CHAPTER III
LITERATURE REVIEW

3.1 Overview of Arboviruses

Arthropod-borne viruses (arboviruses) are viruses that require an arthropod a as vector for transmission among hosts (22). Beside midges, ticks or biting flies, the significant vector of arboviruses is mosquitoes because of their capability of spreading disease in most of tropical and subtropical countries (23). Arboviruses are classified into *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Reoviridae* and *Orthomyxoviridae* families depending on the genomic material, antigenic complex, morphology, and replication strategy (24, 25). The *Flaviviridae* and *Togaviridae* families are the most important families involving in human pathogens. Especially for genus *Flavivirus* in the *Flaviviridae* family, this genus contains more than 70 positive single stranded RNA viruses. Even though humans are a generally dead-end host (26) some of *Flavivirus* can cause human disease including Dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV). These viruses can create high viremia level in humans and they can be also transmitted among humans by different genus of mosquitoes (9). DENVs have become a major public health problem in the endemic areas and tends to be substantially increase annually (6) because of warming temperature from climate change (27).

3.2 Molecular background and structure of DENVs

DENVs are enveloped positive sense single stranded RNA viruses in *Flaviviridae* family and *Flavivirus* genus. Their particles are approximately 50 nm diameter (9). Their RNA genome is approximately 10.6 kb in length (2) is capped by 5'-type 1 structure (m^7G5'ppp5'A) with no polyadenylated (poly A) tail at the 3' terminus (28). The 5'-UTR contains 2 domains which are necessary for viral

replication. The first domain consists of approximately 70 nucleotides which folds into a large stem-loop similar to other members in the *Flavivirus* genus. This region is the place of the promoter for the viral RNA dependent RNA polymerase or NS5. The second domain forms into a short stem loop which plays a role in viral RNA replication. These two domains are separated by an oligo (U) sequence to act as spacer of the two stem loops for their proper function (2). The 3'-UTRs is not only necessary for protein translation and viral replication but also contains conserve sequences of DENVs (28) . The open reading frame of the DENV genome encodes for a single polyprotein which is cleaved by cellular and viral proteases into 3 structural proteins and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) **(Figure 3.1).** Structural proteins consist of Capsid (C), pre-membrane/membrane (prM/M) and Envelope (E) proteins which are the building block of viruses (3, 7). DENVs E glycoprotein of approximately 55 KDa is one target in the host immune response (29). Therefore, DENV E protein is an antigenic determinant and it is a target in the development of antiviral treatments. E protein consists of domain I, II and III which are involved in membrane fusion, E protein dimerization and cell receptor binding, respectively (30, 31) **(Figure 3.2).** Whereas DENV prM/M glycoprotein is responsible in viral maturation in the secretory pathway (9), non-structural proteins facilitate RNA replication, assembly and modulation of the host immune response (3). Dengue viruses are differentiated into 4 serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) according to their antigenic determinants (1, 4).

3.3 DENV transmission cycle

DENVs are mosquito borne viruses. They are transmitted among human by domestic mosquito in genus *Aedes,* principally *Aedes agypti (Ae. aegypti)* and *Ae. albopictus* depending on geographic area (1). These mosquitoes are small, black-and-white color and abundant in domesticity. They always lay their eggs in standing water containers around the house, for example, water tank, flower vase or rainwater bucket. Primarily, DENVs transmit among *Aedes* spp. mosquitoes and primates in rain forest of Asia and Africa, called enzootic transmission. The spread out of DENVs to

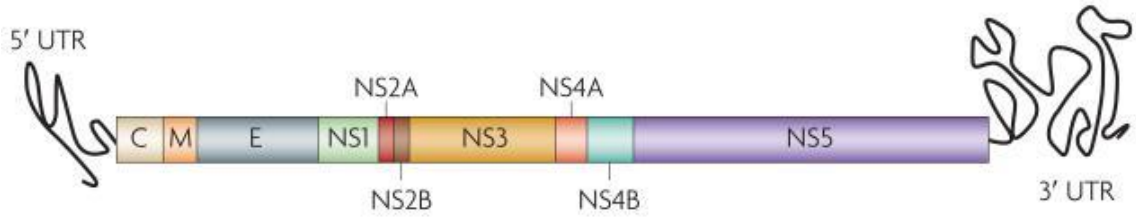

Figure 3.1 The RNA genome of DENVs with a length of approximately 10.6 kb.

It encodes for a single polyprotein which is cleaved by cellular and viral proteases into 3 structural proteins, C, prM/M, E protein and 7 non-structural proteins which are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. (Reprinted by permission from Nature Publishing group: [Nature review microbiology] (7), copyright 2010)

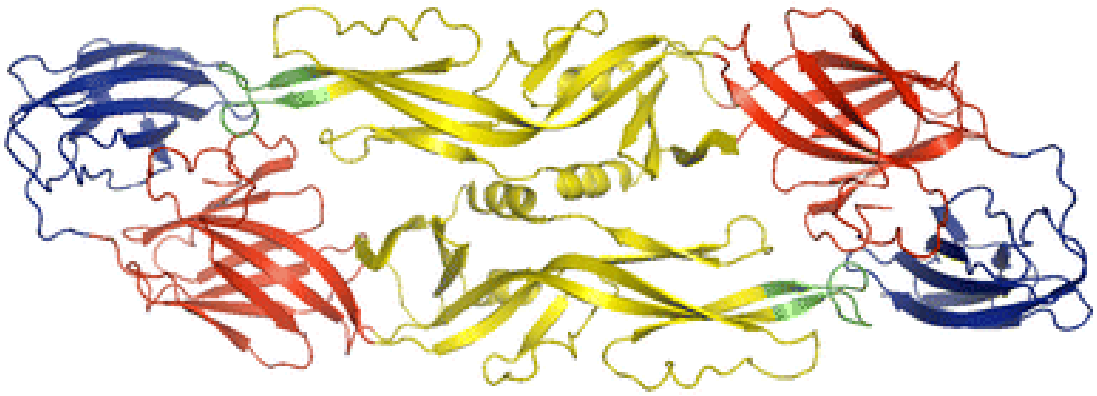

Figure 3.2 Structure of E protein to show the arrangement of E protein domain.

