

Available online at www.sciencedirect.comComparative Immunology, Microbiology
& Infectious Diseases 30 (2007) 329–340

C O M P A R A T I V E
I M M U N O L O G Y
M I C R O B I O L O G Y &
I N F E C T I O U S
D I S E A S E S

www.elsevier.com/locate/cimid

Dengue hemorrhagic fever with special emphasis on immunopathogenesis

Ichiro Kurane*

*Department of Virology 1, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku,
Tokyo 162-8640, Japan*

Received 8 December 2006

Abstract

Dengue virus infections are a serious cause of morbidity and mortality in most tropical and subtropical areas of the world; Southeast and South Asia, Central and South America, and the Caribbean. Dengue virus infection can be asymptomatic or causes two forms of illness, dengue fever (DF) and dengue hemorrhagic fever (DHF), which is the severe form of dengue illness and often fatal. Pathogenesis of DHF has been analyzed, and two mechanisms are considered to be responsible. These include dengue serotype cross-reactive immune responses and virulence of the virus. The immunopathological mechanisms include a complex series of immune responses. Rapid increase in the levels of cytokines, especially TNF- α , and chemical mediators play a key role in inducing unique clinical manifestations of DHF such as plasma leakage, shock, and hemorrhagic manifestations. It is understood that the process is initiated by infection with a virulent dengue virus, often in the presence of antibodies that enhance dengue virus infection in secondary infection, and then triggered by rapidly elevated cytokines and chemical mediators that were produced by intense immune activation. However, complete understanding of the entire pathological mechanism is far from complete, and further studies are still needed.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Dengue virus; Dengue fever; Dengue hemorrhagic fever; Immunopathogenesis

*Tel./fax: +81 3 5285 1169.

E-mail address: kurane@nih.go.jp

Résumé

Les infections de la fièvre dengue sont une cause sérieuse de morbidité et de mortalité dans la plupart des zones tropicales et subtropicales du monde, l'Asie du sud-est, l'Asie du sud, l'Amérique centrale et du sud, et les Caraïbes. L'infection du virus de la dengue peut être ou asymptomatique, ou causer deux formes de maladies, la fièvre dengue et la fièvre dengue hémorragique (DHF), forme grave de la maladie de la dengue qui peut être mortelle. L'analyse de la pathogénie de la DHF a montré deux mécanismes qui sont considérés responsables. Sont inclus dans ces derniers les réactions croisées des réponses immunitaires entre sérotypes de dengue différents, et la virulence du virus. Les mécanismes immunopathologiques comprennent une série complexe de réponses immunitaires. Une augmentation rapide des niveaux de cytokines, en particulier la TNF, et les facteurs médiateurs jouent un rôle clef dans l'induction de manifestations cliniques spécifiques de la DHF comme la perte de plasma, les manifestations hémorragiques et de choc. Il est entendu que le processus est initié par une infection d'un virus de dengue virulent, parfois en présence d'anticorps qui augmentent l'infection du virus de la dengue en infection secondaire, puis déclenché par l'augmentation rapide de cytokines et de médiateurs chimiques produits par une activation immunitaire intense. Toutefois, le mécanisme pathologique est loin d'être entièrement compris, et des études ultérieures sont nécessaires.

© 2007 Elsevier Ltd. All rights reserved.

Mots clés: Virus de la dengue; Fièvre dengue; Fièvre dengue hémorragique; Immunopathogénie

1. Dengue viruses

Dengue viruses are transmitted to humans by infected mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus* [1]. Humans are natural hosts of dengue viruses, and dengue viruses are maintained between mosquitoes and humans in nature. Dengue viruses belong to the family *Flaviviridae*, the genus *Flavivirus*. There are four serotypes, dengue virus types 1, 2, 3, and 4 [2]. Although dengue virus types 1, 2, 3, and 4 are called four serotypes of dengue virus, it is generally accepted that these four dengue viruses, dengue virus types 1, 2, 3, and 4, are antigenically related different species.

