The pathology of dengue hemorrhagic fever

Anthony S-Y. Leong, MBBS, MD, FRCPath, FRCPath, FCAP, FASCP, FHKAMed (Pathol), Hon FHKCP, Hon FRCPT, K. Thong Wong, MBBS, FRCPath, Trishe Y-M. Leong, MBBS (hons), FRCPA, FCAP, Puay Hoon Tan, MBBS, FAMS, MD, FRCPath, FRCPath, Pongsak Wannakrairot, MD, FRCPT

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The global problem

The first record of a disease clinically resembling dengue fever (df) can be found in a Chinese medical encyclopedia dated 992. With the expansion of the world shipping trade in the 18th and 19th centuries, both the viruses and their principal mosquito vector, Aedes aegypti, were spread to new geographic regions, particularly along the tropical trading routes and shipping ports. Because spread was slow and via sailing ships, long intervals of 10 to 40 years separated epidemics of the disease. The expansion of dengue was closely related to development and economic growth in tropical countries and the mode of the viral transmission.
Economic growth is associated with transmigration of large numbers of the population into cities and the development of new cities; this urbanization resulted in a concomitant increase in breeding sites for the mosquito. Accelerated modern travel, particularly by airplanes, allows the introduction of the virus from an endemic area to a dengue-receptive area where both the vector and susceptible population exist. The virus is usually carried in an infected but asymptomatic person during the incubation period of the disease, a method of transmission that often accounts for explosive outbreaks of \( df \).

In the latter part of the 20th century, globalization and rapid urbanization of many developing tropical countries produced increased transmission and hyperendemicity of the disease. Today, dengue is the most frequent arbovirus produced increased transmission and hyperendemicity of the disease. Today, dengue is the most frequent arbovirus disease in humans today. The magnitude of the global problem is compounded by the fact that there is currently no specific treatment or vaccines for the disease, and control is dependent largely on public health measures directed against the vectors.

**Presentation, pathogenesis, and pathology**

The Dengue virus (DV) is a positive-sense, single-stranded RNA surrounded by an icosahedral nucleocapsid. The virus is composed of three structural proteins [the capsid (C), premembrane (prM), and envelope (E) proteins] and seven nonstructural (NS1-7) proteins. With infection, the DV is endocytosed and acidic pH triggers a conformational change of the E protein. This allows fusion of the virus envelope to the endosomal membrane and release of the viral genome into the cytoplasm, where it is translated into viral proteins that replicate in the host endoplasmic reticulum. The newly synthesized viral genomes are packaged as viral core, envelope, and membrane proteins into immature virions before being secreted from the cell. The virus belongs to the family of Flaviviridae and the genus Flavivirus, which includes yellow fever, West Nile, Japanese encephalitis, and St. Louis encephalitis viruses. There are four distinct serotypes of DV (DEN1-4), and infection with any one of the serotypes confers lifelong immunity to that serotype. Whereas DVs exhibit 65% to 70% sequence homology, serotype cross-reactive immunity is only transient and wanes after 6 months so that the host remains susceptible to the remaining three heterologous dengue serotypes.7

**Presentation**

DV infection results in protean manifestations ranging from a mild nonspecific or subclinical febrile illness to DHF or dengue shock syndrome (DSS). \( df \) uncomplicated has an incubation period of 5 to 9 days. The clinical features are age-dependent. Infants and young adults may only have simple fever with a maculopapular rash. In older children and adults, \( df \) is characterized by the sudden onset of high fever of about 40°C, chills, severe headache that is mostly frontal or retro-ocular, skin rash, general malaise, and severe muscle ache in the lumbar region, legs, and joints. There is loss of appetite with nausea and vomiting, and photophobia with puffiness of the eyelids and cervical lymphadenopathy may be present. The fever lasts for 2 to 4 days, and after an afebrile period of about 1 day, a second febrile period may follow, producing the typical “saddle back” temperature curve. At this stage, an itchy maculopapular rash develops, often spreading from the extremities to the trunk to involve the entire body, except for the face. The palms and soles may also be red. Convalescence can take several weeks or months, but there is virtually no mortality associated.8 The triad of fever, rash, and arthralgia requires separation from other nonarbovirus infections, such as measles, rubella, Epstein–Barr virus, cytomegalovirus, and protozoan infections such as toxoplasmosis. Due to the wide clinical variation, it is not possible to make a definite diagnosis on clinical findings alone. A positive tourniquet test (\( \geq 10 \) petechiae/inch\(^2\) read 1 minute after release of 5 minutes of pressure midway between systolic and diastolic) and leukopenia (WBC \( \leq 5000 \) cells/mm\(^3\)) in a febrile patient is highly predictive but still nonspecific for \( df \). Real-time polymerase chain reaction (RT-PCR) to detect the virus remains the gold standard, although this test is not available in many developing nations.

