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Feature Article

Foreward: Molecular epidemiology of viral infection in Asia

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Since the late 20th century, new viral infections, that is emerging virus infections, have been spreading across the world. Due to the effect of globalization and the ease of international travel, local viruses have spread to countries where they were previously unheard-of. West Nile fever/encephalitis, HIV, Nipah virus infection and severe acute respiratory syndrome (SARS) are typical examples. In Asia, Japanese encephalitis, dengue fever/dengue hemorrhagic fever, hantavirus infection, rabies, hepatitis B and C, enterovirus encephalitis including polio, and virus diarrhea are also important infections.[1]

Molecular epidemiological studies on viruses using genome amplification by polymerase chain reaction (PCR) and gene analyses began in the 1990s.[2,3] The source and route of infections are better understood, and the effect of therapy, existence of resistant viruses, and seasonal or geographic distributions have also become more clearly defined and understood. The results of these investigations have contributed to vaccine development. In this series of feature articles, specific viral infections in children such as rubella virus, measles virus, hepatitis B and C viruses, HIV, picorna viruses and diarrheal viruses are individually described. In this forward to the feature article series on viruses in Asia, other viruses such as dengue virus, Japanese encephalitis virus, yellow fever virus, West Nile virus, rabies virus, hantavirus, Nipah virus, metapneumovirus and SARS corona virus are also discussed with special reference to Asia.

Flavivirus

Flaviviridae is divided into four genuses based on antigenic and genetic analyses: dengue virus, Japanese encephalitis virus, tick-borne encephalitis virus and yellow fever virus. Dengue virus, Japanese encephalitis virus and tick-borne encephalitis virus are further divided into 4, 5 and 5 species, respectively (Table 1), through mainly envelope gene analyses.[4,5] Envelope protein and gene analyses are important for understanding pathogenesis and classification. These viruses are arthropod-borne and difficult to control because the viruses are usually transmitted through vectors such as mosquitoes and ticks. Moreover, birds and vertebrates also act as natural hosts and along with mosquitoes and ticks they travel easily.[4,5]

Dengue virus

Dengue virus belongs to *Flaviviridae* and is transmitted in a cycle involving humans and *Aedes* mosquitoes. In forest areas, a cycle including primates and mosquitoes also exist.[6] To date, two manifestations, dengue fever and dengue hemorrhagic fever are known.[4,7] The symptoms are same among the four serotypes. Once immunity is developed in a human, it remains life-long and re-infection with the same serotype does not occur. However other serotypes may then cause disease because of the incomplete protection of the first infection. More than tens of millions of people have dengue fever and more than hundreds of thousands of people are reported to have dengue hemorrhagic fever every year. Dengue virus is approximately 11 kb positive single stranded RNA virus. It consists of structural proteins of core, membrane and envelope, and seven non-structural proteins. Envelope protein is the target of neutralizing antibody and hemagglutinin inhibition antibody. Serotypes of dengue virus 1, 2, 3, and 4 are originally classified using antiserum, but recently they have also been classified with envelope gene analyzes. The similarity of envelope gene is more than 90% at the same serotype and 65% at a different serotype. Moreover, dengue virus serotype 1, 2, 3 and 4 are divided into three, six, four and one genotypes, respectively.[8] There are common and different genotypes in serotypes 1–3 in Asia, Africa, Pacific islands and Caribbean islands. Some genotypes appear in specific areas during a limited period each year. Variation of envelope gene affects the virulence of these

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Table 1 Classification of presented viruses