The genome of dengue viruses is a single-stranded RNA nearly 11 kb in length. It is plus-stranded and infectious [3]. The uninterrupted open reading frame codes for three structural proteins, the capsid (C) protein, preM, the precursor to the membrane (M) protein, the envelope (E) protein, and seven non-structural proteins, the NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [3]. Dengue virion has a spherical shape with 40–50 nm in diameter [4]. The envelope is a lipid bilayer containing two envelope-associated proteins: the E and M proteins. The nucleocapsid is about 30 nm in diameter and covered by envelope.

The E protein is glycosylated and responsible for attachment to the cellular receptors and fusion with cell membrane. The E protein contains the main epitopes recognized by neutralizing antibodies. These epitopes are dengue virus-specific and cross-reactive for other flaviviruses. It is understood that neutralizing antibody plays

a key role in protective immunity against dengue viruses. Thus, the E protein plays the most important role in induction of protective immunity. The role of cellular immunity in the protection against dengue viruses has been analyzed, but has not been completely understood yet.

2. Dengue fever (DF) and dengue hemorrhagic fever (DHF); two types of clinical manifestation of dengue virus infection

Dengue virus infection can be asymptomatic or causes two forms of illness, DF and DHF [4,5] (Table 1), although the majority of dengue virus infections are asymptomatic. DF is a self-limited febrile illness [4,5]. After an incubation period of 2–7 days, a sudden onset of fever occurs. The fever is usually accompanied with retro-orbital or frontal headache. Myalgia and bone pain occur soon after the onset of fever. A transient macular rash that blanches under pressure, nausea, vomiting, lymphadenopathy, and taste aberrations can develop. These symptoms are accompanied by leucopenia and variable degrees of thrombocytopenia. One to 2 days after defervescence, a generalized morbilliform maculopapular rash appears. The rash spares palms and soles. Patients usually recover from the symptoms without complications about a week after the onset of disease.

Some patients infected with dengue virus demonstrate plasma leakage into interstitial spaces, thrombocytopenia, and also hemorrhagic manifestation [5,6]. This severe life-threatening syndrome is called DHF. The incubation period of DHF is similar to that of DF. Illness starts with fever, malaise, vomiting, headache, anorexia, and cough. Rapid clinical deterioration and collapse follows after 2–5 days. In the second phase, the patients demonstrate cold clammy extremities, warm trunk, flushed face, restlessness, irritability, middle gastric pain, and may progress to a rapid weak pulse, hypotention, and narrow pulse pressure. The crisis lasts for 24–36 h and the patients recover rapidly once convalescent starts. The hematological manifestations include an increase in hematocrit, thrombocytopenia, a prolonged bleeding time, and an increased prothrombin time. The temperature returns to normal when capillary leakage occurs. The occurrence of capillary leakage differentiates DHF from DF. The World Health Organization (WHO) categorizes DHF into four grades, from less severe (grade 1) to severe (grade 4). Grades 3 and 4, in which plasma leakage is so profound that shock occurs, are also referred to as dengue shock syndrome (DSS) [5].

3. Epidemiological features of DF and DHF

Dengue virus infections are a serious cause of morbidity and mortality in most tropical and subtropical areas of the world: mainly Southeast and South Asia, Central and South America, and the Caribbean [1,4] (Fig. 1). There are approximately 2.5 billion people at risk in the world for infection with dengue viruses. Nearly 100 countries and areas have a risk for domestic dengue virus infections. Dengue cases are

Table 1

Case definition for dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (from Ref. [5])

Dengue fever (DF)