Domain I II and III are represented in red, yellow and blue, respectively. The fusion loops in domain 2 are represented in green. The image was performed using PyMol Molecular Graphics program. (Reprint from Cellular and Molecular Life Sciences, Vol 67, Rodenhuis-Zybert I. A. *et al*., Dengue virus life cycle: viral and host factors modulating infectivity, 2773–2786, Copyright 2010, with permission from Springer) (30)

urban areas may occur in the place with small population like a village or island. Various serotypes of DENVs often circulate in the same area. Only female mosquitoes bite people because the lipid in blood helps their egg development (32). If they bite an infected person, they will be remain infected throughout their life and they transmit virus to persons shortly. After *Aedes spp.* take up infectious blood, viruses replicate in the midgut epithelium then spread to infect other tissues. The most important organ to reservoir the virus is the salivary gland. In transmission cycle, DENVs in *Aedes spp*. salivary gland contaminate in their saliva and virus can distribute to another hosts by mosquito biting. All of this process or the incubation period takes 7-24 days (1). After the infected mosquito bites a human, viruses head to their primary target that is monocyte-derived dendritic cells (DCs) and human skin Langerhans cells (LCs). Finally, viruses release to bloodstream and viremia remains for an average of 9 days, (33) then spread to naive mosquitoes again if mosquitoes bite the infected person (22, 33) **(Figure 3.3).**

3.4 Emerging and epidemiology of DENVs

Four serotypes of DENVs are believed to have arisen in Africa and South East Asia more than 1000 years ago. Recently, they are endemic in more than 100 countries and most of countries are in tropical and subtropical regions (6). Two-fifths of the world's population live in endemic areas and they have chance to be infected with DENVs, particularly in South-East Asia and the Western Pacific (6, 23) **(Figure 3.4)**. The main reason for DENVs wide distribution is the travel of *Aedes spp.* by cargo transportation. It makes viruses spread to new areas and increased their intensity in their own endemic area. In Thailand, dengue infection is a major public health burden in the population with annual average 76,978 reported case from 2001-2010 (34). Nowadays, climate change or global warming is concerned as a factor of increasing dengue incidence. Because of higher temperature, rainfall increasing and the available of piped water are advantages of *Aedes* mosquitoes life cycle, therefore the success of DENVs prevention by vector control may not be accomplished in the next decade (35).

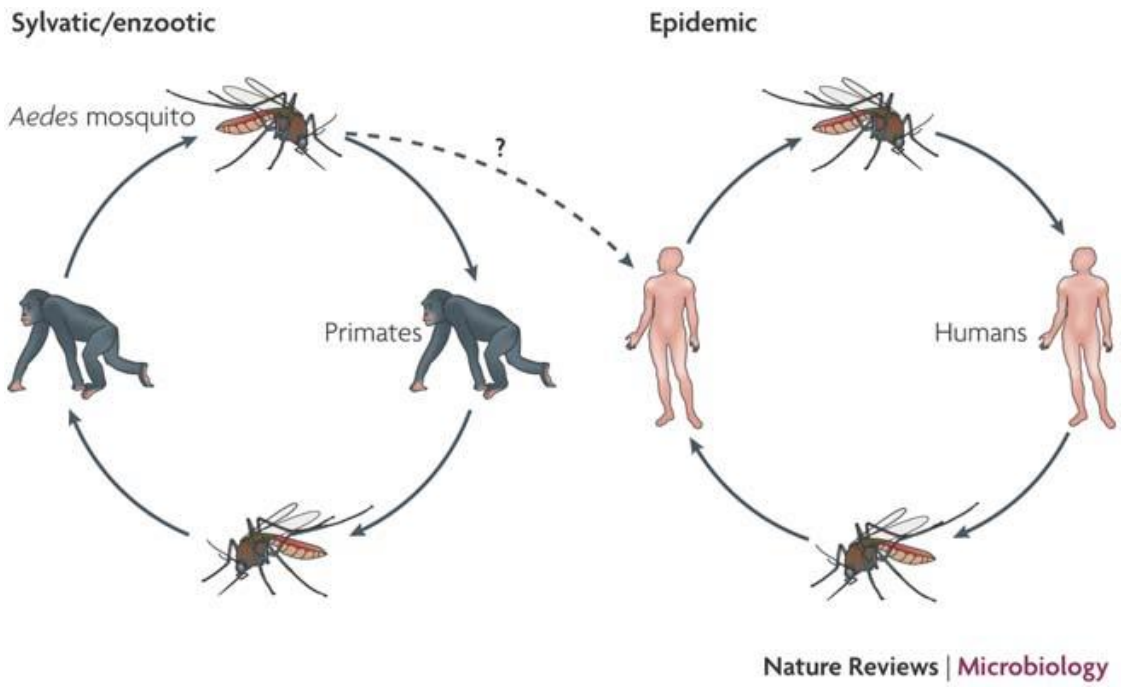

Figure 3.3 DENVs transmission cycle.

