MEDICAL VIROLOGY

Maria Luisa G. Daroy

Scientist, Research and Biotechnology Division, St. Luke’s Medical Center, and Assistant Professor, MS Molecular Medicine Program, St. Luke’s College of Medicine, WHQ Memorial

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I. Mosquito-borne viruses
   A. Dengue virus
      1. Epidemiology

   Dengue virus (DENV) is a mosquito-borne flavivirus that has a positive, single-stranded RNA genome, approximately 11 kb long. The first report of dengue virus infection in the Philippines was in 1954. Infection with dengue virus is characterized by both endemic and epidemic occurrences in the Philippines, which peak in the rainy season months of June to September. All four serotypes (DENV 1-4) have been isolated from the blood of dengue patients from all regions of the country. Moreover, multiple infections of two or more serotypes have been recorded. The most prevalent serotype detected in 2001 was DENV-2, followed by DENV-1, and DENV-3 and -4 (Cinco et al. 2003). Thus, a regular nationwide surveillance of dengue virus infection may be needed to determine the prevailing serotypes during an outbreak.

   2. Pathophysiology

   Manifestations of dengue infection range from asymptomatic or mild self-limiting febrile illness to severe, life-threatening disease. Platelet count is the most common diagnostic tool to evaluate hemostatic status. However, there is evidence that accelerated platelet clearance by phagocytosis leading to thrombocytopenia plays a role in acute dengue infection (Honda et al. 2009, Dimaano et al. 2007). Data suggest that platelet-associated immunoglobulins involving anti-dengue virus activity play a key role in the development of infections (Oishi et al. 2007, Saito et al. 2004). Clinical signs and symptoms accompanying dengue hemorrhagic fever, which was confirmed by laboratory tests, include epistaxis, abdominal pain, increased hematocrit levels, thrombocytopenia and increased fibrinolysis (Carlos et al. 2005, Oishi et al. 2006). In a clinical study of dengue, acute secondary infection and viral serotype did directly affect severity of dengue infection. Furthermore, a nested case-control study of dengue in infants failed to lend support to the prevailing theory of antibody-dependent enhancement in severe secondary dengue infection.
3. Laboratory Tests

Several methods have been developed for the detection of dengue virus in patient sera. Dengue virus can be isolated from infected sera by culture in Aedes albopictus C6/36 cells and direct detection of live virus in infected cells by immunofluorescence antibody test (IFAT), antigen sandwich ELISA and reverse transcriptase-polymerase chain reaction (RT-PCR) of viral RNA (Pimentel, et al. 2003, Buerano et al.2008). Serotyping is also performed by RT-PCR. In 738 samples collected from 1995-1998, 95 were positive for DENV, with DENV-2 and -3 being the most prevalent (Buerano, et al. 1998, Natividad 1997). The infection can also be confirmed through the detection of anti-DENV IgM by ELISA. Laboratory testing of dengue cases in 1999 confirmed the infection in 30/63 cases by IgM-capture ELISA and only 5/63 by RT-PCR (Matias 2003). IgM-capture ELISA gave the highest detection rate (93%), followed by IFAT of buffy coat (25%) and other techniques (2-7%). However, these results depend on the time of blood collection relative to disease onset (Pimentel et al. 2003). A realtime RT-PCR assay developed using a fluorescent dye demonstrated increased sensitivity for dengue virus detection (Tan, et al. 2010).

Flow cytometry confirmed the presence of dengue virus-infected cells in the blood. DENV antigen was detected in CD19 (B-cells) cells from a dengue hemorrhagic patient. Furthermore, two DENV-2 isolates were cultured from his serum in human hematopoietic cells (K562), and can be distinguished by differences in their ability to grow in C6/36 cells and a E-62 mutation in the envelope protein (Kinoshita et al. 2009, Baclig et al. 2010).

The recent introduction of point-of-care testing for dengue has been evaluated for the early diagnosis of dengue. The Bio-rad Platelia Dengue NS1 Ag test was superior to PanBio’s Pan-E Dengue Early ELISA test in sensitivity as well as specificity in a multi-country study. A combined panel of NS1 Ag and IgM detection tests is recommended for increased overall dengue diagnostic sensitivity.

