GDEST China-US Workshop

The Genomics Revolution: New Tools for Combating Infectious Diseases

Workshop Abstracts and Biographies

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Panel I: Surveillance and Detection

Practical Tools to Combat Infectious Diseases in the Developing World: A Model of Technology Transfer That Works

By Maria Elena Peñaranda

Sustainable Sciences Institute (SSI) is an international non-profit organization that works to improve public health in developing countries by helping local scientists gain access to the resources needed to combat infectious diseases in their communities. Through on-site training workshops, small grants for research, donations of equipment and supplies, and long-term mentoring and scientific advice, we help transfer modern laboratory and epidemiologic technologies and build public health capacity in underserved areas. We know from 17 years of experience that empowering local scientists in developing countries hastens improvements in the health outlook of these nations.

Infectious diseases still account for nearly one in two deaths in the developing world, while this figure is one in 10 in industrialized countries. At the same time, The Global Forum for Health Research reports that only 10% of global funding for health research and development is directed to the problems that affect the poorest 90% of the world's population.

SSI aims to help bridge the gap between resource availability in developing countries and resource need in the developed world. According the Genomics and World Health Report of October 2002, SSI has pioneered an effective model: "The work of SSI has demonstrated how the techniques of modern molecular biology may be readily transferred and adapted to local conditions in developing countries and harnessed to address local priorities for improving public health."

SSI's Model

SSI uses a five-tiered approach to build scientific capacity in developing countries:

Our On-Site Training Program gives public health professionals tools to fight diseases locally through workshops on disease diagnosis and characterization, laboratory epidemiology, manuscript- and grant-writing.

Our Small Grants Program provides seed funding for these same scientists to develop and implement their own locally generated research projects and thus improve the health of their communities.

Our Material Aid Program facilitates the donation of equipment and supplies to laboratories in

developing countries from biomedical corporations and universities in the US and other industrialized countries.

Our Networking & Consulting Program expands scientific networks worldwide and provides technical advice and mentoring for developing country researchers. This provides a venue for collaborations and scientific and cultural exchange that are rare in professional settings.

Our more recently developed Centers of Excellence Program focuses long-term support in countries with high disease burdens, where local scientists are committed to conducting research projects that have the potential to help solve their community's health problems.

SSI's future ventures in low cost technology:

In collaboration with scientists at the University of California, Berkeley, we are working to adapt cutting-edge technologies to the realities of developing world laboratories. One of these projects involves the development of a novel low-cost diagnostic platform. As a potential rapid, point-of-care tool for early detection of infection, the ImmunoSensor (IS) could help scientists and public health professionals better manage infectious diseases in their communities.

The IS's functions are based on Micro-Electro-Mechanical-Systems (MEMS) technology, which uses integrated circuit (IC) processing techniques to create sensors on a sub-micron scale. The IC chip surface is modified with a gold overlay to allow efficient interaction with biological molecules (e.g. antibody, antigen, DNA) that determine the disease specificity The ImmunoSensor detects the target with reagent-specific magnetic beads, which create a micron-scale magnetic field that interacts with the magnetic sensor in the chip. This information is then transmitted to a hand-held device, such as a PDA, as easily-interpretable numeric results. SSI's colaborators are currently adapting the ImmunoSensor technology to detect the genetic material of infectious agents and developing prototype devices for dengue and HIV diagnosis.

Intelectual Property:

In the formative stages of the ImmunoSensor project, SSI negotiated a "Development Agreement" with the University of California at Berkeley which credits this collaboration with SSI as being the progenitor of the University's socially-responsible licensing initiative, a concept that is now gaining popularity in the academic world. "Socially responsible licensing" aims to promote global availability of technologies invented at universities through royalty-free agreements, revenue-sharing, and other mechanisms. The goal is not to maximize revenues but rather to contribute to the public good. Usual

license agreements of university-held patents can be negotiated with companies in the “for-profit world” while allowing the therapies and diagnostics to be made available at-cost in developing countries.

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Dr. Maria Elena Peñaranda is the Scientific Director of Sustainable Sciences Institute (SSI), a non-profit organization dedicated to helping scientist in developing countries improve public health in their communities. Dr. Peñaranda is responsible for organizing technology transfer workshops that provide education and training; facilitating donations of equipment and supplies; and supervising the making of small research grants to help local scientists in developing countries combat infectious diseases.

Dr. Peñaranda started her professional training at the University of Costa Rica as a Clinical Microbiologist and in 1978 received a Master’s degree, studying antibiotic resistant plasmids in enterobacteria. In 1983, she received her Ph.D. from the University of Texas, Houston for her work to characterize plasmids mediated virulence factors such as the colonization factor agents CFA-I and CFA-II from enterotoxigenic E.coli. During her postdoctoral training at the Virology Department at Baylor College of Medicine (1983-1986), Dr. Peñaranda studied the molecular biology of rotavirus, cloned and expressed the dsRNA viral genes into eukaryotic and baculovirus vectors, and performed microinjection of dsRNA, in order to rescue viral reasortants by genetic manipulation.

Subsequently, Dr. Peñaranda worked at the Centers for Disease Control studying the epidemiology of infant rotavirus and of other adult diarrhea rotavirus that caused large epidemics in China (Rotavirus B and C). Dr. Peñaranda worked at the Oral AIDS Center of the University of California, San Francisco for 10 years, studying viral opportunistic infections in HIV-positive individuals, primarily Herpesvirus, such as EBV and human papilloma virus (HPV). Her work focused on the molecular mechanisms of pathogenesis of EBV and HPV from oral lesions present in HIV-positive individuals

such as hairy leukoplakia, oral cancer and Kaposi's sarcoma. Dr. Peñaranda has published articles throughout her carrier in numerous scientific journals on the molecular biology and epidemiology of infectious agents.

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Microarray-based Methods for Detecting and Tracing Bacterial Pathogens

By Ruifu Yang

Advances in omics sciences provided great opportunities to develop microarray-based methods, including DNA and protein microarrays, for detecting, identifying and tracing bacterial pathogens. The complete sequencing of a good many of bacterial genomes allows us to transform from the traditional one gene or protein study activity to a world in which whole organelles and pathways are studied simultaneously. For counteracting the bacterial infectious diseases, rapid detection and recognition of the pathogen is prerequisite for timely control of the disease. Traditionally we use one method, such as ELISA, IFA and PCR, for detecting one pathogen, however, with the great advances in genome world we can detect different pathogens or different antibiotic resistant genes in one assay by using microarray-based techniques, which is our expected way for long to diagnose infectious diseases or other kinds of diseases, including cancers.

DNA microarray has been developed for detecting bacterial pathogens and their antibiotic resistant genes and protein microarray for profiling antibodies in the patient's serum. In our lab we have developed a microarray for identifying 80 medically important bacterial pathogens and a microarray for detecting, identifying and tracing Yersinia pestis.

Here we present the development a genetic polymorphism database and the gene chip technology for tracking Y. pestis. It is becoming increasingly clear that bacterial genome constantly undergoes structural changes due to horizontal gene acquisition, gene loss, intra-genome recombination, and mutational events. Investigation on genetic diversity based on these mechanisms will lead to a whole picture of genomic diversity to microevolution of Y. pestis during its focus expansion. In our laboratory we used synonymous SNPs and pseudogene profile to investigate the mutational events; DNA rearrangement profile for intra-genome recombination, whole genome microarray hybridization for horizontal gene acquisition and gene loss and VNTR and CRISPR analysis for minute variations of small repeat sequences. These data

provided us an opportunity to analyze the genetic diversity of Chinese Y. pestis during its focus expansion and found the relationship between the genome structural changes, key metabolic genes' mutation and microevolution.

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Graduated from Hebei Medical University in 1985 for his Bachelor degree of Sciences, from Academy of Military Medical Sciences in 1998 and 2002 for his Master and Ph.D. degree, he is currently a professor in Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, China.

His major research areas cover rapid detection and identification by molecular methods, including DNA and protein microarray, and biosensor; development of DNA, fatty acids and mass fingerprinting-based databases for microbial forensics, and bacterial genomics and pathogenesis. The new techniques for detection and identification of pathogenic bacteria developed in his laboratory include DNA microarray for identification of 80 medically important bacteria, protein microarray for antibody profiling for plague bacteria and SARS-CoV, the optic biosensor for sensitive detection of Y. pestis, anthrax spore and botulinum toxin, and up-converting phosphor technology-based biosensor for field detection of Y. pestis, anthrax spore, SARS-CoV and E.coli O157:H7. His group also analyzed the genetic polymorphisms of Y. pestis isolated in China and developed the DNA polymorphisms database for tracing the source of the bacteria. A microarray was also developed for detection, identification and tracking of Y. pestis. Through collaborating with Beijing Genomics Institute (BGI), Chinese Academy of Sciences, his group has finished whole genome sequencing of a human-avirulent strain of Y. pestis and a new biovar, microtus, was proposed by detail comparative genomics study. The transcriptional regulation of pathogenesis of Y. pestis was their active research aiming at screening vaccine candidates and diagnostic markers.

Professor Yang is director of Laboratory of Analytical Microbiology (LAM), National Center for Biomedicine Ananlysis (NCBA) and one of directors in Beijing Society for Microbiology (BSM), committeeman in Committee of Analytical Microbiology, Chinese Society for Microbiology (CSM) and committeeman in Ministry of Health Consultant Committee for Natural Epidemics.

Using a Microarray to Detect Viral Pathogens Associated with Human Disease

By Charles Chiu

The identification of novel pathogens has greatly advanced our understanding of infectious diseases over the past quarter-century. Such pathogens include the hepatitis C virus in hepatitis, HIV in acquired immunodeficiency syndrome (AIDS), and the bacterium Helicobacter pylori in peptic ulcer disease. It has also been speculated that infection may play an active role in many devastating chronic diseases such as diabetes, cancer, and atherosclerosis. Traditional approaches to detecting novel pathogens are limited in their scope and are labor intensive. Thus, new strategies are needed to increase the spectrum and "throughput" of microbial pathogen detection from clinical specimens.

Our laboratory has developed a comprehensive and unbiased genomics-based method to screen for both known and novel viruses in clinical specimens. The viral microarray, or Virochip, is a DNA microarray containing over 22,000 oligonucleotides designed using partial sequences or fully-sequenced genomes from over 1,200 viral species. This platform has been successful in detecting viruses derived from virus-infected tissue culture cells as well as clinical specimens from patients with human respiratory infections. The Virochip has also been used to identify novel pathogens such as the Severe Acute Respiratory Syndrome, or SARS, coronavirus.

Our goal is to use the Virochip to screen clinical samples from a variety of human diseases for viruses that may play a role in the etiology of these conditions. The Virochip enables screening of a large number of different viruses simultaneously. After a putative candidate pathogen is elicited by the Virochip, additional specific testing is carried out to pinpoint the diagnosis. Two recent applications of this new technology have been in (1) identification of a novel strain of enterovirus associated with aseptic meningitis and (2) comparison of the Virochip with the standardized direct fluorescent antibody (DFA) test in diagnosing upper respiratory infections in a pediatric cohort. To assist in viral detection, we have developed an automated tool called E-Predict, which identifies specific viral species based on observed microarray hybridization patterns.

We have successfully applied this technology to diagnose human parainfluenza virus 4 infection in an immunocompetent adult presenting with an acute severe respiratory illness requiring mechanical ventilation. Prior to the Virochip assay, an extensive panel of microbiological assays (n>70) for the causative agent was unrevealing. The finding of human parainfluenza 4 is unexpected because this

pathogen is thought to cause only a benign and self-limiting illness. The findings on the microarray were subsequently confirmed by specific polymerase chain reaction (PCR) and serology for human parainfluenza virus 4. This clinical case indicates that the Virochip may play a key role in aiding diagnosis in patients with critical illnesses of unknown etiology.