Flavivirus[4]
Dengue virus,[1–4] ***Japanese encephalitis virus*** (West Nile virus, Kunjin virus, Murray Valley encephalitis virus, Japanese encephalitis virus, St. Louis encephalitis virus), ***Yellow fever virus*** (yellow fever virus), ***Tick-borne encephalitis virus*** (Powassan virus, Langat, louping ill virus, Central European encephalitis virus, Russian spring-summer encephalitis virus)
Rabies[19,21]
Lyssavirus (rabies virus, Lagos bat virus, Mokola virus, Duvenhage virus, European bat lyssavirus 1, European bat lyssavirus 2, Australian bat virus)
Hantavirus[24]
Hantaan group (Hantaarn virus, Seoul virus, Dobrava virus, Saaremaavirus)
Puumala group (Puumala virus, Prospect Hill virus, Tula virus, Khabarovsk virus, Topograv virus, Isla Vista virus, Bloodland Lake virus)
Sin Nombre group (Sin Nombre virus, New York virus, Bayou virus, Black Creek Canal virus, Laguna Negra virus, Andes virus, Rio Mamore virus, Rio Segundo virus, El Moro Canyon virus)
Subfamily Paramyxovirunae[27]
***Morbi1ivirus*–Henipavirus** (Nipah virus, Hendravirus)
Subfamily Pneumovirinae[30]
Metapneumovirus (avian pneumovirus, human metapneumovirus)
Coronavirus[35]
group I (human coronavirus 229E, canine coronavirus, feline infectious peritonitis virus, porcine transmissible gastroenteritis virus, porcine epidemic diarrhea virus)
group II (human coronavirus OC43, bovine coronavirus, porcine hemagglutinating encephalomyelitis virus, rat sialodacryoadenitis virus, mouse hepatitis virus)
group III (turkey coronavirus, avian infectious bronchitis virus)
group IV (severe acute respiratory syndrome coronavirus)

Bold italics represents genus. Subfamily **Paramyxovirinae** and Subfamily **Pneumovirinae** belong to Family **Paramyxoviridae**. See references for more information on these viruses and for phylogenetic trees.

viruses, and other structural and non-structural proteins might also affect the virulence.[7] Host immune response also affects the virulence of these viruses. At present, there is no clear explanation for the dengue and dengue hemorrhagic fever.

Japanese encephalitis virus

Japanese encephalitis virus belongs to *Flaviviridae* and is the prototype of Japanese encephalitis antigenic complex.[4,9] Humans are infected through the virus-infected *Culex* mosquito bites. One of 300–1000 infected persons can develop encephalitis. Nearly 20% of encephalitis cases die and approximately 50% recover with sequel. Approximately 50 000 encephalitis cases and 10 000 death cases are annually recorded. Life cycle of *Culex*-pig/bird-*Culex*-human is well known but there is no evidence of human to human transmission. Pigs and birds are effective viral amplification hosts, serving as natural hosts for infection. The gene of Japanese encephalitis virus is approximately 11 kb positive single-stranded RNA. The composition of structural and non-structural proteins are the same as in dengue virus. The serotype is one, but between four and six genotypes exists in the envelope gene. Geographic variation has also been reported.[10,11] Although the induction of mutation in envelope gene affect the difference in neurovirulence, other structural and non-structural genes also affect neurovirulence. Phylogenetic tree of nucleic acids are mainly envelope gene. Antigen of antigen-antibody reaction is also an envelope antigen.

Yellow fever virus

Yellow fever virus belongs to *Flavivirus*.[4,8] Hemorrhagic fever type has a high mortality rate. *Aedes* mosquitoes act as vectors in Africa and *Haemagogus* mosquitoes are vectors in South America. Humans are natural hosts in Africa and the virus is maintained through the cycle of *Aedes*-human-*Aedes* transmission. In contrast, monkeys are natural hosts in South America and the virus is maintained through the cycle of *Haemagogus*-monkey-*Haemagogus*. Humans are mainly infected in forest areas, by mosquito bites. The virus spread to the United States with the transportation of Africans to the United States.[1] At present, yellow fever is not prevalent in Asia. Its structural and non-structural compositions are same as dengue virus. Two genotypes are found from envelope gene analysis: Group I was isolated in east and central Africa, Group IIA in west Africa and Group IIB in America.[12,13] Although the envelope gene makes the differences of infectivity and pathogenicity, other structure and non-structure genes also affect infectivity and pathogenicity. Envelope gene and proteins are used for diagnosis and molecular epidemiology.[8]

West Nile virus

The virus survives through the cycle of bird and *Culex* mosquito. Usually there are no symptoms in humans, however this virus can cause encephalitis, especially in the elderly.

The virus can also cause death in humans, although this is rare and deaths are more common in birds and horses. West Nile virus has been found mainly in Africa, and often in the Middle East, South Europe and West Asia. However, it was reported in the United States in 1999.[14] At the time of writing there are no reports of West Nile fever disease in Asia except India. Considering the spread of West Nile virus in the United States, outbreaks in Asian countries are highly likely to occur in the near future.