- Probable—an acute febrile illness with two or more the following manifestations:
 - Headache
 - Retro-orbital pain
 - Myalgia
 - Arthralgia
 - Rash
 - Hemorrhagic manifestation
 - Leucopenia and
 - Supportive serology (a reciprocal hemagglutination-inhibition antibody titer ≥ 1280 , a comparable IgG enzyme-linked immunosorbent assay titer or a positive IgM antibody test on a late acute or convalescent serum specimen); or
 - Occurrence at the same location and time as other confirmed cases of dengue fever.
- Confirmed—a case confirmed by laboratory criteria (see below).
 - isolation of the dengue virus from serum or autopsy samples; or
 - demonstration of fourfold or greater change in reciprocal IgG or IgM antibody titers to one or more dengue virus antigens in paired serum samples; or
 - demonstration of dengue virus antigen in autopsy tissue, serum or cerebrospinal fluid samples by immunohistochemistry, immunofluorescence, or ELISA; or
 - detection of dengue virus genomic sequences in autopsy tissue or cerebrospinal fluid samples by polymerase chain reaction (PCR).

Dengue hemorrhagic fever (DHF)

The following must all be present:

- Fever, or history of acute fever, lasting 2–7 days, occasionally biphasic.
- Hemorrhagic tendencies, evidenced by at least one of the following:
 - a positive tourniquet test
 - petechiae, ecchymoses or purpura
 - bleeding from the mucosa, gastrointestinal tract, injection sites or other locations
 - hematemesis or melaena
- Thrombocytopenia ($100,000/\text{mm}^3$ or less).
- Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
 - a rise in the hematocrit equal to or greater than 20% above average for age, sex and population;
 - a drop in the hematocrit following volume-replacement treatment equal to or greater than 20% of baselines;
 - signs of plasma leakage such as pleural effusion, ascites and hypoproteinaemia.

Dengue shock syndrome (DSS) (DHF grades 3 and 4)

All of the above four criteria for DHF must be present, plus evidence of circulatory failure manifested by:

- rapid and weak pulse, and
 - narrow pulse pressure ($< 20 \text{ mmHg} (2.7 \text{ kPa})$)
or manifested by:
 - hypotension for age, and
 - cold, clammy skin and restlessness.
-

estimated to occur in up to 100 million population annually. A total of approximately 500,000 cases of DHF occur annually, and the case fatality ratio is 1–5%. Thus, dengue presents one of the most serious human infectious diseases. The areas where dengue

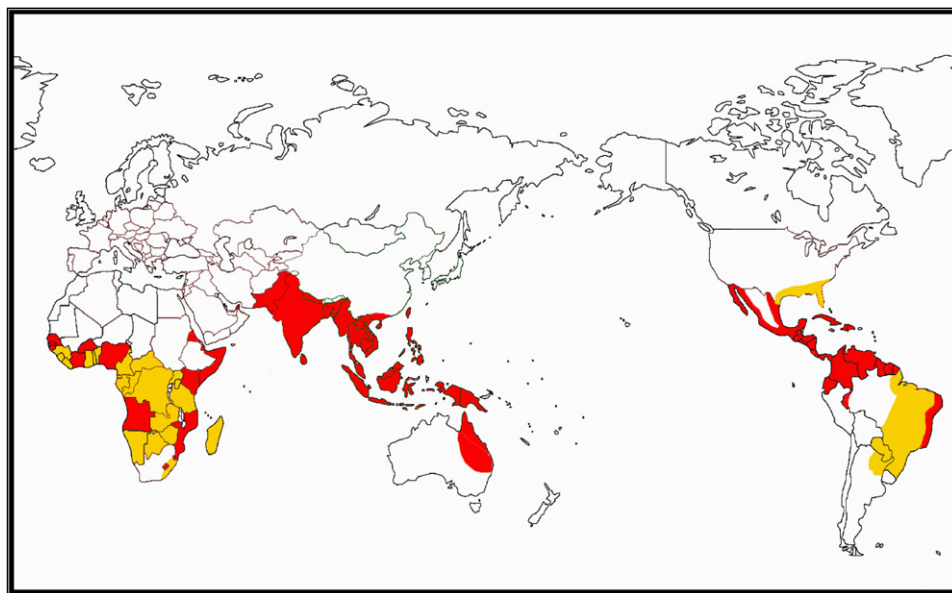


Fig. 1. Distributions of dengue epidemic in the world. Red: areas where dengue epidemics are reported. Yellow: areas where presence of *Aedes aegypti* are confirmed.

virus infection is a serious public health problem have been expanding, and the number of DHF cases has been recently increasing.