The World Health Organization (WHO) distinguishes DHF/DSS from \( df \) with hemorrhage, which is considered a mild disease.9 DHF/DSS is associated with poorer outcomes and a mortality rate that approaches 5%.10 The classical features of DHF are high fever, increased vascular permeability with hemorrhagic manifestations, thrombocytopenia (platelet count \( \leq 100,000/mm^3 \)), and hemoconcentration or signs of plasma leakage. DSS is defined as DHF plus either hypotension for age or narrow pulse pressure in the presence of clinical signs of shock. The increased capillary permeability is generally not accompanied by morphological damage to the capillary endothelium, and there are altered numbers and functions of leukocytes, increased hematocrit, and thrombocytopenia. Raised levels of aspartate-amino-transferase (AST) and alanine amino-transferase (ALT) have been observed in 98% and 37% of patients, respectively, indicating that liver dysfunction is a very common occurrence in DHF.11 As dengue spreads worldwide, there is evidence that the four key criteria of severe disease employed in the WHO grading system (shock, plasma leakage, marked thrombocytopenia, and internal hemorrhage) may not be universally
applicable, and many severe cases (including those that involve shock and fatality) may be missed, especially as the criteria were based on initial observations in children with the disease in Southeast Asia.12

Other less common manifestations include neurological symptoms from cerebral edema and encephalopathy from hepatic failure, complications of disseminated intravascular coagulopathy, diastolic dysfunction, abdominal compartment syndrome, adult respiratory distress syndrome,13 acute renal failure,14 postinfectious fatigue syndrome,15 intracranial hemorrhage,16 fulminant hepatitis,17 acute abdomen,18 hemophagocytic syndrome and dyserythropoiesis,19 and myocarditis.20

Pathogenesis

The pathogenesis of DHF/DSS is poorly understood and has been the subject of much research interest in recent years. Although dengue fever is a self-limiting disease in the great majority of cases, the problem becomes a significant one as the large numbers of infected persons result in about half a million cases per year of potentially life-threatening DHF/DSS. Up to 90% of DHF/DSS cases occur in secondary heterologous DV infection; the remaining are primary infections, usually in infants between 6 and 12 months of age. Clearly, secondary infection with a heterologous dengue serotype is a major risk factor. To date, several immunological aspects of DV infection have been studied in detail. These relate to the target cells of the virus and their immunological effects and the effects of antibody-mediated mechanisms in heterologous secondary infections.

Antibody-enhanced viral duplication

After introduction of the dengue virus through the bite of an infected *Aedes* mosquito, local viral duplication is thought to take place in target epidermal dendritic cells that are up to 10 times more permissive to dengue infection than monocytes or macrophages. A C-type lectin expressed by the dendritic cells can bind to the dengue envelope (E) protein and probably serves as a coreceptor for viral entry.21,22 Migration of interdigitating dendritic cells to regional lymph nodes also allows transfer of the virus to T cells. IgG antibodies and enhancement via IgM and complement C3 receptors have been implicated in dendritic cell infection.23,24 The infection of CD14-positive dendritic cells to regional lymph nodes also allows transfer of the virus to T cells. IgG antibodies and enhancement via IgM and complement C3 receptors have been implicated in dendritic cell infection.23,24 The infection of CD14-positive dendritic cells as well as bone marrow dendritic cells leads to the production of TNF-α, IFN-α, and IL-10 and inefficient maturation of infected dendritic cells, which undergo apoptosis. The dendritic cells display impaired ability to upregulate cell surface expression of costimulatory, maturation, and major histocompatibility complex molecules, resulting in reduced T cell stimulatory capacity. There is also an impaired ability to stimulate allogenic T cells, which is accompanied by further enhanced IL-10 production.25,26 These findings suggest a possible immune evasion strategy of the virus by impairing antigen-presenting cell function through maturation blockade and induction of apoptosis.27 Natural killer cells, which comprise another arm of the innate immune system, may also play a role. These cells can clear virus-infected cells either by direct cytotoxicity or via antibody-dependent cell-mediated cytotoxicity. Dengue immune serum has been shown to mediate antibody-dependent cell-mediated cytotoxic lysis of DV-infected cell lines.28

Research into the effects of primary DV infection has been hampered by the absence of a true animal model of the disease. Nonhuman primates display viremia but not DHF. Several promising murine models of DV infections have recently been developed, including interferon-α/β and γ receptor-deficient mice that developed encephalitis and nonobese diabetic/severe combined immunodeficient mice reconstituted with human hematopoietic stem cells with skin erythema.29,30

Not surprisingly, because of the severity and mortality associated with DHF immunological reactions, secondary heterologous DV infections have been extensively studied. Antibody-dependent enhancement is one mechanism that has been proposed to explain the severity of DHF/DSS. This mechanism evokes the binding of preexisting dengue antibodies at nonneutralizing conditions to heterologous DV to enable viral entry into FcRII-bearing target cells.31 The target cells are predominantly cells of the reticuloendothelial system of spleen, liver, and bone marrow, including monocytes, lymphocytes, Kupffer cells, and alveolar macrophages.32 The enhanced viral entry into such cells produces an increased viral burden in the host. Anti-E antibodies enhanced infection via FcRII, whereas antiprM antibodies enhanced infection of both FcRII- and non-FcRII-bearing cells.33 Some studies have demonstrated an association between viral burden and disease severity.34,35 with the ability of preinfection plasma to enhance infection of monocytes corresponding to disease severity.36 However, a more recent study contradicts those findings by showing no association between the ability of preillness plasma to enhanced infection in vitro and subsequent dengue viremia or disease severity in secondary dengue-2 or dengue-3 virus infections.37

