CHAPTER I

INTRODUCTION

Mosquitoes are one of the most important pests of mankind and are vectors of disease pathogens. At the beginning of the process of blood feeding, mosquitoes inject saliva (1,2,3) which controls and limits blood clotting (4,5,6,7). Deposition of saliva can lead to adverse consequences for the victim, such as production of local hypersensitivity reactions to severe systemic reaction (8,9), moreover, transmission of dengue virus, the ethiologic agent of dengue hemorrhagic fever and dengue shock syndrome, one of the most important public health problem around the world. Dengue viruses are transmitted between human primarily by *Aedes aegypti* and *Aedes albopictus* mosquitoes and are endemic in most area in which the vectors occur (10,11).

Dengue virus is classified in Family Flaviviridae, Genus Flavivirus and occur as four antigenically distinct serotypes. Infections with any of them generally lead to a mild, self-limitting febrile illness, however, a more severe form of the disease, involving vascular and hemostatic abnormalities is responsible for a high mortality rate (12,13,14,15). As each year, millions of human dengue infection occured, and over two billion people are at risk of infections (16,17,18,19,20). At present, there are 3 models of severe pathogenesis of dengue infection, the antibody-dependent enhancement model, the nutritional status model and specific dengue serotype model. First model is antibody-dependent enhancement model (21,22,23,24,25,26,27), associated with prior immune sensitization by a heterotypic virus. This kind of dengue infection provides lifelong homotypic immunity, but only transient cross-protection against other serotypes is achieved, making sequential infection possible. Severe

disease is postulated to be the result of heterologous, non-neutralizing antibodies facilitating virus infection of mononuclear cells via immune complex and Fc receptor.

An alternative hypothesis is nutritional status, the study in Thailand by Usa Thisyakom and Suchitra Nimmannitya reported that most patients with DHF are not undernourished (28). This may be related to strong immune response in patients with good nutritional status.

The last hypothesis is specific dengue serotype model. Some specific viral serotypes may be risk factors in the production of more severe disease (29). There are some virus strains that have greater epidemic potential. Phenotypic expression of genetic changes in the virus genome may increased virus replication and viremia, severity of the disease, and epidemic potential, as several studies showed an increasing of dengue disease severity with DEN-2 virus type (30,31,32,33). However, severity of the disease is also related to other dengue serotype.

The first reports of major epidemics of an illness thought to possibly be dengue occurred in three continent (Asia, Africa, and North America) in 1779 and 1780. It is likely that dengue viruses became endemic in many tropical urban centers during, 1780 to 1940, because during interepidemic period, when there was no apparent disease transmission, nonimmune visitors invariably contracted a dengue-like illness within months of their arrival (34).

Aedes aegypti prefers to feed on human blood, and can spread the dengue virus most effectively when people are living in community. There are so many factors influent the geographic distribution of *Aedes* mosquitoes. Technological advances also have a major impact on the emergence of infectious diseases, they have driven many of the societal changes that can be associated with increased infectious disease incidence. Automobiles have contributed to more rapid spread of many diseases, but have also been a major factor in the increased incidence and geographic spread of *Aedes* mosquito which breeds preferentially in automobile tires that collect

water(35,36). The distribution of these mosquitoes are being studied in parts of the United states. Scientists are almost positive that these mosquitoes entered this country in shipments of used tires from Northern Asia (37). In addition, many cases of dengue introduced into the United State come from the American and Asian tropics, reflecting the increased number of persons traveling to and from those areas. In 1994, an estimated 40 million persons departed the United States by air, over 50% of whom traveled for business or holiday to tropical countries where dengue is endemic. Many travelers become infected while visiting tropical areas but become ill only after returning home, resulting in a constant movement of dengue viruses in infected humans to all areas of the world and ensuring repeated introductions of new dengue virus strains and serotypes into areas where the mosquito vectors occur (38).

Interestingly, most of adult person living in the endemic area of dengue infection such as Thailand, rarely show severe manifestation of dengue infection which are opposite to group of children or travelers who usually show severe illness with dengue infection(39,40,41,42,43,44,45,46). Thus, it is possible that long term exposure to mosquito bite may have some impact on reducing risk of severe dengue infection.

In the studies of sand fly, Leishmaniasis's vector, there are reports that mice bitten by sand flies do not carry Leishmania, resist infection later. These might be the result of some factors or component in the flies's saliva revs up the animals' immune response (47,48,49,50). David Sacks of the National Institute of Allergy and infectious Disease (NIAID) suggested that the exposure to the uninfected sand flies's saliva might lead to some immune response that could neutralize the enhancing effects. Besides, the results of Sacks and his colleague showed that the prior bites of uninfected sand flies do not only decrease the incidence of disease, but also reduce the capacity of serving as reservoirs of infection. Although, the mechanism of resisting infection is not clear, but he found the increased production of Interferon γ in the experimental animals. Interferon γ is one of many cytokines that regulates immune

responses and stimulates cell-mediated immunity, a specialized response for thwarting pathogens that reside within host cells. As Leishmania does. Because the majority of flies in areas where Leishmaniasis is endemic are uninfected, the protective effect of prior bites might explain why the severity of Leishmania infections varies from one individual to another. It might also explain anecdotal reports why children and newcomers to part of the world where the parasites and sand flies live tend to suffer more serious illness than natives adults (51,52,53,54,55,56).

Surprisingly, the same phenomenon has been observed in dengue infections, which children and travelers tend to suffer more serious illness than native adults (57,58). These data suggest that prior exposure to mosquito or sand fly bite may decrease the incidence, severity and the outcome of the disease. The studies also suggested that characterization of components in vector's saliva can lead to the discovery of the important component and, at the same time, to an important vaccine target.

Many studies have revealed 20 peptides in the saliva of the adult mosquito *Aedes aegypti*, such as vasodilators, anti-platelet aggregation factor, and anticoagulant-factor facilitate blood feeding (59,60,61,62,63). Moreover, the sensitization to mosquito saliva by mosquito bites can vary from local mild immediate to delayed type of skin reactions (64,65,66,67).

The study in snake bite-site swabs by PCR reveals that snake venoms contain the secretory epithelial cells, which were released during milking of the venoms. These secretory epithelial cells from the lumina of the venom gland and duct might have been released during gland contraction, then deposited at the bite-site (68).

Likewise mosquito bite, the secretory epithelial cells from mosquito saliva might have been injected into the mosquito bite-site. There are some data to support this idea, the report from Taipei showed IgE and IgG anti-mosquito antibodies in human serum reacted with mosquito whole body extract by using immunoblotting

technique (69). This might be an effect of some secretory cells which were injected with mosquito saliva then resulted in an induction of the specific anti-mosquito cell antibodies..

According to basic acknowledge of dengue virus replication, the virus replicate within host cell using cell membrane as its envelope (70,71,72).

If uninfected mosquitoes bite anyone for a long period or with heavily bite, this will activated immune system to produced specific anti-mosquito cell antibodies which will react to virus itself when the host is bitten again with infected mosquitoes. The pathway may described above could be an explanation of the resistance of native adults to dengue infection in endemic areas as same as Leishmania.

In this study, experimental rabbits were immunized with *Aedes albopictus* mosquito cell line, C6/36, to prove whether mosquito cell could activate host immune system to produce specific anti-mosquito cell antibodies. Then, anti-mosquito cell antibodies were determined from serum of high exposed group; such as guards and dengue infection patients and compared the difference of anti-mosquito cell antibodies between these two groups to find the relation between risk of mosquito bites and dengue infection resistance. Cord blood's sera were used as negative control serum for anti-mosquito cell antibodies detection.