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25th Clinical Virology Symposium
And
Annual Meeting of the
Pan American Society for Clinical Virology
April 19–22, 2009

And
16th Molecular Virology Workshop
April 17-18, 2009
Daytona Beach, Florida

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- www.cme.hsc.usf.edu/virology/
2009 PASCV Award Recipients

Ed Nowakowski, Senior, Memorial Clinical Virology Award
Sponsored by BION Enterprises, Ltd.

Robert G. Webster, PhD, FRS

Dr. Robert G. Webster is Professor in the Division of Virology, Department of Infectious Diseases at St. Jude Children’s Research Hospital and holds the Rose Marie Thomas Chair. A native of New Zealand, Dr. Webster received his B.Sc. and M.Sc. in Microbiology from Otago University in New Zealand. In 1962, he earned his Ph.D. from the Australian National University and spent the next two years as a Fulbright Scholar working on influenza in the Department of Epidemiology at the University of Michigan, Ann Arbor.

Dr. Webster is Director of the Center of Excellence for Influenza Research and Surveillance. His interests include the emergence and control of influenza viruses, viral immunology, the structure and function of influenza virus proteins and the development of new vaccines and antivirals. The major focus of his research is the importance of influenza viruses in wild aquatic birds as a major reservoir of influenza viruses and their role in the evolution of new pandemic strains for humans and lower animals. His curriculum vitae contains over 500 original articles and reviews on influenza viruses. He has trained many scientists who now contribute to our understanding of the evolution and pathogenesis of influenza.

Dr. Webster is a Fellow of the Royal Society, London and a member of the National Academy of Sciences of the United States of America.
Dr. Guy Boivin is a medical microbiologist/virologist and an infectious disease specialist working at the "Québec City University Hospital Center" (CHUQ-CHUL) in Canada. Dr. Boivin is also a professor of medical biology (division of microbiology) at Laval University and a senior researcher in virology at the "Research Center in Infectious Diseases" of the same University.

Dr. Boivin holds a MD from Laval University, a master (MSc) degree in microbiology from University of Montréal and a 3-year specialized research training (Fellowship) in Molecular Virology from University of Minnesota.

Dr. Boivin is currently the holder of the Canada research chair on Emerging Viruses and Antiviral Resistance (2006-13). He is also the director of the Canadian Center of Excellence on Herpesviruses and he holds numerous research grants from governmental health organizations (Canadian Institutes of Health Research, Quebec Health Research Foundation) and private companies. His main research interests concern the diagnosis, pathogenesis and treatment of viral diseases caused by herpesviruses (mainly cytomegalovirus and herpes simplex virus) and respiratory viruses (mainly influenza virus, human metapneumovirus and human respiratory syncytial virus) and the mechanisms of resistance to antiviral drugs.

Dr. Boivin is member of several distinguished societies including the American Society for Microbiology, the American Society of Transplantation, the Canadian Association of Medical Microbiology and Infectious Diseases, the Canadian Society for Clinical Investigation and the Quebec AIDS and Respiratory Diseases Networks.

In addition, Dr. Boivin has published more than 150 peer-reviewed manuscripts and presented more than 200 communication abstracts since 1993. He is on the Editorial board of the Journal of Infectious Diseases, Herpes Journal and the Canadian Journal of Infectious Diseases.
David Wang, Ph.D.

David Wang received his B.S. degree from Stanford University in Chemistry in 1992 and his PhD in Biological Chemistry from MIT in 1998. During his postdoctoral training at UC San Francisco, he worked with Don Ganem and Joseph DeRisi to develop the first pan-viral DNA microarray (ViroChip) designed for detection of both known and novel viruses. The first successful application of this approach was during the SARS epidemic of 2003, when the ViroChip was used to help identify SARS as a novel coronavirus.

Since 2004, he has been an assistant professor in the Departments of Molecular Microbiology and Pathology and Immunology at Washington University in St. Louis. The focus of his laboratory’s research is the identification and characterization of novel pathogens. In 2007, his laboratory used high throughput sequencing methods to discover the novel WU polyomavirus in respiratory secretions from a child with unexplained pneumonia. In addition, his laboratory identified the novel astrovirus MLB1 in a stool specimen from a patient with diarrhea of unknown etiology in 2008. His laboratory continues to develop and refine new technologies for pathogen discovery and to study the molecular and clinical features of newly identified viruses.
Human Metapneumovirus
– a Minireview