DENVs are transmitted among human by domestic mosquito in genus *Aedes*, principally *Ae. agypti*, *Ae. Albopictus* or *Ae. polynesiensis* depending on geographic area. Enzootic transmission cycle of DENVs is the transmission of virus between *Aedes* mosquitoes and primate in rain forest of Asia and Africa. The spread out of mosquitoes lead to DENVs distribution to urban area. *A. aegypti* females bite viremia person and they will remain infected and they transmit virus to persons shortly. (Reprint by permission from Nature Publishing group: [Nature microbiology review] (36), copyright 2007)

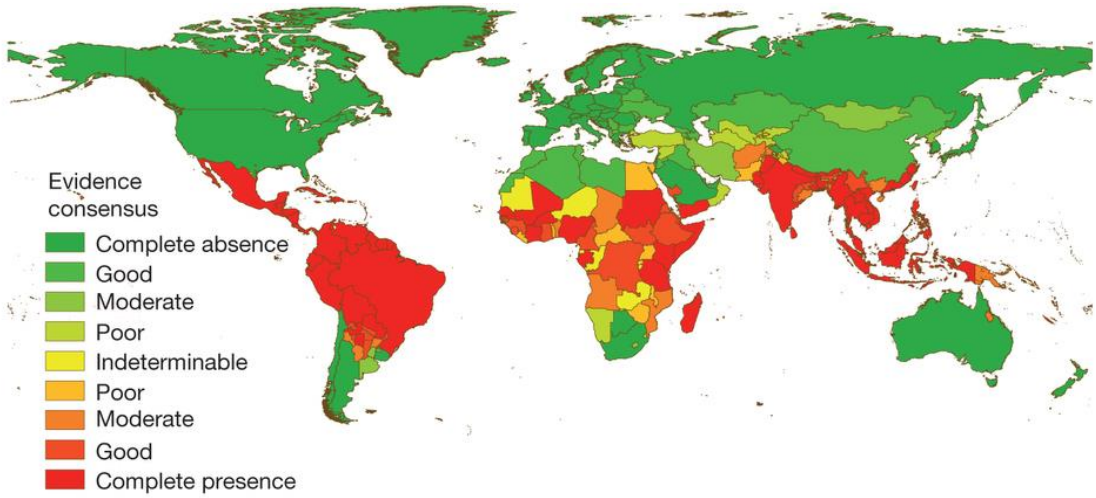

Figure 3.4 The DENVs distribution map in 2010.

The strength of evidence is shown in different colors. Complete presence of DENV is represent in red while complete absent is represented in green color. (Adapted and reprint by permission from Macmillan Publishers Ltd: [Nature] (6), copyright 2013)

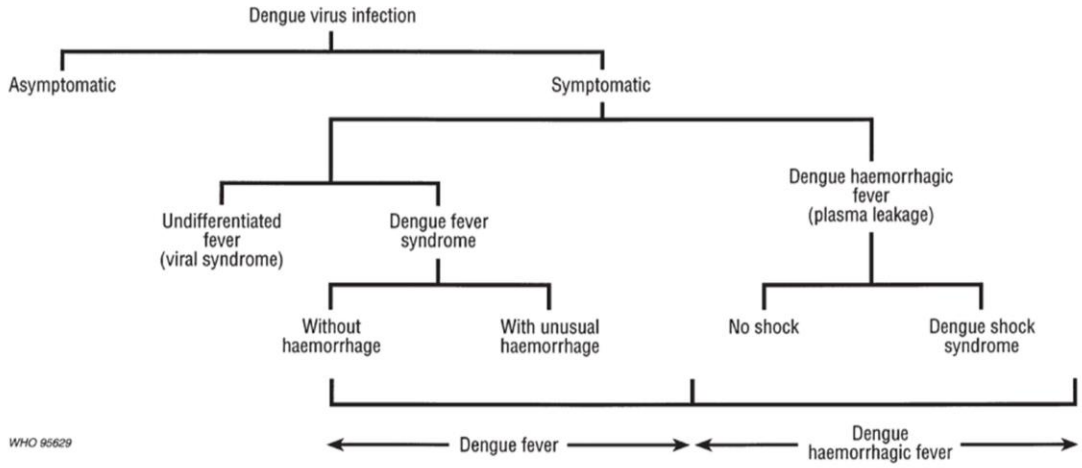

Figure 3.5 Classification of DENV infection.

It can be classified into undifferentiated fever, dengue fever (DF) and life threatening dengue infection, dengue hemorrhagic fever (DHF) and dengue shock syndrome. (DSS) (Dengue: Dengue haemorrhagic fever Diagnosis, treatment, prevention and control. Geneva: World health Organization; 1997. Avaliable from: http://whqlibdoc.who.int/publications/1997/9241545003_eng.pdf)

3.5 Clinical manifestation of DENVs

Dengue symptoms show a wide spectrum of clinical manifestation and it can be classified into undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) **(Figure 3.5)** (40, 41). In incubation period, fever can occur for 2–7 days with common sign of DENVs infection such as rash, petechiae, sore throat, headaches or joint pain. Mostly DF can be cured by patient's immune response and they do not progress to more severe symptoms. For DHF, the patients show some fever with complications including hemorrhagic manifestation (positive tourniquet test result), skin and mucosal bleeding, gastrointestinal bleeding, thrombocytopenia, and evidence of plasma leakage (37). If vascular permeability is uncontrollable together with myocardial dysfunction and dehydration, it will lead to DSS (38) which is the main reason causing life threatening dengue infection. The mechanism of DHF and DSS development is still unclear. Some hypotheses mention that it might be involving in the secondary infection with different serotype called antibody dependent enhancement (ADE) phenomenon (39). Antibody dependent enhancement is the phenomenon that lifelong antibody in the primary infection cannot neutralize different serotype of DENVs antigen in the secondary infection and form a complex between viruses and phagocytes via Fc receptor (40). Therefore, viruses can enter to phagocytic cells and initiate their replication (36) (**Figure 3.6)**. It leads to more severe disease and higher viremia level compared with those in whom ADE has not occurred (41). Recently, approximately 390 million cases of DENVs infection was reported per year and 96 million cases developed to some symptoms (5). Dengue hemorrhagic fever and DSS are the main cause of hospitalization and death of children in Southeast Asia. Successful treatments of DHF and DSS patients need an intensive care and are costly. Mortality rate may increase to 20% if patients are not treated properly (5, 6).

