravenous route. Anti-HCV found together with anti-HIV may be a good evidence that HIV is transmitted by sharing of needles and syringes. Sera positive for anti-HIV from routine service at our hospital were tested for anti-HCV and 24.4% were found positive for anti-HCV. As approximately 50% of IVDU are positive for anti-HCV then it can be calculated that half of HIV infection in our hospital population were transmitted through IVDU. Despite the increasing evidence of HIV transmission by sexual contacts and perinatal transmission, transmission via IVDU still account for about half of the anti-HIV positive population at our hospital.

This work is supported by the Japanese Foundation for AIDS Prevention.

P5-17

MODES OF TRANSMISSION FOR THE HIGH RATE OF HIV INFECTION AMONG MALE STD PATIENTS AND MALE BLOOD DONORS IN CHIANGMAI, THAILAND

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OBJECTIVE: HIV rates in Thailand rose rapidly among IVUDs in 1988 and female prostitutes in 1989 and 1990. In June 1990 in Chiangmai 18% (36/200) of male STD patients and 3.5% (10/289) of blood donors were HIV+. We studied this "third wave" of the HIV epidemic.

METHODS: We compared 69 HIV+ and 105 HIV- male STD patients, and 12 HIV+ and 180 HIV- male blood donors by interview, physical exam and urine testing for opiate metabolites.

RESULTS: Of 69 HIV+ STD patients 55 (80%) had no risk factors for HIV other than heterosexual transmission, 84% (46/55) of these men reported sex with female prostitutes in the prior 12 months (median of 6 times in 1987, 5 times in 1990, compared to medians of 3 times in both years for 87 HIV- patients without other risk factors ($p= 0.03$ for 1990, $p<0.0001$ for 1987). Of 14 HIV+ with other risk factors, 12% (8/69) had tattoos since 1985, 7% (5/69) were IVDUs, 3% (2/69) had homosexual sex, and 1% (1/69) received transfusions.

The 55 heterosexual-risk-group HIV+ reported genital ulcer disease (GUD, $p<0.0001$), gonorrhea (GC, $p=0.005$),and "groin mass" infectious ($p=0.0003$) more often than the 87 HIV- without risk factors other than heterosexuality (X for trend).

Among 12 HIV+ male blood donors, 11 (92%) had no other known risk factors other than heterosexual contact. These 11 were more likely to have history of GUD (OR=23, $p<0.0001$) and GC (OR=6, $p<0.01$), compared to 154 HIV- heterosexual-risk-only blood donors.

CONCLUSION: Female-to-male heterosexual transmission from prostitutes is the probable route of infection for HIV+ male STD patients and male blood donors in Chiangmai.

225- A

P6-1

SEROPREVALENCE OF HTLV 1 IN NEW CALEDONIA (SOUTH PACIFIC)

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11825 sera, collected from persons living in New Caledonia (11,674) and in Vanuatu (151) were screened for antibodies to HTLV1. These were : blood donors, Melanesian people randomized during a cluster sample survey and during sexually transmitted diseases watch. Screening was performed with a particle agglutination test for blood donors and with ELISA for others.

Confirmation was done by Immunofluorescence or Western blot. The screening by particle agglutination showed 50 reactive sera while ELISA found zero. None of these sera (except one which remains doubtful) was positive by confirmation tests.

We conclude that seroprevalence of HTLV1 in New Caledonia is nearly 0%, and very different of those observed in the other islands of Melanesia. Therefore, the screening of blood donors from New Caledonia for HTLV1 seems to be unuseful.

This observation introduces an epidemiological question: why seroprevalence is so different between the neighbour islands of South Pacific?

P6-2

PRELIMINARY RESULTS OF SCREENING ON THE ANTIBODY TO HUMAN T-CELL LEUKEMIA VIRUS TYPE 1 (HTLV-1) IN MONGOLIAN POPULATION

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Antibodies to HTLV-1 was tested by passive agglutination-assay in total 323 sera including: 29 sera trom hepatocellular carcinoma patients, 28 sera from chronic hepatitis patients, 44 sera from healthy carriers of HBsAg, 190 sera from healthy population in Ulaan-baatar, 32 blood donors sera from 4 different districts (aimaks). Only one positive care in the sera of hepatocellular carcinoma patient was observed by passive agglutination assay but not combined by immunofluorescence assay.

Results of this study show absence of spread of HTLV-1 in mongolian population but it must be combined by wider seroepidemiological survey.

P7-1

SEROVARS OF UROGENITAL *CHAMYDIA TRACHOMATIS* ISOLATES IN JAPAN

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Chlamydia trachomatis (C. trachomatis) isolated from humans are classified into two biovars and 18 serovars. Major serovars of *C. trachomatis* isolates from industrial countries in North America and Europe are D, E, F and G, and two thirds of the isolates from the countries have been found to fall into those four serovars. In Asia, however, distribution of serovars of *C. trachomatis* is not clear. Therefore, we attempted to study serotyping of *C. trachomatis* isolates in Japan. A total of 96 urogenital *C. trachomatis* isolates from male and female patients attending the clinics were examined by the micro-immunofluorescence method using immune sera to the isolates produced in mice and/or monoclonal antibody typing kit (Washington Research Foundation). Of these isolates, 44 (45.8%) were types D and E, and 20 (20.8%) were types G and F. The serovars of the remaining 32 isolates were B, H, I, J, and K, and the proportions of these serovars were from 1.0 to 7.3% of the total isolates. As a result, just as in North America or Europe, two thirds of *C. trachomatis* isolates in Japan were found to fall into the four serovars, D, E, F and G. In addition to this, we found the relative distribution of serovars of *C. trachomatis* isolates from male and female patients somewhat differed. Serovars D, E, and serovars G, F were isolated in a same ratio from male patients, while the isolation ratio of the former serovars was four times or more higher than that of the latter serovars in female patients. None of serovar K was found in male patients, while 11.6% of isolates from female patients were typed in this serovar.

P7-2

HIGH SEROPREVALENCE OF PAST GONOCOCCAL AND CHLAMYDIAL INFECTION IN INFERTILE COUPLES.

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Infertility is mainly related to infection and particularly to sexually transmitted diseases. The relationship with infertility has been demonstrate for some microorganisms especially Neisseria gonorrheae and Chlamydia trachomatis. In this study, the prevalence of previous gonococcal and chlamydial infections in 26 infertile couples were investigated in comparison with the four selected control groups i.e. 50 pregnants and their husbands, 45 family planning acceptors and 62 female commercial sex wokers. The cross-sectional seroprevalence study was performed using ELISA system. The pili gonococcal antigen and chlamydial membrane protein antigen supplied by M. Ward, UK were used throughout the study. Higher prevalence of IgG antibodies to gonorrhea and chlamydia were demonstrated in female sex workers and infertile couples when compared to pregnants and their husbands and family planning acceptors. This study supports the hypothesis of previous gonococcal and chlamydial infections play important role in infertility.

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P8-1

ROLE OF AEROSOL IN TRANSMISSION OF EHFV AMONG LABORATORY RATS

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In order to elucidate role of aerosol transmission of EHFV among laboratory animals a special research on this problem was made from 1987-1990.

1. Air samples in animal room of EHF were collected with type GC-1 air-collector. A strain of virus was isolated in vero-E_6 cell and identified by IFA as EHF.
2. Eight Wistar rats were inoculated with EHFV. After inoculation, all the animals were reared in a special area with 1 m^3. EHF antibodies were demonstrated in rat sera by IFA, indicating that the artificial animal infection was successful.
3. A strain of EHF virus was obtained from the aerosol collected 34 days after challenge.
4. When virus challenged rats were placed in the rearing area. 10 contact rats were put on the area of challenged rats and assured to not direct contact with each other and possible vector contact between different groups. After rearing for 34 days, sera of three Wistar rats were detected for EHFV specific antibodies, 54 days seropositive rats 50%, 90% on 136 days and EHFV antigen were also found in brains and lungs of all three rats detected.

This results demonstrated that EHFV may present in aerosol and can infect Wistar rats. This provides evidence for aerosol spread in the laboratory animal type of EHF.

P8-2

HANTAAN VIRUS RELATED STRUCTURE FOUND IN KIDNEYS OF ACUTE PHASE HFRS CORPSES

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For seeking clues in pathogenesis of HFRS virus, two corpses died of acute phase HFRS were studied by means of ordinary thin section and immune colloidal gold electron microscopies. Along with destructive pathologic changes such as edema, necrosis, detachment of epithelia of renal tubules etc, some Hantaan virion-like structures, inclusion bodies, viral antigen layer-like structures as well as electron dense deposits, probably representing antigen-antibody complex, were evidently visualized within and around the epithelia and the basement membranes of the renal tubules. The nature and roles of these structures in pathogenesis of HFRS were discussed.

P8-3

A SURVEY ON COLD BLOOD RESERVIORS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME

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In order to investigate the cold blood animals as reserviors of hemorrhagic fever with renal syndrome (HFRS), 305 snakes belonging to 4 species and 535 frogs belonging to 3 species were captured in Liangping county where is an endemic area of HFRS, Sichuan Province, China, during 1989-1990. The Hantavirus (HTV) antigens in freezing section specimens of lung, liver, and kidney of snakes and frogs were detected by indirect immunofluorescent antibody technique (IFA) and no positive results were obtained.

The specific antibodies against HTV in the sera collected from snakes and frogs were examined by reversed passive hemagglutination inhibition (RPHI). The positive results were: Elaphe taeniura, 7.24% (10/138); Zaocys dhumnades, 8.33% (5/60); Elaphe mandarina, 0.0% (0/1); R. nigroma- culata, 10.0% (1/10); R.P. plancyi fukienensis, 9.0% (9/100); R. guentheri, 0.0% (0/1). The results of serotyping for 15 positive sera of snakes by hemagglutination inhibition test (HI) were: Rattus type, 20.0% (3/15); Apodemus type, 20.0% (3/15); and unidentified type, 60.0% (9/15). The above results suggested that the snakes and frogs living in the HFRS endemic area of Liangping county, Sichuan province had the chances to be infected by HTVs but not permanent reserviors of HFRS.

P8-4

RECENT STUDY OF SIMPLE AND RAPID DIAGNOSIS OF HFRS

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The antibody in the blood of H.F.R.S. patient often appears not early enough to make early diagnosis and so our attention focused to the detection of virus antigen in the blood and urine.

The specially prepared latex sensitized with potent H.F.R.S. monoclonal antibody was found to be specifically agglutinated by H.F.R.S. virus antigen and the result well coincided to that tested with other methods, and the sensitivity is much higher than that tested with other methods.

With this method, a serious examinations of blood and urine of H.F.R.S. patients have been made in more than 100 cases. The virus antigen could be detected in the blood and urine within the 3rd day of illness and the virus antigen concentration in the blood is very high, the highest titer may reach over 40,000 and persists high level relatively long time as long as more than 40 days while it decreases quickly in the urine within 3 weeks.

No non-specific reaction has been found in normal blood examined and in 8% of non H.F.R.S. urine shown non-specific reaction in low titer which can be eliminated by treating the urine with ether.

P8-5

ANTIGENIC POTENCY TEST OF HEMORRHAGIC FEVER WITH RENAL SYNDROME-INACTIVATED VACCINE FROM CHALLENGE OF VACCINATED ANIMAL WITH HANTAAN VIRUS

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HFRS vaccine inactivated Hantaan virus with formalin prepared from infected suckling rat brain was developed in Korea. The antigenic potency of the vaccine and the Hantaan virus infectivity challenge test was studied using hamster as a model to assay induction of protective immunity by HFRS vaccine.

8-10 weeks old normal hamsters were immunized with 4096 ELISA unit of vaccine by intraperitoneal route two times at a 10-day intervals, then were challenged 7 days after booster injection with live Hantaan virus (5×10^5 PFU). The animals were bled by cardiac puncture 3 weeks after virus challenge and various organ were collected for antigen detection using indirect immunofluorescence test (IFT) and Enzyme linked immunosorbent assay (ELISA). Mean antibody titer against Hantaan virus after vaccination was 1:210 by High density particle agglutination (HDPA) test and the neutralizing antibody titer was 1:16 by plaque reduction neutralization test (PRNT). Hantaan virus antigen was detected in the lung of hamster 3 weeks after virus challenge, however the antigen was not detected in the lung of vaccinated hamster. The above results suggested that HFRS inactivated vaccine was effective for the induction of neutralizing antibody and to protection against Hantaan virus infection in experimental animal.

Key words : Hantaan virus challenge test, HFRS inactivated vaccine.

P8-6

STUDIES ON THE LANDSCAPE STRUCTURE OF NATURAL NIDII OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) IN CHINA

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The landscape structure possesses characteristics of topography, hydrology, climate, soil, vegetation, animals, etc. From the aspect of topography divisions, the natural nidii and the epidemic areas are distributed mainly in plain and hilly regions under 500 meters above the sea level. From the aspect of hydrology divisions, they are distributed chiefly in the northeast region, north region, Qin Mountain Range-Dabie Mountain Region, southeast region and southwest region. From the aspect of runoff zone divisions, they are distributed mainly in plentiful-water zone and transitional zone as well as the eastern half of abundant-water zone. From the aspect of agriculture-climate divisions, they are chiefly distributed in temperate zone and subtropical zone of the eastern part of monsoon region. From the aspect of soil divisions, they are distributed mainly in sial soil regions and abundant aluminum soil regions. From the aspect of vegetation divisions, they are distributed chiefly in the northeast forest regions and most of the agricultural regions over the country. From the aspect of animal distribution divisions, they are present mainly in dampness-enduring animal population distributed regions. *Apodemus agrarius, Apodemus peninsulae* and *Rattus norvegicus*, etc. are the thriving animal population, predominant species or common species in landscape regions of HFRS, as well as the main reservoir animals and sources of infection in the natural nidii and epidemic areas of HFRS.