4. Pathogenesis of DHF

Major manifestations of DHF include (i) plasma leakage through elevated vascular permeability, (ii) hemorrhage, and (iii) thrombocytopenia. Some of the patients infected with dengue virus develop DHF, while most with symptomatic infections end up as DF. The pathogenesis of DHF has been explained by two theories. One theory is based on the virulence of infecting dengue viruses; virulent dengue virus strains cause DHF, while avirulent dengue virus strains cause DF. The other is based on immunopathogenesis. This theory suggests that DHF is mediated by host immune responses including dengue virus-cross-reactive antibodies that augment infections. These two theories have been considered as opposing each other for a long period of time. However, it appears that they represent different aspects of the pathogenesis of DHF.

5. Role of non-neutralizing antibodies in the pathogenesis of DHF

Epidemiologic data have suggested that DHF is mediated by host immune responses. The studies done in Thailand demonstrated that up to 99% of DHF cases

had heterotypic antibody to the serotype of dengue virus that caused DHF. These DHF cases were divided into two groups. Nearly 90% of them were children who were older than 1 year and in a secondary infection with a serotype of dengue virus different from that which caused the primary infection. The other 10% were less than 1 year old and undergoing a primary dengue virus infection [7,8]. They were infants who were born to the mothers with dengue virus antibodies.

In dengue epidemic of dengue virus type 2 in Cuba in 1981, most DHF cases were those who had acquired dengue antibodies in the previous epidemics by dengue virus type 1 in 1977 and 1978 [9]. These results suggest that the presence of heterotypic dengue virus antibodies before secondary infections is a risk factor for developing DHF.

Cross-reactive antibodies that lack neutralizing activity are induced in the primary infection. In secondary infection, dengue virus and non-neutralizing antibodies form virus–antibody complexes. Virus–antibody complexes bind to Fc γ receptors on target cells and result in enhancement of dengue virus infection. The non-neutralizing, cross-reactive antibodies thus markedly augment dengue virus infection of Fc γ receptor-positive cells [10]. This phenomenon is called antibody-dependent enhancement (ADE).

Infants born to the mothers develop DHF in the primary infections. The levels of maternal dengue virus antibodies in the infants needed to decline to the levels that can enhance dengue virus infection and lead to DHF [11,12]. These observations are consistent with the idea that enhancing antibodies increase the number of dengue virus-infected cells and the levels of viremia and lead to DHF.

6. Virulence of viruses as a cause of DHF

Although DHF occurs more frequently in secondary infection than in primary infection, DHF also occurs in primary infection. This suggests that virulence of the virus contributes to the development of DHF. It has been assumed that virulent dengue virus strains cause DHF, while avirulent dengue virus strains cause DF. There are multiple genotypes in each of four dengue viruses.

The introduction of the Southeast Asian genotype coincided with the appearance of DHF in different countries in the Americas, while the original American genotype was only associated with DF, but not with DHF [13,14]. Other epidemiological studies demonstrated that there were no DHF cases reported in Peru where the Southeast Asian genotype of dengue virus type 2 was not introduced [15].

Various groups have attempted to define the molecular determinants of dengue virus virulence. It was reported that the determinants for virulence resided at the amino acid 390 of the E protein, in the 5' non-translated region and in the upstream 300 nucleotides of the 3' non-translated region [16]. The other group demonstrated non-synonymous amino acid replacements in the preM, NS1, NS2a, NS3, and NS5 by analyzing multiple strains of dengue virus type 2 [17,18].

