CHAPTER III

LITERATURE REVIEW

I. Dengue infection

1. Dengue virus

Dengue virus belongs to the family Flaviviridae. Four serotypes of dengue virus (designated DEN-1, DEN-2, DEN-3, and DEN-4) can be distinguished by serological methods. Infection in humans by one serotype produce life-long immunity against reinfection by that same serotype, but only temporary and partial protection against the other serotypes. Dengue viruses share many characteristics with other flaviviruses, having a single-stranded RNA genome surrounded by an icosahedral nucleocapsid and covered by a lipid envelop. The virion is approximately 50 nm in diameter. The flavivirus genome is approximately 11 kb in length, and the complete genome sequence is known for isolates of all four serotypes of dengue virus. The genome is composed of three structure protein genes, encoding the nucleocapsid or core protein(C), a membrane-associated protein(M), an envelop protein(E), and seven non-structural(NS) protein genes. The domains which is responsible for neutralization, fusion and interactions with virus receptors are associated with the envelop protein. The order of proteins encoded is 5'-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'. The ultrastructural studies indicated that virion morphogenesis occur in association with intracellular membranes. In many studies, virions appear to accumulate within disorderly arrays of membrane-bound vesicle. Budding of virions at the plasma membrane has been observed occasionally (73,74,75,76,77).

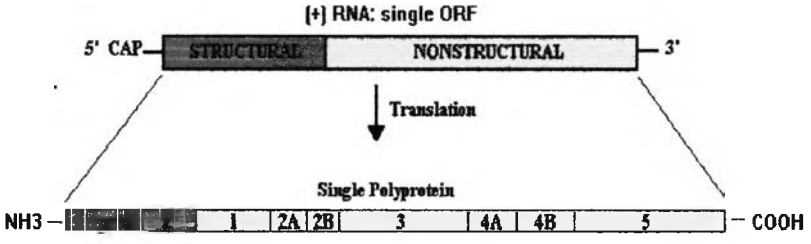

Figure1 Dengue virus genome

2. The vector

Aedes aegypti is a tropical and subtropical species of mosquito found around the globe, usually between latitudes 35 °N and 35 °S, approximately corresponding to a winter isotherm of 10 °C. Although *Aedes aegypti* has been found as far north as 45 °N, such invasions have occurred during the warm season, and the mosquitoes have not survived the winters. Distribution of *Aedes aegypti* is also limited by altitude. It is usually not found above 1000m but has been reported at 2121m in India, at 2200m in Colombia, where the annual temperature is 17°C. *Aedes aegypti* is one of the most efficient mosquito vectors for arboviruses, because it is highly anthropophilic and thrives in close proximity to humans and often lives indoors. Dengue outbreaks have also been attributed to *Aedes albopictus*, *Aedes polynesiensis*, and several species of the *Aedes scutellaris* complex. Each of these species has its own particular geographical distribution; however, they are less efficient epidemic vectors than *Aedes aegypti*. While vertical transmission of dengue virus has been demonstrated in both the laboratory and the field, the significance of this to maintenance of the virus has not been established. A factor complicating eradication of the vector is that *Aedes aegypti* eggs can withstand long periods of desiccation, sometimes for more than a year (78).

3. Transmission of dengue virus

Dengue virus are transmitted to human through the bite of infected *Aedes* mosquitoes, principally *Aedes aegypti*, and are therefore considered to be arboviruses (arthropod-borne viruses). Once infected, a mosquito remains infected for life, transmitting the virus to susceptible individuals during probing and feeding. Human are the main amplifying host of virus, although many studies have been shown that monkeys in some part of the world may become infected and perhaps serve as a source of virus for feeding mosquitoes. The virus circulates in the blood of infected humans at approximately the time that they have fever, and uninfected mosquitoes may acquire the virus if they feed on an individual when a victim is viremic (79). The virus then develops in the mosquito for a period of 8-10 days before it can be transmitted to other humans during subsequent probing or feeding. The length of time required for extrinsic incubation depends in part on environmental conditions, especially ambient temperature (80).

