New developments in the pathophysiology and diagnosis of dengue fever and its complications

V.T.K. Chow*.

Infectious Diseases Program & Department of Microbiology, School of Medicine, National University of Singapore, Singapore

Early diagnosis of dengue fever contributes towards better management of the disease and its complications. Molecular strategies are leading to more rapid techniques for detecting dengue viral RNA, antigens and antibodies. RT-PCR using consensus primers and four type-specific downstream primers based on the NS3 gene can discriminate all four virus serotypes. While RT-PCR is extremely sensitive, specific and identifies the specific serotype in serum samples, it generally yields positive results in viremic sera collected within 2–5 days of fever. Viral load is an obvious factor in pathogenesis since high viremic titers correlate with severe complications, and can be assayed by quantitative real-time RT-PCR. Microfluidic chips simplify and accelerate dengue viral RNA extraction and amplification. Detection of dengue NS1 antigen in serum also facilitates earlier diagnosis. Sera obtained after viremia are more likely to be positive by serological tests such as IgM capture ELISA. Point-of-care kits are available for direct patient testing. Tracking dengue virus in field-caught adult Aedes mosquitoes and correlating with outbreak areas can enhance vector surveillance and control. Phylogenetic trees and sequence differences even between circulating homotypic strains from patients and field-caught mosquitoes emphasize the molecular epidemiology and evolution of geographically and temporally separated dengue viruses within these hosts. Virological, host immunological and cellular mechanisms of pathogenesis influence the outcomes of dengue infection, ranging from mild fever to hemorrhagic phenomena, shock syndrome and even death. The high mutation rates of dengue viral RNA genomes culminate in their genetic variability and diversification, thus encouraging the emergence of strains with heightened virulence. The numerous dengue viral genotypic variants differ in their virulence, infectivity and replication, and are associated with varying disease severity. Specific mutations within structural and non-structural proteins confer greater pathogenicity. The risk of hemostatic defects and hypotensive events is greater in secondary infection, and is explained by antibody-dependent enhancement of infectivity mediated by cross-reactive but non-neutralizing dengue viral antibodies. Macrophages and mononuclear cells are permissive for dengue viral replication, culminating in the release of inflammatory mediators and cytokines. The ensuing complement activation, excessive production of vasoactive cytokines and other molecules contribute to thrombocytopenia and increased vascular permeability. Unusual clinical manifestations such as hepatitis, encephalitis, myocarditis, acalculous cholecystitis as well as marked variation in the susceptibility of dengue virus infection among cell types have been observed. Certain dengue-virus infected cells undergo programmed cell death or apoptosis, e.g. dengue virus infection of human endothelial cells results in apoptosis, activation of complement and chemical mediators. Gene transcription profiles of human cells infected with virulent dengue virus strains, as well as interactions between dengue viral and host cell proteins have implicated the role of host gene products such as STAT3, NRBP, DC-SIGN, and TRAIL. Dengue “infectomics” can potentially contribute towards cellular pathophysiology, novel prognostic markers, and potential targets for vaccine prevention and antiviral treatment of this reemerging disease.


Q.Y. Liu1,2*, H.L. Lin1. 1National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, 2State Key Laboratory for Infectious Disease Prevention and Control, Beijing, China

Objective: To analyze the temporal–spatial distribution characteristics of dengue fever during 2001–2006 in Guangdong Province, China, and to identify key areas for future public health planning and resource allocation in Guangdong Province.

Methods: The epidemic data of dengue fever on the county level during 2001–2006 of Guangdong Province were collected, and a geographical information system database was established using ArcGIS 9.0, and then the temporal–spatial cluster analysis was implemented by SaTScan7.0 to find out the temporal–spatial highly epidemic areas.