3.6 DENVs life cycle

DENVs attach to target cells by interaction with specific receptors or cell-surface binding molecules. The first identified molecule that is involved in DENVs entry was heparan sulfate. This molecule participates in viral attachment

because it is abundant on the surface of several cell types (42). Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) on dendritic cell is one of the reported molecule as a major attachment receptor of DENVs. Envelope protein dimer of DENVs contains glycans and they recognize the carbohydrate recognition domains of DC-SIGN. Furthermore, there is evidence show that C-type lectin or mannose receptors on dendritic cells and macrophages surface act as co-receptor for this mechanism. Others reports of *Flavivirus* receptors are laminin receptor, GRP78 (BiP), CD14, lectin, heat shock protein and other related molecules (42, 43). Then, virus is internalized to target cell by triggering a cellular process called "receptor mediated endocytosis" in a clathrin- dependent manner. Viral particles enter to host cells as an endosomal vesicle. Proton pumps change the pH of the endosome into an acidic condition. Uncoating of the virus or the fusion between viral membrane and endosome membrane occurs to release the viral RNA (11). The pH threshold and uncoating of virus can be influenced by cholesterol and oleic acid (44). After viral RNA release, translation begins at the rough endoplasmic reticulum (rER) to giving a single polyprotein. The polyprotein is co-translationally and post-translationally modified in the lumen of the rER by cellular and viral proteases. These proteases cleave the single polyprotein into 3 structural proteins (C, prM and E) which form the shell of the viral particle and 7 non-structural proteins (3). The newly synthesized RNA of viral progeny assembles with the capsid protein to form a capsid-RNA complex (8, 9). Then, this complex buds into ER lumen covering with lipid membrane associated with heterodimers of 2 trans-membrane proteins, E and prM proteins. These immature particles travel to the trans-Golgi network using DENVs native signal sequence for facilitating translocation, transportation and secretion from rER to Golgi apparatus (9). The 19-aa region at 3' end of capsid sequence and the 15-aa region at 3' end of prM sequence represent the native signal sequence for prM and E, respectively **(Figure 3.7)** (10). Inside the trans-Golgi network, the immature viral particles become mature after furin-dependent cleavage. The pr domain cleavage product remains associated with the E protein to prevent premature fusion of the particle and pH induced reorganization during secretion, and pr domain is liberated only after the particle has been released from the cell to infect other cells (8, 9, 11, 30) **(Figure 3.8)**.

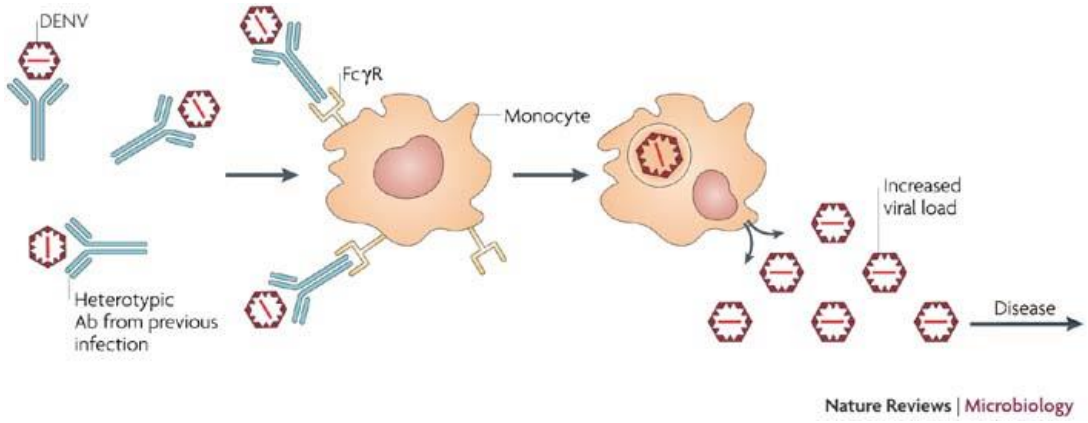

Figure 3.6 ADE phenomenon of secondary DENVs infection by different serotype.

Primary infection results a lifelong antibody to that serotype. If secondary infection occur with different serotype, the primary antibody cannot neutralize DENVs antigen and form a complex. Fc receptors on phagocytic cells bind to these complex. Viruses can enter to phagocytic cell and initiate the viral progeny production. (Reprint by permission from Nature Publishing group: [Nature review microbiology] (36), copyright 2007)

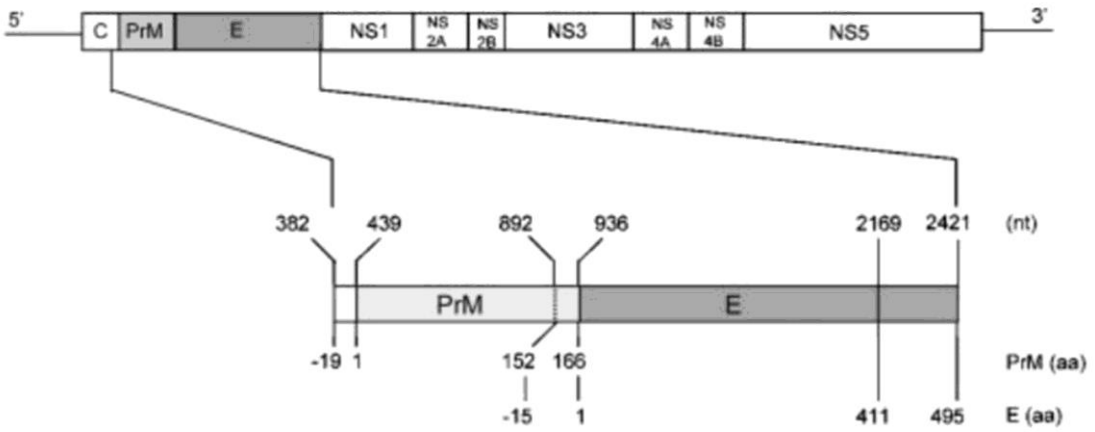

Figure 3.7 Native Signal sequence for prM and E protein of DENVs.