4. Epidemiology

Dengue virus occur worldwide in tropical regions. Their distribution is determined by the presence of the principle mosquito vector (*Aedes aegypti*). In tropical areas, the vector is active year-round and dengue occurs throughout the year, with increased transmission during the rainy season. This is due to higher mean temperatures and attendant shorter extrinsic incubation period in the vector (interval between feeding on infectious blood and ability to transmit on refeeding) and to higher humidity and enhanced survival of adult mosquitoes. In temperate zones, transmission is limited to the summer months.

It is estimated that over 2.5 billion people inhabit tropical areas at risk of infection. Since World War II, dengue has expanded in incidence and geography, due principally to urbanization and the attendant increase in domestic *Aedes aegypti*

populations, and to the increased movement of infected people by airplanes. Dengue infection are most prevalent in Southeast Asia, where all four serotypes are continuously present. It is estimated that over 100 million cases of dengue fever occur annually throughout the world, but reports underestimate the true incidence (81). In hyperendemic areas of Southeast Asia, the annual incidence of infection is 10% to 20%, and most children have experienced at least one dengue infection by the age of 7 years. In these areas, dengue is a childhood disease, and adults are protected by cumulative immunity (82).

5. Pathogenesis

The pathogenesis of dengue infection is still controversial. Three hypothesis about these, which are not mutually exclusive, are frequently cited to explain the pathogenetic changes that occur in DHF and DSS.

1) **Immune enhancement**

The most commonly accepted is known as the secondary-infectious or immune enhancement hypothesis. This hypothesis developed extensively by Halstead (83) implies that patients experiencing a second infection with a heterologous dengue virus serotype have a significantly higher risk for developing DHF and DSS. Preexisting heterologous dengue antibody recognizes the infecting virus and forms an antigen-antibody complex, which is then bound to and internalized by immunoglobulin Fc receptors on the cell membrane of leukocytes, especially macrophages. Because the antibody is heterologous, though, the virus is not neutralized and is free to replicate inside the macrophage (84,85,86). Thus, it is hypothesized that prior infection, through a process known as antibody-dependent enhancement (ADE), enhances the infection and replication of dengue virus in cells of the mononuclear cell lineage. It is thought that these cells produce and secret vasoactive mediators in response to dengue

infection, which cause increased vascular permeability leading to hypovolemia and shock (87,88).

2) **specific viral serotype**

Dengue hemorrhagic fever or dengue shock syndrome only occurs in a relatively small proportion of patients with secondary infections. Despite the co-circulation of several dengue serotype in the Americas, it was not until the 1981 epidemic in Cuba that the first DHF cases occurred in the region. This event coincided with the introduction of a new genotype of dengue virus serptype 2 from South-east Asia. Subsequent epidemics with DHF in South America also coincided with the occurrence of South Asian dengue virus strains. In contrast, in Peru, no evidence of DHF was found during an epidemic caused by dengue 2 virus, five years after an epidemic of dengue1. Evidence of secondary dengue virus infection was found in 60.5% of subjects tested. The absence of DHF in this population has been attributed to the America origin of the dengue 2 strain causing the epidemic. These and other findings suggest that viral virulence factors may play an essential role in the pathogenesis of DHF/DSS (89).

3) **Nutritional status.**

The previous study by Usa T. and Suchitra N. showed that there are relationship between nutritional status and clinical manifestation of dengue infection (90). They had suggested that patients with DHF had better nutritional status when compared with patients with other infection disease and healthy children. Besides, they confirmed that patients with DHF are not usually malnourished.

6. Clinical manifestation

Dengue infection characteristically results in fever, headache and rash. The clinical spectrum can vary, however, from asymtomatic to more severe infections with

bleeding and shock. They can be classified into four presentations: non-specific febrile illness, classic dengue, dengue hemorrhagic fever and dengue hemorrhagic fever with dengue shock syndrome. Asymtomatic and classic dengue is more commonly seen among older children, adolescents and adults. Dengue is abrupt in onset, typically with high fever accompanied by severe headache, incapacitating myalgias and arthralgias, nausea and vomiting, and rash. A positive tourniquest test may be found in over one-third of patients with DF. Clinical findings alone are not very helpful to distinguish DF from other febrile illness such as chikungunya and measles.