4. Molecular biology

A molecular epidemiology study of DENV-2 isolated from Filipino patients from 1995 to 2002 revealed a gradual shift from the Asian
2 genotype to Cosmopolitan genotype based on a phylogenetic analysis of the envelope gene (Salda et al. 2005).

5. Vaccine trials

A phase I study of a recombinant, live, attenuated tetravalent dengue vaccine showed that vaccination resulted in a balanced antibody response to all four dengue serotypes (Capeding et al. 2011).

6. Public health strategies

Mosquito control measures have been tested at the community level as a means to limit dengue infection and spread. One of the strategies reported on the transient efficacy of treating standing water with anti-larvicidal tablets.

B. Japanese encephalitis virus

Japanese encephalitis is a major cause of viral encephalitis in Asia. Laboratory confirmation, done using IgM-capture ELISA on cerebrospinal fluid samples, detected infection in 72/614 CSF samples collected from 2002-2004. Co-infection with dengue virus was determined in 17 samples (Natividad et al. 2006). In another report, serum samples collected from dengue-suspected cases were positive when tested by IgG indirect ELISA for Japanese encephalitis. The utility of this test for detection of infection with other flavivirus, such as dengue, was demonstrated (Inoue et al. 2010).

Japanese encephalitis chimeric virus vaccine was given to children in the Philippines. One year after receiving the JE-CV booster dose, 99.4% of children remained seroprotected (Feroldi et al. 2013).

C. Chikungunya virus

Like dengue fever, CHIKV is transmitted by the bite of a mosquito. Chikungunya virus (CHIKV) infection was found in patients suspected of having dengue when tested by immunofluorescence antibody test, IgM-capture ELISA and RT-PCR for chikungunya virus. Out of 192 dengue-suspected cases, seen in January to August 1999, 10 were ser-positive for chikungunya fever alone, 8 were positive for both CHIKV and DENV, and 98 were positive for dengue alone.
These three arboviruses, DENV, JEV, and CHIKV, were also found in healthy *Macaca fascicularis* monkeys in the Philippines, indicating possible sylvatic transmission of these viruses (Inoue *et al.* 2003).

II. Hepatitis viruses
   A. Hepatitis B virus
      1. Epidemiology

      75% of all new cases of HBV infection worldwide occur in Asia where it is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. In the Philippines, there appears to be two types of age-specific HBsAg prevalence, suggesting different modes of transmission (Merican *et al.* 2000).

      A recent study of acute hepatic failure in Filipino children revealed that the predominant etiologic agents were hepatitis A virus (5/26) and hepatitis B virus (1/26) (Bravo *et al.* 2012).

      2. Laboratory tests

      In 182 patients studied, only 3.3% were infected with precore mutant HBV while the rest were with wildtype viruses. Serum ALT levels were directly related to the concentration of viral load in patients reactive for HBV DNA and HBeAg. Quantification of viral load using DNA hybridization assay is thus recommended in managing chronic HBV infection. Another study showed that a high proportion of Filipino chronic hepatitis B patients harboured the virus in the replicative stage (26/61 HBeAg(+)). Precore mutant infections were found in 8.5% of the study population. Yearly cumulative HBeAg clearance and anti-HBe seroconversion were low.

      3. Molecular biology

      Genotyping of HBV was conducted by several groups. Phylogenetic analysis based on the sequences of nine complete genomes and 100 core promoter/precore regions showed genotypes A (aa/A1), C and B, in 51, 27 and 22 samples, respectively. Furthermore, a novel subtype C5 was identified. Genotypes B and C were significantly more prevalent than genotype A, in cirrhosis and hepatocellular carcinoma patients (Sakamoto *et al.* 2006). Research into HBV
genotypes associated with clinical parameters of HBV infection conducted in St. Luke’s Medical Center in 2005 revealed genotype A was the most predominant (37.8%), followed by genotypes B, D and C. The patients with genotype A appeared to have better response to treatment than those with genotype D. In another study conducted between St. Luke’s Medical Center and Tohoku University, complete nucleotide sequences of HBV genotype B, subgenotype B5, from 5 Filipino patients was analyzed (Nagasaki et al. 2006). Genotype analysis of 28 samples from asymptomatic HBV carriers in the Philippines gave the following profile: 54% B, 18% C5, 7% D, and 7% A1. Double infections with genotypes B and D were detected in another 7%. One of the isolates seemed to belong to a new subgenotype, C6 (Cavinta et al. 2009). Results showed that these belonged to a new subgenotype, B5. A recent study conducted in Cebu City showed HBV genotypes A and C to be predominant in chronic hepatitis B patients (Batoctoy et al. 2011).