The 19-aa region at 3' end of capsid sequence and the 15-aa region at 3' end of prM sequence represent the native signal sequence for prM and E, respectively. (Reprint from Journal of Medical Virology, Vol 80, Puttikhunt C. *et al*., Novel antidengue monoclonal antibody recognizing conformational structure of the prME heterodimeric complex of dengue virus, 125-133, Copyright 2007, with permission from John Wiley and Sons) (10)

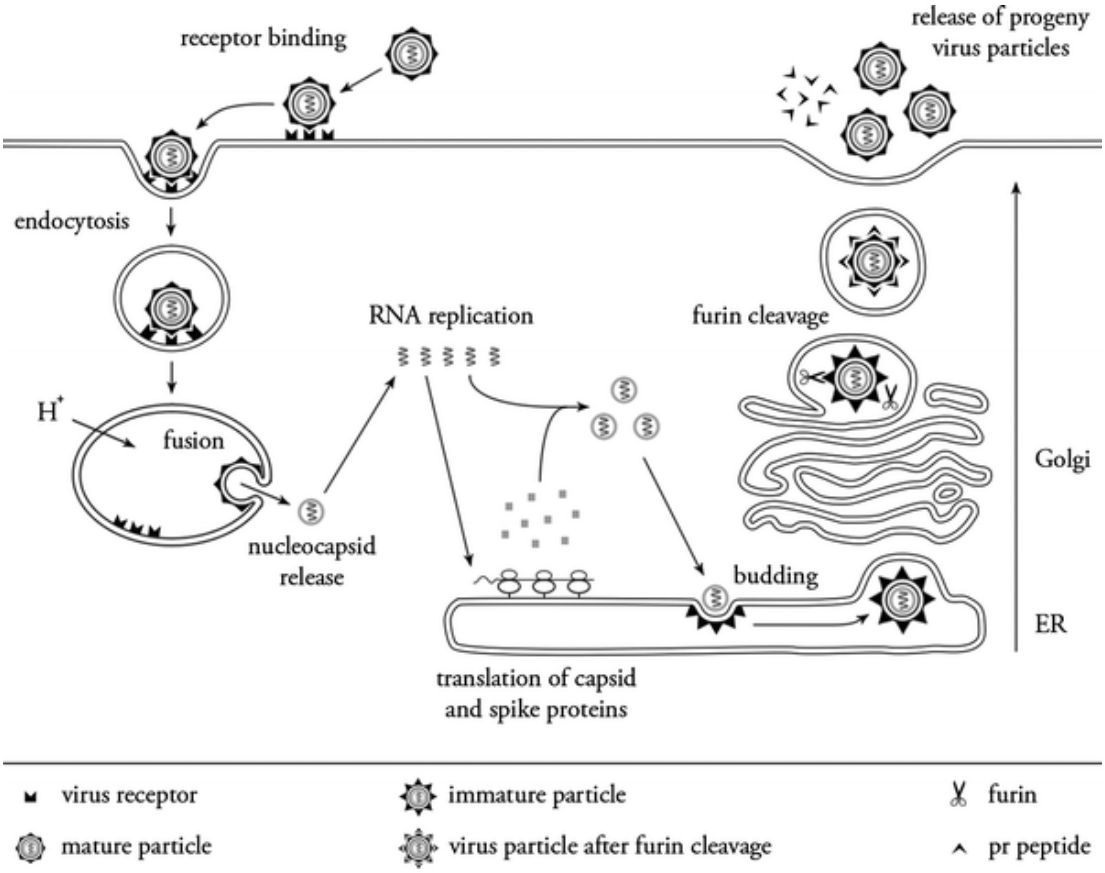

Figure 3.8 Mechanism of DENV entering into the host cell.

DENVs enter to the host cell by receptor mediated endocytosis. Acidic condition of endosome catalyzes uncoating of virus. Viral RNA can be a template of RNA replication and translates to a single polyprotein by associating with rER. The newly immature particles are formed and complete their maturation at tran-Golgi network. Then, the mature viral particles release out from the cell to infect other cells. (Reprint from Cellular and Molecular Life Sciences, Vol 67, Rodenhuis-Zybert I. A. et al., Dengue virus life cycle: viral and host factors modulating infectivity, 2773–2786, Copyright 2010, with permission from Springer) (30)

In viral budding step of yellow fever virus (YFV), the viral nonstructural protein 3 (NS3) facilitates viral budding step by interacting with proteins in the endosomal sorting complex required for transport machinery (ESCRT). NS3 recruits a host cellular protein called Alix to aid in budding process of the infectious particles. In previous study, Alix is involved in several *Retroviruses* budding. For YFV, Alix can bind with the YPTI (a conserved peptide sequence composing of Tyrosine, Proline, Threonine, Isoleucine) on NS3 starting at residue 399 and recruit other ESCRT associated proteins for viral budding (12, 13). Furthermore, Alix can recruit a protein that facilitates remodeling of membrane structure, called endophilin (45). Endophilin can create membrane curvature in the initiation step of viral budding. However, YPTI sequence is only present in the domain II of NS3 helicase of YFV whereas these residues in other closely related viruses were formed to be similar sequence **(Figure 3.9)**. Mutations on helicase domain or domain II of NS3 in YFV had no effect in viral RNA replication but resulted in viral production blocking and lost their infectivity (12). The closely associated motif YIKT and YPKT were found in DENV-2 and DENV-4, respectively. However, the capacity of these DENVs motifs in interacting with alix facilitating viral budding is not known (12, 13).