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Charles Chiu, MD/PhD, is a clinical infectious diseases fellow and postdoctoral researcher at University of California, San Francisco (UCSF). He was born in the United States in the city of Columbia, Missouri. Chiu is the elder son of 1st-generation Chinese immigrant parents and has a brother named Ben. His research career began in high school when he participated in the CAST (Committee for Advanced Science Training) program. While working in the laboratory of Dr. Jerome Siegel at the Sepulveda Veterans Administration Medical Center in California, Chiu conducted single-unit recordings in narcoleptic dogs to identify a population of neurons discharging during cataplexy (one of the cardinal signs of narcolepsy), with his work being published in Science. In high school, Chiu also pursued one of his passions, computer programming, winning first prize in the Los Angeles Unified School District-Rockwell Computer Science Competition three years in a row.
Chiu graduated from Van Nuys High School in 1989 and attended University of California, Berkeley as an electrical engineering and computer science major. He completed his Bachelor's degree in 1993 with highest honors and then entered medical school at University of California, Los Angeles (UCLA) as part of a combined MD/PhD program. His thesis research under the mentorship of Dr. Phoebe Stewart involved the use of cryo-electron microscopy and three-dimensional image

reconstruction to investigate structures of an adenovirus-receptor complex, an adenovirus gene therapy vector, and a DNA repair enzyme. This research led to numerous publications in journals such as EMBO, Journal of Virology, and Journal of Molecular Biology, as well as the 1999 Sylvia Sorkin Greenfield Award for Excellence in Biophysics. Chiu was granted his MD/PhD degree from UCLA in June of 2001.

Chiu is currently working in the laboratory of Dr. Joseph DeRisi on the use of microarrays for viral pathogen detection and discovery. He is in his 5th year of the UCSF Molecular Medicine Training Program, a program designed to develop clinical scientists on the forefront of new advances in medicine and technology. His work on microarrays is funded by the Doris Duke Charitable Foundation and a National Institutes of Health Infectious Diseases Training Grant.

Chiu is a board certified physician in Internal Medicine and works as a part-time hospitalist at three community hospitals. He is a member of the Speaker's Bureau for Pfizer Incorporated and is also collaborating with a psychiatrist on a computerized software program to assist in psychiatric diagnosis.

Chiu lives in San Francisco and is happily married to Wendy, his wife of two years. In his spare time, he is an avid poker player and enjoys reading, basketball, and social dancing.

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Laboratory-based subtype surveillance of infectious diseases

By Biao Kan

Laboratory detection of microbial pathogens is critical in the infectious disease treatment, outbreak control and prevention. Public health laboratories play important role in detection and confirmation of clinical case and outbreaks, exploration and confirmation of special or emerging pathogens, determination of immunological status of the case and human group in a community, and the selection of vaccine and its evaluation.

Nowadays microbial pathogens are spread more rapidly through the different locations of the world, and their patterns of epidemiology, microbiology, and geographical distribution have diverged in a certain extent from the previously reported. As the cases stands, laboratory network, in which the detection and surveillance information could be collected, compared and shared, should be established in different regions and technical departments of relative social administration. The network

surveillance based on laboratories may provide the pathogen evidence or clue, combined with other epidemiological information, to detect, survey and alert the outbreaks and epidemics. It may help to find the internal association or clusters of the outbreaks and epidemics occurred in different regions and dates.

After obtaining the isolates from the patients and transmission related materials such as animals, food and water, the subtype and other biological characters of the strains should be investigated to identify the identity of them. In these analysis processes, the standard technical methods, work protocols and equipments are required, and then the data from different regions could be exchanged and compared. The comparison and cluster analysis is based on the principia that the isolates are identical from the common source of infection. Therefore the apparently sporadic cases also might be linked together, and the unrelated cases might be separated from the related ones. Some cases of laboratory surveillance network have showed its significant role in the outbreak detection, source tracing and outbreak alert. With the timely exchange of the comparable data from different laboratories, and with the combination of information from laboratories and epidemiological investigations, the laboratory based surveillance provide a useful means in infectious diseases control and prevention.

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Biao Kan, born in 1969, is the Professor, Director of Department of Diarrheal Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (China CDC). In 1999, he got the Ph.D. in Microbiology in Chinese Academy of Preventive Medicine (now China CDC). From April 2002 to December 2002, he worked as a scientific visitor in Max-Planck Institute for Infection Biology, Germany. He is responsible for laboratory surveillance of cholera and typhoid fever in China, and development of the laboratory surveillance network on diarrheal diseases. His research focuses on medical microbiology and molecular genetics of Vibrio cholerae, including toxigenicity related genes and their transference,

bacteriophage genome and functional genome analysis, live oral cholera vaccine development, and molecular epidemiology of V. cholerae and Salmonella typhi. He also took charge in the SARS laboratory detection and surveillance in animals in 2003 and 2004.

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Panel II: *Predicting Pathogenesis and Adaptation to Human*

New insights into the Interactions between Hepatitis Viruses Iwith IFN Using cDNA Microarray: implications in Identifying New Tools for Combating Infectious Diseases

By Zhenghong Yuan

In the past two decades, alpha interferon (IFN-α) has been widely used in treatment of chronic Hepatitis B virus infections, but only partial responses obtained. Until now, the biological function of many IFN-α induced genes as well as the molecular mechanisms underlying failure of IFN-α treatment have not been fully elucidated. To uncover the global effect of IFN-α on cellular genes and its interaction with HBV, we used cDNA microarray filters dotted with 14,000 human genes to analyze transcriptional changes between an HBV DNA transfected hepatoblastoma cell line (HepG2.2.15) and its parental cell line (HepG2) pre- and post IFN-α treatment. Analysis of gene profiles revealed that, compared with HepG2 cells, the mRNA expression level of myeloid differential primary response protein MyD88 (MyD88) was reduced in the HBV persistently replicating HepG2.2.15 cells, and this could be restored by the treatment with antiviral such as lamivudine. This result suggested that the expression of MyD88 was inhibited by HBV and the interplay between MyD88 and HBV might account for the establishment of viral persistence. This also suggested that MyD88 protein could play an important role in antiviral activity against HBV. To examine the role of MyD88 in the antiviral activity of IFN-α against hepatitis B virus (HBV), we established MyD88 stably expressing cell lines and studied HBV replication in these lines after transient transfection. The levels of HBV proteins and viral replicative intermediates were effectively reduced in MyD88 expressing cells. And a significant reduction of total and cytoplasmic viral RNAs in MyD88 stable expressing cells was also observed. Using a nuclear factor-kappaB (NF-kB) dependent reporter assay, it was shown that activation of NF-kB was moderately increased in the presence of expression of

MyD88. As dominant negative mutant of IkappaB kinase-alpha effectively inhibited NF-kappaB activation and restored the inhibition of HBV replication by MyD88, it is inferred that NK-kB activation is essential for inhibition of HBV replication by MyD88. Further study with HBV PRE-dependent reporter assay showed that the export of HBV RNA was blocked by over-expression of Myd88. These results suggest a novel mechanism for the inhibition of HBV replication by IFN-α via expression of MyD88 protein involving activation of NF-kB signaling pathway and inhibition of HBV RNA export. Another important implication of the above results is that the novel antiviral activity of MyD88 could be potentially developed as new anti-HBV drug to interfere with HBV infection.

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Dr. Zhenghong Yuan was born on Jan 17, 1966 in Qidong county of Jiangsu Province, P.R.China. He graduated with MD. and Master degrees in Clinical Medicine and Microbiology from Nantong Medical College in 1985 and 1988, and earned his Ph.D degree in molecular virology from Shanghai Medical University in 1993. During 1995 and 1996, supported by EC Mary Curie Fellowship, he conducted his postdoctoral research in Department of Medicine, St Mary's Hospital Medical School, Imperial College of Science, Technology and Medicine. He then worked as visiting Professor in Washington University School of Medicine at St Louis in 1999 and Rockefeller University in 2002. Now, Dr. Yuan is a professor of medical molecular virology, chair of the Key Lab of Medical Molecular Virology, Vice-dean of Shanghai Medical College, Fudan University and Vice-director of Shanghai Public Health Center.

For the past ten years, Dr Yuan has been engaged in research focused on mechanism of viral replication and antiviral innate immunity. For the mechanism of viral replication, his main interest is

to study the interaction of cellular proteins interacting with viral structural and other non-structural proteins to uncover possible components of viral replication complex and identify novel targets for antiviral intervention. His group has found that α-actinin is a component of the HCV replication complex, and other cellular factors, including the La protein, are required for the enhancement of IRES-mediated translation by the polyU/UC tract. For the anti-viral innate immunity, his interest is on the roles of Toll like receptors (TLR) and IFN in innate immunity and vaccination. He found that the TLR9, while not present basally in skin, can be induced by physical trauma and then mediate responses to the CpG motif, and the CpG motif can act as a 'danger signal' and Th1 immune response enhancer in DNA vaccination. Besides, his group also found that, myeloid differential primary response protein MyD88, an important adaptor protein in innate immunity, was involved in the inhibition of HBV replication by IFN-α via activation of NF-kB signaling pathway. The down regulation of MyD88 expression by HBV might account for the establishment of viral persistence.
So far, Dr. Yuan has more than 40 academic papers published in domestic and international jourals, of which 18 papers are published in journals cited by SCI and the cited numbers of his papers has exceeded 200. Due to his prominent work and achievements in scientific research, Dr Yuan has earned several scientific awards sponsored by the State Council, State Science and Technology Committee, Ministry of Education as well as the Shanghai Municipal Government.

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Genome Sequence Analysis as a Tool for Understanding and Controlling Infectious Diseases

By Eric Eisenstadt

The Institute for Genomic Research is a not-for-profit research institute established in 1992 with interests in structural, functional, and comparative analysis of genomes and gene products in viruses, bacteria, archaea, and eukaryotes (both plant and animal). I will provide a brief overview of research and development activities at TIGR, the services we provide the research community, and current efforts to improve genome sequencing technologies.. To illustrate the practical implications and applications of genome sequence information, I will also present some examples of recent work on the sequencing of multiple isolates of Group B Streptococcus and the full-length genome sequence

analysis of hundreds of influenza A genomes, both human and avian. Our work on Group B Streptococcus is a vaccine development effort performed in collaboration with Chiron. The TIGR Human Influenza Sequencing Project is an NIH/NIAID funded effort whose primary goal is to generate whole genome flu sequence data for the international research community. All flu sequencing data is released immediately to the public domain: http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html. As of February 2006, this project has sequenced 831 complete flu genomes.

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Dr. Eric Eisenstadt is Vice President for Research at The Institute for Genomic Research since August 2005. Prior to his appointment, Dr. Eisenstadt served as Program Manager at the Defense Advanced Research Projects Agency (from1999 to 2005) where he developed and managed research programs in biotechnology. Dr. Eisenstadt also served as a Program Officer at the Office of Naval Research (1988-1999). Prior to government service, Dr. Eisenstadt taught and did research on mutagenesis and DNA repair as a member of the faculty of the Harvard University School of Public Health in the Department of Microbiology and the Laboratory of Toxicology.

Dr. Eisenstadt earned his bachelor's degree and doctorate in biology from Washington University in St. Louis, Missouri. Following his graduate studies, he completed a one-year NSF-NATO Postdoctoral Fellowship at the Université de Paris, Orsay, and a two-year Deutsche Forschungsgemeinschaft Postdoctoral Fellowship at the Universität zu Köln. Dr. Eisenstadt was also a NIH Staff Fellow at the Laboratory of Molecular Biology, NINDS.

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Genomics: A New Tool For Combating Infectious Disease

By Huanming Yang

Beijing Institute of Genomics, Chinese Academy of Sciences "What has China achieved in the life sciences so far? A good place to begin answering that question is Beijing Genomics Institute" (*Far Eastern Economic Review*, 22 March 2001). Beginning by its participation in the Human Genome Project, BGI contributed significantly to the rice, chicken and silkworm genomes. In early 2003, genomics, with its large scale and high throughput platforms, provided a strong tool for identification and detection of SARS virus, as well as to the genome analysis on *Streptococcus suis* and avian influenza virus in the past years.