Ted E. Schutzbank, PhD, D(ABMM), Associate Director of Genomics, Covance Central Laboratory Services, Indianapolis, IN

Historical Perspective

In 2001, van den Hoogen and her colleagues at the Erasmus Medical Center in Rotterdam announced the discovery of a new respiratory virus. The new agent was isolated from nasopharyngeal aspirates obtained from children suffering from respiratory tract infections. Inoculation of these specimens into tertiary monkey kidney cells (tMK) resulted in CPE, but no specific virus could be identified after immunofluorescent antibody (IFA) staining for a wide variety of viruses including adenovirus, influenza A, B, and C, measles, mumps, respiratory syncytial virus (RSV), and parainfluenza types 1 – 4 (1). The unidentified virus isolates would replicate only in tMK cells, albeit slowly, poorly in Vero and A549 cells, and not at all in MDCK or chicken embryo fibroblasts. The CPE was identical to that induced by RSV, about 10 – 14 days after inoculation. Electron microscopic examination of the cell-free culture medium showed enveloped particles with morphology consistent with paramyxoviruses. Antisera from experimentally infected guinea pigs did not react by IFA with cells infected with a variety of ortho- and paramyxoviruses. Nucleic acid sequencing of the viral RNA demonstrated that the genomic organization of the new virus was similar to that of avian metapneumovirus. The inferred amino acid sequence for several of the viral proteins showed significant homology with their avian counterparts, but little homology with RSV. The name given this new agent was human metapneumovirus (hMPV).

The Virus

As we now know, hMPV is a member of the Paramyxoviridae family, Pneumovirinae subfamily, and Metapneumovirus genus. The genome consists of a single negative-stranded RNA molecule about 13.3 kb, consisting of eight genes coding for at least 9 proteins (2). The virus has been classified into 2 major serological groups (A and B) and at least 4 subgroups (A1, A2, B1, and B2) based on phylogenetic analysis of F and G protein sequences (2a).

Epidemiology

Although not actually identified until 2001 (1), retrospective studies indicate that hMPV has been present (at least in the Netherlands) since 1958 (1), and as long as 15 – 20 years in Canada and the US (3, 4). Most infections occur during the winter months, paralleling the appearance of RSV. Infections are reported in all age groups, but, similar to RSV, more severe disease occurs in young children <2 years old, immunocompromised children and adults, and the frail elderly. Most children are infected at an early age (<1 year old), and nearly all children are infected by the ages of 5 – 10 years old (1, 5). The incidence of hMPV infection in children varies from 5% – 10% in those hospitalized for severe acute respiratory tract infections, to 12% – 20% in children seen at physicians’ offices and out patient clinics (2, 6). Again, as with RSV, there is no long lasting immunity, and re-infections are common throughout life.

hMPV is also a significant cause of community-acquired respiratory disease in adults, including pneumonia (7, 8). Although hMPV infections occur primarily during the winter months, at least one summer outbreak has been reported in the elderly in a long term care facility. (9).

Clinical Presentation

The clinical presentation of hMPV is identical to RSV, ranging from the common cold and mild upper respiratory tract infection, to lower respiratory tract infections such as bronchiolitis and pneumonia. As with RSV, the latter can result in hospitalization, and patients may require mechanical ventilation (6). Common symptoms in pediatric cases are cough, wheezing, coryza, fever, diarrhea, vomiting and dyspnea, as well as acute otitis media (1, 10). Infection with hMPV has also been implicated in exacerbation of asthma (2, 10).

Pathogenesis

The vast majority of information regarding the pathogenesis of hMPV has been gathered through animal studies. Airway epithelial cells are the primary target of infection (11, 12). Infection by hMPV induces pulmonary inflammation, characterized by alveolitis, interstitial inflammation, and increased peribronchiolitis (11, 14 15). Infection of primary small alveolar cells, or alveolar type II-like epithelial cell lines (A549), induces expression of a variety of chemokines, RANTES, and IL-8 (13).

Neurological manifestations have also been associated with hMPV infections (16), with one reported case of fatal encephalopathy possibly attributable to hMPV (17).