The virus has been detected in *Culex* mosquito, birds and animals in urban areas in the United States. Viremia is found in the early stages of the infection, and the virus is transmitted through the placenta and blood transfusion also. Two genotypes are known: Genotype I is found in Europe and the United States and Genotype I and II are found in Africa.[15] Genotype I viruses can be further subdivided based on the origins of the viruses; European and the United States, Australia and India. Kunjin virus is common in Australia (Table 1).[16]

St. Louis encephalitis virus is common in the United States and belongs to genus *Japanese encephalitis virus*.[4] The virus is carried through the cycle of bird and *Culex* mosquito. Human and cattle are often infected.

Zoonotic virus

Being the largest continent, most Asian countries can be connected via land to European and African countries, and animals can move easily from one country to another. In this section, rabies virus and hantavirus are described. Lassa virus, Ebola virus and Marburg virus are not included because the diseases are not found or are quite rare in Asia.[1,17,18]

Rabies virus

Rabies virus belongs to *Rabdoviridae* and is approximately 12 kb negative single stranded RNA virus. The virus causes zoonosis among human, animals and birds.[19] The virus in the salivary glands of animals invades from the bitten site. Other routes such as corneal transplant, eye injury, and respiratory infection have also been reported.[20] The virus infects muscle cells and moves to peripheral nerves, and eventually moves and grows in central nervous cell. It is fatal when it infects the brain. In Japan, rabies infections were first reported in humans in 1954, and in animals in 1856.[21] Wild foxes, wolves, skunks, raccoons and bats are natural hosts in 'forest type' rabies and it has spread worldwide with the exception of a few countries including Japan. Dogs are a common cause of the spread of 'urban type' rabies in Asia, Africa and middle/south America.

Pre- or postexposure prophylactic vaccinations are useful for the prevention of virus invasion to the central nervous system. The virus consists of nucleoprotein (N protein), non-structure protein (NS protein), membrane protein (M protein), glycoprotein (G protein) and large protein (L protein). G protein is closely related to infection (neurotoxin) and is a target for infection control. Recombinant vaccine has been developed using the G gene. Negri body in neuronal cells is found in the hippocampus of infected animals. The Negri body is composed of virus nucleoprotein. The nucleoprotein gene is conserved well and isuseful for molecular epidemiological analyses of the infected area. Genotype 1 (serotype 1) of classical rabies in Asia, Europe, Africa and the United States develop in separate clusters. The cluster is also differentiated within Asia. Sequence also has further small variations in the Philippines between Luzon Island and Mindanao Island.[22]

Rabies virus was previously thought to have only one serotype, but a new type which shows the symptom of classical rabies has been found in bats. One example is Australian bat virus of genotype 7, and a case of human death involving this genotype has been reported.[7]

Hantavirus

Hantavirus belongs to genus Hanta of *Bunyaviridae.* It is divided into two groups; the first group includes the virus which spreads in Eurasian Continent and causes hemorrhagic fever with renal syndrome. The other group is widespread in South and North America and causes hantavirus pulmonary syndrome. There are 24 species (serotypes/genotypes); four species cause hemorrhagic fever with renal syndrome and six species cause hantavirus pulmonary syndrome.[23] The virus is secreted through urine, stool and saliva from rodents. Humans who aspirate or contact the virus can have the disease. In infected rodents, infection persists without symptoms. Hantavirus causes zoonosis except for one species. Bunyavirus consists of three negative stranded RNA (L, M and S). Each segment codes L protein (polymerase), envelope protein (G1 and G2) and Nucleoprotein (NP). The molecular epidemiological analysis is mainly done in nucleotides of M gene. Three different clusters were discovered: (1) Hantaan virus, Dodrava virus, Seoul virus and Thailand virus from Murinae rodents; (2) Puumala virus from Arvicolinae rodents which cause hemorrhagic fever with renal syndrome; and (3) Sin Nombre virus, New York virus, Bayou virus, Black Creek Canal virus, Andes virus, Lagna Negra virus from Sigmodontinae rodents which causes hantavirus pulmonary syndrome.[24,25]

New viruses

Outbreaks of Nipha virus and SARS coronavirus occurred in 1998–1999 and 2002–03, respectively. These viruses were found mainly in Asia, although they were not common in

children. Methapneumovirus of unknown origin was reported as the cause of respiratory infection in children in 2001. These emerging viruses warn us of the challenges humanity faces in the new century.