Dengue hemorrhagic fever is primarily a disease of children under 15 years in hyperendemic areas. DHF is defined as an acute febrile illness with minor or major bleeding, thrombocytopenia ($\leq 10^5/\mu l$), and evidence of plasma leakage documented by hemoconcentration, pleural or other effusions, or hyperalbuminaemia or hypoproteinaemia. The major pathophysiological change that determines the severity of disease in DHF and differentiates it from DF is the leakage of plasma (91). Transudate due to excessive capillary permeability collects at the pleural and abdominal cavities. The development of DHF provides warning of an increased probability of shock. The first to ascertain is the time elapsed since onset of illness. DHF/DSS usually develop around day 3-7 of illness, at the time of defervescence, which is an indication for intensified observation. A progressively decreasing platelet count and a rising hematocrit indicate increased probability of impending shock. When all four criteria for DHF are fulfilled, intravenous fluids may be all that is requires for treatment. Dengue shock syndrome is defined as DHF with signs of circulatory failure, including narrow pulse pressure ($\leq$20 mmHg), hypotension or frank shock. The four warning signs for impending shock are intense, sustained abdominal pain; persistent vomiting; restlessness or lethargy; and a sudden change from fever to hypothermia with sweating and prostration (92).

7. Laboratory diagnosis (93)

A definitive diagnosis of dengue infection depends on isolating the virus, viral antigen detection, viral nucleic acid detection, specific antibody detection from patient's specimen, often be serum. Acute-phase blood sample is a choice for diagnosis of dengue infection. So, blood sample should be taken in during onset of suspected dengue illness and ideally, should be taken for convalescent-phase sample 2-3 weeks later, however, it is often difficult, but should be taken blood sample before discharged from hospital.

1) Serologic diagnosis

Routinely serologic test for dengue infection's diagnosis are hemagglutination-inhibition(HI), immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA), indirect immunoglobulin G ELISA, neutralization test (NT) and complement fixation (CF).

Hemagglutination-inhibition assay (HI) has been the most frequently used; it is sensitive, is easy to perform, requires only minimal equipment, and is very reliable if properly done. Because HI antibodies persist for long periods, the test is ideal for seroepidemiologic studies. HI antibody usually begins to appear at detectable levels, about titer of 10, after 5-6 days of illness. In primary infection, antibodies titers in convalescent-phase serum often be less than 640. But in secondary infection, reciprocal antibody titers increase rapidly during the first few days of illness by immediate anamnestic response, often reached to more than 5,120. Thus, a titer of $\geq$ 1,280 in an acute-phase or early convalescent-phase serum sample is considered presumptive evidence of a current dengue infection. The disadvantage of HI test is lack of specificity, unreliable for identifying the infecting virus serotype.

An indirect IgG-ELISA, this assay is comparable to HI test and can also be used to differentiate primary and secondary dengue infection. The test is simple and easy to perform for high-number testing. It's very nonspecific and exhibits the same

broad cross-reactivity among flavivirus as the HI test does. Therefore, it can not be used to identify the infecting dengue virus serotype. However, it has a slightly higher sensitivity than HI test. As more data are accumulated on the IgG-ELISA, it is expected to replace the HI test as the most commonly used IgG test in dengue laboratories.

2) Viral Isolation

By period of viremia, isolation of dengue virus from clinical specimens is frequently possible. There are different methods (table.1) of confirming the dengue virus, choice of method up to local availability of mosquito, cell culture and mice.

Table.1 Methods for dengue virus isolation

Method	Result confirming presence of dengue virus
Inoculation of mosquito (Adult or larvae)	Detection of antigen in head squash by serotype-specific immunofluorescence
Inoculation of various Mammalian or insect Cell cultures	Detection of antigen by antibody staining Cytopathic effect; identification of virus upon subpassage Plaque formation; identification of virus upon subpassage
Intracranial inoculation of Sucking mice	Presence of antigen in brain detected by antibody staining Symptoms or signs indicating encephalitis Identification of virus upon subpassage

Baby mice, intracerebral inoculation of 1 to 3 day old baby mice, although all four dengue serotypes were initially isolated from human serum by using baby mice, this method is very time-consuming, slow, and expensive. Moreover, because of the

low sensitivity of the method, many wild type viruses cannot be isolated with baby mice. Those that are isolated frequently require numerous passages to adapt the viruses to growth in mice. This method is no longer recommended for isolation of dengue viruses, but some laboratories continue to use it. One advantage of using baby mice, however, is that other arboviruses that cause dengue-like illness may be isolated with this system.