3.7 DENVs pathogenesis

The mechanism of host cells to response DENVs infection is a complex network. Dendritic cells, macrophages and monocytes are primary target of DENVs infection prior to taking DENVs to T and B lymphocyte (46). B cells produce IgM and IgG antibodies to neutralize DENVs. Then, serotype specific T cells are selected, activated, and clonally expanded to overcome DENVs infection. Viruses are killed by cytotoxic T cells after binding to antigen presenting cells. On the other hand, antibody-antigen complex is phagocytosed and eliminated by macrophage. In case of mast cells, they are sensitized to virus-antibody complexes and produce cytokines to facilitate in viral containment (47).

In primary infection, CD4+ and CD8+ memory T cells are induced and they generate both serotype-specific and serotype cross-reactive memory T cells. The production of these memory T cells generates long-term immunity to the same

serotype and gives effective viral elimination after recalling. Unfortunately, memory T cells offer short-term immunity to different serotype. These CD4 T-cell clones from DENVs infected patients were found to cross-react with other *Flaviviruses* (33). Moreover, DENVs elimination involves in inflammatory cytokine/chemokine responses, for example, interferon gamma (IFN-ɣ), tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-2, IL-6, platelet-activating factor (PAF), complement activation products and histamine. For secondary infection, CD8+ is recalled and responsible for getting rid of viruses by targeting their non-structural antigens (48). Different serotype or strain of virus in secondary infection creates antibody-dependent enhancement as mentioned above. All produced cytokines can induce a complex network to increase other cytokines and chemical mediators. Excessive cytokines in secondary infection with different serotype has effect on vascular permeability and cause plasma leakage or circulatory failure in DHF and DSS (49) **(Figure 3.10)**. Higher concentration of many cytokines can be detected in peripheral blood of DENVs patients. There are some publications suggesting that natural killer cells (NK cells) play an important role in early-stage of infections and the suppression of the NK cells responses increases a chance to develop to more severe disease (47).

3.8 DENVs prevention and control

Nowadays, DENVs are endemic in more than 100 countries with 100 million reported cases each year (6). It makes DENVs infection become an urgent public health problem in DENVs endemic area. The key of DENVs prevention is vector control. Hence, people in dengue endemic area should be well informed about personal protection and the environmental management. People have to avoid biting of mosquitoes. Especially, children and people who have experience of DENV infection, these people should not be in an environment that has a chance of mosquitoes biting. For household protection, mosquito-proof mesh on doors and windows and mosquito nets in sleeping area are necessary to DENVs prevention. On the other hand, mosquitoes breeding have to be avoid by empty and refill the outside water container

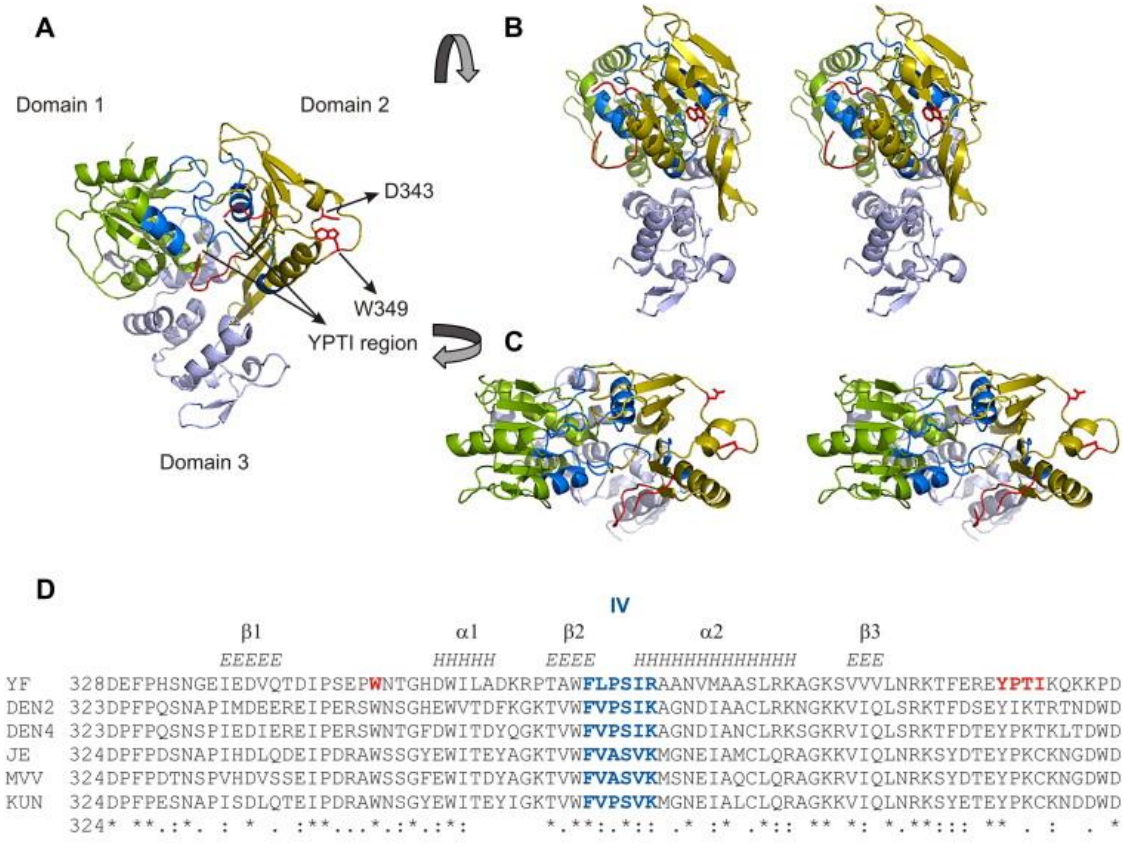

Figure 3.9 YPTI motif on NS3 protein of YFV.