The studies done in Thailand demonstrated that viral loads in peripheral blood are higher in patients who develop DHF than in those with DF [19]. However, it is not

determined why and how higher levels of viremia lead to the pathology and symptoms seen in DHF. The virulence of dengue virus has been determined by the characteristics *in vitro*: plaque size, virus titers, etc. However, because of the absence of animal models, it is difficult to confirm that some “virulent” strains actually induce DHF *in vivo*. Further studies are needed to elucidate the molecular bases underlying these possible different phenotypes.

7. Role of cytokines in the pathogenesis of DHF

A series of studies have suggested that plasma leakage, which differentiates DHF from DF, is caused by malfunction of vascular endothelial cells induced by cytokines or chemical mediators rather than by destruction of the small vessels [20–22]. Plasma levels of various cytokines are significantly higher in DHF than in DF. The cytokines elevated in patients with DHF include TNF- α , IL-2, IL-6, IL-8, IL-10, IL-12, and IFN- γ . The levels of IL-8 and MCP-1 are also elevated in the pleural effusion from DHF patients [23]. However, it is not clearly understood how these cytokines are induced and how these cytokines cause malfunction of vascular endothelial cells and lead to plasma leakage.

Of these cytokines, TNF- α has attracted attention because of its well-known activity in inducing plasma leakage. It was reported that dengue virus-infected monocytes and endothelial cells produce some of multiple cytokines including TNF- α [21,22]. It was also reported that monocytes produce TNF- α when they were infected with dengue virus in the presence of enhancing antibody and that the produced TNF- α induces plasma leakage in an *in vitro* experimental system using endothelial cells [24]. It has also been reported that a mast cell/basophile line infected with dengue viruses can produce IL-1 and IL-6 [25]. Some of the cytokines, IFN- γ , IL-2, and TNF- α , were also produced by virus-specific T lymphocytes upon activation [21,26]. It is likely that both dengue-virus-infected monocytes and activated specific T lymphocytes are responsible for increased levels of cytokines in DHF.

8. Complement activation in patients with DHF

Activation of complement is another important clinical manifestations in DHF. It was reported that the levels of C3a and C5a, complement activation products, are correlated with the severity of DHF and the levels of C3a and C5a reached the peak at the time of defervescence when plasma leakage becomes most apparent [27]. This is consistent with the assumption that complement activation is also responsible for the pathogenesis of DHF.

The mechanism of complement activation in DHF is not completely understood. Circulating immune complexes are present in the DHF patients, and it has been assumed that the complement is activated by the immune complexes [27,28]. High levels of secreted NS1 and pre-existing cross-reactive antibody may also mediate

complement activation. Further, it was reported that dengue virus-infected monocytes and endothelial cells activate complement via classical and alternative pathways [23]. It is likely that complement is activated by various mechanisms in DHF patients, and that complement activation also contribute to the pathogenesis of various clinical features, especially plasma leakage.

9. Further researches

There have been many studies that target the pathogenesis of DHF (Table 2). However, our understanding is not complete yet. In this section some questions and projects that remain to be addressed toward a fuller understanding of DHF pathogenesis are listed.

9.1. Animal models

Natural host of dengue viruses are humans. Lack of animal models is one of the biggest factors that prohibited the analysis of the pathogenesis of DHF. Development of animal models has been attempted using various types of animal models by many groups. Monkeys can be infected with dengue viruses, but do not develop symptoms, except for limited success. Murine models for dengue virus infections have been reported by several groups. A variety of strains of mice were used including SCID mice reconstituted with human peripheral blood mononuclear cells (PBMC), and interferon alpha and beta receptor-deficient mice. These mice showed limited success, but did not demonstrate increased vascular permeability. Promising murine model was recently reported by Shresta et al. [29]. Mouse-adapted dengue virus strains were used and the mice showed increased vascular permeability,

Table 2
Major factors that are considered to contribute to the pathogenesis of DHF