P8-7

THE STUDY OF HEMAGGLUTINATION ACTIVITY ON NUCLEAR STRUCTURAL PROTEIN OF HFRS VIRUS

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McAb A35 against EHFV-NP was linked to sepharose 4B to carry out affinity chromatography (AC) for purifying EHFV A9 strain antigen. The AC-NP was further isolated and purified by high pressure liquid chromatography (HPLC). HPLC-NP was EHFV specific with sera and McAbs by Indirect-ELISA, its MW was 50Kd by SDS-PAGE, EHFV nucleic acid was not contained in HPLC-NP by dot-hybridization test. And its rate activity was 13.6 times higher than AC-NP. HPLC-NP had also hemagglutination activity which was inhibited by specific antibody and hemagglutination inhibition antibody not neutralization antibody have been detected in its immunosera. We consider that there are hemagglutination binding epitopes which can induce hemagglutination inhibition antibody present in NP of EHFV A9 strain.

Key words : hemorrhagic fever with renal syndrome; nucleocapsid protein; high pressure liquid chromatography; hemagglutination binding epitopes.

P8-8

RIFT VALLEY FEVER IN MADAGASCAR : A RECENT EPIZOOTIC OUTBREAK IN CENTRAL HIGHLANDS

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Although Rift Valley fever (RVF) epizootics occurred in East and South Africa since the beginning of the century, RVF virus was not known in Madagascar until 1979 where it was isolated from 12 pools of mosquitoes. Despite that fact, no RVF episode occurred among the susceptible livestock (mainly cattle) and human population. In March 1990 during the rainy season a RVF epizootic was described surrounding the city of Fenerive on the East Coast of Madagascar. Since January 1991, numerous foci of bovine abortion were reported in the Central Highlands where cattle movements and density are important, and one human case with fever, jaundice and coma before death was described.

Seventeen strains of RVF were isolated on Vero cells and suckling mice from bovine abortion product, and one strain from serum of the human case. RVF virus antibody was detected by ELISA, immunocapture in 25/40 specimens. In bovine sera IgM prevalence performed by ELISA test was very high 35/47 (74.4%) in recovering aborting female and 230/938 (24.5%) inhabitants of villages where bovine abortions were observed. Mosquitoes catches were done in the different villages (n = 1597, 58% of which been *Culex antennatum),* but no virus was isolated from these potential vectors. These data permit to assert existence of RVF epizootic among cattle in Central Highlands and risk for Public Health. Epidemiological studies will be done for understanding of this epizootic outbreak.

P8-9

SEROEPIDEMIOLOGIC STUDY OF HANTAVIRUS INFECTION OF WILD BIRDS AND BATS IN KOREA

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Hantaan virus, the etiologic agent of Korean hemorrhagic fever (KHF) was discovered from the lungs of *Apodemus agrarius* caught in Songnae-dong, Dongduchun city, Kyunggido province in 1976 for the first time in Korea and it was designated Hantaan virus after Hantaan river in 1980. Seoul virus, Puumala virus, Prospect Hill virus and Leaky virus that are members of genus Hantavirus were isolated from lungs of house rats, *Clethrionomys glareolus, Microtus pensylvanicus* and *Musmusculus*, respectively. Now we know that natural reservoirs of hantaviruses are not only field mice but also house rats in many parts of the world. This experiment was designed to find the distribution of Hantavirus infection among the wild birds and bats in Korea from 1989 to 1990.

The results were as follows :

1. Among the 166 wild birds of 15 species, 14 *Paradoxomis webbiana* and 1 *Emberiza elegans* were immunofluorescent (IF) antibody positive against Hantaan virus. IF antibody titers against Hantaan virus of seropositive sera from wild birds ranged between 1:16 to 1:256.

2. Among the 143 wild bats of 2 species, 3 *Rinolophus ferrum-equinum* and 1 *Vespertilo abramus* were IF antibody positive against Hantaan virus, and 2 *Rinolophus ferrum-equinum* were IF antibody positive against Seoul virus. IF antibody titers against Hantaan and Seoul virus of seropositive sera from wild bats ranged 1:16 to 1:256.

The above results are the first evidences of hantavirus infection among wild birds and bats in Korea.

P8-10

HIGH DENSITY PARTICLE AGGLUTINATION (HDPA) FOR RAPID SERODIAGNOSIS OF HANTAVIRUS AND JE VIRUS INFECTIONS

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We have developed new high density particles agglutination (HDPA) for rapid serodiagnosis of Hantavirus and JE virus infections. HDP have silica core surrounded with dyed layer, and second silica layer covered the dyed layer. It's surface of the particles are covered with functional groups designed for adsorption of antigen. Antigens for these reactions were prepared from suckling rat brain using ultracentrifugation, protamine and ethanol treatment etc. For preparations of antigen coated HDP, HDP suspensions were added to an equal volume of antigen, and incubated for one hour at room temperature. Then the suspensions were washed with PBS, and lyophilized. Microtiter techniques were applied throughout all these reactions. To serial two fold dilutions of sera, every one drop of antigen coated HDP was added, and then let stand for more than forty minutes at room temperature. Positive agglutination patterns using the antigen coated HDP were distinctly demonstrated against positive sera for antibody while negative ones were found in both negative sera and diluent. It was also found that these reactions were more sensitive compared with IFA test for Hantavirus and CF test for JE virus. Accordingly, as antigen coated HDP were lyophilized, these reactions are easily used for measurements of Hantavirus and JE virus antibodies without any technical complexity within one hour, it is expected that these reactions will be applied for clinical and epidemiological use

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P9-1

ROTAVIRUS INFECTION IN ACUTE DIARRHEAL CHILDREN IN CHIANG MAI, THAILAND 1987-1990

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Nine hundred and eighty four stool specimens were collected from diarrheal children of age less than 4 years old admitted in hospitals between October 1987 to September 1991, and 280 non-diarrheic children under 3 years of age, in Chiang Mai Thailand. An ELISA for antigen detection and RNA electrophoresis follow by silver staining of the RNA band were both performed to demonstrate rotavirus infection.

The results showed that 394 out of 984(35.5%) of stool specimens obtained from diarrheal children were positive for rotavirus antigen by ELISA while only 8 out of 280 (2.8%) stool specimens from normal children were detected. It was found that the children of 10-12 months age group were most affected (37.9%). Among 291 ELISA positive specimens, 288 were positive for rota virus RNA. Of these, 155 were in the long pattern and 139 were in short pattern. It was found that the incidence of rotavirus infection in children was prevalent in winter.

P9-2

ANTIBODIES TO HUMAN ROTAVIRUS IN COLOSTRUM AND MATERNAL SERUM

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The antibody levels to human rotavirus (HRV) in maternal and cord serum and colostrum were measured to elucidate the protective effect of breast-feeding against diarrhea in infants. Maternal sera at the third trimester of pregnancy and cord sera and colostra were obtained from 443 mothers. HRV (Wa strain) specific IgG antibodies in maternal and cord serum and HRV specific IgA antibodies in colostrum were measured by ELISA. Three or 4 years after birth of the infants, the questionnaires on the period of breast feeding and the infant's history of "winter gastroenteritis" were sent to their mothers.

The results were summarized as follows:

1. Positive rates of HRV antibodies in maternal serum and colostrum were almost similar, 93.0% and 91.3% respectively. The distribution of antibody titers in maternal serum of primipara showed the peak at 20 GEU of titer, while the peak was seen at 60 GEU for multipara. Higher titer levels were observed in colostra of multiparas than those of primiparas.
2. The mean levels of HRV-specific-immunoglobulin classes were significantly higher in maternal sera and colostra of multiparas within 2 years after delivery than those of primiparas. However, mean levels of antibodies of multiparas with 4 years and more interval of delivery were similar to those of primiparas. This results suggest that mother's HRV antibodies might be increased by re-infection with HRV from their children.
3. The morbidity of "winter gastroenteritis" in infants fed with formula-milk alone or breast-milk in a short period (less than 2 months) were significantly higher than that of infants with long period (over 6 months) breast-feeding.

P9-3

ISOLATION OF BOVINE ROTAVIRUSES IN PUNE, INDIA

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In India, preliminary studies reveal that about 30 per cent of hospitalized acute diarrhoea cases are due to rotaviruses. There is no estimate of rotavirus diarrhoea cases occurring in the community. The need for vaccine against rotavirus is an urgent one. Although candidate vaccines of animal rotavirus strains are known to offer some protection against human rotavirus diarrhoea, there is need to develop more effective vaccines. The present study has been undertaken to isolate indigenous strains of bovine rotavirus with a view to evaluate and select suitable strains as vaccine candidates.

Faecal specimens were obtained from ten cow calves suffering from diarrhoea at Tathwade farm, Pune, in 1988. Specimens from seven calves were positive for rotavirus group A ELISA.

Bovine rotaviruses were isolated by serial passages in tissue culture from the faecal specimens of calves positive for rotavirus in ELISA, employing roller cultures. Primary lamb kidney cultures and Madin Darby bovine kidney (MDBK) cell line were employed for initial passages. Further passages were made in MA 104 cell line.

All the tissue culture grown isolates are positive for rotavirus group A ELISA. Three of the isolates have been confirmed as rotaviruses by electron microscopic examination.

P9-4

PHYLOGENETIC ANALYSIS OF COXSACKIEVIRUS A24 VARIANT : THE MOST RECENT WORLDWIDE PANDEMIC WAS CAUSED BY PROGENIES OF A COMMON VIRUS WHICH WAS PREVALENT AROUND 1981

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[1]National Institute of Health, Tokyo, [2]National Children's Medical Research Center, Tokyo, Japan.

Nucleotide substitutions in the viral-encoded proteinase 3C region of the genome of a coxsackie- virus A24 variant (CA24v), one of the agents causing acute hemorrhagic conjunctivitis, was studied using 32 isolates collected from eastern hemisphere in 1970-1989.

Based on the regression analysis of the nucleotide difference of each isolate from the 1975 strain, we estimated the nucleotide substitution rate, namely evolutionary rate, of CA24v to be 3.895 x 10^{-3}/nucleotide/year. A phylogenetic tree constructed by the modified unweighted pair group method using arithmetic averages (UPGMA) indicates that CA24v had evolved from a common ancestor which appeared in one focal place in July 1964 + 20 months, about 5 years before the first pandemic of acute hemorrhagic conjunctivitis in Asian countries.

The tree also revealed that allthe recent epidemic isolates in 1985-1989 including Asian and Ghanian isolates were branched each other after 1981. The result is consistent with the evidence that AHC due to CA24v had been confined in Southeast Asia and Indian subcontinent until1985, and then it suddenly and explosively spread to other areas where no CA24v isolations had ever been reported.

The isolates were provided from following researchers:

Mr. J.A.M. Brandful, Noguchi Memorial Institute for Medical Research, Accra, Ghana;

Dr. W.K. Chang, Queen Mary Hospital, Hong Kong

Dr. A. Ghafoor, National Institute of Health, Islamabad, Pakistan;

Dr. J. Chuinrudee and Mrs. K. Supanaranond, Virus Research Institute, Nonthaburi, Thailand;

Dr. Yin-Murphy, National University of Singapore, Singapore;

Dr. J.S. Tam, the Chinese University of Hong Kong, Hong Kong;

Dr. M. Guifan, Institute of Chinese Academy of Medical Sciences, Beijing, China.

P9-5

PHYLOGENETIC ANALYSIS OF ISOLATES OF THE COXSACKIEVIRUS A24 VARIANT REVEALED ITS DISCONTINUOUS CIRCULATION AFTER REPEATED INTRODUCTION TO JAPAN

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The nucleotide sequence variation of 3C proteinase region of coxsackievirus A24 variant (CA24v) was used to analyze the route of transmission and the spread of the viruses which were introduced to Japan during 1985-1989.

The strains isolated from a large epidemic in Okinawa Prefecture both in 1985 and in 1986 had an almost identical nucleotide sequence, indicating that the epidemic over two years was caused by the viruses of a common source. All isolates from other areas in 1986 were closely related genetically to the Okinawa strain. Taking account of the previously estimated evolutionary rate of the virus, isolates from Chiba Prefecture in 1986 were shown to be the same phylogenetic lineage as the isolates from other areas including Singapore in 1987, Beijing in 1988 and Hong Kong in 1988. While, the Chiba isolates in 1989 belonged to the other lineage together with the Taiwan isolates in 1988. In addition, one Japanese isolate from a case who contRacted acute hemorrhagic conjunctivitis in Singapore in 1988 was found to have a closely related sequence to the 1989-Japanese isolates.

The results explained that, so far, CA24v was introduced to Japan in each occasion independently from different focal places rather than being endemically in this country.

The isolates were provided from following researchers:

Drs. T. Yamanaka and K. Kasuga, Public Health laboratory of Chiba Prefecture;

Dr. K. Oda, Kanagawa Prefectural Research Center of Environment and Public Health;

Dr. K. Imai, Wakayama Prefectural Public Health Laboratory;

Dr. Y. Yamamoto, The Tokushima Prefectural Institute of Public Health;

Dr. K. Uchida, The Fraternity Memorial Hospital, Tokyo;

Dr. H. Nakagawa, Tokyo Women's Medical College, Tokyo

P9-6

THE COMPLETE NUCLEOTIDE SEQUENCE OF A VARIANT OF COXSACKIEVIRUS A24

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2. Central Virus Diagnostic Laboratory, National Institute of Health, Japan.