Response predictive markers for interferon treatment for hepatitis B virus infections were studied based on mutation patterns in the basal core promoter and precore regions using PCR and molecular cloning techniques. HBV variants, T1762 and A1764, and HBV genotype A provide advantage in response to pegylated IFN in Filipino patients (Agdamag et al. 2010).

4. Vaccine and therapeutic studies

A survey of HBV vaccine coverage, 7 months after the Philippines adopted a hepatitis B vaccine birth dose policy, showed that 54% of infants had a documented birth dose and only 22% were vaccinated within 24 h of delivery (Sobel, et al. 2011). Timely administration of hepatitis B vaccine beginning at birth, simultaneously with diphtheria-pertussis-tetanus vaccine would increase vaccination coverage and completion of vaccine dose series (Wallace et al. 2012). Investigational vaccine, recombinant HBsAg-1018 demonstrated faster, superior, and more durable seroprotection in adults, ≥ 40 years, than a licensed comparator HBV vaccine (Engerix-B), with a similar safety profile (Sablan et al. 2012).

A randomized, double-blind, placebo-controlled study demonstrated the potential efficacy of oromucosal IFN-α-n1 in chronic HBV infection with therapeutic benefit equal to
parenterally administered IFN-α, without the side effects of myelosuppression (Tupasi et al. 2002).

B. Hepatitis C virus

1. Epidemiology

In 2002, 560 subjects from Metro Cebu were tested for HIV, HCV and HBV infections. The seroprevalence of anti-HCV and HBsAg were highest among injecting drug-users, 61/87 and 9/87, respectively (Agdamag et al. 2005). An overall prevalence of 0.12% anti-HCV (+) cases was obtained among 6,560 healthy donors tested at East Avenue Medical Center.

2. Molecular biology

From 2002 to 2007, 444 samples out of 1,590 individuals enrolled were positive for HCV RNA. Phylogenetic analysis of the NS5B and E1-E2 regions showed the most dominant subtype to be 1a, followed by 2b, 2a and 1b (Kageyama et al. 2009). A second inter-genotypic (2b/1b) recombinant of HCV isolated from Metro Manila was identified based on the nucleotide sequence of the 5’-UTR-Core and NS5B regions (Kageyama et al. 2006). The concordance of subtyping genotype 1 HCV based on the NS5B, NS5A and 5’UTR regions was determined. NS5A sequencing can identify HCV-1a and -1b subtypes with predictive values of 86% and 70%, respectively. The overall concordance with NS5B sequencing was 73%. The predictive value of 5’UTR sequencing for subtype 1a was 73% while for subtype 1b, it was 87%. Overall concordance between 5’UTR and NS5B sequencing was 80%. NS5B sequence analysis remains to be the reference method to identify HCV-1 subtypes (Baclig et al. 2010).

C. Hepatitis G virus

Using reverse transcriptase-PCR, 11 out of 120 chronic liver disease patients were positive for HGV-RNA, compared to 6 out of 240 healthy adults. This difference was not significant. Ten out of 11 HGV-infected cases were positive for HBsAg, and 45% had undergone previous blood transfusion. A similar study using RT-PCR of the 5’ UTR of the HGV genome followed by hybridization ELISA to detect the amplified product, showed HGV RNA in 6/516 healthy blood donors, 11/138 chronic liver disease patients, 7/207 hemodialysis patients and 14/227
multiple transfused patients. HCV RNA was also detected in 14 subjects and HBsAg in 7 subjects who were positive for HGV RNA. There was one chronic liver disease subject who tested positive for HGV RNA, HCV RNA and HBsAg.