1	Dengue viruses *Virulent dengue virus strains
2	Anti-dengue virus antibodies *Enhance dengue virus infection by developing virus-antibody immune complex (non-neutralizing, serotype cross-reactive) *Activate complement
3	T lymphocytes (serotype cross-reactive CD4+ and CD8+ T lymphocytes) *Produce lymphokines (cytokines) *Lyse dengue virus-infected cells
4	Complement *Activation products induce plasma leakage
5	Platelet *Thrombocytopenia plays a main role in hemorrhagic manifestation
6	Endothelial cells *Play a main role in plasma leakage *Produce cytokines

which is one of the characteristic clinical features in DHF patients. The mice died without the encephalitic symptoms. They also demonstrated that TNF- α is responsible for early death of mice. This study suggests that development of small animal model for elucidating the pathogenesis of DHF will be possible. Further studies are needed to develop new animal models for elucidating the pathogenesis of dengue virus infection.

9.2. *Progress of dengue viral infection in vivo after mosquito bite*

Although this may be the most fundamental question in infectious diseases, the progress of dengue virus infection after introduction of dengue virus *in vivo* is not well understood. It was reported that the monocytes, macrophages, and the other cells of reticuloendothelial origin are the cells that mainly support dengue virus infections [30,31]. It was also reported that dendritic cells, namely Langerhans cells, and dermal and interstitial dendritic cells, are more permissive for dengue virus than monocytes and macrophages, using DC-SIGN as a receptor for dengue viruses [32]. Recently, it was reported that vascular endothelial cells and hepatocytes were infected with dengue virus *in vivo* in patients with DHF [33,34]. Although there were reports that fibroblasts and B cells can be also infected with dengue viruses *in vitro* [35–37], whether these cells were also infected with *in vivo* is not known.

Based on these data, it is likely that multiple cell types including dendritic cells and monocytes/macrophages and hepatocytes are the cells that mainly support dengue virus infection *in vivo*. Main target organs of dengue virus infections are not known. Liver appears to play a role in dengue virus propagation *in vivo*; however, whether liver is the only main organ of dengue virus propagation has not been defined yet.

Further, the questions unanswered are (i) how do the viruses actually spread? (ii) which organs and cells contribute to the viremia? and (iii) how important is the infection of vascular endothelial cells by dengue virus in the pathogenesis of DHF; especially plasma leakage and hemorrhagic manifestations.

9.3. *Vaccine development*

There are no dengue vaccines available for human use. At this point various types of dengue vaccines are under development [38,39]. They include live attenuated vaccines, chimeric vaccines that consist of yellow fever 17 D strain (vaccine strain) as a backbone and prM and E genes of dengue viruses, other type of chimeric vaccines that consists of attenuated dengue virus and prM and E genes of other dengue viruses serotypes. With any types of these live attenuated or chimeric vaccines, the final product will be tetravalent vaccines, which induce protective immunity against homologous serotypes. These vaccine candidates finished pre-clinical studies and in the phase I and phase II studies [38,39].

Although protective immunity against dengue viruses is not completely elucidated, it is generally agreed that neutralizing antibodies play most important roles in protection against dengue virus infections. Especially, neutralizing antibodies against homologous dengue serotypes are considered to be the most important protective

immunity. This concept is based on the epidemiological data that humans develop life-long protection against homologous dengue virus serotype, while protection against heterologous serotypes is short lived. The role of cellular immunity in the protection and recovery from dengue virus infection has been studied; however, further studies are needed for complete understanding.

9.4. Dengue viral phenotypes and cytokines

There are differences in the growth capacity *in vitro* among dengue virus isolates. The strains that were isolated from patients with DHF or DSS are often called “virulent strains”. However, their virulence cannot be confirmed because of lack of adequate animal models. It has not been determined whether some strains are virulent because they grow well *in vivo* with or without enhancing antibodies, because they are highly cytopathic, or because they induce high levels of cytokines. It has been reported that viral titers are higher in DHF patients than in DF patients. However, it has not been determined why higher viral titers lead to unique clinical manifestations of DHS: plasma leakage, hemorrhagic manifestations, etc.