Results: The temporal–spatial distribution of dengue fever in Guangdong Province was not random. When the biggest moving radius was set as 50% of all population, there were two significant clusters, the most likely cluster was in the middle districts of Guangdong Province, July–October 2002, the annual incidence was 36.7/100,000, and the risk ratio (RR) was 114.27. The secondary cluster was located in the west, September–October 2006 with the annual incidence 18.7/100,000 and the RR 38.68. When the radius was 30%, there were also several clusters, the most likely cluster was the same as the 50% analysis, and the secondary cluster included three districts, one was at the mid-south region, August–November 2001 with the annual incidence 11.4/100,000 and the RR 25.04, another was at the west districts, September–October 2006 with the annual incidence 18.7/100,000 and the RR 38.68, and the last was at the south districts, September–October 2002 with the annual incidence 4.0/100,000 and the RR 7.83. Other results were also relevant.

Conclusions: Some epidemic clusters of dengue fever were found in Guangdong Province, which reminds us that we should implement specific and geographically appropriate risk-reduction programs, and the use of such spatial analysis tools could become a useful component in epidemiology research and risk assessment of dengue fever.

Phylogenetic status of Pneumocystis derived from Mongolian gerbil

X.M. Feng1, C.J. Wei1, R.D. Adam2, Z.H. Li1,2, F.Y. Wang1, S.Q. Lu1*. 1Department of Parasitology, Capital Medical University, Beijing, China, 2Departments of Medicine and Immunobiology, University of Arizona College of Medicine, Tucson, USA, 3Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing, China

Background: Pneumocystis spp. infect the lungs of multiple mammalian species and cause disease in immunosuppressed individuals. There is at least some degree of host specificity with primate Pneumocystis spp. (humans and monkeys) forming one group and rodent Pneumocystis forming another group.

Aim: To understand the phylogenetic status of Pneumocystis derived from Mongolian gerbils (Meriones unguiculatus).

Methods: Animals were immunosuppressed by subcutaneous injections of dexamethasone (0.04 mg/g weight) twice a week for 8 weeks. After the animals were sacrificed, lung
impression smears and lung tissue sections were made and stained to observe the extent of infection. Also, the samples of lung tissue were observed with transmission electron microscopy for morphology study. Genomic DNA was extracted from the lung tissue of the infected animals. 5.8S and ITS1 and ITS2 regions of the rDNA, a portion of the mitochondrial large subunit (mtLS) rDNA and entire fragment of 18S rRDA were amplified and sequenced. The sequences were compared with those from other isolates of *Pneumocystis* and those from fungal out groups accessed in GenBank. Phylogenetic trees based on the sequences were constructed with Neighbor-joining (NJ) method.

**Results:** The current study showed that dexamethasone immunosuppression consistently resulted in histologically apparent lung infection in gerbils (28/35). Analysis of the Sequences and phylogenetic trees demonstrated that the gerbil *Pneumocystis* grouped with, but was distinct from other rodent *Pneumocystis*. Our results suggest that the gerbil *Pneumocystis* is distinct enough from the *Pneumocystis* species from in other rodents to consider it as a separate species. According to the criteria recommended in 2006 (Redhead et al., 2006), the appropriate species name would be *Pneumocystis unguiculatii*.

**I-82 Proteomic analysis of CSF in patients with neurocysticercosis**

Y.P. Xue1 *, X.J. Tian1, J.Y. Li1, S.Q. Lu2, 1Beijing Tropical Medicine Research Institute, Beijing Friendship Hospital, Beijing, China, 2Capital Medical University, Beijing, China

**Aim:** To investigate the proteomic profile of CSF in patients with active cysticercosis with that of healthy subjects in order to further search the potential biomarkers related to neurocysticercosis.

**Methods:** 2D-DIGE (two-dimensional difference in-gel electrophoresis) was used to investigate differential expressed proteins in CSF of patients with active neurocysticercosis by comparing the protein profile of pooled CSF from patients with active neurocysticercosis and healthy subjects respectively. The Coomassie blue stained protein spots correspondent to the differentially expressed protein spots in DIGE analysis gel were excised and identified with MALDI-TOF-TOF.