Mammalian cell cultures have many of the same disadvantages as baby mice for isolation of dengue viruses; they are expensive, slow, and insensitive. As with isolation systems that use baby mice, viruses that are isolated frequently require many passages before a consistent cytopathic effect can be observed in the infected cultures. Although the use of this method continues in some laboratories, it is not recommended.

Mosquito inoculation is the most sensitive method for dengue virus isolation. Isolation rates of up to 100% of serologically confirmed dengue infections are not uncommon, and this is the only method sensitive enough for routine successful virologic conformation of fatal DHF and DSS cases. Moreover, there are many endemic dengue virus strains that can be recovered only by this method. Four mosquito species have been used for virus isolation, *Aedes aegypti*, *Aedes albopictus*, *Toxorhynchities amboinensis*, and, *Toxorhynchities splendens*. Male and female mosquitoes are equally susceptible; dengue viruses generally replication to high titer in as little as 4 to 5 days. Dengue virus replicate in most mosquito tissues, including the brain. Virus detection in the mosquito, regardless of the species, is generally performed by the direct fluorescent-antibody (DFA) test on mosquito tissues, usually brain or salivary glands. The mosquito inoculation technique has the disadvantages of being labor-intensive and requiring an insectary to produce large numbers of mosquitoes for inoculation. Also, unless strict safety precautions are maintained, the

chance of laboratory infections increases, although this risk can be eliminated by using male *Aedes* mosquitoes or nonbiting *Toxorhynchites* species for inoculation.

Mosquito cell culture are the most recent additional to dengue virus isolation methodology. Three cell lines of comparable sensitivity are most frequently used. The first cell line developed, and still the most widely used, is the C6/36 clone of *Aedes albopictus* cells. The use of these cell lines has provided a rapid, sensitive, and economical method for dengue virus isolation. Moreover, many serum specimens can be processed easily, making the method ideal for routine virologic surveillance. However, this system is less sensitive than mosquito inoculation. However, the sensitivity of the mosquito cell lines may vary with the strain of virus. Dengue antigen can be detected in infected-cell cultures by DFA or IFA tests with the conjugates used for mosquito tissues. Some workers, however, prefer to use cytopathic effect to detect infection. However, this method alone will miss many dengue viruses that do not replicate rapidly in mosquito cells.

3) Viral antigen detection.

This is a new method of immunohistochemistry, it is now possible to detect dengue viral antigen in a variety of tissues. Although immunofluorescence tests were used in the past, newer methods involving enzyme conjugates such as peroxidase and phosphatase in conjunction with either polyclonal or monoclonal antibodies are greatly improved. Because tissue can be fresh or fixed, autopsies should be performed in all cases of suspected DHF with a fatal outcome.

4) Viral nucleic acid detection.

In recent years, several new method of diagnosis have been developed and have proven very useful in dengue diagnosis, such as PCR and hybridization probes.

PCR. Reverse transcriptase PCR (RT-PCR) has been developed for a number of RNA viruses in recent years and has the potential to revolutionize laboratory diagnosis; for dengue, RT-PCR provides a rapid serotype-specific diagnosis. The assay is rapid, sensitive, simple, and reproducible if properly controlled and can be used to detect viral RNA in human clinical samples, autopsy tissues, or mosquitoes. Although RT-PCR has similar sensitivity to virus isolation systems that use C6/36 cell cultures, poor handling, poor storage, and the presence of antibody usually do not influence the outcome of PCR as they do virus isolation. A number of methods involving primers from different locations in the genome and different approaches to detect the RT-PCR products have been developed over the past several years.

Hybridization probes. The hybridization probe method detects viral nucleic acids with cloned hybridization probes. Probes with variable specificity ranging from dengue complex to serotype specific can be constructed depending on the genome sequences used. This method is rapid and relatively simple and can be used on human clinical samples as well as fixed autopsy tissues.