III. Respiratory viruses

Nasopharyngeal aspirates from 465 patients with influenza-like illness were obtained in 2006-2007 and tested by PCR and RT-PCR. Human metapneumovirus, human bocavirus, human coronavirus HKU1, KI virus, and WU virus were detected for the first time in the Philippines (Furuse et al. 2010). In a study conducted from May 2008 to May 2009 in Tacloban City, the following viruses were detected in 501/819 pediatric cases of severe pneumonia by PCR: human rhinoviruses (n=189, types A, B, C), respiratory syncytial virus (n=165), influenza A virus, and novel viruses such as human metapneumovirus, human coronavirus NL63, human bocavirus, human polyoma viruses WU and KI. Bacteria were also isolated (Suzuki et al. 2012).

A. Influenza viruses

Influenza viruses A/Philippines/341/2004 (H1N2) and A/Thailand/271/2005 (H1N1) isolated from two males provide evidence of sporadic human infection by contemporary swine influenza. Both viruses were antigenically and genetically distinct from influenza A H1N1 and H1 N2 viruses circulating in the human population (Komaladina et al. 2007).

In the Philippines, pandemic influenza A/H1N1 2009 was first detected in May 2009 and by July 2009, 3207 cases and 6 deaths were reported. Using RT-PCR as the gold standard, clinical sensitivity and specificity of Quidel QuickVue (QV) Influenza A+B was shown to be 63% and 96%, respectively, demonstrating moderate sensitivity for the pandemic influenza A/H1/N1 infection (Velasco et al. 2010).

A comparison of hemagglutinin sequences from the pandemic 2009 H1/N1 viruses with that of the 1918 H1/Ni virus reveals a serine (2009 strain) substitution for proline (1918 strain) at position 200, which may affect receptor-binding ability (Padlan 2010).
B. Respiratory syncytial virus

Human RSV was detected in 415 out of 2,150 nasopharyngeal swabs, collected from infants and children hospitalized for severe pneumonia, by nested PCR of the M2 gene and C-terminus of the G gene. Phylogenetic analysis identified HRSV-A (genotype NA1) in 65% of positive samples, and HRSV-B (genotype BA) in 35%. The emergence of a new variant of HRSV-B was also reported (Ohno et al. 2013).

IV. Human immunodeficiency virus

HIV sentinel surveillance conducted among injecting drug users in Metro Cebu detected 0-0.52% positivity in 2002-2007, which increased 10-fold in 2009, reaching 75% in January 2010 (Telan et al. 2011). HIV/AIDS rates were below 2% in surveyed groups in the Philippines (Mateo, Sarol and Poblete, 2004). HIV-1 RNA was detected in plasma (80%) and cervicovaginal lavage (60%) samples of Filipino women indicating significant association of RNA shedding in genital tract with plasma viral load (Natividad-Villanueva et al. 2003). The first case of HIV-2 infection in the Philippines was reported (Leano et al. 2003).

V. Herpesviruses

A. Herpes simplex viruses

Herpes simplex virus type 2 seroprevalence in Filipino women (9.2%) was much lower than in Brazilian women (42%), due to differences in sexual behaviour of the women and their husbands (Smith et al. 2001).

B. Varicella zoster virus

Two studies of the reactogenicity to varicella zoster vaccine in initially seropositive subjects versus seronegative subjects showed no difference, being immunogenic and safely tolerated in both groups across all ages (Barzaga, Florese, and Bock, 2002, Macaladad et al. 2007).

C. Human cytomegalovirus

A case of acute retinal necrosis due to cytomegalovirus infection was reported (Baclig, Daroy and Abano 2008).
VI. Human papillomavirus

Forty-six HPV types were identified among 211/369 female sex workers who tested positive for HPV DNA by PCR. HPV-52 was most common followed by HPV-16, -58, and -67. Multiple-type infection was detected in 44.5% (Miyashita et al. 2009). A broad spectrum of mucosal HPV types was detected from cervical tissue biopsies by PCR targeted to the major capsid gene. Cloning and sequence analysis of 11 samples out of 169 that tested positive, showed close similarity to HPV type 6 (Matias et al. 2003). HPV DNA testing is recommended for confirmation of cervical screening test, especially for patients with atypical cells of undetermined significance.

Systemic lupus erythematosus cases were reported following vaccination for HPV (Soldevilla, Briones, and Navarra, 2012).

Mother to infant transmission of antibodies to HPV following vaccination with quadrivalent HPV virus-like particle vaccine was demonstrated (Matys et al. 2012).