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Environmental Influences on Emerging Pathogens, Development of a High-density Microarray to Measure Microbial Community Dynamics

By Gary L. Andersen

Considering the importance of its potential implications for human health, agricultural productivity and ecosystem stability, surprisingly little is known about the composition or dynamics of ecosystems that may serve as reservoirs for emerging diseases. These environments include the atmosphere, marine and freshwater bodies, and soil. Most studies to date have used culture-based methods for determining microbial composition. For this reason, there is little known of what constitutes the breadth of diversity of "typical" organisms in the atmosphere (as opposed to those capable of growth in laboratory media) and what influences their composition. To address these methodological limitations and to augment our view of microbial diversity and dynamics we have designed a microarray for the comprehensive identification of bacterial organisms. We target the variation in the 16S rRNA gene, possessed by all prokaryotes, to capture the broad range of diversity of microorganisms that may be present in any complex environment. This tool allows bacteria to be identified and monitored in any type of sample without the need for microbial cultivation.

The two greatest obstacles to designing a 16S rRNA gene-based microarray to identify individual organisms in a complex environmental mixture are sequence diversity and potential

cross-hybridization. Sequence diversity is an issue as we sample new and distinctive environments. There may be many organisms, including close relatives of known pathogens with 16S rRNA gene sequences similar, but not identical to the sequences that were used for array design. Microarrays based upon single sequence specific hybridizations (single probes) may be ineffective in detecting such environmental sequences with one or several polymorphisms. To overcome this obstacle, we have designed a minimum of 11 different, short oligonucleotide probes for each taxonomic grouping, allowing for the failure of one or more probes. On the other hand, non-specific cross hybridization is an issue when an abundant 16S rRNA gene shares sufficient sequence similarity to non-targeted probes, such that a weak but detectable signal is obtained. We have found that the perfect match-mismatch (PM-MM) probe pair approach works very well in minimizing this effect. Widely used on expression arrays as a control for non-specific binding, the central nucleotide is replaced with any of the 3 non-matching bases so that the increased hybridization intensity signal of the PM over the paired MM indicates a sequence-specific, positive hybridization. By requiring multiple PM-MM probe-pairs to have a positive interaction, we greatly increase the chance that the hybridization signal is due to a predicted target sequence.

Using this approach we identified potential pathogens, or pathogen close relatives in atmospheric samples concentrated on collection filters in multiple locations. To assess temporal, spatial and meteorological factors that influence microbial composition of these urban aerosols, two cities, Austin, and San Antonio, in Texas, USA, were sampled for 17 consecutive weeks. The results of the high-density microarray were compared with a more laborious and costly method, DNA sequencing of 16S rRNA clone libraries. We found a higher level of microbial diversity by the high-density 16S microarray than when measured by 16S rRNA clone library sequencing. As an example, the majority of microbial sub-families documented by cloning aerosol amplicons were also detected in 4 of 4 replicate microarray hybridizations. Conversely, the array detected over double the number of sub-families than did the clone library. The phyla Nitrospira and Spirochaetes were uniquely detected by the array and were verified with specific PCR primers and subsequent amplicon sequencing. Changes in hybridization intensity of specific probe-sets in 17 consecutive weekly aerosol samples correlated well with quantitative (Q)-PCR results. Compared with sequencing a 16S rDNA clone library, the microarray allowed samples to be rapidly evaluated with replication.

Among the potential pathogens identified in the atmospheric samples were two sub-families

Campylobacteraceae and Helicobacteraceae, both of which contain human and animal pathogens. The exact Campylobacter cluster detected by the array contained the genus Arcobacter which we subsequently confirmed by cluster specific PCR and sequencing. This genus has been known to cause bacteremia and severe gastrointestinal illnesses in humans and along with Helicobacter (a causative agent of gastric ulcers) could be considered indicators of fecal contamination known to occur through aerosolization. Clostridium botulinum types C and G were detected regularly. Also, two near neighbors of the Brucella assemblage (agents of Brucellosis) and near-identical sequences to tick-borne Rickettsia were identified frequently. Multivariate regression tree analysis suggest that sampling location (in this case two geographically proximate cities), was less of a factor in determining aerosol bacterial composition than temporal influences with underlying differences in temperature, air pressure, wind speed and particulate matter concentrations.

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Dr. Gary Andersen is the Group Leader for Molecular Microbial Microbiology within the Earth Sciences Division of Lawrence Berkeley National Laboratory. His current research focuses on using molecular approaches to investigate a variety of natural microbial communities. Dr. Andersen is a leader in the use of microarray technology for the study of microbial diversity and the natural distribution of bacteria in complex ecosystems. His laboratory is currently looking at the distribution and spread of pathogens from natural reservoirs in bioaerosols using 16S-targeted microarrays. Recent work has also focused on the epidemiology of specific pathogens, including the identification of genetic variation in Yersinia pestis, Bacillus anthracis, Salmonella enteritidis and Helicobacter pylori. Prior to joining LBNL, Dr. Andersen was a Principle Investigator at Lawrence Livermore National Laboratory for six years. He received his Ph.D. in Plant Pathology at the University of

California at Berkeley in 1993 where he worked on the molecular characterization of stress survival of bacteria on leaf surfaces. His post-Doctoral work on bacterial pathogen epidemiology was at Duke University. While at Duke University, he developed a system for typing strains of Bacillus anthracis using variable number tandem repeats (VNTRs) that is still in use today.

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Panel III: *Diagnostics-US and China Perspective*

Surveying Gene Expression in Whole Blood: Host Response and Classification of Infection

By Stephen Popper

Sequencing of the human genome, development of cDNA microarrays, and advances in computational analysis, present the possibility to gather data about complex systems and interpret these findings in meaningful ways. Microarrays have been successfully used for in vivo studies of human cancer, including ones that focus on cells of immune origin; these studies identified gene expression patterns that define biological processes and distinguish pathological and clinical outcomes indistinguishable by traditional methods. We propose that the use of cDNA microarrays can be extended to identify host gene expression "signatures" in the context of clinically-based studies of infectious diseases and will allow us to identify pathogens or classes of pathogens, segregated by phylogeny or biological processes, as well as targets from which to develop reagents for diagnosis and treatment of infectious diseases.

In a study examining the transcription profiles of circulating leukocytes in healthy volunteers, we demonstrated that the overall variation in gene expression in the blood of healthy donors is lower than in infected febrile donors, as well as in leukocytes isolated from patients with lymphoma. Only a few of the genes measured in this study exhibited significantly more variation between individuals than within individuals ("intrinsic" genes), indicating that person-to-person differences are unlikely to affect our ability to identify gene expression "signatures" common to classes of infection. We also identified clusters of genes with coherent biological themes that appear to be characteristic of cell subsets, gender, and time of day – demonstrating the potential for uncovering fundamental biological

processes among the gene expression patterns found in blood samples, despite the inherent complexity.

In a second study set in Kathmandu, Nepal, we compared gene expression patterns in whole blood samples obtained from individuals who appeared in the local emergency room with fever, and subsequently had a positive microbiological diagnosis. Examination of the data indicated that the species of the infecting organism constituted an important, though not universal, component of the variation in gene expression. Most noticeably, 6 of the 7 bacterial infections caused by the etiologic agent of scrub typhus, Orientia tsutsugamushi, had a distinct gene expression profile marked by particularly strong expression of genes related to aspects of the cellular immune response. In addition, using statistical methods developed for analysis of microarray data, we were also able to identify a set of genes which led us to correctly predict infection with Salmonella typhi 83% of the time.

A study of Kawasaki Disease is also enabling us to test the utility of profiling host gene expression patterns in a setting where an etiologic agent has not yet been identified. Kawasaki Disease (KD) is a leading cause of pediatric acquired heart disease, and is characterized by an acute, self-contained vasculitis, sometimes leading to formation of coronary artery aneurysms. The epidemiology is strongly suggestive of an infectious process, but the lack of a known pathogen and similarities in clinical presentation to other diseases leads to delays in diagnosis and treatment. In addition, the mechanism of action of the current standard of treatment, intravenous gamma-globulin (IVIG), is not known, and roughly 5-10% of affected children fail the treatment regimen. By examining gene expression patterns prior to treatment, we have identified genes whose expression is associated with the risk of IVIG non-response, and also suggest possible molecular events involved in the KD pathogenesis and treatment. We have also identified sets of genes whose expression in acute KD differs when compared to several “look-alike” infections, including adenoviral and streptococcal infections.

In summary, we have conducted a series of studies that have demonstrated several necessary conditions for the application of host gene expression profiling during infection: variation in gene expression among healthy donors is low, and person-person differences do not dominate the profiles, obviating the need for each individual to serve as his or her own control; we can identify biologically coherent patterns of gene expression in multiple studies, and microbiological diagnosis

is a significant factor determining the degree of variation in gene expression. Continued development of technologies leading to higher throughput and lower costs, and new analytical approaches, promise to promote the feasibility of profiling of host gene expression as a method of identifying potential diagnostic and prognostic markers in infectious diseases.

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Dr. Stephen Popper is a postdoctoral research associate at the Stanford School of Medicine, where he works with Dr. David Relman and Dr. Patrick Brown. His work is focused on characterizing the host response to infection using DNA microarrays, and exploring the potential for transcriptional profiling to lead to identification of new diagnostic and prognostic markers. He is currently working on a number of clinically-based projects; collaborations include studies of dengue infection, cross-sectional studies of the etiology of fever, and a project focused on identifying diagnostic and prognostic markers for Kawasaki Disease. Dr. Popper has also been involved in the development of a next-generation oligonucleotide-based microarray at Stanford.

Dr. Popper received his Doctor of Science degree from the Harvard School of Public Health in 1999, where he received training in both virology and epidemiology. His thesis work focused on the pathogenesis of HIV-2, and included development of an assay which is used to measure viral loads in infected patients. Dr. Popper has worked in both Senegal and Brazil, and sits on the Board of Directors of the Sustainable Sciences Institute, an organization that has focused on development of tools and skills needed to address infectious diseases at a local and regional level.

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The Diagnostic Reagent Development in National Institute of Diagnostics and Vaccine Development (NIDVD) in Infectious Diseases

By Wenhao Ng and Ningshao Xia

NIDVD was established as a part of national effort to improve response to newly emerged infectious diseases and to improve forecast of infectious diseases emerging. The Institute's goal is to ensure that appropriate and reliable diagnostics and efficacious vaccines are available in time for surveillance and to facilitate control of newly emerged infections and prevent emergence of new infections. We aim to achieve this goal by working in collaboration with academia, industries and the healthcare sectors within and outside of China.

Two programs conducted by the institute on hepatitis E and the bird flu, respectively, are highlighted here. The hepatitis E program is at an advanced stage of development. The effort so far yielded three diagnostics for use under different settings and a candidate vaccine, which is in advanced stage of clinical evaluation. Large epidemiologic studies conducted in eastern and southern China have revealed a chain of events that led us to believe that the virus is likely to present a significant health and economic threat world wide. But, with the necessary tools to combat the disease already at hand before this eventuates, we can now follow the development closely, and if we so wish, arrest the development through vaccination and prevent the virus from becoming a threat.

We initiated a program on bird flu in 2004 in response to the looming threat of an influenza pandemic. We have raised a panel of 114 H5-specific monoclonal antibodies and produced 3 prototype H5-specific tests using antibodies selected from this panel, which recognize virtually all the current and past H5N1 virus isolates. One of these is a rapid test intended for use in the field to identify outbreak of H5N1. The other two tests are intended for use in surveillance of this virus in the laboratory. All three tests are being independently evaluated by different centers and results so far are encouraging. Currently, we are extending this effort to all influenza viruses to strengthen influenza surveillance in the future.

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Professor Ng is currently Appointed Professor of National Institute of Diagnostics and Vaccine Development in infectious diseases (NIDVD), Xiamen University. He got his PhD degree (Microbiology) in New York University in 1970. After a short training in the same University, he started to work in Department of Microbiology, the University of Hong Kong in 1972 as Lecture and then Senior Lecture, and became Chair Professor in 1978 till retired in 2002. Prof. Ng is also the head of the Department from 1981 to 1996. He has joined Xiamen University since 2002.

Prof. Ng's interests are wide and include hepatitis viruses, EB virus and their related diseases. He has published over 200 peer-reviewed papers, books/chapters and reviews. He has strong background in virology, molecular virology, epidemiology, diagnostics and vaccine development in infectious diseases. As a senior consultant, Prof. Ng makes many contributions to the establishment and the development of the laboratory both in scientific guidance and in public affairs.