Laboratory Diagnosis

The laboratory diagnosis of hMPV infections is difficult for several reasons. First, hMPV grows poorly in most cell lines commonly used in the clinical laboratory, and the CPE is indistinguishable from that of RSV. Second, until recently, there were no commercially available hMPV-specific monoclonal antibodies; laboratories had to rely on polyclonal and monoclonal reagents produced by research laboratories (1, 18). Due to the lack of standardized biological reagents real-time PCR assays were developed. Most of the reported methods targeted the N, P, F, or L gene, due to the presence of conserved nucleotide sequences common to all four subgroups and the ability to determine subtypes using DNA sequencing (19 – 22). One recent report demonstrated the utility of a pan-virus microarray (Virochip) for hMPV detection in a critically ill patient (23).

Molecular diagnostic reagents are now commercially available, either as analyte specific reagents (ASR’s) based on nucleic acid sequence-based amplification (NASBA) (NuclISENS hMPV, BioMerieux Durham, NC) (24) or PCR-based research use only kits (MultiCode-Plx, Eragen Biosciences, Madison WI, ProhMPV and Hexaplex, Prodesse Inc., Madison WI, and Resplex II, Qiagen Inc., Germantown, MD). One multiplex PCR-based respiratory panel, xTAG™ RVP (Luminex Molecular Diagnostics, Toronto, Ontario), has recently been cleared by the FDA as an in vitro diagnostics kit. This kit detects
up 12 different viruses or virus subtypes, including hMPV (25).

Standardized biological reagents are also now commercially available for detection of hMPV by cell culture, or directly from patient specimens by direct fluorescence immunoassay (DFA). FITC-conjugated antibodies are available as ASR’s from both Diagnostic Hybrids (Athens, OH), and Light Diagnostics (Temecula, CA). Figure 1 shows upper respiratory tract epithelial cells obtained from an hMPV-infected patient, stained with such a monoclonal antibody. Various cell lines, including A549, HEP-2, and LLC-MK2, have been evaluated for rapid shell viral centrifugation culture (26) with acceptable results. Several poster presentations at previous Clinical Virology Symposia (as well as other scientific meetings), have also demonstrated the utility of these reagents using the above cell lines, as well as shell vial culture (R-mix and R-mix Too, Diagnostic Hybrids) (24, 27).

Prevention and Treatment

No vaccines are currently available for the prevention of hMPV infection. Several approaches are being evaluated for the development of live attenuated virus, as well as virus subunit vaccines (28, 29). Antibodies directed against hMPV F protein have been shown to neutralize the virus in vitro (30). Monoclonal antibodies directed against the neutralizing epitopes of this protein have been developed that prevent hMPV infection in hamsters (31). Another recent study has shown that a humanized monoclonal antibody directed against the F protein is therapeutically effective in experimentally infected cotton rats (32). The antiviral agent, ribavirin, has also been studied, but with little success (33).

Conclusions

hMPV is now recognized as an important pathogen, and a major cause of respiratory infections, especially in young children. The virus is a relative of RSV, and is very similar to this close cousin in terms of its epidemiology, spectrum of disease, and pathogenesis. Much research is still needed before an effective vaccine can be produced. Efforts to develop immunotherapeutic agents are on-going.

References

The Re-emergence of Mumps: More Questions than Answers

Todd F. Hatchette MD FRCPC, Director of Virology and Immunology, Division of Microbiology, Department of Pathology and Laboratory Medicine, QE II Health Science Center, Halifax, NS

Mumps is a highly infectious, vaccine preventable viral illness, spread by respiratory droplets. Since routine vaccination was instituted in 1969, North America has seen a 99% decline in mumps cases. Within the last decade, however, outbreaks of mumps have occurred in the United Kingdom, the US, and in Canada (1-3). As of March 5, 2008, 1,284 confirmed cases of mumps were reported in 2007 from ten of 13 Canadian provinces and territories; 61% of cases occurred in Nova Scotia. 

We recently described two successive outbreaks of mumps in Nova Scotia in 2005 (3). The majority of Nova Scotia cases were in adolescents and young adults. In the first outbreak, 69% of cases had received 2 doses of mumps vaccine; the median age of those infected was 14 years. In the second outbreak, which affected older individuals among a university population (median age 23 years) only 5% received two vaccine doses. The second outbreak was similar in epidemiology to outbreak cases seen in the US in 2006 in that the highest age-specific rate was among individuals aged 18-24 years, many of whom were college students (3, 4). However the second Nova Scotia outbreak differed notably in vaccination status from the US cases; 84% of US cases between the ages of 18 and 24 years had received 2 doses of vaccine (4).

Why these outbreaks occurred in partially or fully immunized populations has not been entirely elucidated. Multiple factors have been proposed as contributing to these mumps outbreaks including primary vaccine failure, waning vaccine-induced immunity, and close living conditions among college students, particularly those living in dormitories.