On the other hand, many studies showed that cytokines and/or chemical mediators contribute to the pathogenesis of DHF. It has been, however, difficult to attribute the pathogenesis of DHF to the sole cytokine. Rather it is more likely that the multiple cytokines function in the synergistic and additive fashion, and induce the pathology and the clinical manifestations of DHF. If it is the case, elucidation of the pathological mechanism of DHF may be quite difficult. Irrespective of this assumption, further studies are still needed for better understanding.

10. Final remark

DF/DHF is one of the most important infectious diseases in tropical and subtropical countries. The areas where DF and DHF are a serious health concern have been expanding, and the number of DHF patients has been increasing. New findings of dengue viruses are accumulating, and a dengue vaccine may be available in the near future. However, understanding of the pathogenesis of DHF still remains a challenge. Better understanding of DHF pathogenesis will lead us to developing better strategies to the treatment and the prevention of DHF.

References

- [1] Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998;11:480–96.
- [2] Burke DS, Monath TP. Flaviviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. Philadelphia, USA: Lippincott Williams & Wilkins; 2001. p. 1043–125.
- [3] Lindenbach BD, Rice CM. Flaviviridae: the viruses and their replication. In: Knipe DM, Howley PM, editors. *Fields virology*. Philadelphia, USA: Lippincott Williams & Wilkins; 2001. p. 991–1041.

- [4] Monath TP, Tsai TF. Flaviviruses. In: Richman DD, Whitley RJ, Hayden FG, editors. *Clinical virology*. Washington, DC: ASM Press; 2002. p. 1097–151.
- [5] World Health Organization. *Dengue haemorrhagic fever: diagnosis, treatment and control*. Geneva: World Health Organization; 1997. p. 12–23.
- [6] Nimmannitya S. Dengue hemorrhagic fever: diagnosis and management. In: Gubler DJ, Kuno G, editors. *Dengue and dengue hemorrhagic fever*. Oxon, UK: CBA International; 1997. p. 133–45.
- [7] Halstead SB. Immunopathological parameters of togavirus disease syndromes. In: Schlesinger W, editor. *The togaviruses*. New York: Academic Press; 1980. p. 107–73.
- [8] Kurane I, Ennis FA. Immunopathogenesis of dengue virus infections. In: Gubler DJ, Kuno G, editors. *Dengue and dengue hemorrhagic fever*. Oxon, UK: CBA International; 1997. p. 273–90.
- [9] Guzman MG, Kouri GP, Bravo J, Soler M, Vazquez S, Morier L. Dengue hemorrhagic fever in Cuba. *Am J Trop Med Hyg* 1990;42:179–84.
- [10] Kurane I, Mady BJ, Ennis FA. Antibody-dependent enhancement of dengue virus infection. *Rev Med Virol* 1991;1:211–21.
- [11] Kliks SC, Nisalak A, Brandt EE, Wahl L, Burke DS. Antibody-dependent enhancement of dengue virus growth in human monocytes as a factor for dengue hemorrhagic fever. *Am J Trop Med Hyg* 1989;40:444–51.
- [12] Kliks SC, Nimmannitya S, Nisalak A, Burke DS. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am J Trop Med Hyg* 1988;38:411–9.
- [13] Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C, et al. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology* 1997;230:244–51.
- [14] Rico-Hesse R, Harrison LM, Nisalak A, Vaughn DW, Kalayanarooj S, Green S, et al. Molecular evolution of dengue type 2 virus in Thailand. *Am J Trop Med Hyg* 1998;58:96–101.
- [15] Watts DM, Porter KR, Putvatana P, Vasquez B, Calampa C, Hayes CG, et al. Failure of secondary infection with American genotype dengue 2 to cause dengue hemorrhagic fever. *Lancet* 1999;354:1431–4.
- [16] Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon, et al. Dengue virus structural differences that correlate with pathogenesis. *J Virol* 1999;73:4738–47.
- [17] Pandey BD, Igarashi A. Severity-related molecular differences among nineteen strains of dengue type 2 viruses. *Microbiol Immunol* 2000;44:179–88.
- [18] Pandey BD, Morita K, Hasebe F, Parquet MC, Igarashi A. Molecular evolution, distribution and genetic relationship among the dengue 2 viruses isolated from different clinical severity. *Southeast Asian J Trop Med Public Health* 2000;31:266–72.
- [19] Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;181:2–9.
- [20] Rothman AL, Ennis FA. Immunopathogenesis of dengue hemorrhagic fever. *Virology* 1999;257:1–6.
- [21] Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. *Curr Opin Infect Dis* 2006;19:429–36.
- [22] Kurane I, Ennis FE. Immunity and immunopathology in dengue virus infections. *Semin Immunol* 1992;4:121–7.
- [23] Avirutnan P, Malasit P, Seliger B, Bhakdi S, Husmann M. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *J Immunol* 1998;161:6338–46.
- [24] Anderson R, Wang S, Osioy C, Issekutz AC. Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. *J Virol* 1997;71:4226–32.
- [25] King CA, Marshall JS, Alshurafa H, Anderson R. Release of vasoactive cytokines by antibody-enhanced dengue virus infection of a human mast cell/basophil line. *J Virol* 2000;74:7146–50.
- [26] Gagnon SJ, Ennis FA, Rothman AL. Bystander target cell lysis and cytokine production by dengue virus-specific human CD4+ cytotoxic T-lymphocyte clones. *J Virol* 1999;73:3623–9.
- [27] Malasit P. Complement and dengue hemorrhagic fever/dengue shock syndrome. *Southeast Asian J Trop Med Publ Health* 1987;18:316–20.