Prof. Ng has won many scholarships and awards. He has been Honorary Professor to Institute of Virology, Chinese Academy of Preventive Medicine, Nanjing Medical College and Shantou University. He has also been serving as Visiting Professor of Sun Yat-Sen University of Medical Sciences and Senior Scientist of Hong Kong Institute of Biotechnology, The Chinese University of Hong Kong.

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The Impact of Genomics on the Diagnosis of Infectious Diseases

By Patrick R. Murray

The diagnosis of infectious diseases is a challenge for both clinicians and microbiologists. New infections such as avian influenza, SARS, and metapneumovirus respiratory disease are now well recognized, and old diseases have evolved into new presentations (e.g., necrotizing pneumonia caused

by oxacillin-resistant Staphylococcus aureus). Likewise, the number of newly recognized microbes responsible for infectious diseases have expanded dramatically in the last few years, in large part due to increased sophistication of detection and identification procedures. For example, 25 Nocardia species and 67 Mycobacterium species have been implicated in human disease, and initially unculturable organisms such as Tropheryma whipplei are now detected by molecular techniques.

Historically, tools for the diagnosis of infectious diseases included microscopy, culture, biochemical testing, antigen detection, and serology. The relative value of each tool was defined by both the technique and the organism. For example, microscopy is most useful for detection of parasites and identification of fungi, culture and biochemical testing are commonly used for the isolation and identification of bacteria, and antigen detection and serology are the tools used for the diagnosis of viral infections. It is noteworthy that many of the techniques in common use in today's labs were developed more than 100 years ago.

It is within this technologic framework that newly introduced molecular tools must be applied. The successful integration of conventional and molecular technology requires knowledge of the limitations of each technology, as well as an understanding of the related but different needs of the infectious disease specialists and microbiologists. Thus, microscopy is a rapid but labor-intensive method for detecting organisms in clinical specimens. Culture is a sensitive tool for the recovery of pathogens, invaluable for antimicrobial susceptibility testing and epidemiologic investigations, but is associated with lengthy delays before growth is observed. Biochemical testing is invaluable for the rapid characterization of some organisms such as S. aureus but imprecise for many fastidious pathogens. Antigen detection is a rapid, inexpensive tool for detecting organisms like Streptococcus pyogenes, Cryptococcus neoformans, and varicella zoster virus, but is dependent upon the quality of reagents available. Serology is the method of choice for documenting many viral, fungal, and parasitic infections, but can lack sensitivity and specificity. Likewise, the selection of the appropriate molecular diagnostic tool is influenced by the diagnostic question under consideration. The infectious disease specialist may ask – Is a specific organism present (e.g., HIV, Mycobacterium tuberculosis, Chlamydophila pneumoniae); What is the infectious etiology of a patient with a sexually transmitted disease, gastroenteritis, meningitis, sepsis; or, Does my patient have an antibiotic resistant organism (e.g., MRSA, VRE)? The microbiologist may ask – What is the identity of an isolated organism? Each

question has value and can be resolved by different approaches. If the question is specific such as in the first example, then the use of specific probes with or without nucleic acid amplification can be effective. It must be appreciated that this example does not address the possibility that other organisms are responsible for the disease. The second example, to identify the etiology of a clinical presentation, is a more complex problem. For some diseases such as meningitis, sexually transmitted diseases, and gastroenteritis the number of different organisms that can produce the disease is relatively small, so the question is resolved by asking if any of the limited number of pathogens is present. The question is also easy to resolve if the presence of the specific organism is always associated with disease. Unfortunately, this question is more complex for other clinical diseases such as lower respiratory tract infections and sepsis. In both examples the number of potential pathogens is large so molecular tools, at least at the present time, can only complement not replace conventional techniques. Additionally, in the example of respiratory infections, detection of some common pathogens such as Streptococcus pneumoniae or Klebsiella pneumoniae may simply represent colonization of the upper airways. In the example of detection of antibiotic resistant organisms, molecular tests are available that detect specific genome sequences that are associated with resistance such as vancomycin resistance in enterococci or oxacillin resistance in staphylococci. However, resistance to these same antibiotics by alternative methods would not be detected. In other words, the current utility of genomics for the detection of antimicrobial resistance is limited by the specific targets that are sought; that is, genomics are useful for defining resistance but not susceptibility. Finally, the question of interest to the microbiologist – what is the identity of an isolate – may be the easiest to address. Indeed, identification of organisms by sequence analysis of specific genes is commonly used in many large clinical laboratories, and is rapidly replacing conventional biochemical testing for the identification of unusual or fastidious organisms. The current limitation of this technology is the accuracy of a limited database.

In summary, molecular tools are rapidly augmenting conventional technologies in the clinical laboratory leading to rapid detection and accurate identification of microbial pathogens.

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Dr. Murray is Chief of the Clinical Microbiology Laboratory in the NIH Department of Laboratory Medicine since January 2002. Previously he was Director of Clinical Microbiology at the University of Maryland (1999-2001) and Washington University in Saint Louis (1976-1999). For his doctoral dissertation, earned in 1974 from the UCLA Department of Medical Microbiology and Immunology, he characterized endogenous oncornaviruses discovered in in-bred guinea pigs with lymphobastic leukemia. After completion of his doctoral studies, he served a two year fellowship in clinical microbiology at the Mayo Clinic in Rochester, MN, and then assumed his position at Washington University.

He is board certified in Clinical Microbiology by the American Board of Medical Microbiology and a member of a number of scientific societies. Dr. Murray has served on many local, regional, and national committees including: American Board of Medical Microbiology (Chairman); American Academy of Microbiology Board of Governors; ASM Division of Clinical Microbiology (Chairman); Washington University Medical School Faculty Rights Committee (Chairman); NIH Clinical Center Scientific Advisory Committee; NIH Promotions and Tenure Committee; NIH Clinical Center Technology Transfer Committee (Chairman); NIH Intellectual Property Working Group; Editor-in-Chief of the ASM Manual of Clinical Microbiology; Editor of the Journal of Clinical Microbiology; and Editor of Topley & Wilson's Microbiology and Microbial Infections. He is also author of a number of textbooks including Medical Microbiology (published by Elsevier), Review of Medical Microbiology (Elsevier), and Pocket Guide to Clinical Microbiology (ASM Press). Dr. Murray has published more than 200 research articles in peer-reviewed journals, and numerous book chapters, monographs, and editorials.

Dr. Murray is the recipient of the Becton Dickinson Award in Clinical Microbiology Research (1993), Washington University, School of Medicine Distinguished Teaching Award (1993, 1994, 1999), BioMerieux-Sonnenwirth Award for Leadership in Clinical Microbiology (2002), University of

Maryland School of Medicine Distinguished Teaching Award (2004,2005), and numerous invited lectureships. As Chief of the Clinical Microbiology Service, Dr. Murray has expanded the existing program in the use of genomics for the detection and identification of medically important microorganisms, and coordinates the core microbiology laboratory for extramural funded, multicenter epidemiology studies.

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Recent outbreaks of avian influenza in migratory birds and Streptococcus infection in China

By Fu Gao

In 2005, two major emerging infectious disease outbreaks occurred in China. This talk will discuss in detail these two outbreaks. H5N1 avian influenza virus (AIV) has emerged as a potential pathogenic entity for a variety of species including humans in the recent years. In May and June 2005, an outbreak among migratory birds on Lake Qinghaihu, China was observed , in which hundreds of thousands of birds were affected. Pancreatic necrosis was the major lesion seen in the affected birds, accompanied by abnormal neurological symptoms. Complete genomes of four of H5N1 AIV strains isolated from the viscera of dead/sick birds were sequenced and found to be reassortants with close relatedness to a peregrine falcon isolate from Hong Kong. Experimental animal infections reproduced typical highly pathogenic AIV-infection symptoms and pathology. Genomic sequencing results revealed known “highly-pathogenic” characteristics in all isolates. Streptococcus suis serotype 2 (S. suis 2, SS2) is a major zoonotic pathogen but with only sporadic cases of meningitis and sepsis in humans. Streptococcal toxic shock syndrome (STSS) was only well documented for streptococcus-caused disease in non-SS2 group A streptococcus (GAS) previously. However, a recent large-scaled outbreak of S. suis 2 in Sichuan Province, China, appeared to be a more invasive deep-tissue infection with STSS, characterized by acute high-fever, vascular collapse, hypotension, shock, and multiple organ failure.

**The description of these two outbreaks has been published in Science (Aug. 19th. 2005) and PLoS Medicine (In press, 2006) respectively.*

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Dr. George Fu Gao, who was born in Nov, 1961, is the director-general and professor of the Institute of Microbiology, Chinese Academy Sciences (IMCAS). He got his BSc and MSc in Shanxi Agricultural University and Beijing Agricultural University respectively. In 1991, He went to the UK, and got his PhD in Oxford University in 1995. Then, he worked in Oxford University (UK), Calgary University (Canada), Harvard University (USA) as a postdoc from 1995 to 2001. During his stay at Harvard, he was supported by the UK Wellcome Trust International Travelling Fellowship. He worked in Oxford University as a lecturer from 2001-2004 before taking up the director-general of the IMCAS. Dr. Gao is interested in protein-protein interactions related to molecular virology and molecular immunology. His work is involved in the application of structure biology in the understanding of the molecular mechanism of T cell molecular recognition and envelope virus entry. He also has interests in molecular epidemiology of avian influenza virus, with a paper recently published in Science. He has 70 publications in the related fields involved in Nature, Science, Nature Medicine, Lancet, Immunology Today, Trends in Immunology, Immunity, Proceedings of National Academy of Sciences-USA, Journal of Experimental Medicine, Journal of Biological Chemistry, etc. His recent work covers the structural basis of interaction of peptide-MHC and T cell receptor (TCR) in the rhesus macaque, esp. related to AIDS vaccine design and immune response to avian influenza virus both in birds and humans (other mammalians as well).

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Panel IV: Vaccine Development

Development of Sendai Virus-based Vaccines to Prevent Pediatric Respiratory Infectious Diseases

By Xiaoyan Zhan

Respiratory syncytial virus (RSV) is the leading cause of hospitalization for viral respiratory tract diseases in infants and young children. Worldwide, RSV is estimated to cause approximately 900,000 deaths per year. Second to RSV as agents of viral respiratory disease are the human parainfluenza viruses (hPIV). hPIV type 1 (hPIV1) is the principal cause of croup. hPIV type 3 (hPIV3), like RSV, causes serious pneumonia and bronchiolitis in infants. hPIVs are responsible for up to 100,000 hospitalizations per year in the US alone. While individuals may endure repeat infections with these respiratory viruses, the first infection is generally most severe. Recurrent infections tend to result in more mild disease. These observations illustrate the importance of immunity to the control of virus disease, and underscore the need for effective pediatric vaccines. Unfortunately, despite decades of work toward the development of RSV and hPIV vaccines, no approved vaccines have been established.

Previous studies in our laboratory have highlighted the profound antigenic and structural similarities between hPIV1 and murine parainfluenza virus type 1 (also referred to as Sendai virus, SV). Based on antigenic similarities identified in small animals, we conducted studies in African green monkeys which demonstrated that priming with intranasal SV uniformly protects animals from challenge with hPIV1. Based on this success we advanced to clinical trials and confirmed the expected tolerability of intranasal SV (Phase I trial) in a cohort of healthy adults. Studies are progressing to confirm that SV is similarly well-tolerated in younger age groups. The utility of SV as a naturally attenuated, xenotropic vaccine for hPIV1 prompted interest in using SV as a vaccine platform to deliver target antigens of RSV and hPIV3. This abstract outlines our preparation of Sendai virus recombinants expressing immunogenic glycoproteins of RSV and hPIV3 and the examination of these chimeric vaccines in a small animal model.

Materials and Methods:

1. Construction of recombinant SV:

SV is a non-segmented RNA virus. Using reverse genetics, recombinant SV expressing cloned G (attachment) or F (fusion) proteins of RSV were rescued. In brief, the F and G genes of RSV were amplified by RT-PCR, and the PCR products were cloned into a cDNA copy of SV at a site between the F and HN genes. Recombinant cDNA was co-transfected into cells with plasmids expressing the NP, P and L proteins of SV to recover fully infectious recombinant SV particles (rSV). rSV recovered from transfected cells was then inoculated into embryonated eggs where abundant replication of SV naturally occurs. Recovered rSV bearing cloned G and F proteins of RSV (rSV-RSV G and rSV-RSV F) was confirmed to express these target proteins by Western blot analysis.