**Results:** Analysis of 2D-DIGE with DeCyder 5.0 software found 94 protein spots were differentially expressed over 1.5 fold between active neurocysticercosis group and control group. Forty four of enzyme digested peptide mass fingerprint were obtained with MALDI-TOF-TOF. Of the 44 proteins, 23 were identified through search of the NCBI protein database with Mascot software, including 19 expressed more than 1.5-fold higher and 4 expressed more than 1.5-fold lower in CSF of patients with active neurocysticercosis.

**Conclusions:** Our approach demonstrates the capacity of proteome approach to analyze cysticercosis at the level of CSF proteins and provides a rational basis for screening biomarkers that may be used for the study of diagnosis of active neurocysticercosis. Further studies are needed to confirm this observation and determine characteristic proteomic profile or potential biomarker.

---

**I-83 A new mouse model for investigating tolerance to hepatitis B virus: The important role of HBV core protein**

P.J. Chen1,2, Y.J. Lin1, 1Graduate Institute of Microbiology, National Taiwan University College of Medicine, Taipei, Taiwan, 2Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

We have successfully developed an immunocompetent mouse model for the tolerance of human hepatitis B virus persistence (Huang et al., PNAS 2006; 103: 17862) using a replication-competent plasmid, pAAV/HBV1.2. By this model, we recently found that mice receiving c/e-null pAAV/HBV1.2, which is defective both in core and e antigen expression, had higher surface antigen persistence rate compared to those receiving wild type (100% and 14–20% respectively at 8 weeks post-injection). On the other hand, mice receiving e-null pAAV/HBV1.2 developed similar surface antigen persistence rate to mice receiving wild type. To dissect which domain, the assembly domain (aa 1–149) and/or the protamine-like domain (aa 150–185), of core protein is important for the HBsAg persistence, we generated a series of core protein mutants in pAAV/HBV1.2 by site-directed mutagenesis. Wild-type and the mutant c/e-null, HBc118, HBc150, HBc166, and HBc175 pAAV/HBV1.2 were hydrodynamically injected into C57BL/6 mice respectively and the persistence of HBsAg as well as the viral transcription and translation were assayed in these mice. At day 3 and 41 post injection, the levels of all viral transcripts were similar among mice receiving the mutants and wild type, while the viral replication could only be detected in mice receiving wild type and HBc175 mutant. Interestingly, mice injected with HBc166 and HBc175 mutants, in which the protamine-like domain of core protein are partially deleted, showed significantly higher HBsAg persistence rate (86–100% and 71–83 respectively) than mice injected with wild type at 8 weeks post-injection. In mice receiving Hbc118 and Hbc150 mutants, core proteins were barely detected and the HBsAg persistence rates in these mice were close to that in mice receiving the c/e-null mutant. Our preliminary data suggested that protamine-like domain, especially the carboxyl-terminal 10-aa region, might be important for HBsAg persistence. The underlying mechanisms for these phenomena are under investigation.

**I-84 Gene therapy of chronic hepadnavirus infection**

J. Prieto*, J. Crettaz, G. Gonzalez-Aseguinolaza. *Clinica Universitaria and Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain*

Chronic hepatitis B is a major cause of liver-related death worldwide. Interleukin-12 (IL-12) induction accompanies viral clearance in chronic hepatitis B virus (HBV) infection. Here, we tested the therapeutic potential of Interleukin-12 (IL-12) gene therapy in woodchucks with chronic woodchuck hepatitis virus (WHV) infection, a condition that closely resembles chronic hepatitis B. Woodchucks were treated by intrahepatic injection of a helper-dependent adenoviral vector encoding IL-12 under the control of a liver-specific RU486-responsive promoter. All woodchucks with viral load below 10^{10} viral genomes (vg)/ml showed a marked and sustained reduction of viremia accompanied by a reduction in hepatic WHV-DNA, loss of e- and surface antigens and improved liver histology. In contrast, none of the woodchucks with viremia higher than 10^{10} vg/ml responded to therapy. In the responder animals, the antiviral effect