A variant of Coxsackievirus A24 (CA24v) is one of the agents causing of acute hemorrhagic conjunctivitis (AHC). Since CA24v emerged as a new human pathogen from a single focus, like enterovirus 70(EV70), the virus provides us a unique example to find a clue to identify the origin of the virus as well as to know the evolutionary relationship among other picornaviruses, we have determined the complete nucleotide sequence of the standard strain of CA24v, the EH24/70 strain.

The genome of CA24v is 7,461 nucleotides long with poly (A) tail at the 3'end. Following a 750 nucleotides 5' non-coding region, there is long open reading frame of 7,392 nucleotides, which serve to encode a viral polyprotein consisting of 2,214 amino acids. Comparison of the deduced amino acid sequence the polyprotein with that of known enteroviruses allowed us to predict the possible cleavage sites.

The overall structure and the organization of the RNA genome is typical for an enterovirus. Based on the similarity of the nucleotide sequence of the 5' and 3' non-coding regions together with the amino acid sequence of the encoded proteins, EH24/70 appeared to be closely related to poliovirus and coxsackievirus A21.

P9-7

NEUTRALISING ANTIBODIES TO COXSACKIE B, POLIO AND ECHO VIRUSES IN CASES OF GUILLAIN-BARRE SYNDROME

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Guillain-Barre syndrome or infective polyneuritis is being increasingly diagnosed clinically at the JIPMER Hospital, Pondicherry, South India. Virus neutralising antibodies were estimated in 7 cases against Coxsackie B1 to B6, Polio 1, 2 & 3, and Echo 6, 7, 9 and 11 using LLC MK_2 in microwell plates. Serological evidence in 5 paired samples showed 2 cases had polio 3 infection, 3 cases had Coxsackie B virus infection. Out of the two single samples one had antibodies to Echo 6 virus.

P10-1

COMPARISON OF RABIES NEUTRALIZING ANTIBODIES DETERMINED BY RAPID FLUORESCENT FOCUS INHIBITION TEST (RFFIT) AND STANDARD MOUSE NEUTRALIZATION TEST (SMNT)

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The study sera were obtained from 6 groups of postexposure vaccinees. The first group of 9 vaccinees received PCEC 1.0 ml IM on days 0, 3, 7, 14, 28 and 90 (PCEC conventional dose). The second group of 11 vaccinees received PCEC 1.0 ml IM on days 0, 3, 7, 14, 28 and 90 and received HRIG 20 IU/kg on day 0 (PCEC conventional dose plus HRIG). The third group of 11 vaccinees received PVRV 0.5 ml IM on days 0, 3, 7, 14, and 28 and received HRIG 20 IU/kg on day 0 (PVRV 5 doses plus HRIG). The fourth group of 15 vaccinees received 2 injections of PCEC 1.0 ml IM on day 0 and 1.0 ml IM on days 7 and 21 (PCEC 2-1- 1). The fifth group of 15 vaccinees received 2 injections of PCEC 1.0 ml IM on day 0 and 1.0 ml IM on days 7 and 21 and received HRIG 20 IU/kg on day 0 (PCEC 2-1-1 plus HRIG). The sixth group of 11 vaccinees received 2 injections of PVRV 0.5 ml IM on day 0 and 0.5 ml IM on days 7 and 28 (PVRV 2-1-1, DO-7-28).

The GMT of neutralizing antibody (NT Ab) on day 0 determined by RFFIT and SMNT was <0.10 and <0.02 in all groups and on days 14, 30, 90, 364 were as follow.

Gr.	Regimen	GMT on days 14		30		90		364	
		RFFIT	SMNT	RFFIT	SMNT	RFFIT	SMNT	RFFIT	SMNT
1	PCEC x 6	6.5	11.6	7.7	12.7	4.9	4.6	3.5	1.9
2	PCEC x 6 +HRIG	5.2	4.3	12.9	11.9	4.1	2.6	2.6	1.6
3	PVRV x 5 +HRIG	7.5	13.6	13.7	19.8	4.0	6.3	1.8	2.2
4	PCEC 2-1-1	5.1	7.3	8.2	13.1	-	-	2.1	1.5
5	PCEC 2-1-1 +HRIG	3.0	3.8	9.4	19.3	-	-	0.7	2.0
6	PVRV 2-1-1	11.8	14.2	13.0	15.4	9.9	14.0	4.2	6.0

The correlation coefficient between SMNT and RFFIT determined by Pearson's test was 0.92 for group 1, 0.93 for group 2, 0.96 for group 3, 0.92 for group 4, 0.80 for group 5, 0.97 for group 6 and 0.91 in 431 sera from all groups of vaccinees. When NT Ab 0.5 IU/ml was taken as the cut off value for determining the positive sera, the specificity and sensitivity of RFFIT were 93.0% and 98.7% respectively.

P10-2

SMALL DOSES MULTISITES CELL CULTURE RABIES VACCINE WITH AND WITHOUT HUMAN ANTIRABIES IMMUNE GLOBULIN : A SIMULATION SCHEDULE FOR POSTEXPOSURE PROPHYLAXIS

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One hundred thirty-three volunteers, 100 males, 33 females, aged 17-24 years (median 19 years) received PCEC intradermally as a simulation schedule for postexposure prophylaxis in August 1990. Eighty-two vaccinees received 0.1 ml ID 2 sites on days 0, 3, 7 and 0.1 ml ID 1 site on days 28 and 90 (group 1). Fifty-one vaccinees received similar schedule plus HRIG 20 IU/kg at gluteal region on day 0 (group 2).

Blood specimens were taken from these vaccinees on days 0, 7, 14, 28, 90, 180 and 365 after the initial dose. Neutralizing antibodies were measured by rapid fluorescent focus inhibition test (RFFIT) used WHO rabies antiserum as reference. All 133 tested sera showed neutralizing antibody less than 0.05 IU/ml on day 0. The GMT and 95% confidence limits of antibodies on days 14 and 90 were 9.79 (3.81-25.11) and 3.91 (1.43-10.70) for group 1 and 8.27 (2.81-24.33) and 3.39 (1.11-10.34) IU/ml for group 2 respectively.

The results showed good immunogenicity of PCEC, no suppression effect when HRIG was given simultaneously. The small doses intradermal is acceptable as an alternative economic regimen for pre- and post-exposure prophylaxis.

P10-3

TOWARDS AN ECONOMIC RABIES VACCINATION

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Rabies threatens everybody, but children and people of low income group are mostly endangered.

Post exposure vaccination is effective but expensive compared with the purchasing power of that group.

Research is in progress of developing more economic vaccination schedules, cheaper vaccines or mass vaccination strategies.

So far, there seems to be no break-through, but by abbreviated vaccine regimens with fewer visits the costs can be lowered considerably without any loss in efficacy of vaccination.

Specific post exposure rabies vaccination with already existing tissue culture rabies vaccines like PCECV seems to be the treatment of the choice for this decade. Nevertheless, research on new adjuvants, new production techniques with higher virus yield, new vaccination regimen is in progress.

THURSDAY 21 NOVEMBER 1991

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Micheal D. Lindsay, AUSTRALIA

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Vincent Chow, SINGAPORE

11.3. Processing of Dengue Virus Type 2 Structral Proteins Containing Deletions in Hydrophobic Domains
A. Greunberg, Peter J. Wright, AUSTRALIA

11.4. Evaluation of the Dengue IgM Enzyme Immuno-Assay and the Dengue Blot Test for the Rapid Diagnosis of Acute Dengue Virus Infections
S. Doraisingham, SINGAPORE

11.5. Dengue IgM Capture ELISA Using Labelled Monoclonal Antibodies
Chui Fui Chong, SINGAPORE

11.6. Japanese Encephalitis Antibody Prevalence Survey in Northern Thailand
Suntharee Rojanasuphot, THAILAND

11.7. An Identification of Japanese Encephalitis Virus Infection in a Non-Epidemic Area of Japan
Nobuyoshi Kobayashi, JAPAN

11.8. Flavivirus-Specified Changes in VERO Cell Plasma Membranes
Yuan-Lu Ho, SINGAPORE

11.9. Low Temperature Affects Replication and Expression of Proteins and Glycoproteins in Flaviviruses Infected VERO and C6/36 Cells
V. Sreenivasan, SINGAPORE

11.10. Entry of Kunjin Virus : Inhibition Studies on the Interaction Between the eceptor and Virion During Attachment
Mah-Lee Ng, L.C.L. Lau, SINGAPORE

11.11. Monoclonal Anti-Dengue Type 3 Antibodies
Rubporn Kittivachra, THAILAND

11.12. Evaluation of JE Vaccine among Lambay School Children in Taiwan
Chwan-Chuen King, TAIWAN

Virus Associated Cancer - P12

12.1. HSV IgA Serum Antibodies in Women with Cervical Intraepithelial Neoplasia and Invasive Cancer and in Their Husbands
M.M. Gupta, INDIA

12.2. Detection of Human Papillomavirus DNA in Penile Carcinoma in Thailand
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Uraiwan Kositanont, THAILAND

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Antiviral Agents - P14

14.1. Novel Antiviral Compounds from Marine Sources
Lance C Jennings, NEW ZEALAND

14.2. An Experiment for Development of a Biotechnological Treatment of Subacute Sclerosing Panencephalitis (SSPE) : Selective Killing of SSPE Virus-Infected Cells by Liposomes Containing Fragment A of Diphtheria Toxin
Shigeharu Ueda, JAPAN

14.3. Amphotericin B Effect on Scrapie Depends on Injection Site and Host Species
Y.S. Kim, KOREA

14.4. Diminished Rhinovirus (RV) Illness Severity Correlates with Increased Leukocyte Ascorbic Acid (AA) Levels
Lance C. Jennings, NEW ZEALAND

14.5. ReplicationofHumanInfluenza Virus Strains in Various Organs of Mice and Lack of Effect of Vitamin C on Replication
H. Polasa, INDIA

14.6. Interferon Therapy in Patients with Chronic Viral Hepatitis C : Trial for One Year
Nice Giachino, ITALY

14.7. Retroviral Reverse Transcriptase Inhibitory Activity in Thai Herbs and Spices
Orasa Suthienkul, THAILAND

P11-1

AEDES CAMPTORHYNCHUS MOSQUITOES ARE THE MAIN VECTORS OF ROSS RIVER VIRUS IN THE SOUTH-WEST OF WESTERN AUSTRALIA

Michael Lindsay, *Cheryl Johansen, Maria D'Ercole, John Mackenzie*

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Ross River virus (RRV) is a mosquito-borne virus which can infect man. Each year, during late spring and summer, human cases of Ross River virus infection (epidemic polyarthritis) are reported from the south-west of Western Australia. The number of cases varies from a few (in most years) to several hundred during epidemic years. Case records show that there is a focus of RRV activity on the Mandurah to Bunbury region of the Swan Coastal Plain. The summer saltmarsh mosquito, *Aedes vigilax*, was thought to be the major carrier (vector) of RRV in the region, based on circumstantial evidence and the results of studies in the Eastern States.

Research was undertaken in order to find out more about RRV, the mosquitoes which carry it and environmental conditions which may affect the mosquitoes and lead to major outbreaks of RRV. Mosquito trapping programs were established in the Mandurah and Bunbury regions. The mosquitoes were identified, ground up and passaged onto C6/36 and Vero cell lines to isolate viruses from them. Virus isolates were then identified using HI and Neutralization tests. Data on tides, rainfall and temperature in the region were collected and analyzed in association with the mosquito data and human case records.

Twenty one isolates of RRV were obtained, seventeen of which were from *Aedes camptorhynchus* mosquitoes trapped in winter, spring and summer. Several of these isolates were from *Aedes camptorhynchus* trapped just before and during the States biggest recorded epidemic of RRV, in the summer of 1988/89. *Aedes camptorhynchus,* which breeds in tidal saltmarshes during the cooler months of the year, was also found to be the dominant mosquito species in the region, comprising over 85% of the totalcatch. No isolates of RRV were obtained from *Aedes vigilax,* even during the 1988/89 epidemic. Higher and more frequent tides throughout the summer, and above average rainfall in the preceding winter and spring were found to have enabled large numbers of *Aedes camptorhynchus* to persist into the summer of 1988/89, leading to the RRV epidemic.

Our results show that *Aedes camptorhynchus* is an important maintenance and epidemic vector of RRV in the south-west. Monitoring of the populations of *Aedes camptorhynchus* and the tide and rainfall patterns in the south-west should enable us to predict future outbreaks of RRV.

P11-2

POLYMERASE CHAIN REACTION AMPLIFICATION OF DENGUE VIRUS SEQUENCES USING CONSENSUS PRIMERS

V.T.K. Chow, *C.L.K. Seah, Y.C. Chan*
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Dengue haemorrhagic fever and its associated syndromes constitute a major public health problem in the Asia-Pacific, with a record 1,733 cases in 1990 in Singapore alone. While established immunological and cell culture assays are available for the diagnosis and typing of dengue virus types 1,2,3 and 4, these tests may be time-consuming and less sensitive. DNA amplification by the polymerase chain reaction (PCR) was thus employed as a sensitive and rapid technique for the evaluation of dengue viruses. cDNAs were synthesized from RNAs of dengue virus types 1,2,3 and 4 (isolated from infected C6/36 *Aedes albopictus* cells) using random or oligo-dT primers, and served as templates for PCR. PCR consensus amplimers were designed based on highly conserved regions within the NS3 non-structural gene. These amplimers flank target fragments of approximately 470 base pairs within the dengue virus NS3 gene. When coupled with DNA sequencing, this RNA-PCR consensus amplimer strategy represents a powerful tool for the diagnosis and epidemiology of dengue viruses.