- [28] Aviruthan P, Punyadee N, Noisakran S, Komoltri C, Thiemmecca S, auethavornanan K, et al. Vascular leakage in severe dengue virus infections: a potential role for the nonstructural viral protein NS1 and complement. *J Infect Dis* 2006;193:1078–88.
- [29] Shresta S, Sharer KL, Prigozhin DM, Beatty PR, Harris E. Murine model for dengue virus-induced lethal disease with increased vascular permeability. *J Virol* 2006;80:10208–17.
- [30] Bhamarapravati N, Tuchinda P, Boonpucucknavig V. Pathology of Thailand hemorrhagic fever: a study on 100 autopsy case. *Ann Trop Med Parasitol* 1967;61:500–10.
- [31] Yoksan S, Bhamarapravati N. Localization of dengue antigen in tissue from fatal cases of DHF. In: Proceedings of the international conference on dengue haemorrhagic fever 1982. Malaysia: University of Malaya; 1983. p. 406–10.
- [32] Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, Putvatana R, et al. Human skin langerhans cells are targets of dengue virus infection. *Nat Med* 2000;6:816–20.
- [33] Huerre MR, Lan NT, Marianneau P, Hue NB, Khun H, Hung NT, et al. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virchows Arch* 2001;438:107–15.
- [34] Jessie K, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues by immunohistochemistry and in situ hybridization. *J Infect Dis* 2004;189:1411–8.
- [35] King AD, Nisalak A, Kalayanrooj S, Myint KSA, Pattanapanyasat K, Nimmannitya S, et al. B cells are the principal circulating mononuclear cells infected by dengue virus. *Southeast Asian J Trop Med Publ Health* 1999;30:718–28.
- [36] Lin YL, Liu CC, Lei HY, Yeh TM, Lin YS, Chen RM, et al. Infection of five human liver cell lines by dengue-2 virus. *J Med Virol* 2000;60:425–31.
- [37] Kurane I, Janus J, Ennis FA. Dengue virus infection of human skin fibroblasts in vitro: production of IFN- γ , IL-6 and GM-CSF. *Arch Virol* 1992;124:21–30.
- [38] Pugachev KV, Guirakhoo F, Monath TP. New developments in flavivirus vaccines with special attention to yellow fever. *Curr Opin Infect Dis* 2005;18:384–94.
- [39] Simasathien S, Watanaveeradej V. Dengue vaccine. *J Med Assoc Thai* 2005;88:363–77.