YPTI locates at residue 399 on NS3 protein and it is a conserved peptide composing of Tyrosine, Proline, Threonine, Isoleucine. YPTI can bind to Alix host protein and recruits other ESCRT associated proteins to facilitate in viral budding. (Adapt and reprint from Microbes and infection, Vol 13, Carpp L.N. *et al*., Interaction between the yellow fever virus nonstructural protein NS3 and the host protein Alix contributes to the release of infectious particles, 85-95, Copyright 2010, with permission from Elsevier) (13)

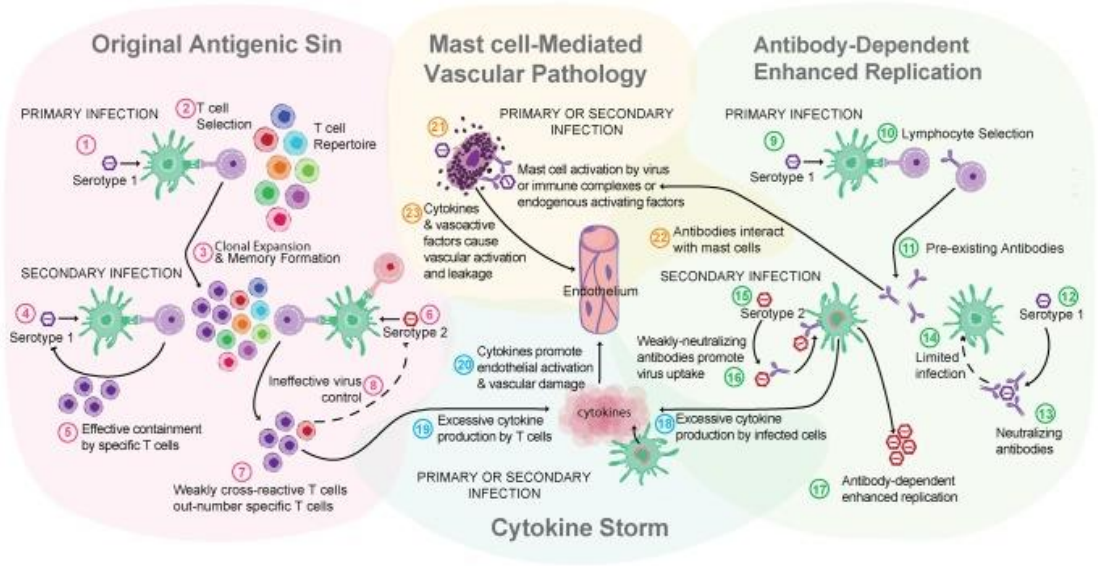

Figure 3.10 Pathogenesis of DENVs infection.

Primary infection of DENVs resulted in B cell or lymphocyte selection occurs, promoting serotype specific antibody production and create serotype specific T cells response. T cells are selected and clonally expanded to overcome DENVs infection. This adaptive immune response is long term protection and effective elimination of virus particles in secondary infection with the same serotype. In contrast, if secondary infection happens with a different serotype or even different strain, non-neutralization of antibody to DENVs epitope will occur. The cells containing Fc receptor on their surface will uptake virus-antibody complexes and promote viral replication, called antibody dependent enhancement. Furthermore, excessive cytokines produce effect on vascular permeability and cause plasma leakage or circulatory failure. (Reprint from PLOS Pathogens, Vol 9, St. John A.L., Influence of Mast Cells on Dengue Protective Immunity and Immune Pathology. PLoS Pathog 9(12): e1003783. doi:10.1371/ journal.ppat.1003783, Copyright 2013) (47)

once a week or cover any water tanks with a sealing lid. A small amount of liquid paraffin or domestic kerosene is efficient to prevent mosquitoes breeding by dropping into water tank. In case of mosquito-repellent or insecticide, they can be used for house-hold prevention but they are not recommended for children under 2 months of age (50). Unfortunately, there is limitation on DENVs prevention. The option of mosquito control is not available in some area and no commercial DENVs vaccine currently available (37).

3.9 DENVs vaccine development

DENVs vaccines have been developed for decades but no commercial vaccine is yet available (29). The first live attenuated vaccine (LAV) for DENVs prevention was developed in 1945 by Sabin and Schelsinger. After adaptation with different strategies, this kind of DENVs vaccine can induce long term immunity and cost effectiveness but the level of immunity against all of dengue serotypes in term of tetravalent vaccine was not balanced. For over 20 years, LAV was clinical tried in human. Side-effect profiles or lacking desirable immunogenicity still was problems (51). After that, several attempts have been made to develop vaccine. Chimeric vaccine, live-attenuated tetravalent vaccine based on chimeric yellow fever-dengue virus (CYD-TDV), was warranted by World Health Organization WHO. CYD-TDV or ChimeriVax technology contained genes from prM-E gene of each dengue serotype together with gene from the yellow fever virus 17D strain (YF17D), 77–100% of vaccinated persons generated neutralizing antibody responses following 3 doses administration and showed evidence of remaining immunity at roughly 4–6 month after vaccination. This vaccine could eliminate the interference between the four serotypes (52). Accordingly, the test of CYD-TDV was halted in a phase III trial because higher efficacy of the vaccine in those who had serological evidence of previous dengue infection and longer term protection still are problems and need a further analysis (53).

Several novel dengue vaccine candidates have been investigated to obtain more effective vaccine including inactivated virus vaccines, replication-incompetent vaccines and DNA vaccine (36). Nowadays, virus like particles (VLPs) is one of