Evidence of waning immunity was demonstrated by Date et al. (5) who measured neutralizing antibody (NA) titers and stratified results by time from receipt of the second dose of MMR vaccine. Geometric mean NA titers were 97 and 58 for those vaccinated 1-5 years previously vs. those vaccinated ≥15 years ago, respectively. While not significantly different, these results demonstrate that antibody levels can decay over time (5). However, because the minimum level of neutralizing antibody required for protection against mumps infection is not known, it is not possible to correlate a decline in antibody levels with increased susceptibility to infection.

Another possible reason that mumps infection occurred in vaccinated populations is that subtle antigenic differences between wild-type mumps (primarily genotype G) and the vaccine strain (genotype A) rendered vaccine-induced immunity less effective against wild-type virus. However, Rubin et al. (6) also demonstrated that vaccination with the Jeryl Lynn mumps vaccine strain elicited neutralizing antibodies against both genotype G (wild-type) and vaccine strains, although titers to wild-type virus were approximately one-half of the GMT NA titers to the Jeryl Lynn virus. Again, because the minimum level of neutralizing antibody required for protection against mumps infection is not known, it is possible that antigenic differences between vaccine and wild-type strains rendered vaccine-induced immunity less effective against a heterologous wild-type strain (6).

Laboratory diagnosis of mumps has proven difficult in a partially or fully immunized population. The CDC recommends capture-IgM serology and a swab from the parotid duct or other affected salivary gland ducts for viral isolation and reverse transcriptase-PCR (2). However, detection of IgM antibodies by capture IgM antibody was successful in only 15-50% of clinical cases (4).
Commercial IgM assays also performed poorly (7). Indeed, Maudlin et al. (8) demonstrated that measurement of neutralizing antibodies is a more sensitive and specific method of measuring serological responses to wild-type mumps virus. Likewise, PCR and virus isolation studies were noted to be “unhelpful” in diagnosis due to the presence of low virus titers in vaccinated persons (4).

Vaccination recommendations have been updated in both the US and Canada as result of mumps outbreaks in these countries. After the first outbreak in 2005, Nova Scotia decided not to offer vaccine to those individuals who had received only a single dose of mumps virus vaccine (3). However, in 2007, the Canadian National Advisory Committee on Immunization modified its recommendations regarding mumps vaccination to include the recommendation for a second dose of mumps vaccine to children before school entry and to high risk individuals such as secondary and post-secondary students and health care workers. (http://www.phac-aspc.gc.ca/media/cpho-acsp/mumps_naci070821-eng.php).

In the US, the Advisory Committee on Immunization Practices (ACIP) updated mumps vaccination recommendations in 2006 in response to the nationwide mumps outbreak (9). 1998 ACIP recommendations (10) required one dose of MMR vaccine at ages 12-15 months and a second at ages 4-6 years. Two doses of MMR vaccine were also recommended for students attending colleges and other post-high school institutions. However, documentation of immunity to mumps required only 1 dose of mumps-containing vaccine for all groups, including health care workers (HCWs). Updated recommendations (9) now define acceptable documentation of mumps immunity as 2 doses of a live mumps virus vaccine for school-aged children and for high-risk adults (HCWs, international travelers, and students at post-high school educational institutions). In addition, routine vaccination recommendations for HCWs have been changed. Two doses of live mumps virus vaccine are recommended for HCWs if born during or after 1957 and 1 dose is recommended in HCWs born before 1957 because birth before 1957 is only presumptive evidence of immunity. In outbreak settings, a second dose of mumps vaccine should be “considered” for children aged 1-4 years, adults at low risk, and HCWs born before 1957 (without evidence of mumps vaccination), if affected by the outbreak (9).

Many questions about control of mumps remain unanswered. By eliminating wild-type mumps from the community, have we increased the number of susceptible hosts though waning immunity because the added immunologic boost from periodic exposure to natural infection is no longer present? Will regular boosters of attenuated vaccines be required to maintain herd immunity? What is the role of cell-mediated immunity in protection against mumps infection (11)? Do antigenic differences between wild-type virus and vaccine render vaccine-induced immunity less effective? Will vaccination during outbreaks interrupt transmission of mumps virus? What is the optimum laboratory test(s) for diagnosis of mumps in a vaccinated population? Answers to these questions are essential for designing effective strategies to control mumps.
Membership dues
Reminder: Annual membership fees were due January 1, 2009. You will not be considered an active member of PASCV if annual dues are not paid. A membership form is on page 14 of this newsletter. Dues are $25 per year. Membership in PASCV entitles you to a reduced subscription price to the Journal of Clinical Virology (see below) and a discount of $50 off the registration fee for the Molecular Virology Workshop ($110 for PASCV members, $160 for non-members).