P11-3

PROCESSING OF DENGUE VIRUS TYPE 2 STRUCTURAL PROTEINS CONTAINING DELETIONS IN HYDROPHOBIC DOMAINS

A. Gruenberg, *P.J. Wright*
Department of Microbiology, Monash University, Victoria, Australia.

The 5' end of the genome of dengue virus type 2 encoding the structural proteins was expressed using recombinant vaccinia virus. The experiments were designed to assess the role of hydrophobic domains in the processing of the viral polyprotein. One construct contained a deletion of nucleotides encoding most of the C protein; nucleotides encoding the hydrophobic domain at the carboxy terminus were retained. Two additional constructs contained deletions of 72 bp or 129 bp encoding hydrophobic domains at the carboxy termini of C and prM respectively. Indirect immunofluorescence and radioimmunoprecipitation were used to detect prM and E in cells infected with recombinant viruses. The results showed that deletion of 90% of C had no apparent effect on the processing of prM and E, and that the signal sequence for E at the carboxy terminus of prM was active in the absence of the upstream signal sequence for prM at the carboxy terminus of C. Deletion of the hydrophobic sequences preceding the amino terminus of E prevented cleavage at the prM-E junction. These results obtained using infected cells were consistent with the published findings for the translation of flavivirus RNA *in vitro*, and indicated the importance of membrane association in the cleavage of structural proteins from the flavivirus polyprotein. In addition, cells infected with the recombinant virus containing the large deletion in the C coding region released the E glycoprotein into the culture medium.

P11-4

EVALUATION OF THE DENGUE IgM ENZYME IMMUNO-ASSAY AND THE DENGUE BLOT TEST FOR THE RAPID DIAGNOSIS OF ACUTE DENGUE VIRUS INFECTIONS

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A study was carried out on 1613 patients with suspected dengue virus infection to evaluate the efficacy of the dengue-specific IgM enzyme immunoassay (EIA) and the Dengue Blot for the rapid diagnosis of dengue virus infection. The EIA was an in-house assay and the Dengue Blot, a commercial test (Diagnostic Biotechnology Pte. Ltd.).

Together, the two tests were able to diagnose 80% of acute infections on acute phase sera. The IgM EIA was the more sensitive, chiefly because of the inability of the Dengue Blot to diagnose most primary infections. However, even with secondary responses, the IgM EIA was more sensitive.

While the Dengue Blot was more than 99% specific, the IgM EIA was less specific, being, at best, 93% specific. This was considered unaccep-table for the diagnosis of seriously ill patients. While the two tests, together, are useful for rapid diagnosis, the specificity of the IgM EIA should be improved.

P11-5

DENGUE IgM CAPTURE ELISA USING LABELLED MOMOCLONAL ANTIBODIES

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An IgM capture ELISA for the rapid diagnosis of dengue virus infection was developed utilizing virus-specific monoclonal antibody labelled with horseradish peroxidase and a shortened procedure. The rapid IgM capture ELISA was compared with the haemagglutination inhibition test, Dengue Blot and standard IgM capture ELISA for early diagnosis of dengue infection using acute-phase sera from patients.

P11-6

JAPANESE ENCEPHALITIS ANTIBODY PREVALENCE SURVEY IN NORTHERN THAILAND

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Japanese encephalitis (JE) antibody prevalence survey was carried out in 3,226 children from 5 selected northern provinces, Thailand during July to September 1989. The study is aimed at examining the immune status of the people in order to determine the target areas and age groups for JE vaccination. The results show that inapparent JE infection occurs in children from under 1 year old as indicated by the presence of JE neutralizing antibody in their sera. The antibody levels gradually increase with increasing ages. The antibody positive rates of children under 1 to 5, 6-9 and 10-14 years old residing in urban areas were 18.4%, 31.5% and 47.2% whereas those in rural areas were 13.3%, 30.7% and 54.1% respectively. The overall antibody positive rates among children residing in uban and rural areas are not significantly different. The GMT of the rural children is rather higher than that of the urban children suggesting higher intensity of JE infection in rural areas. However, it is concluded that the children under 14 years are equally susceptible to JE infection regardless of where they live, urban or rural areas. Children under 6 years old should be the first priority group for JE vaccination since they are the highest risk group.

P11-7

AN IDENTIFICATION OF JAPANESE ENCEPHALITIS VIRUS INFECTION IN NONEPIDEMIC AREA OF JAPAN

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No cases of Japanese encephalitis had been observed in Yokohama for years. However, a case occurred in this area on August 22, 1990 and compromised in symptoms. Since the detection of this case, a series of laboratory tests have been run trying to confirm the herpes virus infection. But encephalitis in following with herpes virus infection was not denied.

There upon, we have been investigating this case for serological and molecular biological evidence of Japanese encephalitis virus (JEV) infection. Using the purified antigens, haemagglutination-inhibition (HI) and ELISA tests in confirmation of the infection were employed. For a period of fourteen to fifty-three days after onset of illness, the results on conventional testing showed no clear cut presence. Then, using clinical samples, fractionation of immunoglobulin class in a density gradient centrifugation was carried out. In the serum collected after fourteen days from the onset of the illness, anti-JEV activities in IgM and IgG fractions on HI and ELISA testing were found. The IgM fractions were then treated with 2ME and reduced to anti-JEV activities below the quarter on HI testing. However, after fifty-three days from onset of illness, no 2ME sensitive antibodies were detected in the IgM fractions. The obtained results on HI testing were the same so with the ELISA testing.

Moreover, we investigated possibility of rapid diagnosis on JEV infection. And then we attempted to analyze the genomes of JEV by PCR method using spinal fluid from the patient.

P11-8

FLAVIVIRUS-SPECIFIED CHANGES IN VERO CELLS PLASMA MEMBRANES

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The morphogenesis and the final mode of exit of flavivirus from host cells are still very much a mystery. The possibility of the plasma membrane playing a role in the final stage of maturation of the virus was studied. Immunofluorescence, polyacrylamide gel electrophoresis (PAGE) and cell fractionation techniques were used. It was found that besides the two structural proteins (E and PrM), three non-structural proteins (N5, NS3 and NS1) were also present on the plasma membrane. These findings were observed using immunofluorescence technique on unfixed and fixed cells. The protein profiles obtained from the PAGE of purified infected plasma membrane ghost also confirmed the findings The non-structural proteins are likely to be transported passively to the plasma membrane due to excessive accumulation in the cytoplasm. From the preliminary results, comparison of the E protein found in the infected plasma membranes and the envelope protein of the purified virus by PAGE showed some variations in their molecular weights. At this stage, one could conclude that the plasma membrane may not be the main contributor of the virus envelope.

P11-9

LOW TEMPERATURE AFFECTS REPLICATION AND EXPRESSION OF PROTEINS AND GLYCOPROTEINS IN FLAVIVIRUSES INFECTED VERO AND C6/36 CELLS

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The advantage of low temperature inhibition (Matlin and Simons 1983; Saraste and Kuismanen, 1984) is applied here to study the expression of flavivirus proteins and glycoproteins. Initial results have shown several interesting findings when Vero cells and C6/36 cells were infected with Kunjin and West Nile (glycosylated and unglycosylated) viruses. Generally at 15°C and 20°C, the yields of intracellular and extracellular viruses were reduced although not inhibited. The yields from Vero cells were also lower at 28°C compared to 37°C. Data from immunofluorescent study and cytopathic effects observation supported the plaque assay results. At ultrastructural level, the Golgi complex appeared to be swollen at 15°C and 20°C in the Vero cells. However the morphology of the Golgi was not affected in the C6/36 cells. Most flavivirus protein bands were also not well resolved at 15°C or 20°C. Shifting-up the cells to 37°C for 3 hours (during the labelling period) did not improve the resolution of the viral proteins. At lower temperatures, the processing of the envelope protein to the expected molecular weights was also delayed. This was shown up more obviously in the C6/36 cells. Current studies are being carried out to follow the processing of the viral glycoproteins at the lower temperatures using ^{3}H-glucosamine and ^{3}H-mannose. From the results so far, one would conclude that these two cell lines frequently used in flavivirus studies have different ability to cope with the lower temperatures. With these inherent host properties, the expression and processing of proteins and glycoproteins of flavivirus also differed in the two cell types.

P11-10

ENTRY OF KUNJIN VIRUS : INHIBITION STUDIES ON THE INTERACTION BETWEEN THE RECEPTOR AND VIRION DURING ATTACHMENT

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Kunjin virus has been shown to use the endocytotic route in the entry into Vero cells. Our studies have also shown that this mode of entry is dependent on the multiplicity of infection. At higher multiplicities (> 20), the pattern of entry seemed to indicate that there was a second, non-specific mode of entry which did not depend on the receptor. In this study, the interaction between the virion and its receptor was considered. In our previous work, we had determined that the receptor was a glycoprotein, but no conclusive information was available on its structure. The use of hexa-peptides to inhibit attachment were not successful. We have determined the ability of amino acids and some sugars (known to be found in glycoproteins) to inhibit virus attachment. Our results showed that there were indeed specific amino acids and sugars which were able to significantly lower the efficiency of virus attachment and adsorption to the host cells. However, the experiments were conducted under different conditions, viz: at 37°C and 4°C, and with the inhibiting solutions mixed with the cells or the virus first before infection of the cells. These showed a marked difference in the list of amino acids and sugars that were able to inhibit attachment and adsorption. These results showed that the interaction between the virions and host cell receptors were multi-factorial and that the process of infection is more complex than had been expected. We are presently in the process of isolating the binding activity (that is, the receptor) from the host cells in order to identify the receptor and its ability to block virus attachment to its host cells.

P11-11

MONOCLONAL ANTI-DENGUE TYPE 3 ANTIBODIES*

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Type specific monoclonal antibodies against the dengue-3 virus were prepared from mouse lymphocyte hybridomas produced by fusion of NS-1 mouse plasmacytoma cells with B-lymphocytes from a mouse immunized with dengue type 3(H87) virus. The fused cells were cultured in the selective (HAT) medium; media from hybrid colonies were screened for antibody activity by using indirect immunofluorescent assay. The positive hybrids were cloned by culturing the cells at limiting dilution in microtiter plates. The subclones were rescreened, most of them produced antibodies specific for dengue-3 antigen, but not dengue-1, dengue-2 and dengue-4 antigens. Type specific monoclonal antibodies are useful for the further study of pathogenesis and serodiagnosis of dengue haemorrhagic fever.

* This work is a part of the project, Application of Monoclonal Antibodies in the study of DHF, studied in 1984.

P11-12

EVALUATION OF JE VACCINES AMONG LAMBAY SCHOOL CHILDREN IN TAIWAN

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Japanese encephalitis (JE) is a major public health problem in Asia, with a case fatality rate of 10-50% and CNS sequela. JE became a notifiable disease in Taiwan since 1955. The nation-wide mass JE immunization program initiated for all 2-yr-old children in 1968 right after a large-scale outbreak of JE occurred in 1967. The 4th dose booster of JE started in 1983 for all grade 1 elementary school children. The incidence rate of JE decreased significantly since 1968 with 0.10, 0.12, 0.08, 0.16 per 100,000 population in 1987, 1988, 1989 and 1990 respectively.

The specific aims of this study are: (1) to quantitate the JEV protective Ab and the duration of its persistence in a Chinese community and (2) to evaluate the JE vaccine efficacy (VE) after different doses of immunization. Two study population were involved: (1) 71 paired sera obtained from the Lambay school children in Feb. 1990 and Jan. 1991 for evaluating the booster effect of the 4th dose of JE vaccine, (2) 55 sera obtained from the Lambay children (aged 2-4 in July 1991) for comparing the JE VE after the 2nd (n = 27) and the 3rd (n = 28) doses. These samples were then quantitated for level of Ab by PRNT with a 70% plaque reduction (titer > 10 for seropositive).

Our preliminary results showed that: (1) seropositivity rates after the 2nd and the 3rd doses were 21% and 93% respectively; (2) the 3rd dose of JE vaccine is crucial for Taiwan children to generate its neutralizing antibody; (3) the 4th dose of JE vaccine has the booster effect on the Ab level but there were about 8% nonresponder rate after the 4th dose in school children; and (4) the JE antibody wanes over time with GMT = 44 (aged 8 right after the 4th dose) and GMT= 8 (aged 15-16). Therefore its persistence and protective effect needs further thorough evaluation.

In conclusions: (1) JE immunization schedule, particularly the interval time between the 2nd and 3rd doses should be changed in Taiwan with the solid epidemiology data; (2) the preparation of JE vaccine in Taiwan should be evaluated by a more comprehensive study because both the seropositivity rate and GMT were not high enough for protection, comparing the data in the U.S.A.

P12-1

HSV IgA SERUM ANTIBODIES IN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE CANCER & IN THEIR HUSBANDS

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The cervical cancer is believed to be a venereal disease and (male factor) plays an important role in its transmission. In addition to other venereal agents e.g. HPV, the synergistic role of latent genital herpes (HSV-2) infection is thought to be important in the genesis of cervical cancer. To gain an insight into the hypothesis, sera were examined from CIN, invasive cancer cases and controls and their husbands for HSV-IgA antibodies*. In cases (women) odds of having HSVIgA antibodies were highest with CIN III (OR = 22.0), followed by invasive carcinoma and CIN I & II (OR = 9.5 & 5.2 resp.). HSV-IgA amongst husbands of cases have significantly high antibody titers as compared to their wives, as well as control women and their husbands. The analysis revealed that HSV-IgA positive husbands whose wife is HSV negative may contribute the risk 16 times to the wives for development of cervical neoplastic lesions. The highest risk of cervical lesion was seen when both spouses were positive for HSV-IgA. The preliminary observation highlights the importance of latent GH infection, as detected by serum HSV-IgA antibodies, in the venereal etiology of cervical cancer.