This same reverse genetics approach was utilized to prepare rSV expressing the F and HN glycoproteins of hPIV3 (rSV-hPIV3 F and rSV-hPIV3 HN).

2. Analysis of immune responses and protection elicited by rSV:

Cotton rats (Sigmodon hispidus) were immunized with wild type (wt) or rSV via the intranasal route (2 x 106 PFU/rat) and blood was collected before and 4 weeks after immunization for ELISA and neutralization assays. To measure the protective efficacy, vaccinated cotton rats were challenged with intranasal RSV five weeks after immunization. Lungs were harvested and pulmonary RSV was measured 3 days after RSV challenge.

A similar approach was followed in the evaluation of rSV vaccines targeting hPIV3.

Results

1. RSV-based rSV vaccines elicited antibody responses against RSV. Using binding antibody assays, specific anti-RSV F or G antibodies were detected from cotton rats immunized with rSV-RSV F or rSV-RSV G (respectively). Serum samples from vaccinated animals also demonstrated potent RSV neutralizing activity compared to that of control animals (receiving wt SV).

2. RSV-based rSV vaccines elicited protection against RSV challenge. Animals primed with intranasal rSV-RSV G, rSV-RSV F or a mixture of both demonstrated equivalent and solid protection from intranasal RSV challenge compared to control animals.

3. Results here suggest that no distinct serotypes of RSV exist. RSV has been proposed to circulate as distinct serotypes (A and B). Studies here show that antibody responses induced following vaccination with an rSV expressing glycoproteins from an RSV identified as type “A” are capable of neutralizing both type “A” and “B” (and vice versa) virus isolates.

4. rSV vaccination elicits durable immune responses. RSV-specific antibody responses could be

detected 45 weeks following a single intranasal rSV-RSV G inoculation.

5. hPIV3-based rSV vaccines elicited antibody responses and protected cotton rates from hPIV3 challenge.

Conclusion

Results demonstrate the utility of Sendai virus as a naturally attenuated vaccine for hPIV1 and as a valuable vaccine platform for RSV and hPIV3. A single intranasal inoculation elicited durable binding and neutralizing responses to target viruses and conferred protection against challenge. Human trials with unmodified Sendai virus as a vaccine for hPIV1 advance through the clinic and future studies of rSV expressing the glycoproteins of RSV and hPIV3 are being planned.

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Xiaoyan Zhan was born and grew up in Hangzhou, China. She received her Bachelor of Science and Master of Science degrees in molecular genetics from Zhejiang University, China, and went on to earn a PhD degree in molecular biology from the same institute. She was awarded a Rockefeller Foundation Fellowship permitting her to conduct her PhD dissertation research at Yale University. Subsequently she pursued postdoctoral training at Yale University, the University of California at Berkeley and St. Jude Children Research Hospital. Currently she is appointed as a Scientific Manager in the Vaccine Program headed by Drs. Julia Hurwitz and Karen Slobod in the Department of Infectious Diseases at St. Jude Children Research Hospital. Her research interests include areas in molecular genetics, virology and anti-viral immune responses. She has published a number of papers regarding the analysis of gene function and the pathogenesis of cytomegalovirus, the molecular analysis of HIV vaccine components, the immune response to a candidate HIV vaccine, and the activity of a multi-envelope HIV vaccine in nonhuman primate. Her current research focuses on the

development of novel vaccines against respiratory syncytial virus and human parainfluenza virus types 1 and 3, important causes of pediatric respiratory disease.

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Studies on HIV Preventive Vaccines in China

By Youchun Wang

Up to the end of Sep. 2005, the cumulative reported HIV infections in China have reached 135,630, including 31,143 AIDS cases and 7,773 death cases. HIV-infected cases have been identified in all 31 provinces of China. The highest cumulative number of HIV infections came from Yunnan Province, consecutively followed by Henan, Guangxi, Xinjiang, Guangdong Provinces. Most of the the HIV-infected cases identified in the southwestern and northwestern parts of China came from the drug-using population. Most in the southeastern China and the major cities of China came from the commercial sex population. Several genotypes such as subtypes B' and BC may existed in China. The vaccine should include the genomic regions of these genotypes to insure the vaccine may prevent infection of those genotypes. A successful HIV vaccine should induce both cell-mediated immune and humoral (cytotoxic T responses and neutralizing antibody) responses. In order to induce neutralizing antibodies, the envelope proteins (gpl60, gpl20 and gp4l) should include in vaccine, but they are very variable. In order to induce cell-mediated immune response, the proteins such as core, pol proteins as well as regulatory proteins such as nef, tat etc should include in preventive vaccine. Both CTL (cytotoxic T responses) and neutralizing antibody responses should be evaluated in animals or in non-human primates before clinical trials. More than 10 research groups focus on HIV vaccine search in China. Several DNA vectors and virus vectors such MVA, Ad5, Replicating and non-replicating Tiantan Vaccinia have been used to develop HIV vaccine. Genomes of subtype B', BC and different fragments of HIV have been used in different groups. The concern for DNA vaccine is the source of the DNA incorporated into the vector including eukaryotic promoters, enhancers, and etc. In order to limit the possibility for chromosomal integration, homology of plasmid DNA sequences to known sequences in the human genome should be avoided. The anti-vector antibody (anti-nuclear antibody) as we1l as specific antibody in the volunteers after vaccination shall be evaluated in laboratory. So far one HIV vaccine had been approved for phase I clinical trial.

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Professor Youchun Wang has worked at Department of Cell Biology, National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). He got Ph.D in virology, University College London (UCL), UK, and MD in epidemiology, Beijing Medical University.He has mainly done research work on viruses such as hepatitis virus, HIV, SARS virus and responsible for quality control of these related biological products such as vaccines and diagnostic assays. So far, more than 80 papers have been published.

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Influenza Pandemics: Their Origin and Control

By Arnold S. Monto

Derivation of Pandemic Viruses

There have been three influenza pandemics in the 20th century, and we now know the identity of the viruses causing them. The 1957 and 1968 pandemic viruses resulted from reassortment. The influenza virus genome is made up of eight segments of RNA, and in reassortment, segments are exchanged between avian and human viral parents. In 1957, three segments derived from the avian parent, those coding for the hemagglutinin (HA) neuraminidase (NA) and polymerase (PB1). In 1968, two segments came from the avian parents, those coding for the HA and the polymerase. The resultant viruses in 1957, A (H2N2) and in 1968, A (H3N2) each had a totally new hemagglutinin. As a result, the population was largely susceptible and a pandemic arose. This process, in which the virus changes by reassortment, is called antigenic "shift."

In 1918, the pandemic virus, A (H1N1) did not evolve by reassortment, but by mutation. It has been known for many years that influenza virus changes gradually from year to year, as a result of mutation, and this process is called antigenic "drift." Drift indicates a minor change in antigenicity, but this

change is sufficient to require a change in the viruses included in the influenza vaccine. The 1918 virus derived not from a previous human virus, but from a fully avian virus. Among the changes in the virus was one in the hemagglutinin which determines receptor specificity, so that it could attach to human cells, not as previously, to avian cells.

The avian ancestral virus has not been identified, but the RNA segments of the 1918 human virus, after being sequenced, have been reconstructed by reverse genetics, and studied in animals. Extreme virulence of the virus is polygenic in origin. In the 1918 pandemic, the virulence resulted in an unusual mortality pattern, and worldwide between 40-60 million people were estimated to have died.

Influenza Prevention and Control

Seasonal or interpandemic influenza is prevented by vaccine. Antivirals can be used both for prevention and treatment. The traditional influenza vaccine is produced in fertile hens' eggs and inactivated by formalin. For pandemics such as 1957 and 1968, this process was followed. However, to produce a vaccine for the A (H5N1) avian strain, the reverse genetics process needs to be used, after the multi-basic cleavage site of the hemagglutinin is removed. To reduce the need for large amounts of antigen, adjuvants such as MF-59 and alum are being studied, as is use of cell culture for virus propagation.

Adamantanes (particularly amantadine) and neuraminidase inhibitors (NAI's) zanamivir and oseltamivir, have been useful in seasonal influenza. However, resistance to amantadine has become so common as to render the drug useless. The NAI's shorten duration of illness and prevent complications in seasonal influenza and therefore, they clearly have a role in control of pandemics. When a pandemic starts, there will be little or no specific vaccine, which means that full reliance will have to be put on the NAI's, particularly oseltamivir, which is orally bioavailable.

While effective in prophylaxis, that use requires a great deal of drug, since it must be taken daily.

For that reason, most approaches will use oseltamivir in treatment, especially since it is known to reduce complications.

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Arnold S. Monto's Biography

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Arnold S. Monto is Professor of Epidemiology at the University of Michigan School of Public Health in Ann Arbor and is Director of the Bioterrorism Preparedness Initiative. The major focus of his work has been the epidemiology, prevention and treatment of acute infections. These activities have included work on the occurrence and characteristics of the infections as well as potential for vaccine prevention and antiviral treatment. Respiratory infections, in particular influenza, have been a major interest, with special reference to the evaluation of vaccines in various populations and the assessment of the value of antivirals such as amantadine, rimantadine and the neuraminidase inhibitors. Dr. Monto was closely involved in the US HCFA-sponsored studies, which made influenza vaccine a covered benefit for older individuals. He has also studied other approaches to influenza vaccine use, particularly to control transmission of virus in the community and in nursing homes. He is currently involved in assessing the efficacy of vaccine in prophylaxis and the neuraminidase inhibitors in therapy of influenza and, internationally, evaluating the relative efficacy of hepatitis A vaccine in post-exposure prophylaxis.

During his tenure at the University of Michigan, Dr. Monto has also served for periods of time in the Acute Respiratory Infection program at the World Health Organization, Geneva, and as Scholar in Residence at the United States Institute of Medicine/National Research Council. He has been a member of the Pulmonary Diseases Advisory Committee of the National Heart, Lung and Blood Institute and of the National Advisory Allergy and Infectious Diseases Council. He has also served on various United States and international advisory bodies addressing the overall response to the problem of emerging and reemerging infections, control of influenza in the epidemic and pandemic situation, and bioterrorism preparedness. He is the past president of the American Epidemiological Society.

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Panel V: *Drug Discovery*

454 Life Sciences Next Generation Sequencing Technology: Applications to Infectious Disease Research

By Bruce E. Taillon

454 Life Sciences has developed a revolutionary sequencing technology that produces over 20 million base pairs of sequence data in a single 5 hour run. This sequencing capacity makes the 454 system an ideal platform for the study of virus, bacteria and other infectious organisms. The 454 Life Sciences sequencing system, the GS20, uses a novel fiber-optic slide of individual wells which are picoliter-sized reaction vessels where sequencing by synthesis is conducted on clonal DNA fragments. These clonal DNA fragments are generated through an emulsion method for DNA amplification. In the initial technology publication (Margulies et al, Nature. 2005. 437(7057):376-80) of the Mycobacterium genitalium was sequenced and the resulting de novo assembly data showed 99.99% accuracy when compared to the published M. genitalium sequence. 454 Life Sciences has conducted a comprehensive study of bacterial sequencing in order to fine tune the sequencing process and de novo assembler for microorganism sequencing. In addition to the data presented in the Nature publication described above, we sequenced E. coli using 3 sequencing runs (~13-fold over sampling) and found the de novo assembled genome to be 99.998% accurate. We also sequenced S. pneumoniae with 2 sequencing runs with similar de novo assembly accuracy (99.991%) and B.licheniformiswith 3 sequencing runs (accuracy 99.993%). The technology is not only suitable for small genomes but we have also found that the technology can be used for sequencing larger genomes as well. We recently completed the resequencing of the yeast Saccharomyces cerevisiae (genome size ~13Mbp) on one machine, in one week, by one individual and achieved de novo assembly accuracy better than 99.99%.