Nipah virus

Nipah virus is a negative single stranded RNA virus and belongs to *Paramyxoviridae*. An outbreak of Nipah virus was recorded in 1998–1999 in Malaysia.[26] This virus was transmitted from pig to humans and it caused encephalitis in approximately 40% of infected persons. Pigs were infected from the urine of fruit bats living in the forest of Nipah. Pig to pig and pig to human transmission occurs through saliva and urine. Human to human transmission is rarely recognized. Respiratory syndrome is common in pigs but convulsions, abnormal movement and miscarriage are also recognized. The mortality rate for this virus is low. The virus infects dogs, cats and horses with the rise of the antibody level. The virus reached Singapore via pigs imported from Malaysia. More than 10 humans were infected and one person died. Hendra virus is similar to Nipah virus and was discovered in horses and spread in Australia in 1994.[27] It was transmitted to humans and caused respiratory symptoms. Currently classification of Nipah virus and Megapneumovirus is recommended as belonging to the genus Henipavirus, because the molecular size of these viruses are approximately 18 kb and different from other viruses of Paramyxoviridae (15–16 kb).[27–29]

Metapneumovirus

A new virus, metapneumovirus, was discovered in 2001 in a child with respiratory symptoms.[30] The same group had been recognized in animals and this was the first report in humans. This virus was included in mononegavirus (negative single strand RNA virus). The virus belongs to genus *Metapneumovirus*, subfamily Pneumovirinae in family Paramyxoviridae, but does not belong to genus *Pneumovirs* which includes respiratory syncytium virus.[30] The virus has characters of *Paramyxoviridae*, that is, glycoprotein and fusion protein. However there are no characteristics of hemagglutinin and pneuraminidase. Respiratory syncytium virus has been known to have one serotype (genotype) but recently it was divided into subgenus (suserotype) A and B.[31] There are differences within one subtype across geographic locations and time. More detailed analysis is required for the complete understanding of metapneumovirus.

Almost all children aged <5 years old have antibodies against metapneumovirus.[30,32] This was found in countries worldwide, including Japan.[33]

Severe acute respiratory syndrome coronavirus

Coronavirus is approximately 27–31 kb plus single stranded RNA virus. There are genus *coronavirivirus* and *torovirus*. Human coronavirus was cultured in human tracheal cells in 1965. Coronavirus was discovered in vertebrates and birds. Membrane protein, envelope protein, spike and glycoprotein with hemagglutinin and esterase function, and nucleoprotein have been observed. Coronavirus causes mainly respiratory infection, however it can also cause gastrointestinal infection and rarely neuronal infection. Outbreaks of gastroenteritis have also been reported. Molecular epidemiological studies, understanding of variation of serotype and re-infection are not well established for this virus. Human coronavirus spreads mostly in children in winter. Infection routes are droplet and/or airborne infection and fecus-oral infection. Human coronavirus 229E and OC43 strains were known long before the recent severe acute respiratory syndrome (SARS) coronavirus outbreak in China.[34,35]

Severe acute respiratory syndrome first occurred in Kuangtung in Guangdong province in China. Three-hundred-and-five people had respiratory disease and five persons died at that time. Following that, a SARS outbreak occurred in hotels, hospitals and condominiums in Hong Kong in March, 2003. Finally it spread to 29 countries including Vietnam, Taiwan, Singapore and Canada, and was spread mainly via travellers. The disease spread in hospitals where health workers and relatives of SARS patients were in close with patients. Fortunately, SARS disappeared in June 2003. More than 8000 people were infected and more than 800 people died of SARS in the 2003 outbreak. Considering the course of this disease, the disease started from the breeder of palm civet in Guangdong province and it is a zoonosis. The transmission route is usually droplet and/or air infection. However, fecus-oral infection and contact infection also occur. Effective management of a new respiratory infection is not easy. Research on diagnosis, treatment and prophylaxis are under way in order to counter the reappearance of SARS in the winter of 2004.[36]

There are four serotypes in Coronaviridae, and they are described in Table 1. There is some variation in the SARS coronavirus, although the variation is minor and at this time there is no need for subtyping.[34,35]

Conclusion

Changes in viral gene and host defense mechanisms are important factors in the spread of infection. Changes in habit and environment also affect the spread of infection. Molecular approach nowadays gives us quick and detailed analysis. In this paper, we have briefly described the following viruses at the molecular level: flaviviruses (dengue virus, Japanese

encephalitis virus, yellow fever virus, West Nile virus); zoonotic viruses (rabies virus, hantavirus); and new viruses (Nipah virus, metapneumovirus, severe acute respiratory syndrome coronavirus).

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