*By IFA test using HSV-2 infected HEp-2 cells as an antigen.

P12-2

DETECTION OF HUMAN PAPILLOMAVIRUS DNA IN PENILE CARCINOMA IN THAILAND

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Association between specific types of human papillomavirus (HPV) and human genital cancer has been postulated, especially in uterine cervical cancer. Investigation of HPV in male genital cancer has been previously reported but the data are still few. In this study, we examined 5 cases of penile carcinoma in Thai patients to detect HPV DNA. Using Southern blot hybridization with radiolabelling (^{32}P) DNA viral probes (HPV type 11, 16 and 18) under nonstringent condition, all specimens revealed no relation with HPV type 11. With HPV 16/18 probes, one out of five cases demonstrated 2 bands at approximate 4.9 and 1.8 kb. Further study needs to be done with other possible HPV DNA probes.

P13-1

RUBELLA OUTBREAK IN BANGKOK DURING 1989-1991 : A STUDY AT SIRIRAJ HOSPITAL

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This is a report of the incidence of rubella infection at Siriraj Hospital during January 1989 to September 1991. Rubella infection was diagnosed by a four-fold rising of antibody titers using hemagglutination inhibition or the presence of specific IgM using IgM capture test in paired serum specimens. Each year, the number of the cases started to increase in August, and reached its peak in March and then declined. It was found that there was a small rubella outbreak in 1990-1991. At the beginning of the outbreak, 21.7% (13/60) of cases in September 1990 was compared to 9.9% (7/71) of cases in September 1989. The number of rubella cases in March, 32.3% (60/186) of cases in 1991 was remarkedly higher than 20% (18/90) of cases in 1989 and 11.3% (12/106) of cases in 1990. The findings indicate that inspite the effective rubella vaccination in some population, rubella is still a problem in Thailand.

P13-2

AN IgM ANTIBODY CAPTURE TEST FOR RUBELLA USING ANTIGEN COATED LATEX

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INTRODUCTION

Test for IgM specific antibody to rubella is useful for diagnosis of rubella infection. The commonly used techniques are based on hemaggluti- nation, hemadsorption, and enzyme linked immunosorbent assay (ELISA). The first two methods are inexpensive but strict quality control and experience are essential for reproducing the results. Regardless of thecost, ELISA test for IgM is well accepted worldwide. We have introduced a rubella IgM antibody capture assay using commercial coated latex as an indicator.

METHODS

Anti-human IgM were coated to microtiter plate. Sera were diluted, incubated and washed. Suspension of 1:50 dilution of polystyrene latex particle coated with rubella antigen was added. The plate was left undisturbed overnight at room temperature and result was read. Positive reaction was shown clearly as mat of latex particle adsorb to the bottom of the well. Negative reaction was shown as a small white button. The test was evaluated with a commercial antibody captured ELISA kit for rubella IgM.

RESULTS

The test compared superbly with the ELISA test for rubella IgM *(Spearman's rank correlation coefficient = 0.9, $p < 0.001$)*. Forty four positive sera by IgM ELISA were also positive by the IgM latex method. Five of the sera came from congenital rubella infants. There were 8 paired sera from acute apparent rubella infection which showed rising IgM antibody to rubella. The rubella IgM were detectable within 1-3 days from onset of rash as determined by both the IgM ELISA and IgM latex methods. In evaluation of specificity, 134 sera from pregnant women and 20 rheumatoid factor positive sera were all negative by the IgM latex.

DISCUSSION

The IgM antibody capture latex agglutination test was not as rapid as the ELISA which could be finished within a working day. However, the new test was very simple and requiring just two main steps. It did not require laborious treatment of test serum. The reagent did not contain IgG conjugate which made it free from possible interference by rheumatoid factor. With its simplified technique and reasonable cost, the test is suitable for large scale screening of rubella IgM antibody.

P14-1

NOVEL ANTIVIRAL COMPOUNDS FROM MARINE SOURCES

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Novel compounds extracted from marine invertebrates, with demonstrated antiviral, antibacterial and/or antitumour activity, have been investigated for their spectrum of antiviral activity. Viral replication in cell cultures was demonstrated by immunofluorescence using specific monoclonal antibodies. Mycalamide A and Mycalamide B, extracted from a *Mycale* species of sponge, showed strong inhibitory action at low concentrations against Herpes simplex virus type 1 (HSV-1) [A-2x 10^{-5}mg/ml, B-4x10^{-7} mg/ml], compared to Acyclovir [0.01 mg/ml] for 90% inhibition of virus replication. No cytotoxicity was observed at these concentrations. Activity against Cytomegalovirus (CMV) was similar [A - 5 x 10^{-5} mg/ml, B - 5 x 10^{-6} mg/ml] compared with Ganciclovir [1 mg/ml] for 90% inhibition of replication. Two further compounds PNI 62.4 and NP15 27.2, both from Southern Ocean species of sponges, inhibited HSV-1 at 5 x 10^{-4} and 8 x 10^{-4} mg/ml respectively. No cytotoxicity was observed with compound NP while some was with compound PNI. None of the compounds have shown any activity against Respiratory syncytial virus.

P14-2

AN EXPERIMENT FOR DEVELOPMENT OF A BIOTECHNOLOGICAL TREATMENT OF SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE) SELECTIVE KILLING OF SSPE VIRUS-INFECTED CELLS BY LIPOSOMES CONTAINING FRAGMENT A OF DIPHTHERIA TOXIN

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Subacute sclerosing panencephalitis (SSPE) is one of slow virus infections. Persistent infection in the central nervous system (CNS) by a defective mutant of measles virus is the cause of SSPE. The measles virus (SSPE virus) does not produce infectious virus particles. Infection spreads by cell-to-cell transmission of viral genomes in the CNS. Diphtheria toxin fragment A (FA) has NAD:EF-2 ADP-ribosyl transferase activity and only one molecule of FA can stop all protein synthesis in the cell. We adopted liposomes containing FA to destroy SSPE virus-infected cells selectively.

Golden hamsters (5-week old) infected intracerebrally with SSPE virus-infected cells developed neurological symptoms such as hyperactivity, myoclonus and generalized seizures around 7 days after infection. Large amount (2 ml) administration of the liposomes directly into the brain of infected hamsters using a peristaltic pump at the rate of 1-2 ul/min during the incubation period reduced the mortality rate to half its control rate.

P 14-3

AMPHOTERICIN B EFFECT ON SCRAPIE DEPENDS ON INJECTION SITE AND HOST SPECIES

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Scrapie agent is a fatal progressive neurological disease in sheep and is similar to the transmissible agent causing certain dementias in humans such as Kuru and Creutzfeldt-Jakob disease. It was reported that Amphotericin B (AmB) increased the incubation period of 263K strain of scrapie injected hamsters by 45 days. We therefore studied the efficacy of AmB by determining the incubation period and two different species (hamsters and mice) injected three different brain areas (cerebellum, hypothalamus, cortex) by stereotaxic method. We also determined the effect of AmB on incubation period and amyloid plaques formation in IM mice brains inoculated with the 87V scrapie strain. Our study shows that AmB extends the incubation period of only 263K injected hamsters by 15 days rather than 45 days. In particular, AmB effect on scrapie infected hamsters depends on injection site. The incubation period for the AmB treatment was significantly longer than PBS treatment in hypothalamus and cortex injection group but not in cerebellum. However, there was no effect of AmB on scrapie infected mice. These findings suggested that AmB effect on scrapie infected animals depends on injection site and host species.

P14-4

DIMINISHED RHINOVIRUS (RV) ILLNESS SEVERITY CORRELATES WITH INCREASED LEUKOCYTE ASCORBIC ACID (AA) LEVELS

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[2]University of Wisconsin, Madison, WI and

In three separate double-blind placebo-controlled trials, use of AA supplements (500 mg, q.i.d.) significantly reduced symptoms ($p < 0.001$) and signs [total mucous weight (MW), $p = 0.028$; coughs, $p = 0.046$] of illness in human volunteers ($n = 24$) naturally exposed [J. Infect. Dis. 150 : 195 (1984); 156:442 (1978)] to RV type 16 (RV 16). It was also found that individuals with greater levels of AA in their sera had significantly ($p = 0.009$) milder illness. However, since leukocyte AA levels are more representative of total body AA stores, the relationship of these levels to illness severity is likely of greater relevance and, thus, was analyzed thoroughly. In each of the above trials, 16 men free of RV16 antibody were given AA ($n = 8$) or placebo with laboratory-induced RV16 colds. Blood was obtained weekly and mixed leukocytes (ML) or mononuclear (MONO) and granulocytic (GRAN) cells were purified for intracellular AA quantitation. Each day, symptom and sign severity scores were logged, nasal washings quantitated for virus, and used tissues collected for MW measurements (trial 3 only). When data from trials 1 and 2 were combined, a significant ($p = 0.025$, $n = 32$, Spearman's rank correlation with Fisher method for combining results) inverse correlation between symptom severity scores and MLAA levels was found. This relationship was examined especially thoroughly in trial 3. Significant inverse correlations were found between symptom severity scores and AA levels in ML ($p = 0.032$) as well as GRAN ($p = 0.037$) and MONO ($p = 0.051$) cells. Total MWs were also significantly inversely correlated with ML ($p = 0.002$), GRAN ($p = 0.026$), and MONO ($p = 0.016$) AA levels. Surprisingly, there were no differences in any trial in the quantity and duration of virus shedding between AA and placebo recipients, nor was there any relationship between virus shedding and leukocyte AA levels. These results suggest that the amelioration of RV16 illness by AA supplementation is related to increased leukocyte AA levels but that AA may not be directly involved in those cellular immune functions related to virus clearance.

P14-5

REPLICATION OF HUMAN INFLUENZA VIRUS STRAINS IN VARIOUS ORGANS OF MICE AND LACK OF EFFECT OF VITAMIN C ON REPLICATION

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Earlier studies in our laboratory demonstrated the replication of A_2 Hong Kong and B strain of influenza virus in lungs and testes of mice infected intraperitoneally (IP) or intranasally (IN).

This communication deals with the comparative study on the ability of the mouse adapted neurotropic (WSN) and the non-neurotropic recombinant X-31 (human vaccine) strains of influenza virus to infect and replicate in various organs of mice which were inoculated IP, a finding which has epidemiological and pathological implications.

Ascorbic acid or vitamin C is widely used as prophylactic and therapeutic agent against the common cold caused by viruses as in the case of influenza virus. Therefore, the effect of vitamin C on the infectivity and replication of influenza virus in mice and chicken embryonated eggs was investigated and found no effect of this vitamin on virus replication.

P14-6

INTERFERON THERAPY IN PATIENTS WITH CHRONIC VIRAL HEPATITIS C : TRIAL FOR ONE YEAR

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In this study the authors have kept 27 patients affected by chronic hepatitis C under observation for one year. These subjects were divided into three groups : 9 of them underwent therapy with interferon-beta with a posology of 3,000,000 U, one for 3 times a week; 9 of them were treated with recombinant interferon-alfa-2b administered with phials of 3,000,000 U, one for 3 times a week; 9 of them, observed as controls, were not treated with interferon. The anti-HCV antibodies in ELISA and in RIBA in patients before, during and after treatment, the autoantibodies in IFA, and some hematochemic parameters (haemochrome, platelets,transaminase and so on) were evaluated. As an instrumental study,the hepatic echography and in some cases, even the hepatic biopsy and esophagogastroscopy were carried out. The purpose of this study has been to evaluate the efficacy of the therapy in patients treated and the untreated ones and the eventual differences between the treatments with two types of interferon.

P14-7

RETROVIRAL REVERSE TRANSCRIPTASE INHIBITORY ACTIVITY IN THAI HERBS AND SPICES

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Fifty-seven Thai herbs and spices were examined for their retroviral reverse transcriptase inhibitory activity. All herbs and spices were extracted with hot-water and methanol. Reverse transcriptase inhibitory activity of the extracts was determined by using Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV-RTase) reacted with ^{3}H-dTTP and measured radioactivity with scintillation counter. Eighty-one per cent (46/57) of hot-water extracts and 54% (31/57) of methanol extracts showed inhibitory activities. Thirteen per cent (6/46) of hot-water extracts, namely *Eugenia caryophyllus* Bullrock ex Harrison (100%), *Phyllanthus urinaria* Linn (91%), *Terminalia belerica* Rxb (75%), *Nelumbo nucifera* Gaertn (74%), *Psidium guajava* Linn (61%) and *Lawsonia inermis* Linn (60%), had the relative inhibitory ratio (IR) over 50%. Only 10% (3/31) of methanol extracts from *T. belerica* (83%), *E caryophyllus* (54%) and *N. nucifera* (54%) had IR value over 50%.

LIST OF CHAIRPERSONS

AUSTRALIA
AASKOV, JOHN G.
FENNER, FRANK
HOLMES, IAN H.
McKENZIE, JOHN S.

CHINA
JIANG, YUTU
YUNDE, HOU

FRANCE
CHERMAN, JEAN-CLAUDE
HANNOUN, CLAUDE M.
PARKIN, D.M.

GERMANY
HAUSEN, HARALD ZUR
WOLF, HANS J.

HONG KONG
SHORTRIDGE, KENNEDY F.

INDIA
BANERJEE, KALYAN

INDONESIA
WURYADI, SUHARYONO

JAPAN
NEROME, KUNIAKI
TAKAHASHI, MICHIAKI
YAMANISHI, KOICHI

KOREA
LEE, HO WANG

MALAYSIA
LAM, S.K.

MYANMAR
THIEN, SOE

PHILIPPINES
TUPASI, THELMA E.