The GS20 system developed by 454 Life Sciences has applications relevant to infectious disease beyond the de novo sequencing of microorganisms. In January, 2005 scientist from Johnson and Johnson PRD published the use of the 454 sequencing technology in the study of diarylquinoline drug resistance in Mycobacterium tuberculosis (Andries et al, Science. 2005. 307(5707):223-7). Furthermore, the technology has been used in the analysis of genetic variation in HIV and finally, the

open nature of the 454 Life Sciences sequencing system allows for the study of complex mixtures such as those sampled from the environment. The use of the technology for metagenomics was demonstrated in the recent analysis of ancient DNA using the GS20 (Poinar et al., Science. 2006. 311(5759):392-4).

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Bruce E. Taillon is a molecular and cellular biologist that has been working in the genomics and functional genomics field for over a decade. He received his Bachelor of Sciences in Biology from the University of South Carolina in 1986 and his Doctoral Degree in Cell and Molecular Biology from Carnegie Mellon University in 1993. His postdoctoral training was done at Washington University in St. Louis, MO as a fellow of the National Institute of Child Health and Human Development. Following his postdoctoral training Dr. Taillon entered the biotechnology field at Vyrex Corporation, a functional genomics start-up company, where he directed the research and development efforts as Associate Vice President of Research. In 1999, he then joined CuraGen Corporation, a genomics based biopharmaceutical company. While at CuraGen Corporation he built and led a team of scientists and engineers dedicated to the development of genomic technologies and the application of these technologies to drug discovery and development. Recently, he has moved this expertise in the applications of genomic technologies to 454 Life Sciences, a majority owned subsidiary of CuraGen Corporation, where he lead the efforts to expand the application of 454's next generation sequencing technology.

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Current Situation of Medicine Used for Infectious Disease in China

By Junzhi Wang

In the aspect of treatment of infectious diseases, China has different kinds of medicines for viral and bacterial infectious diseases, being the same with other advanced countries. These medicines include chemical drugs, biologicals for therapeutical use and the Chinese traditional medicine. All these medicines have played an important role on treatment of infectious diseases and preventing its spread.

Chemical Drugs: 1. Antibiotics for treatment of bacterial infectious diseases such as penicillin, streptomycin, erythrocin, cephalosporin, Gatifloxacin, Antofloxacin Hydrochloride, Caderofloxacin Lactate; 2. ribonucleotide medicine for treatment of viral infectious diseases such as zidovudine, Lamivudine, strvudine didanosine, nevirapine; 3. Proteinase inhibitors for treatment of AIDS such as Indinavir, Saquinavir, Ritonavir, Nelfinavir, Amprenavir.

Biologicals for therapeutical use: 1. Human immunoglobulins. Products derived from normal human pooled plasma : human immunoglobulin, Human immunoglobulin for intravenous use. Products derived from immunized normal human pooled plasma :human Hepatitis B immunoglobulin, human rabies immunoglobulin, human tetanus immunoglobulin. Products derived from recovery patients: human SARS immunoglobulin. Human Hepatitis B immunoglobulin for intravenous use is under clinical trial. 2. Antiserum biologicals such as anti-tetanus toxin, anti-rabies virus serum, yolk immunoglobulin , anti-botulinum serum; These kinds of anti-serum have been used for the treatment of corresponding diseases. 3.rDNA products: such as viral macrophage inflammatory protein-II, Lysozyme, IFNα1b、IFNα2a、IFNα2b、IFNω. 4.Bacterial vaccines for therapeutical use such as Freeze-dried M.vaccine for Therapeutic use, Mycobacterium Phlei F.U.36 Injection, Brucella Vaccine for Therapeutic Use.

The Chinese traditional medicine: Chinese traditional medicines, such as folium isatidis, rhizoma coptidis, Flos lonicerae, have been proven to have anti-bacterial and viral roles.

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Functional Genomics of Virus - Host Interactions

By Paul Ahlquist

Most or all steps in virus infection depend on interactions of virus components with specific host factors, which thereby are crucial determinants of virus replication, host range, tissue tropism and pathology. Thus, not only viral functions but also appropriate host functions can be valuable targets for antiviral therapy. Moreover, modulating the expression or activity of relevant host factors can create protective, antiviral states equivalent to enhanced innate immunity. For these and other reasons, the identification and study of host genes required for virus replication is a critical frontier in virus research. The advent of functional genomics has opened significant new opportunities for analyzing such virus-host interactions, and below we consider two diversified examples.

Positive-strand RNA ((+)RNA) viruses are the largest genetic class of viruses, encompassing over

one-third of all virus genera and many important pathogens such as hepatitis C virus, the SARS coronavirus, and others. (+)RNA viruses invariably replicate their genomes on host membranes, often in association with vesiculation or other membrane rearrangements. Our results imply that the viral RNA replication complexes are virus-induced, membrane-bounded compartments that act as mini-organelles to concentrate and sequester viral replication factors and templates, coordinate successive replication steps, and protect potentially dsRNA replication intermediates from host defenses. We also find that the structure, assembly and function of these intracellular RNA replication complexes share multiple parallels with the replicative cores of extracellular virions from reverse-transcribing and dsRNA viruses, revealing functional and potentially evolutionary links across three of the six genetic classes of viruses.

As one approach to identify and characterize host genes and functions that participate in such virus replication processes, we showed that the genetically tractable yeast S. cerevisiae supports the normal pathways of genome replication, gene expression and virion assembly by two well-studied (+)RNA viruses: the model alphavirus-like virus, brome mosaic virus (BMV) and the best-studied animal nodavirus, flock house virus (FHV). For BMV, we used classical yeast genetics to identify over a dozen host genes contributing to diverse replication steps. For more global analysis, we performed the first genome-wide analysis of host functions in virus replication, analyzing 4500 strains of a genome-wide yeast single-gene-deletion library (~80% of yeast genes). This functional genomics approach revealed nearly 100 genes whose absence inhibited or, in some cases, enhanced BMV RNA replication by from >3-fold to >30-fold. For many of these host genes, we have identified the viral replication step(s) affected and characterized the mechanism of action, revealing insights to virus and host function. Our results show that unexpectedly diverse host functions control viral translation, the recruitment of viral RNA templates from translation to RNA replication, chaperone-mediated activation of RNA replication complexes, viral RNA and protein stability, membrane characteristics essential for RNA replication, and other steps. Most of these are common, unifying themes among (+)RNA viruses, and all are potential antiviral targets. Further analyses are revealing contributions from additional host genes.

In addition to other diseases, viruses cause at least 15-20% of human cancers. Epstein-Barr virus (EBV), e.g., is a widespread DNA herpesvirus that causes lymphomas and carcinomas, including most nasopharyngeal carcinomas (NPCs), a tumor with a high incidence in parts of China. To identify the

molecular mechanisms by which EBV-associated epithelial cancers develop, we measured the expression of essentially all human genes and all latent EBV genes in a collection of laser capture microdissected nasopharyngeal carcinoma (NPC) tissue samples and normal nasopharyngeal tissues provided by collaborators in the US and Asia. Global gene expression profiles clearly distinguished tumors from normal healthy epithelium. Expression levels of multiple viral genes were correlated among themselves and with changes in the expression of a large subset of cellular genes. Particular associations were noted between EBV gene expression and changes in the expression of specific genes involved in cell cycle checkpoints, apoptosis, immune surveillance and metastasis. The results imply that EBV makes varied contributions to the development and maintenance of NPC tumors, and suggest viral and cellular targets for therapy.

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Paul Ahlquist is the Kaesberg Professor of Molecular Virology and Oncology at the University of Wisconsin - Madison and an Investigator of the Howard Hughes Medical Institute. His research interests include the mechanisms of virus replication and gene expression, virus-host interactions, and viral pathology, oncogenesis, and evolution. These activities have included the early development of methods for genetically manipulating RNA virus genomes, production of the first engineered RNA viruses, and functional studies of various aspects of RNA virus replication, including genome replication, gene expression, RNA recombination, virus assembly and others. He has also studied the replication and virus-host interactions of retroviruses and DNA viruses, including human papillomaviruses and herpesviruses.

Dr. Ahlquist also is a member of the McArdle Laboratory for Cancer Research, the head of the Human Cancer Virology Program at the University of Wisconsin Comprehensive Cancer Center, and a past chair of the University of Wisconsin Institute for Molecular Virology. He has served on the

Executive Councils of the American Society for Virology and the International Commission for Taxonomy of Viruses, and on a recent team that reorganized all US National Institutes of Health research grant review panels in Infectious Diseases and Microbiology. He is a member of the US National Academy of Sciences.

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Discovering Anti-SARS Compounds from the Existing Drugs

By Hualiang Jiang

The 3C-like proteinase (3CLpro) of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) is one of the most promising targets for anti-SARS-CoV drugs due to its crucial role in the viral life cycle. In this study, a database containing structural information of more than 8,000 existing drugs was virtually screened by a docking approach to identify potential binding molecules of SARS-CoV 3CLpro. As a target for screening, both a homology model and the crystallographic structure of the binding pocket of the enzyme were used. Cinanserin (SQ 10,643), a well-characterized serotonin antagonist that has undergone preliminary clinical testing in humans in the 1960s, showed a high score in the screening and was chosen for further experimental evaluation. Binding of both cinanserin and its hydrochloride to bacterially expressed SARS-CoV 3CLpro was demonstrated by surface plasmon resonance technology. The catalytic activity of the enzyme was inhibited with IC50 values of 5 μM, as tested with a fluorogenic substrate. Antiviral activity of cinanserin was further evaluated in two tissue culture-based assays, a replicon system of human coronavirus 229E and a quantitative test assay with infectious SARS-CoV. Both assays revealed a strong inhibition of coronavirus replication at nontoxic drug concentrations. The level of SARS-CoV RNA and infectious particles was reduced by up to 3-log units with an IC50 of 31 μM and 34 μM for cinanserin and cinanserin hydrochloride, respectively. These findings demonstrate that the old drug cinanserin is a potent inhibitor of SARS-CoV replication, acting most likely via inhibition of the 3CL proteinase.

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Hualiang Jiang obtained his bachelor's degree from the Department of Chemistry, Nanjing University in 1987. In September 1989, he entered East-China Normal University, and received his Master degree in physical chemistry (quantum chemistry) in 1992. From September 1992 to July 1995, he studied in Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences for his Ph.D. degree, and in 1995 he received the Ph.D. degree in organic chemistry. He is currently a professor of SIMM. He also assumes the associated director of the institute. From 1997 to 2002, he worked in Hong Kong University of Science and Technology, Max-Plank Institute for Solid Physics, and Weizmann Institute of Science as visiting scholar and visiting professor, respectively.

Dr. Jiang's research interesting is focusing on discovering new drugs and related basic research synthetically employing approaches of computational chemistry & biology, computer-aided drug design, organic chemistry, and molecular biology and structural biology. Dr. Jiang has published more than 160 papers, edited one monograph, written chapters for 10 monographs, and applied 25 patents.

Panel VI: Case Studies – Prevention and Treatment

HBeAg Negative Hepatitis B: The Mutation Genotype, Genotyping Relationship and Outcome

By Lai Wei

Since the finding that the discrepancy between the positive HBV DNA and the negative HBeAg was the infection with HBV variants at position 1896 of the pre-core region and 1762,1764 core promoter, it was suggested the concept of HBeAg negative chronic hepatitis B. Now, it was accepted that HBeAg negative chronic hepatitis B is characterized by positive HBV DNA and continued

necroinflammation in the liver, it was also found that HBeAg negative chronic hepatitis B has been reported in all parts of the world.