VII. Enteroviruses

Coxsackieviruses B infection of cardiac tissue was detected in 14 samples out of 28 obtained from patients with dilated cardiomyopathy.

VIII. Animal viruses

A. Rabies virus

A two-stage clinical trial confirmed the safety and immunogenicity of chromatographically purified Vero cell rabies vaccine in post-exposure rabies treatment (Quiambao, et al. 2000). Phylogenetic characterization of Philippine isolates of rabies virus based on partial nucleotide sequences of the nucleoprotein gene showed a different lineage to Asian isolates (Nishizono et al. 2002). The diagnosis of a rabies case imported from the Philippines into the UK was reported (Smith et al. 2003). Reverse transcription loop-mediated isothermal amplification (RT-LAMP) method for the detection of rabies viral RNA was developed (Boldbaatar et al. 2009). A retrospective review of clinically diagnosed rabies cases admitted in 1987 to 2006 at San Lazaro Hospital was reported (Dimaano et al. 2011).
B. Ebola-Reston virus

The complete genome of Ebola virus subtype Reston isolated from the Philippines in 1996 showed variations in the glycoprotein of Ebola-Reston virus isolated in 1989 and 1992 (Ikegami et al. 2001). An indirect immunofluorescence assay to detect antibodies to Ebola-Reston virus was developed using HeLa cells stably expressing viral nucleoprotein (Ikegami et al. 2002). Histopathological investigations of 24 Macaca fascicularis naturally infected with Ebola-Reston virus indicate systemic coagulopathy and increase in blood-derived macrophages/monocytes (Ikegami et al. 2002). Domestic swine with unusually severe outbreaks of porcine reproductive and respiratory disease syndrome were found to harbour Ebola-Reston virus. The emergence of the virus in the food chain is of concern (Barrette et al. 2009; Miranda and Miranda, 2011).

IX. Other viral illnesses

A study evaluated the immunogenicity and reactogenicity of the combined administration of live attenuated JE vaccine and measles vaccine showed that both were well tolerated and immunogenic in infants. Surveillance for measles viruses in 2000 to 2008, showed no measles cases in 2005 following mass vaccination in 2004, however, re-emergence of measles cases was reported in 2007 caused by other genotypes (Fuji et al., 2011).

Laboratory-based surveillance of diarrheal and respiratory illnesses was conducted during the 2009 RP-US Balikatan exercise. ELISA and PCR (realtime and MassTag) showed samples positive for norovirus and human parainfluenza virus 3 (Velasco et al. 2011).

X. Future researches

Research on emerging and re-emerging viral infections should be a major focus of molecular epidemiology efforts in the country. The acquisition of advanced genomics capabilities in both public (Philippine Genome Center) and private (St. Luke’s Medical Center) sectors, can spur the production of genetics and bioinformatics data on local pathogenic viruses, such as dengue, influenza, other human respiratory and diarrheal viruses, and zoonotic viruses. Genomic information can then guide the: (1) monitoring of disease outbreaks by determining the prevalent strains, (2) management of infected patients by identifying drug-resistant strains, and (3) aid in the design of vaccines and antiviral drugs by identifying molecular targets.
The development of vaccines and antiviral compounds to eradicate and control the spread of viral infections is a promising area of research, that may have significant socioeconomic impact.

A fertile field for research is the development of rapid molecular diagnostics for the detection, characterization and quantification of viruses in biological specimens. This area of research combines the disciplines of immunochemistry, molecular biology, materials science and bioengineering. It requires the production of nucleic acid probes, monoclonal antibodies, reporter molecules, and artificial vehicles.

Studies on host-virus interactions utilizing in vitro cellular techniques, imaging analysis, and in vivo animal models will lead to the elucidation of the molecular basis of virus pathology, such as animal-to-human transmission, histopathological changes, and effects of disease severity.

The identification of non-culturable human pathogenic viruses can be proposed using metagenomics strategies to mine the gut, the respiratory tract, and the central nervous system.

I have only delineated here the cutting edge research that can be undertaken in medical virology, and have not mentioned the vast array of conventional virology research that is now underway, albeit underutilized in the science industry of our country. It is hoped that this review will spur young researchers and students into studying life in its smallest form.

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