SINGAPORE
CHEONG, CHAN YOW
LING, AI EE
NG, MAH-LEE

SRI LANKA
VITARANA, TISSA

SWEDEN
GRANDIEN, MONICA

SWITZERLAND
GHENDON, YURI

TAIWAN
LIU, WU-TSE

THAILAND
BOONYAPRAKOB, URABALA
BUKKAVESA, SOMBODHI
CHAINUVATTI, TERMCHAI
CHUMDERMPADETSUK, SOAWANEE
JAYAVASU, CHUINRUDEE
KIDSON, CHEV
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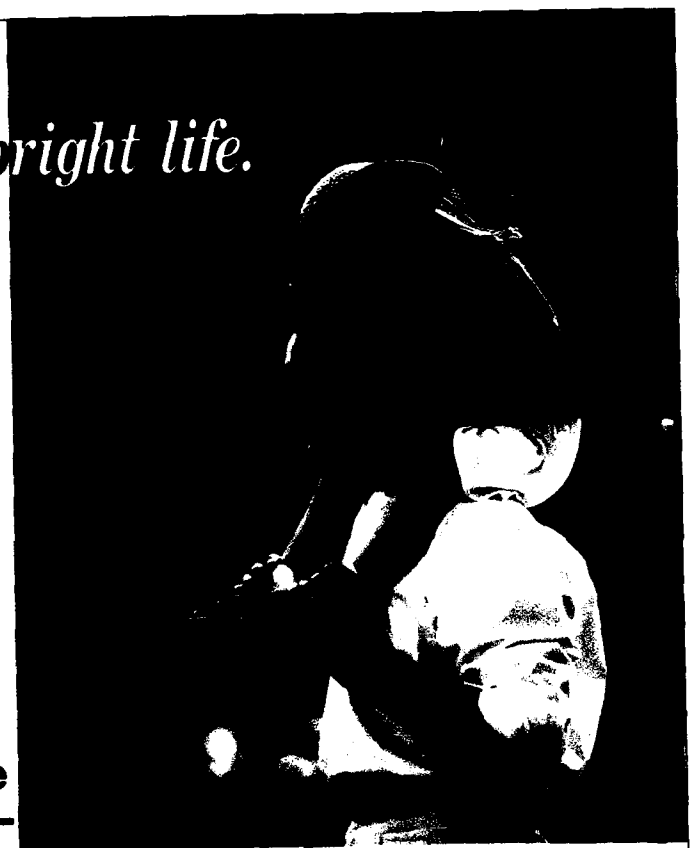

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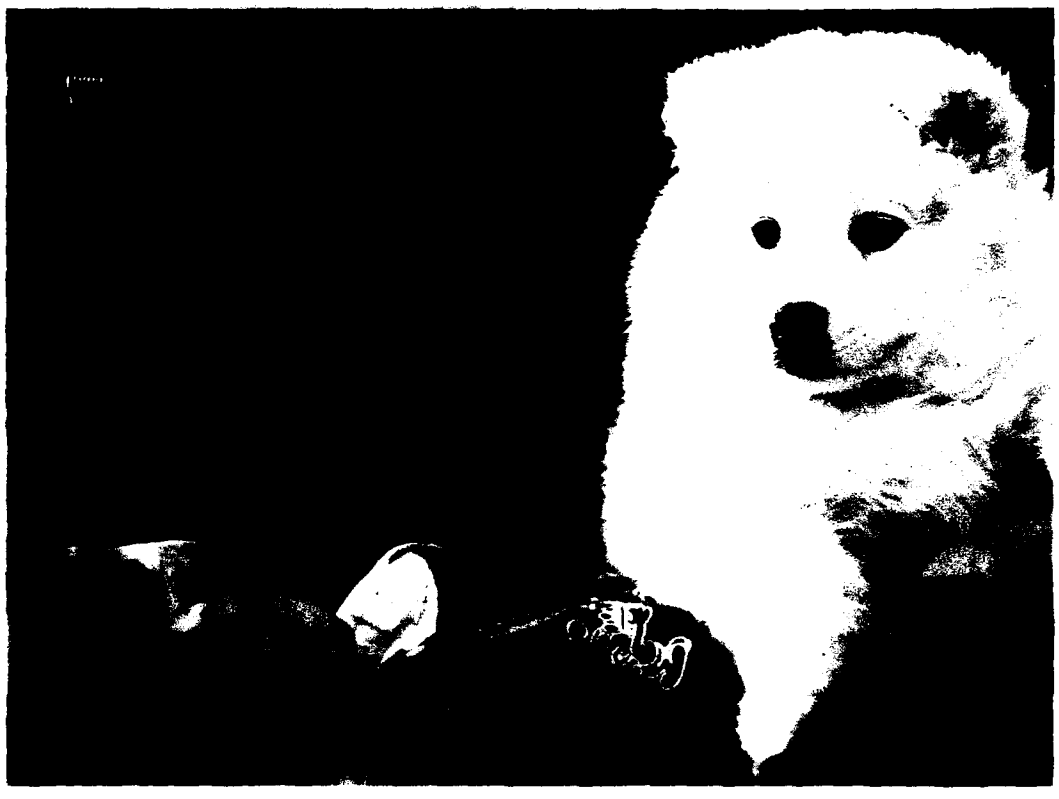

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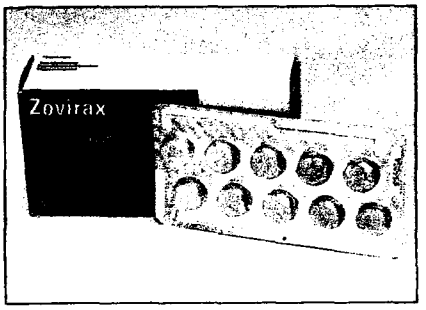

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REFERENCES
1. Data on file, The Wellcome Foundation Ltd., 1990.
2. Huff JC, Bean B, Balfour HH Jr, et al Therapy of herpes zoster with oral acyclovir. Am J. Med. 1988 ; 85 (suppl 2 A) : 84-89.
3. Morton P, Thomson AN. Oral acyclovir in the treatment of herpes zoster in general practice. NZ Med J. 1989 ; 102 : 93-95.

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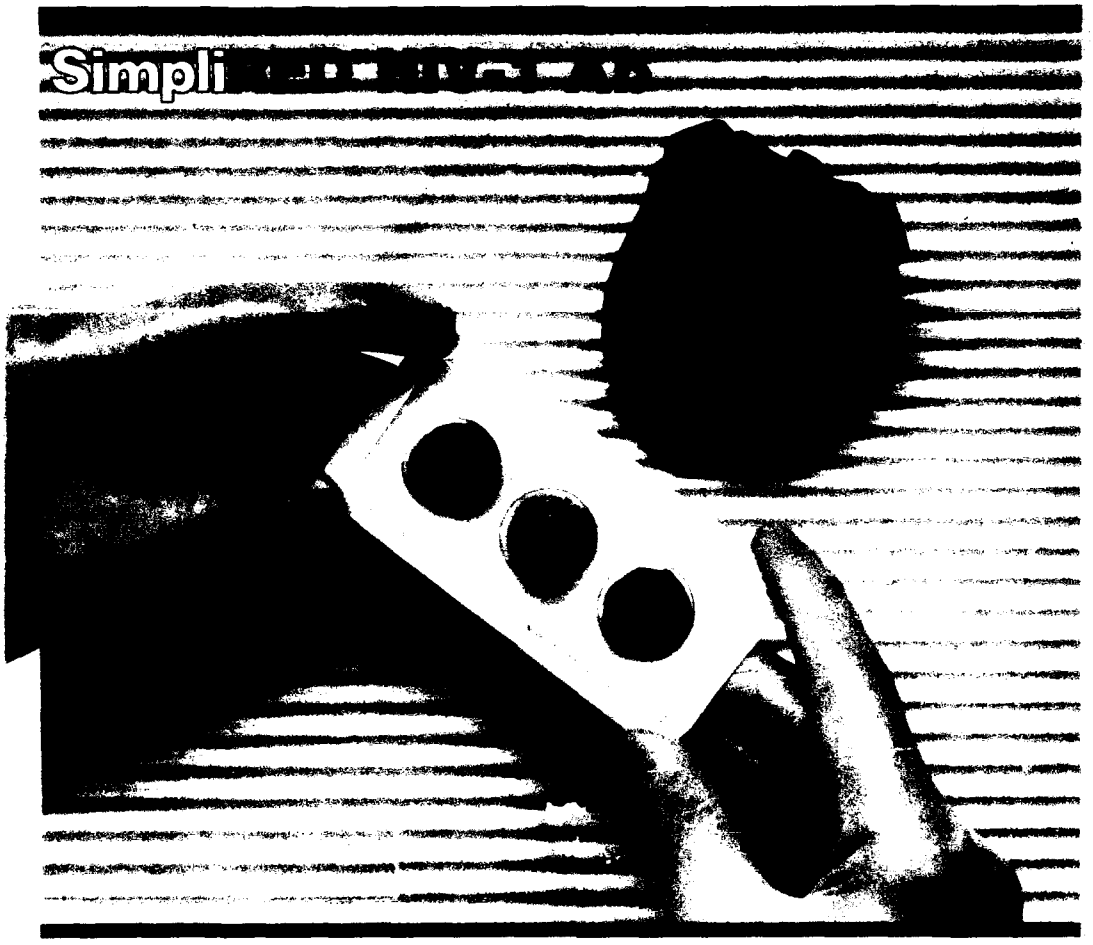

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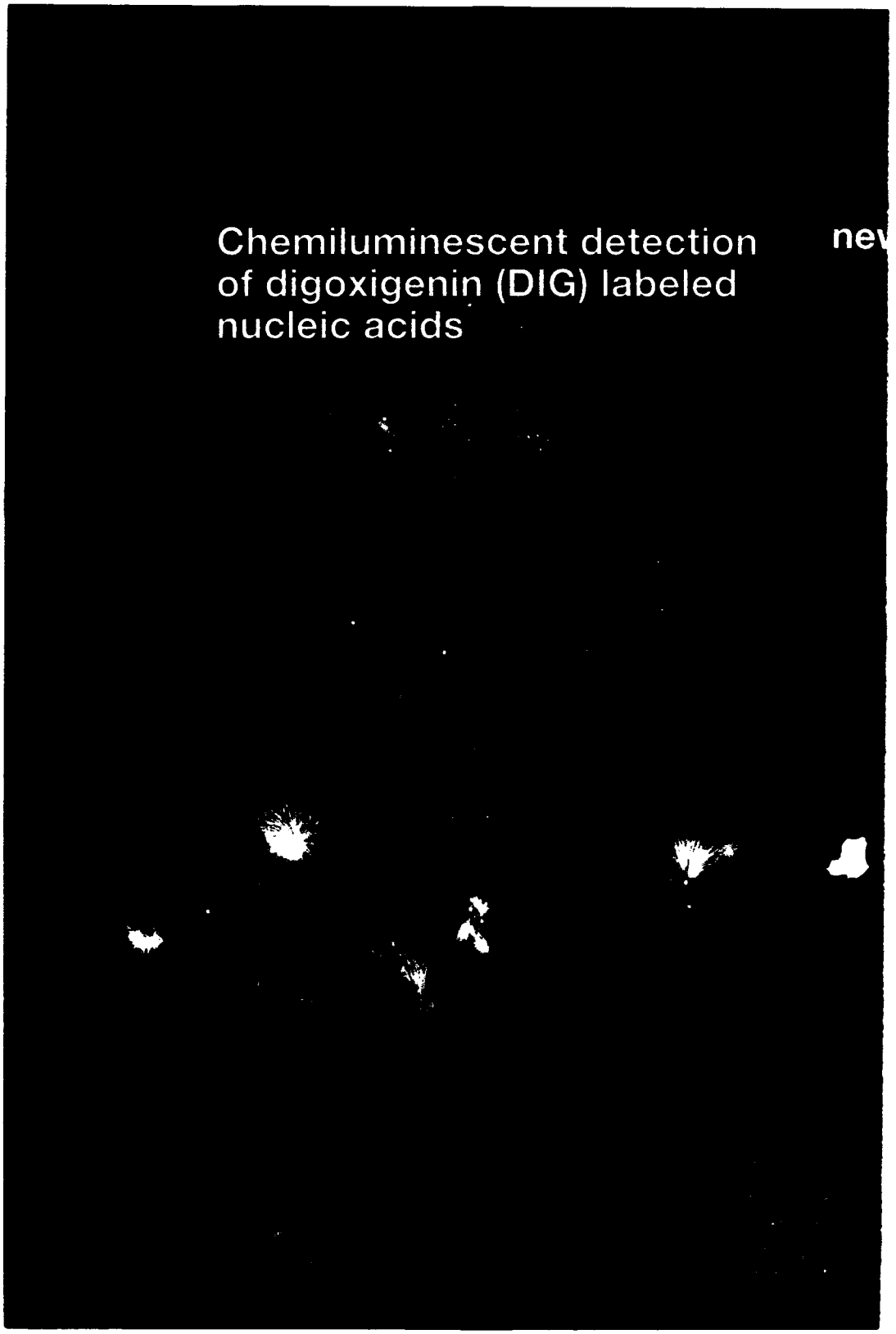
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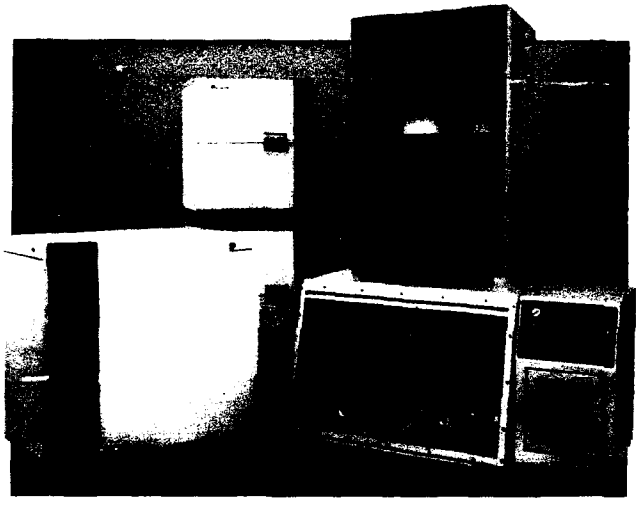