vaccine candidate for DENVs. VLPs are shell-like pseudoviruses, composed of the organization and conformation as native viruses but lack of replicative viral genetic materials. From these reasons, they are non-infectious particles but can trigger strong immune responses from their remaining antigenic determinants as the native virus. Two types of structural proteins that are frequently used in DENVs VLPs construct are coding region of envelope proteins (E) and pre-membrane/membrane proteins (prM/M) because human antibodies raised against the DENVs are mostly targeted at the E and prM (15). VLPs vaccines are more safely than other vaccines and it can also be used to explore the mechanism of virus infection (14). In previous study, VLPs could produce high immunogenicity against various pathogens so DENVs VLPs was tried to construct in a similar method. Firstly, DENVs VLPs was in a form of fusion protein with Hepatitis B virus surface antigen and expressed in *Pichia pastoris (P. pastoris)* system. They were successfully expressed in *P. pastoris* and they could induce specific antibodies against all DENV1-4 antigens when immunized in mice. However, it still had a problem with VLPs that were constructed from E protein in domain III because they could not form intact VLPs (54). Thus, construction of fusion protein composed of ectodomain of E protein and 30 amino acids residues of the prM protein was performed. It also successfully expressed in *P. pastoris*. Mammalian cell culture system is an interesting system of DENV-2 VLPs expression. Transient transfection of insect cells resulted in 10–100-fold yields when compared with mammalian cell (15). Therefore, the limitation of the VLPs production is low yield in cell-based systems. As mentioned above, newly synthesized membrane and secretory proteins will assemble in ER then transport to the Golgi complex. Some proteins such as DENVs E protein has ER retention signal (19). Intracellular accumulation of prM-E proteins lead to inefficient VLPs transportation from ER to Golgi complex. For this reason, signal sequence is added at 5’ terminus of the targeting gene to improve protein expression.

3.10 Signal sequence

Proteins secretion from rER to Golgi apparatus has different rate in mammalian cells. One hypothesis involves in retention signals of secreted protein

altered them from normal bulk-flow pathway. An evidence suggested that ER resident or retention signal occurred in post-ER translation. Furthermore, KDEL (Lysine-Aspartic acid-Glutamic acid-Leucine) or typical mammalian ER retention motif played an important role in protein accumulation in ER and their mechanism could recycle when compared with lysosomal sorting signal on the same hybrid protein (55). To solve this problem, signal sequences, which facilitate protein escaping from bulk-flow pathway from ER to Golgi complex (20) are used. Signal sequence is usually 16 to 30 amino acid residues at N terminal of protein and it composes of a central hydrophobic region and latter cleavage site for signal peptidase. In eukaryotes, N- terminus of protein remains in cytoplasm (56) and signal sequence facilitate binding of ribosomal complex. Then, signal sequence is recognized by the signal recognition particle (SRP). The ribosome–nascent chain–SRP complex is targeted to the membrane by a GTP-dependent interaction. Then, the complex docks onto translocon using the SRP receptor, and the nascent polypeptide resume translation process again (57). Finally, signal sequence is cleaved off by signal peptidase prior to travel to Golgi complex (17) and SRP is recycled to the cytosol by GTP hydrolysis (58) **(Figure 3.11)**. The signal sequences of vesicular stomatitis virus G-protein (VSV-G) is a type I membrane protein (18). It was identified as one of the shortest efficient signal sequence, containing 16 amino acid sequence (Methionine-Lysine-Cysteine-Leucine-Leucine-Tyrosine-Leucine-Alanine-Phenylalanine-Leucine-Phenylalanine-Isoleucine-Glycine-Valine-Asparagine-Cysteine), and it specifically facilitates ER translocation (17).

3.11 Codon optimization

One popular strategy to improve VLPs expression is codon optimization. A codon is a series of three nucleotides that encodes for one amino acid. Most amino acids can be coded by more than one triplet of nucleotides. There are 64 different codons, 61 codons encoding for 20 amino acids plus 3 stop codons to terminate translation process. For translation process in individual organism, specific codon usage is preferred differently. It was also suggested that translation of the coding

sequence containing abundant codons is faster than mRNAs with rare codons (59). This phenomenon called codon bias (21). Codon bias play a role in natural selection because it has evidence that there was different pattern of codon usage between thermophilic prokaryotes and mesophilic prokaryotes, result of natural selection linked to thermophily. Moreover, external environmental factors could affect codon usage of organisms (60). The optimal codons could improve rate and accuracy of translation. Therefore, the codon optimization is strategy to adapt most of codon into compatible codon usage of their host and increase level of protein expression in different cell culture system (21).

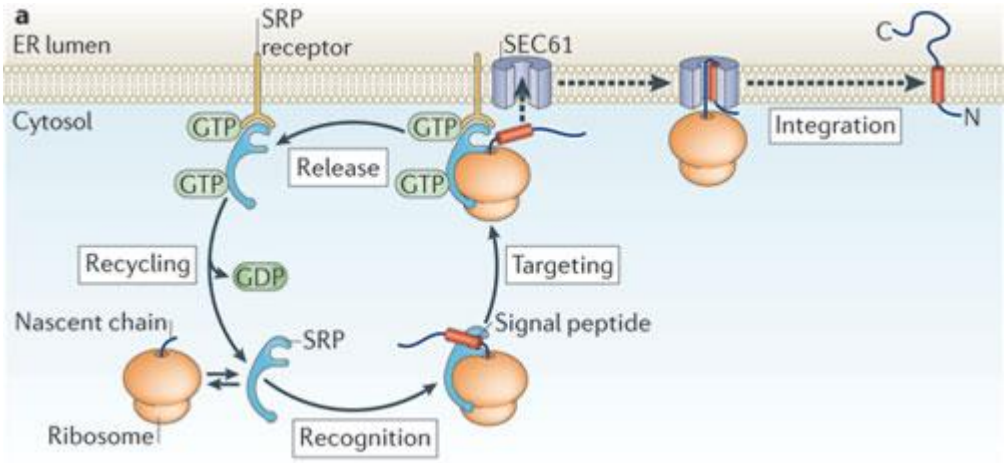

Figure 3.11 Signal sequence in protein translocation.

Hydrophobic peptide on Signal sequence is recognized by signal recognition particle (SRP). After SRP-SRP receptor binding on ER, the complex docks onto translocon and nascent polypeptide resume translation process again. Finally, signal sequence is cleaved off by signal peptidase prior to travel to Golgi complex and SRP is recycled to the cytosol by GTP hydrolysis. (Reprint by permission from Nature Publishing group: [Nature Molecular Cell Biology], copyright 2011) (58)