8. Vaccine development

The World Health Organization designated the development of a tetravalent dengue vaccine a priority for the most cost-effective approach to dengue prevention. Effective vaccination to prevent DHF will most probably require a tetravalent vaccine, because epidemiologic studies have shown that preexisting heterotypic dengue antibody is a risk factor for DHF. With WHO supported, developing dengue vaccine has been made in recent years. Promising candidate attenuated vaccine viruses have been developed and has been evaluated in phase I and II trials in Thailand as monovalent, bivalent, trivalent, and tetravalent formulations (94). A commercialization contract has been signed, and the tetravalent vaccine formulation is currently undergoing repeat phase I trials in the United States. Current progress on he live attenuated dengue vaccine has been recently reviewed.

In recent years, molecular technology was used to modify vaccine strategies. Additional, inactivated whole virion vaccine, synthetic peptides, subunit vaccine, vector expression, recombinant live vector systems, infectious cDNA clone-derived vaccines and naked DNA were developed in vaccine strategies. The last two approaches appear to be the most promising. An infectious clone of the DEN-2, PDK-53 vaccine candidate virus from Thailand has been constructed, and work is in progress to construct chimeric viruses by inserting the capsid, premembrane, and envelope genes of DEN-1, DEN-3, and DEN-4, into the DEN-2 PDK-53 backbone. Despite the promising progress, it is unlikely that an effective, safe, and economical dengue vaccine will be available in the near future (95).

II. Sand fly and Leishmania infection

Protozoas of the genus Leishmania are causative agent of Leishmaniasis. All leishmanial disease are transmitted to their vertebrate hosts by phlebotomine sand flies, which acquire the pathogen by feeding on infected hosts and transmit them by regurgitating the parasite at the site of a subsequent blood meal.

As same as mosquito, while obtaining a blood meal, sand flies salivate into the host's skin. Their saliva contains many substances such as anticlotting, antiplatelet, and vasodilatory compounds that increase the hemorrhagic pool when sand flies feed. David S. and his colleagues (96,97) reported that previous exposure to sand flies or immunity to salivary gland homogenate (SGH) prevents Leishmaniasis infectivity either by coinoculation of Leishmania with SGH or by infected sand flies bites. They contemplate the new strategies for Leishmania vaccine development. There was proposed that immunity to sand fly saliva may confer protection to subsequent Leishmania infection. They characterized the main proteins in the salivary glands of sand fly from SDS-PAGE and construct DNA vaccine containing the coding infection of the main protein. Mice vaccinated with constructed DNA vaccine and challenged

with Parasites plus SGH were efficiently protected against the manifestations of the disease at the site of the inoculation. Both of pathology and the number of parasites were significantly reduced (98,99,100,101). So, there may be a new strategy of vaccine's development.

III. Anti-mosquito antibody

Mosquitoes are one of the most important pests of mankind and are vectors of disease pathogens. Many studies showed mosquito bites caused allergic reaction in a host. Antigenic components of saliva are injected continuously after the skin is penetrated during the probing and feeding process (102). Some studies have shown that there are as many as 20 peptides in saliva of the adult mosquito *Aedes aegypti*. Some of these molecules such as the vasodilators, anti-platelet aggregation factors and anti-coagulant-factor facilitate blood feeding (103). Their bite cause immediate and delayed local cutaneous and rarely, systemic reaction. Reactions to mosquito bites are immunologic in nature, and are caused by specific sensitization to mosquito saliva.

By SDS-PAGE and immunoblotting technique have revealed that some protein band of mosquito's saliva bound to the IgE of subject with skin reaction to mosquito bites (104). The studies reported mosquito-specific IgE correlated with immediate skin reactions. The delayed reaction appeared to be consistent with lymphocyte-mediated hypersensitivity, which appears only after several hours and peaks between 24 and 48 hours (105). Moreover, there are few reported about human IgG antibodies to mosquito proteins (106).

There are important idea from the study in snake found that in snake bite-site, could detect toxin-encoding gene in swab specimen from snake bite-site. It showed that some snake cell were released during biting into their prey. In another bitting, it's possible that there are cell released to the bite-site as same as in snake bite-site.

So, from these data, we could suggested that some mosquito cell were released into bite-site to induced human immune response to produced anti-mosquito protein antibody, detected from many studies (107,108,109,110,111,112). Then mechanism of dengue replication depend on host's cell membrane, mosquito cell, they must take some protein antigen of mosquito's cell coat their particle. If human was immunized with mosquito bite to produce anti-mosquito cell antibody, so, it may be a new strategy of dengue vaccine development.