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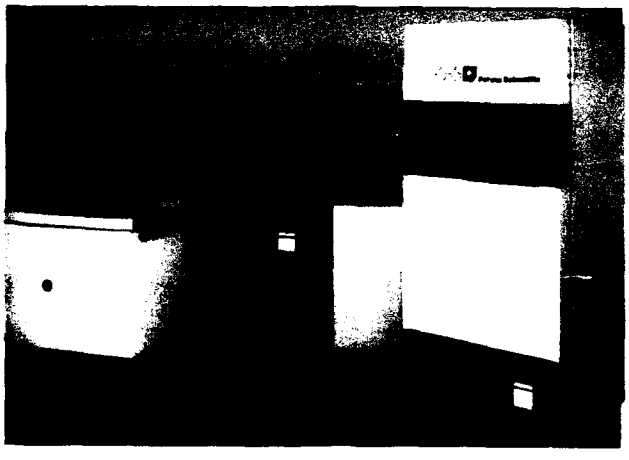

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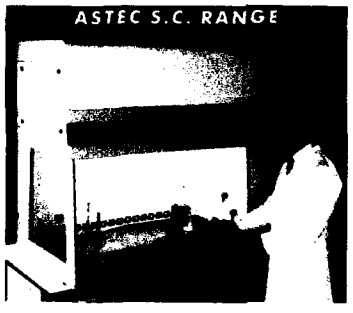

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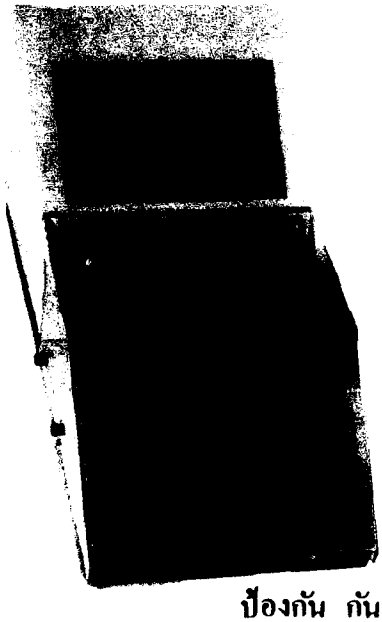

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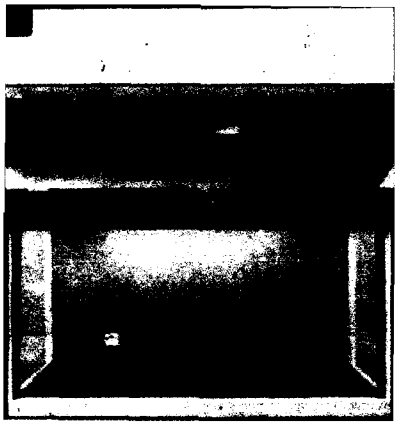

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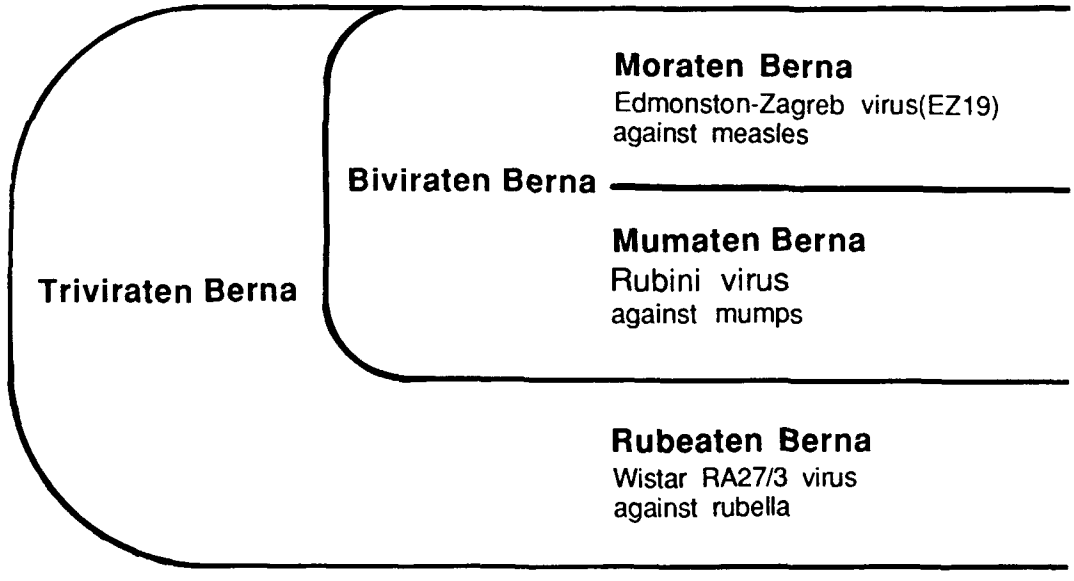
32. Swiss Serum and Vaccine Institute Berne
33. TAT Duty Free Co., Ltd.
34. Thai Airways International Ltd.
35. The Thai Military Bank Ltd.
36. Thai Nakorn Patana Co.,Ltd.
37. The Third World Academy of Sciences
38. Tourism Authority of Thailand
39. United Nation Children Fund
40. Union Drug Laboratories Ltd.
41. Wellchem Pharmaceutical Co.,Ltd.
42. Wellcome Thailand Ltd.
43. World Health Organization under by project THA DPC 001
44. World Travel Service Ltd.

BERNA vaccines
against measles, mumps and rubella



The vaccines are produced on a human diploid cell line (HDC);
they do not contain antibiotic additives nor protein of animal origin

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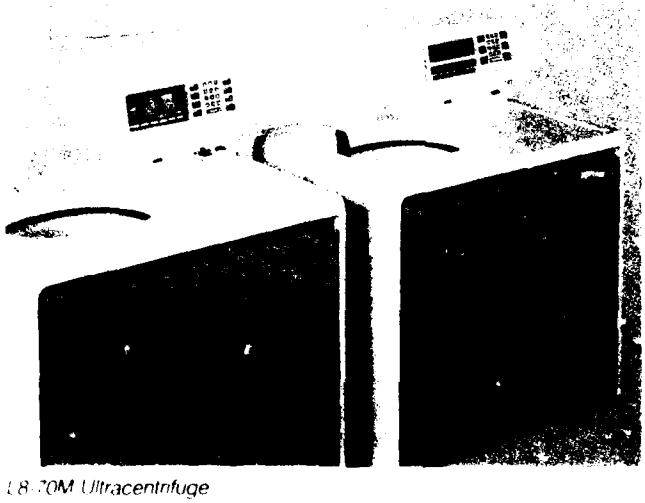
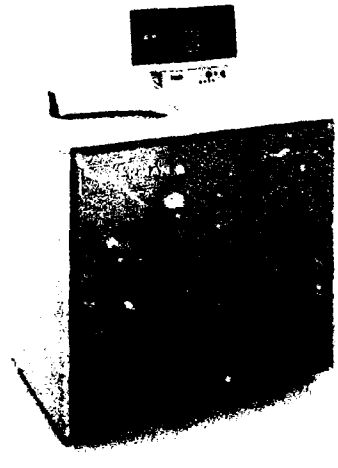


L8-70M Ultracentrifuge



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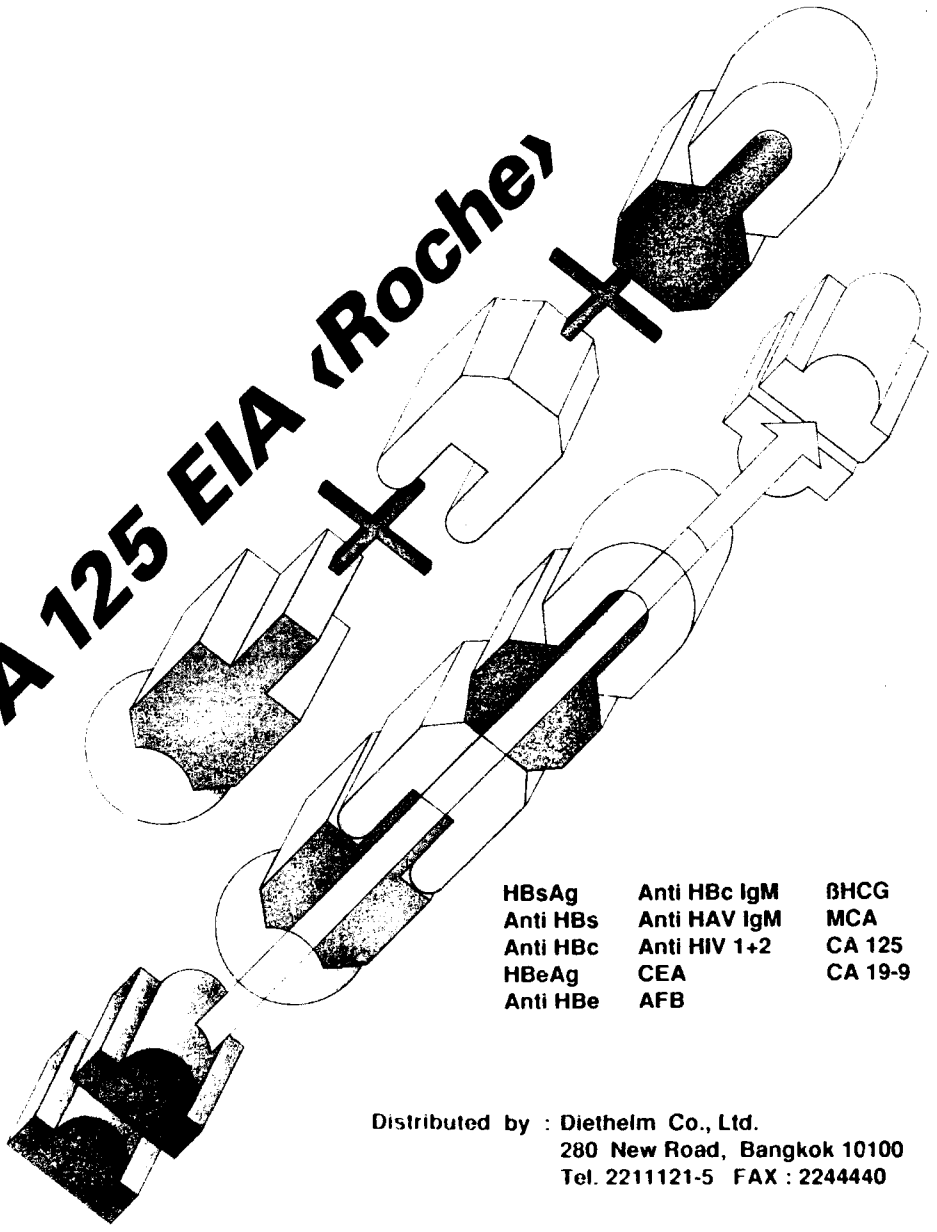

HBsAg	Anti HBc IgM	ßHCG
Anti HBs	Anti HAV IgM	MCA
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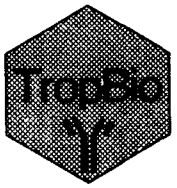

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Canine parvovirus

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Alphaviruses (Ross River, Sindbis, Getah and Barmah Forest)
Canine parvovirus
Newcastle disease virus

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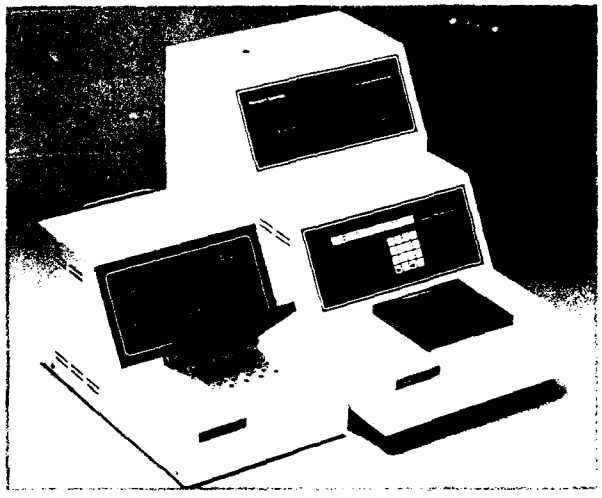
Precision Scientific

Model GTC-1 and GTC-2 Genetic Thermal Cycling Systems



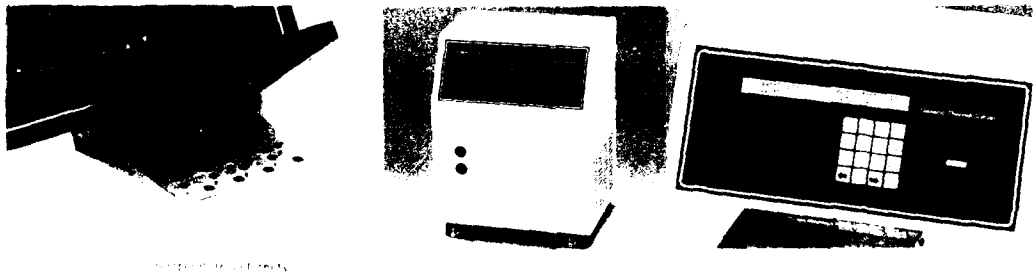
- *Choice of 2 microprocessor controlled cycler models and a low temperature cooling module*
- *Machined aluminum sample block holds forty eight 0.5 ml microfuge tubes.*
- *Microprocessor control is programmable for multiple temperature steps or programs*
- *0-100° temperature range with external cooler, ambient+100°C with tap water cooling.*
- *Temperature uniformity is ±0.5°C with 1.0°C per second heat up rate.*
- *GTC-2 features an RS232 port for printer interface and an analog output for chart recorder hook-up*

Genetic Thermal Cycling Systems from Precision Scientific are used for DNA and gene amplification, enzyme digesting, enzyme catalysis, DNA denaturing, and other temperature cycling of biological matter.