We collected Sera from 113 patients chronically infected with HBV. Among them, 36 patients were asymptomatic HBsAg carriers(AsC), 54 chronic hepatitis B(CHB), 14 liver cirrhosis(LC), and 9 hepatocellular carcinoma(HCC). HBV genotype was determined by S gene sequencing. Double mutation in BCP(T1762/A1764) and PreC mutation(A1896) were determined by INNO-LiPA. The double mutation in BCP (T1762/A1764) was significantly more frequent in genotype C than B patients(34.2% vs 10.0%, P <0.01). However, there was no significant difference in the distribution of PreC mutant with A1896 between genotype B and C patients(2.5% vs 4.1%, P >0.05). Compared with AsC, the double mutation in BCP(T1762/A1764) was more common in patients with CHB, LC, and HCC(24.1% vs 2.8%, P <0.01; 71.4% vs 2.8%, P <0.01; 55.6% vs 2.8%, P <0.01, respectively). However, PreC mutation(single A1896) did not develop in patient with CHB and LC, and the prevalence of PreC mutation (single A1896) was low in patients with AsC and HCC (8.3% and 11.1%, respectively). Compared with AsC, genotype C infection in patients with LC and HCC was more common(92.3% vs 52.7%, P <0.01 and 88.9% vs 52.7%, P <0.05, respectively). And compared with patients with CHB, genotype C infection in patients with LC was more common (92.3% vs 61.1%, P <0.05). Identical genotyping results were found in 90 of 108 samples(83.3%) by INNO-LiPA and sequence analysis. Contradictory results were found only in four cases(3.7%) by the two methods. In eight cases(7.4%), the genotyping results were indeterminate by INNO-LiPA.

Also, 211 chronic Hepatitis B patients with cirrhosis were prospectively followed up for 19 months (5-34 months). Comparing with HBeAg positive cirrhosis patients. The numbers of HBeAg negative cirrhosis patients were more than HBeAg positive cirrhosis; the median ALT and AST levels of HBeAg negative patients were lower than HBeAg positive patients（P=0.025,P=0.004）; the median WBC, HB and PLT levels of HBeAg negative patients were lower than HBeAg positive patients（P=0.009,P=0.015,P=0.025）; the MELD and Child-Pugh scores between the two groups had no difference; HBV DNA positive rate and proportion of HBV DNA >10^5 copies/mL of HBeAg negative patients was lower than HBeAg positive patients（P=0.002, P=0.000）; among two groups the numbers of patients with complication did not differ significantly and the number of complications also did not differ significantly; The mortality rate of the two groups patients did not differ significantly.

During the prospective study, 166 HBeAg negative patients with decompensated liver cirrhosis was observed the relationship between the death rate and the gender, age, ALT activity patterns, HBV DNA level, MELD formula, Child-Pugh. Univariate analysis showed that the mortality of male patients（26.23%）was higher than female patients（11.36%, χ2=4.126, P=0.042）;The deaths's average ages （57.84±11.07,years old）was higher than the survivors's（52.26±11.69,years old, t=2.585, P=0.011）; the mortality of MELD≥18 patients（30.95%）was higher than the MELD<18（13.41%。χ2=7.368, P=0.007）; the mortality of Child C（46.34%）>Child B（19.75%）>ChildA（4.54%, χ2=21.992, P=0.000）.Multivariate analysis showed that death rate was independently predicted by old age、higher Child-Pugh and the ALT persistent elevation in hepatitis B e antigen-negative patients with liver cirrhosis（P <0.05）.

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Lai WEI is a Professor of Hepatology & Molecular Virology at the Peking University Health Science Center. His research interests involve in virus gene expression, protein expression related with virus infection, virus-host interactions of hepatitis virus, the mechanisms of virus replication and inhibition by interferon, and stem cell in liver fibrosis. These researches included expression of HCV RNA-dependent RNA polymerase, HCV NS3 and it's activity, proteome of HBV infection and differentiate proteomics of hepatitis B patients during anti-virus therapy, HBV pre-c mutant and BCP mutant resistance to interferon.

Dr. Wei also is the Head of Peking University Hepatology Institute and Chief of Department of Hepatology at Peking University People's Hospital. His clinical research included HBV, HCV epidemiology in China, Natural history of hepatitis B and hepatitis C in China.

Dr. Wei also is a attending member of the Chinese Association For Liver Disease. He served to develop Guideline for management of Hepatitis B, and Management, diagnosis and Prevention of

Hepatitis C virus Infection. He also serves to Kidney Disease Improving Global Outcome to develop a Guideline for HCV infection diagnosis, prevention and management in Kidney Disease.

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Genetic Architecture of Complex Infectious Diseases: Lessons from AIDS

By Stephen J.O'Brien

The progression of HIV disease in a dynamic demographic and genetic process involving the interaction of viral and host gene products. The host functions that interact with viral replication and spread are characterized with endemic genetic diversity that potentially can be rate limiting steps in HIV-1 infection, in disease progression, in the specific AIDS defining illness that develops and in the consequence of therapy. Since the discovery of CCR5-delta 32 in 1996, we have employed the technology, scope, and power of the human genome project to discover and characterize over twenty AIDS Restriction Genes, ARGs, in large genetic association studies. In my presentation I will describe the validity, attributes and applications of these genes for the AIDS epidemic. In addition further applications of ARGs based on selection signatures, mitochondrial DNA and Y-chromosome influences will be described. Finally a preview of a whole genome scan for undiscovered ARGs in the context of the recently annotated human Hap Map will be discussed.

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Dr. Stephen J. O'Brien is Chief of the Laboratory of Genomic Diversity and head of the Section of Genetics. He studied Drosophila genetics at Cornell University where he received a Ph.D. degree in 1971. He came to NIH as a postdoctoral fellow and built a program based on mammalian somatic cell genetics. He is co-chairman of the International Committee on Comparative Gene Mapping and

Editor of Genetic Maps. The principal focus of his investigation concerns the collaborative interaction of mammalian cellular genes operative in concordant evolutionary descent of the immune system, retroviruses, and cancer onset. The goal is to determine the comparative mammalian genetic principles that participate in these processes. Three sharply focused research projects are currently in progress.

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Avian Influenza: Preparing for and Responding to a Potential Human Pandemic

By Lance Gable

Highly pathogenic influenza A (H5N1) is endemic in avian populations in Southeast Asia, with serious outbreaks now in Africa, Europe, and the Middle East. Although the H5N1 virus is highly contagious among birds, relatively few human cases have been found due to a significant species barrier. Human cases continue to increase, with high reported case fatality rates exceeding 50%. However, due to possible under-reporting, the prevalence, transmissibility, and fatality of H5N1 remain uncertain.

The threat of a human pandemic from H5N1 influenza has spurred multiple emergency preparedness efforts around the world. These efforts seek to develop plans, strategies, resources, and expertise to avert public health emergencies from the pandemic influenza threat. The complexity of these initiatives requires the integration of scientific and medical expertise, augmented public health and medical capacity, good logistical planning, and clear laws and policies. In the context of a highly pathogenic strain of influenza, the principle strategies for prevention and response include authorizing medical countermeasures (e.g., vaccines, antiviral medications) and public health interventions (e.g., surveillance, quarantine, isolation). Each of these strategies presents complex problems that must be considered prior to their implementation. These issues are particularly important since pandemics can be deeply socially divisive and the politically-sensitive.

This presentation will focus on the major legal and policy challenges that affect emergency preparedness for a pandemic outbreak of human influenza. The presentation shall highlight the national and international approaches being considered to address emergency preparedness and response. Furthermore, the presentation shall consider how national and international systems can be

used to develop effective, sensible, and humane policies to prepare for and respond to this threat.

Medical countermeasures

In the face of a pandemic influenza outbreak, the international community will need a stable, economically viable supply of vaccines and antiviral medications to meet extensive public needs. However, a number of potential barriers may impede this goal. Market forces create disincentives to produce vaccines resulting in a limited number of vaccine manufacturers and a risk of severe vaccine shortages. Intellectual property laws may encourage innovation among pharmaceutical companies and researchers, but may also limit the production of and access to generic drugs under compulsory licensing provisions. Liability protections for pharmaceutical manufacturers may encourage these entities to produce vaccines and antiviral medications, but do not provide compensation for harmed patients. Regulatory requirements on pharmaceutical production and distribution can lead to burdensome delays or prevent rapid mass commercial production in a health crisis. Logistical hurdles may also impede effective deployment of medical countermeasures absent a cogent governance structure. Finally, assuming the international community is faced with a shortage of medications, it will be challenging to ration scarce, life-saving resources among many nations and their people.

Public Health Interventions

Population-based public health interventions are an important component of a preparedness plan. The key question is which measure, or combination of measures, works best at each stage of the pandemic. Multiple, targeted approaches are likely to be most effective, but can have deeply adverse consequences for the economy and civil liberties. Public health surveillance poses privacy risks as government collects sensitive health information from patients, travelers, and other vulnerable populations. This risk to privacy should be balanced with meaningful and responsible access to information by emergency responders. Agriculture and trade practices may need to be regulated to reduce animal-to-human transmission. However, such regulation can result in negative economic effects that must be balanced against the threat to the public’s health. Infection control is challenging and must be used reliably until the risk subsides. Policymakers will also have to address the problem of critical shortages in infection control and patient care equipment. Influenza vaccination can be critically important in preventing transmission, particularly among health care workers. Voluntary measures (education, incentives, peer advocacy, and easy access) could increase the vaccination rates. Mandatory vaccination also has been attempted, but the legitimacy and effectiveness of such a

strategy needs to be critically examined. Interventions such as social separation, community restrictions, isolation, and quarantine raise complex questions of law, ethics, and policy. Enforcement and assurance of population safety remain critically important but unanswered questions. Isolation and quarantine are extreme measures that require rigorous safeguards: scientific assessment of risk and effectiveness, safe and habitable environment, procedural due process, and the least restrictive alternative. Above all, the rule of law must ensure that state power is exercised fairly, and never as a subterfuge for discrimination. Finally, restrictions on international travel and border controls may be invoked and require careful scrutiny.

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Mr. Gable is a Professorial Lecturer at the Georgetown University School of Foreign Service, where he teaches a course on the politics of international health. He is the Project Director for the Emergency System for Advance Registration of Volunteer Health Professionals (ESAR-VHP) – Legal and Regulatory Issues project, administered by the Health Resources and Services Administration (HRSA). From 2003-2004, Mr. Gable was the Alfred P. Sloan Fellow in Bioterrorism Law and Policy at the Center for Law and the Public's Health at Georgetown and Johns Hopkins Universities. Beginning in fall 2006, Mr. Gable will be an Assistant Professor of Law at the Wayne State University Law School in Detroit, Michigan.

Mr. Gable received a Juris Doctor from Georgetown University Law Center and a Masters in Public Health from the Johns Hopkins Bloomberg School of Public Health. He also received a Bachelors Degree with a double major in political science and biology at the Johns Hopkins University. Prior to joining the Center for Law and the Public's Health, Mr. Gable practiced health care law at a major international law firm in Washington, DC.

Mr. Gable has extensive experience speaking on public health law and ethics issues at international, national, regional, and state conferences. He has also published numerous articles on topics related to public health law and policy.

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Clinical Outcomes and Immune Reconstitution in Advanced Chinese AIDS Patients Undergoing 12 Months of Hhighly Active Antiretroviral Therapy

By Taisheng Li

Objectives: To investigate the efficacy and side effects of highly active antiretroviral therapy (HAART) in Chinese AIDS patients.

Methods: 45 antiretroviral drug-naïve AIDS patients were enrolled and divided to two groups by their baseline CD4 count < 100/µl or ≥100/µl. Clinical, virological and immunological outcomes were followed at baseline and at the end of months 1, 3, 6, 9, 12 after receiving HAART, as well as side effects.

Results: As for the 45 HIV/AIDS patients included, by the end of 12 months of HAART, the plasma viral load (VL) got a mean reduction by 2.8 lg copies/ml, CD4 count had a mean gain of 187 /µl , among which the naïve phenotype increased by 68/µl and the memory phenotype by 119/µl; the CD4+CD28+T cell percentage went up from 62.5%±25.8% to 82.6%±15.6% ($p<0.001$); as well as there was a significant reduction of CD8+ T-cell activation. In 31 patients with their baseline CD4 count< 100/µl, 11 had a VL < 50 copies/ml, and 14 had fluctuations in their VL; while in 14 patients with their baseline CD4 count≥100/µl, 10 and 2, respectively, which had statistic significance between.

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Taisheng LI is a professor of infectious diseases in the Peking Union Medical College Hospital. His research interests include the pathogenesis of HIV/AIDS, immune response to acute and long term viral infections and influence of human gene expression on virus-host interactions. These activities have included the development of the immune reconstitution theory, the description of the characteristics of immunophenotypic alteration in Chinese HIV/AIDS patients, and of changes of peripheral T lymphocyte subsets in SARS patients. He also wrote the first version of "Guidelines for Diagnosis and Treatment of HIV/AIDS in China" as corresponding author.