Membership directory on line
A feature on the PASCV website (www.virology.org) is the membership directory. When you log onto the website, select “membership”, then “member services, web interface”. Under member login, enter your email address and password. (Follow instructions to retrieve a temporary password if you don’t remember your password). You can edit your profile, search for an individual member, or list the entire membership by typing an asterisk (*) in the box for last name. Only active members, who have paid dues for 2009, are listed.

PASCV members meet with FDA
On October 1, 2008, a subcommittee of PASCV members along with members of the Association of Molecular Pathology (AMP), and the Association of Public Health Laboratories (APHL) met with members of the FDA’s Office of In Vitro Diagnostic Device (OIVD) Evaluation and Safety in Rockville, Maryland.

PASCV initiated a request to meet with OIVD members following the FDA’s notice to manufacturers of Class II or Class III in vitro diagnostic devices that were inappropriately labeled and marketed as analyte specific reagents (ASRs), to comply with the law by September 15, 2008. Certain molecular assays, particularly viral load assays, have been marketed for years as ASRs but did not meet the FDA’s definition of an ASR.

Because many of these assays are routinely used for managing patient care, PASCV and its partners sought to offer assistance to the FDA to expedite clearance of these products as in vitro diagnostic devices. We also sought to convey our concerns that the new enforcement eliminated certain “ASRs” that had become crucial elements of laboratory test menus. The meeting was cordial and the input of PASCV, AMP, and APHL welcomed. The PASCV subcommittee is in the process of drafting a “white paper” on the design of clinical trials of commercial molecular diagnostic tests for detection and quantitation of viral diseases for follow-up meetings with the OIVD members.

Journal of Clinical Virology Update
The Journal of Clinical Virology is the official journal of the PASCV and the European Society of Clinical Virology. Its current impact factor has increased from 2.630 to 3.468. The Journal’s ranking has also increased from 15th out of 23 last year to 7th out of 25 in the Virology category. There are two editors in chief: Christine C. Ginocchio, Ph.D., MT (ASCP), Director of Microbiology, Virology and Molecular Diagnostics, North Shore-LIJ Health Systems Labs, Lack Success, NY, who is replacing Myron Levin, and Professor William F. Carman, Director West of Scotland Specialist Virology Centre and Chair, UK Clinical Virology Network. The Editorial Board has been reinstated which should facilitate turn around times of manuscript reviews.

A new addition to the Journal is a pilot scheme called VIROQAS (Virology Question and Answer Scheme). This is an extension of a scheme already available to members of the UK Clinical Virology Network. Each issue will have a clinical case deemed to be of interest or imparting an important learning point. The reader has the opportunity to consider their diagnostic approach and which tests they would perform. A diagnosis and an expert commentary will be provided in the same issue. We encourage the members of PASCV to submit their interesting cases to joy.kean@ggc.scot.nhs.uk who will be coordinating the consideration of the cases and submitting them on-line. This is an excellent opportunity to share our interesting cases with our colleagues.

The 2009 individual subscription price for members of PASCV is $99 including postage and handling. A subscription is for the complete calendar year, from January to December. When requesting a subscription mid-way through a calendar year, you will receive all previously published issues for that year. When ordering, be sure to mention that you are a member of PASCV. Also, include your complete bill-to and ship-to address, plus the title of the journal, and the subscription year.

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**Future Virology Meetings**

19th Annual Meeting of the "Gesellschaft für Virologie" (GfV, Society for Virology)
March 18-21, 2009, Leipzig, Germany
http://www.virology2009.de/

22nd International Conference on Antiviral Research
May 3 – 7, 2009 in Miami Beach, Florida, U.S.A.

25th International Papillomavirus Conference
May 8 -14, 2009 in Malmö, Sweden.

Drug-resistant and Vaccine-escape Hepatitis B Virus Mutants: Emergence and Surveillance
June 4-5, 2009
Centers for Disease Control, Atlanta, GA
http://www.cdc.gov/hepatitis/hbvsymposium2009/

12th Annual ESCV meeting
Sep 27- 30, 2009 in Istanbul, Turkey
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