The GTC System is comprised of a choice of two cycler models and a low temperature cooling module. GTC-1 is the base cycler model and is ideal for routine applications in research or teaching labs. GTC-2 is the deluxe version with a more powerful microprocessor, programming keypad, alpha/numeric LCD display, RS232 interface, programmable autoextension feature that allows you to extend soak points, and much more. The low temperature cooling module can be used with either model and will accommodate more than one cycler unit (either GTC-1 or GTC-2) so you can economically expand capacity in the future. It can even be placed in a remote location to preserve valuable benchtop space.

Both cycler models feature menu-driven programmable microprocessor control capable of running multiple temperature steps and/or programs. Each program can be cycled or repeated thousands of times, resulting in a geometric increase in sample yield. Programs can be stored in the controller and memory is protected from power loss by a lithium battery backup.

Reduce amplification time, increase yield, and maintain more consistent and reproducible results with a GTC System from Precision Scientific. We've been making high quality temperature control products for over 70 years.



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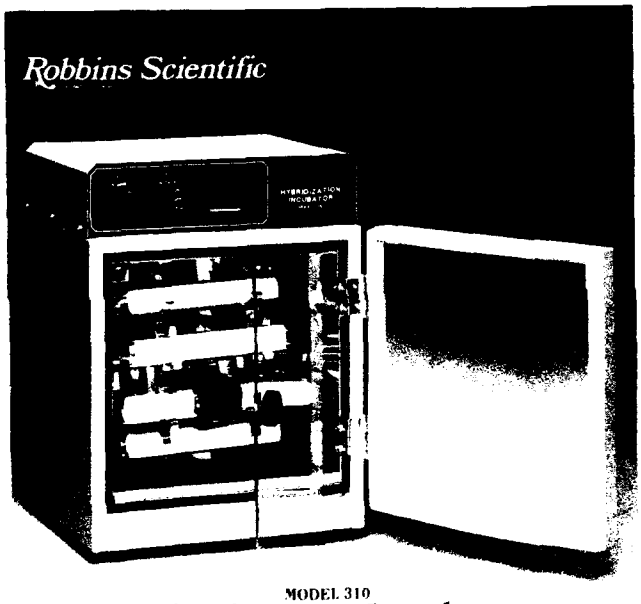
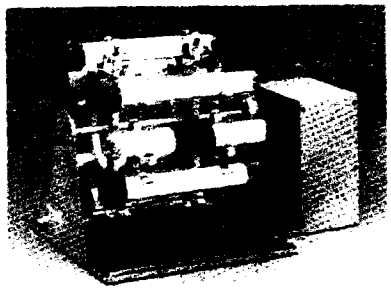
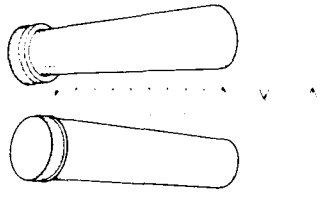


MODEL 310
Hybridization Incubator





The tri-directional motion of the rotator moves the fluid back and forth around the inside of the glass tubes and assures that the blots are uniformly wetted with a continuously moving thin film of probe solution.

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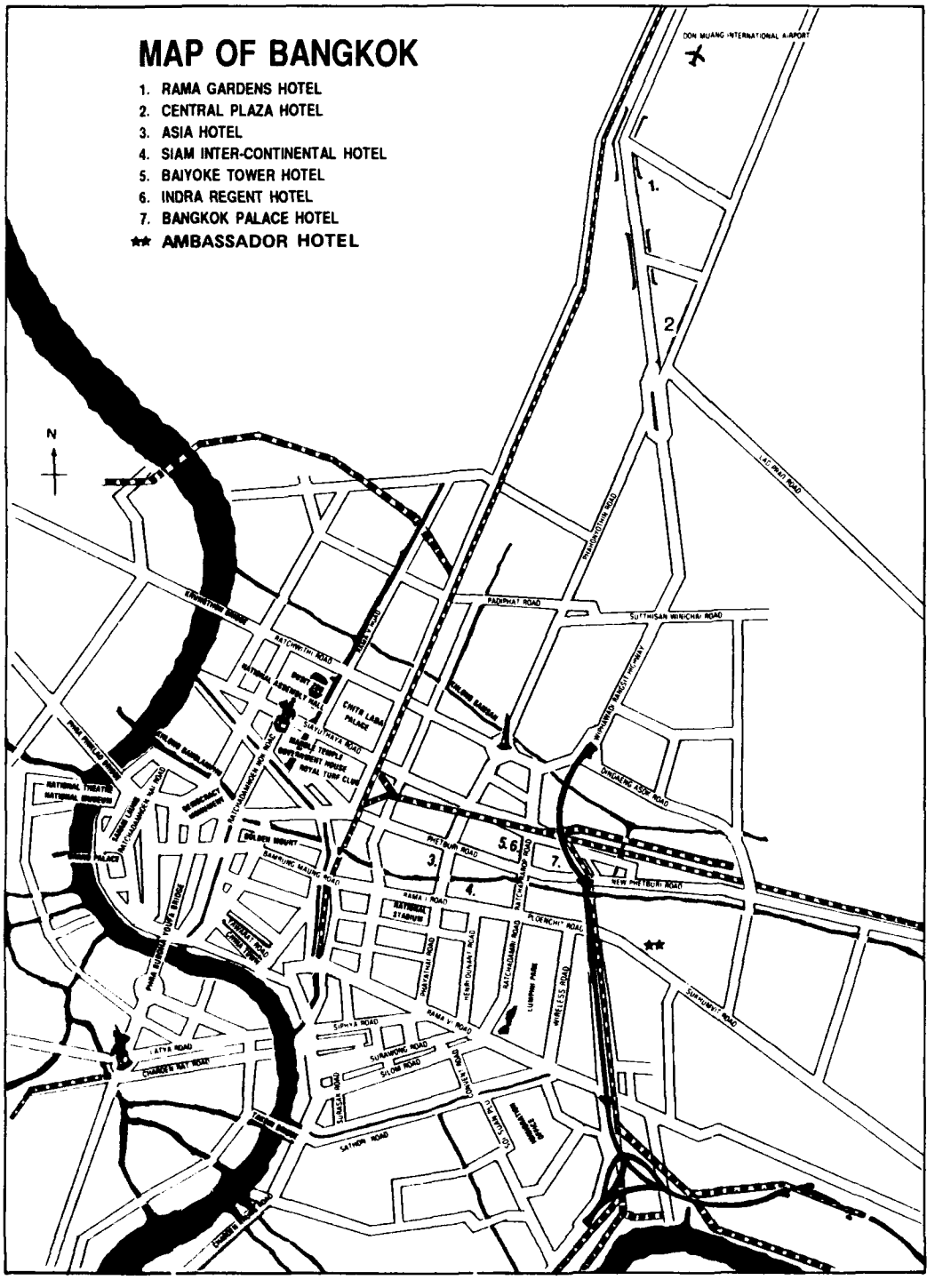
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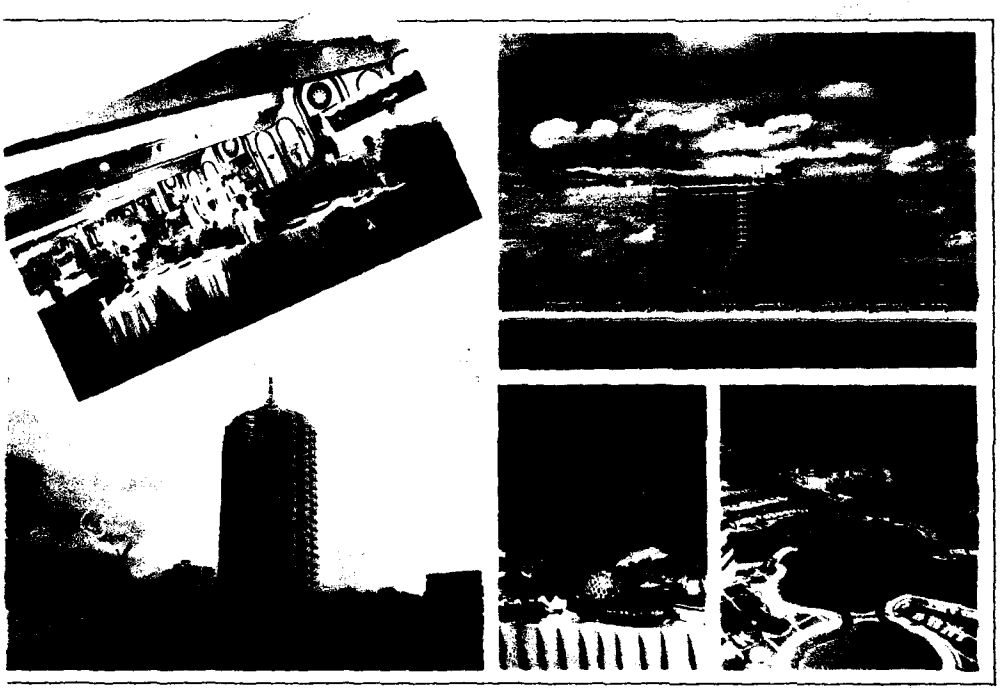

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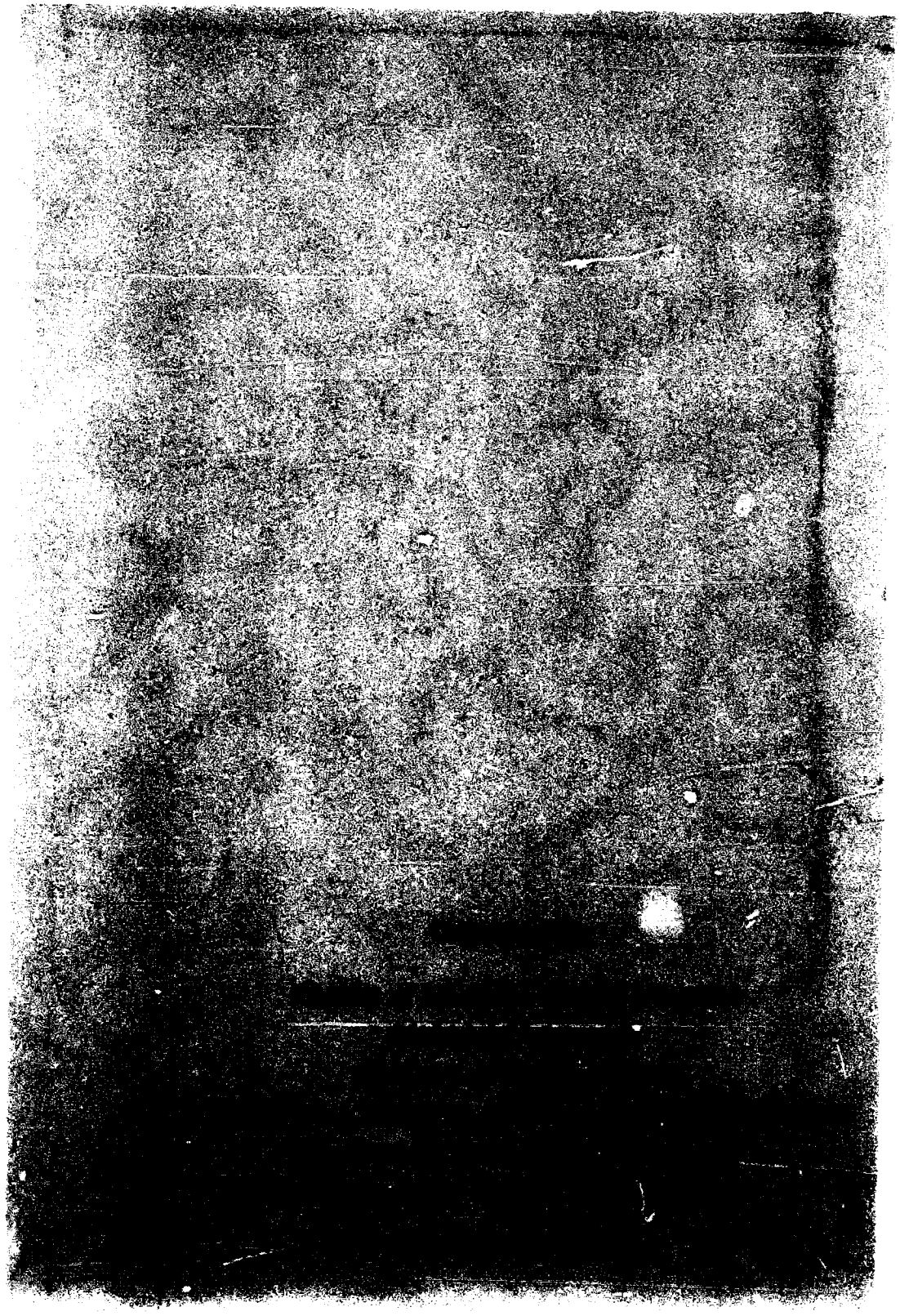
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