Dr. Taisheng LI is also dean of the "HIV/AIDS center for treatment and diagnosis" in the Peking Union Medical College Hospital. He does outstanding work in fighting against HIV/AIDS epidemic in China, with many actions in both prevention and treatment.

Student Dialogue Abstracts:

SARS Coronavirus-infected Animal Model in Macaca Mulata

By Qin Chuan, Wei qiang et al

Development of drugs/vaccines against severe acute respiratory syndrome (SARS) depends mostly on a reliable and sensitive animal model. We successfully established a SARS animal model by inoculating SARS coronavirus (SARS-CoV) into Rhesus Macaque (Macaca mulata) through nasal cavity and bronchi and successively detected the pathological changes of lungs from macaques on 5, 7, 10, 15, 20, 30, and 60 days after virus inoculation, respectively. Although all the animals did not show clinical signs as severely as humans except for a transient fever 2 to 3 days after inoculation, there were three pieces of evidence that makes such SARS animal model convinced: firstly, the SARS-CoV-specific IgGs were positively detected in the sera of macaques from 11 to 30 days after inoculation; secondly, the SARS-CoV RNA in pharyngeal swab samples could be detected in all of 8 cases 5 days after inoculation via nested RT-PCR, while SARS-CoV particles were observed in lungs, spleens and lymph nodes under a transmission electron microscope, respectively; at last, pathological changes of interstitial pneumonia in lungs were dynamically found during 60 days after viral inoculation via autopsies, with characteristic of a human SARS patient-like process from exudative inflammation to fibrosis. Taken together, our data demonstrated that SARS-CoV could infect Rhesus Macaque to induce remarkable pathogenesis with obvious replication/excretion of SARS-CoV and induction of specific immune response, which might provide insight into the mechanism of SARS infection and greatly facilitate development of vaccines and therapeutics against SARS.

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China (SAC/TC281); Co-PI of Core D in CIPRA project; Professor of Pathology and supervisor of PhD of Peking Union Medical College. Major researches were focused on establishing and identifying the laboratory animal models of human disease. The Alzheimer's disease mouse model that was established by transgenic technique, and won the fruit prize of National Ministry of Health in 2002. More than thirty papers had been published on famous journals such as "Nature Medicine", "Journal of Pathology", "Journal of Virology", "Neurobiology Disease"," Journal of Pineal Research"," Free Radical Biology Medicine". PI in many grants of "the ninth five-year project" and "nature science fund"; "National 973 program"、"National 863 program" etc. The projects of "the establishment of SARS-CoV susceptive animal model" and "the development of inactivated SARS vaccine" supported by National 863 momentous project contributed very much.

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Novel class of HIV-RT inhibitors

By Lan Xie

In previous research, 3 ,4 -di-O-(S)-camphanoyl-(+)-cis-khellactone (DCK, 1) and its analogs were identified as a novel kind of potent anti-HIV agents that inhibit the production of double-stranded viral DNA from the single-stranded DNA intermediate[4], in contrast to current HIV-RT inhibitors. Due to the low solubility and poor bioavailability of first-generation DCK analogs, our research returned again to novel modification of DCK analogs to identify new drug candidates with potent anti-HIV activity and acceptable pharmaceutical properties.

Based on the structures and bio-data of previous DCK analogs, 3D-QSAR studies have been

performed which resulted in two reliable computational models, CoMFA and CoMSIA, with r2 values of 0.995 and 0.987, and q2 values of 0.662 and 0.657, respectively. In accord with these 3D-QSAR models, 16 new DCK analogs with polar functional groups at the 3-position were subsequently designed, synthesized, and evaluated against wild-type HIV-1 strain replication in H9 and MT4 cell lines. Seven DCK analogs with predicted log P values from 0.90 to 5.19 showed promising potency with EC50 values ranging from 0.11 to 0.0002 M in both cell lines[5-6]. Furthermore, 2 promising compounds also exhibited inhibitory activity against mutant viral strains (K103N+Y181C) in MT-2 cell line. Preliminary ADMET evaluation indicated that both compounds have lower toxicity and better pharmacokinetic properties than first-generation DCK analogs. They showed good cell permeability (Caco-2 assay) and enough metabolic stability. Their acute oral toxicities are >2000 mg/kg in rats, and no teratogenicity and mutagenicity were observed. Further studies are in progress.

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Lan Xie received her B.S. degree in Organic Chemistry from Wuhan University, P.R. China in 1982. She then worked on drug discovery and development at Institute of Materia Medica, Chinese Academy of Medicinal Sciences, P.R. China as research assistant and research associate for a number of years. She returned to graduate school in 1995 and received Ph.D. degree in Medicinal Chemistry in 1999 from the University of North Carolina at Chapel Hill under the direction of Professor Kuo-Hsiung Lee. Dr. Xie joined Triad Therapeutics, Inc., San Diego, CA, in 2000. She is now a Professor in the Department of Chemical Synthesis, Beijing Institute of Pharmacology and Toxicology, P.R. China. Dr. Xie's research interests include the design and synthesis of anti-AIDS and other antiviral agents. She has published over 40 articles and obtained 5 patents dealing with anti-HIV agents.

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Bartonella vinsonii berkhoffii Isolated from Blood of Native Dogs in China

By Qi-yong Liu

Dong-mei Li，Feng-xia Meng， Xiu-ping Song， Zeng-jun Qin， Xiao-ran Yang， Hai-xia Wu，Dong-sheng Ren， Qi-yong Liu
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Objective: To isolate and identify Bartonella strains from native dogs in Shandong Province in China. Methods The EDTA-anticoagulated blood samples were collected from 71 native dogs in Yanggu County of Shandong Province in March 2005. All isolates were grown on brain heart infusion agar plates containing 5% defibrinated rabbit blood. The agar plates were incubated at 37 in a humidified with 5% CO2 environment for 4 weeks at least. All Bartonella-like isolates were examined by routine Gram and Giménez staining and then followed by PCR and PCR-RFLP analysis to identify and differentiate the isolates. Sequencing 16S rRNA, citrate synthase (gltA) gene and 16S-23S rRNA ITS were carried out and sequence similarities was calculated using the DNASTAR5 software package. The phylogenetic tree was inferred from each bootstrap sample by using the neighbor-joining methods as executed in the MEGA3.1 software. The translation from DNA to protein were determined by using the DNASIS2.5. results The two Bartonella-like oganisms(strains Q52SHD and Q64SHD) were isolated from the blood of 71 dogs. Light microscopic examination of the Gram and Giménez–stained micro-organisms showed small, short and slightly curved pleomorphic Gram-negative bacilli. Amplified products of the four pairs of Bartonella genus- specific primers were the same size as the predicted of those Bartonella species. PCR-RFLP analysis show that the two strains have same profiles were all different from the B.henselae type strain. 16S rRNA, gltA and 16S-23S rRNA ITS sequences from the two isolates were 100.0%, 99.7% and 97.2% homologous to B. vinsonii berkhoffii. Conclusions On basis of these findings, the two isolates Q52SHD and Q64SHD are demonstrated B. vinsonii berkhoffii. To our knowledge, this is the first report on the presence of Bartonella infection in native dogs from China, which constitute a large reservoir of Bartonella species in this country.

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Identification of new Neisseria Meningitides serogroup C clone from the Anhui Province of China

By Zhujun Shao

Background Outbreaks of a new serogroup C meningococcal disease emerged during 2003–04 (five outbreaks with 43 cases) and in 2004–05 (five outbreaks with 29 cases), all in Anhui province, China. We describe the molecular epidemiology and features of the causative bacterial strains. Methods We used pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) to analysethe strains. Findings Of 34 strains of Neisseria meningitidis cultured during 2003–04 from Anhui province, 31 were group C meningococci, 28 of which were associated with three of five outbreaks; one from a patient and 27 from close contacts of eight patients. Of 30 strains isolated from Anhui province during 2004–05, 17 were identified as serogroup C meningococci, ten of which were associated with four of five outbreaks. In a nationwide survey, 542 strains were isolated during 2004–05; 58 were serogroup C meningococci interspersed among 11 other provinces where no serogroup C outbreak occurred. Of the 106 serogroup C strains analysed, 89 had identical PFGE patterns, designated AH1. Of 28 strains selected for MLST analyses, 25 were sequence type 4821 (ST-4821), which did not belong to any of the previously reported sequence types that can form a new hypervirulent lineage. Interpretation ST-4821 seems to be unique and caused the serogroup C meningitis outbreaks during the two seasons from 2003 to 2005 in Anhui province. The emergence of this sequence type has epidemiological importance that should be monitored for future spread in China and the rest of the world.

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Zhujun Shao, born in 1971, member of the State Key Laboratory of Communicable Disease Prevention and Control, is majoring in the bacteria-associated respiratory infectious disease control and prevention, including Meningococcal Disease, Legionellosis and Hib et al. Now , Shao is in

charge of one key technologies R&D program grant of research of meningococcal disease and respiratory disease, from the Ministry of Science and Technology, People's Republic of China and responsible for the laboratory work of national meningococcal disease surveillance. In 2005, meningococcal disease outbreaks occurred in Anhui province, China. Shao and other colleagues found that the serogroup C meningococcal disease outbreaks were caused by one new clone strains, named ST-4821. The results were published by The Lancet on Feb, 4th 2006.

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Proteome analysis and the finding of biomarkers related to infectious diseases detection and surveillance

By Jianzhong Zhang

To find the biomarkers related to infectious diseases detection and surveillance is the key step to infectious control and prevention. Proteome analysis is a methodology, based on the core techniques of two-dimensional gel electrophoresis and mass spectrometry (MS), which is directed towards the resolution, display and identification of the whole complement of proteins expressed by an cell or tissue. Some studies on bacteria proteins have suggested that the identification of the proteins might provide important insights into the molecular mechanisms of pathogenesis, find protein marker candidates, which could be useful for laboratory tests, infectious diseases detection and surveillance.

2-DE and MALDI-TOF-MS

The first dimension (isoelectric focusing, IEF) of 2-DE was performed on a Pharmacia Biotech IPGphor Electrophoresis System.. After IEF, the second dimension was performed on 12.5% polyacrylamide gels at 25 in a Pharmacia Biotech Ettan DALT system using a continuous Tris-glycine buffer. The second dimension electrophoresis program was as follows: 2.5W per gel for 0.5h; 18W per gel for 4.5 h (all set for constant watt). Proteins in the slab gel were quantitatively visualized by silver staining. Gels were digitized by scanning with Pharmacia Biotech ImageScanner, using a resolution of 400 bpi. The gels were analyzed by 2D-Image Master 4.0 software (Pharmacia Biotech). Protein spots were excised from the gel (Coomassie Brilliant Blue R250 staining) by Spotspick (Pharmacia Biotech)and each spot was transferred into one tuber. Samples were washed in

60%, 50mM ammonium bicarbonate, 40% acetonitrile for 1 hour or until all traces of Coomassie Brilliant Blue had been removed. Gel pieces were then dried under vacuum for 30 min, rehydrated in 15μl trypsin solution (20μg/ml in 25mM ammonium bicarbonate) and incubated for 30 min ant 4 . Unabsorbated trypsin solution was replaced with 10 ml the same buffer but without the protease to keep the gel pieces wet during the enzymatic cleavage (37 overnight). Trypsin peptides were analyzed in a Reflex (Bluker) matrix-assisted laser desorption/ionization time of-flight (MALDI-TOF) mass spectrometer.

Finding of biomarkers related to infectious diseases detection and surveillance

V.cholerae strains were cultured in media with and without sorbitol respectively. Proteins were separated by 2-DE and those showed different expression in the two media were identified by MALDI-TOF. It was demonstrated that 15 proteins in epidemic strains and 11 proteins in non-epidemic strains showed different expression in sorbitol medium. Among them 4 proteins were in common.

As well as Vibrio cholera, different strains belong to Campylobacter jejuni, Yersinia, Helicobacter pylori,TB and Neisseria meningitidis serogroup C were analyses, and some biomarkers related to infectious diseases detection and surveillance were found.