In the small percentage of DHF cases that manifest during primary DV infection, usually in infants, it has been postulated that maternal transmission of nonneutralizing DV antibodies result in the same phenomenon as described above in adults with secondary infections.38

Although the viral envelope glycoprotein E is responsible for viral attachment and entry, and is the antigenic target for neutralizing antibodies, there are also several viral non-structural proteins that are involved in viral replication and have other effects in vivo. The dengue nonstructural protein NS1 has both a secreted and cell-associated form. Secreted NS1 levels have been found to be associated with viremia levels in secondary dengue-2 infection.39 Anti-NS1 antibodies appear to have both a protective as well as pathogenic role in DV infection. In animal models, anti-NS1 antibodies protect against lethal flavivirus challenge,40,41 but these
antibodies to NS1 have also been suggested to have a direct role in the pathogenesis of vascular leakage. Sera from acute dengue-infected individuals are able to bind to human umbilical cord endothelial cells, and this process can be blocked by the addition of recombinant NS1.42 Furthermore, the treatment of such umbilical cord cells with murine antidengue NS1 antibodies, but not Japanese encephalitis NS1 antibodies, induced the production of IL-6, IL-8, and monocyte chemo-attractant protein type 1, an inflammatory response that was abrogated in the presence of recombinant dengue NS1.43 Anti-NS1 antibodies have also been implicated to cause damage to endothelial cells by inducing nitric oxide-mediated apoptosis.42 Antibodies to NS1 persist long after dengue infection has resolved but do not cause pathology. Furthermore, the persistence and kinetics of development of anti-NS1 antibodies during and after secondary DV infection do not correlate with the timing of plasma leakage, raising questions as to the clinical relevance of this in vitro evidence for molecular mimicry.

On primary infection with DV, antibodies are generated against NS1 and the viral envelope protein E. Serotype-specific and serotype cross-reactive neutralizing antibodies are directed against the E protein. Enhancing antibodies can affect the severity of the disease as long as 20 years after the primary infection, especially during dengue-2 and dengue-3 infection following a primary infection with dengue-1 virus.44 However, all serotypes have been shown to be capable of causing severe disease,45 and milder disease is associated with lower viral loads and high levels of preexisting heterotype neutralizing antibodies during the secondary infection. This is not necessarily always true; in the case of secondary dengue-3 virus infection, higher levels of these cross-reactive memory humoral immune responses appeared to be beneficial as demonstrated by reduced viremia levels and decreased disease severity, but the same did not hold true for secondary dengue-1 or dengue-2 virus infection.46

Immunological mechanisms

The observation that onset of plasma leakage occurs up to several days following significant reduction or clearance of viremia suggests that, instead of the proposed antibody-dependent enhancement due to viral burden, an immune-mediated mechanism may be responsible for the extreme capillary permeability that is characteristic of DHF/DSS. Furthermore, despite high viral loads in secondary infections, progression to more severe forms of the infection occurs only in <5% of cases. Serotype-specific and cross-reactive T cells have been detected in the peripheral blood of individuals with acute DV infections. It is suggested that these serotype cross-reacting T cells show low affinity for the infecting virus and a higher affinity for another virus serotype (presumably from a previous infection), a phenomenon called the “original antigenic sin.”47 However, definite identification of the primary infecting DV serotype is difficult because neutralizing antibodies can be highly serotype cross-reactive after secondary infection, a caveat that questions the validity of this hypothesis.47 Importantly, it has been demonstrated that cross-reactive dengue-specific T cells induce high levels of cytokine production that may lead to increased vascular permeability.46

CD4 and CD8 T cell responses after primary DV infection have shown interesting differences. CD4+ T cells produced greater amounts of IFN-γ to homologous DV antigens, but the ratio of TNF-α to IFN-γ was higher after stimulation with heterologous serotype antigens or CD4+ T cell epitopes.49 CD8+ T cells have shown partial agonist responses in vitro in which a heterologous serotype variant could sensitize target cells for lysis but not cytokine production or proliferation.

Several studies have suggested a possible role for “immunodominant” variants of dengue epitopes in which the sequence of DV serotypes may influence the risk of DHF/DSS. Human leukocyte antigen (HLA) A2-restricted CD8+ T cell responses in primary dengue-immune individuals have shown both quantitative as well as qualitative differences in their cytokine responses to variant dengue epitopes, suggesting that previous infection as well as the sequence of heterologous DV infections may affect subsequent clinical outcome.50 For some epitopes, a single dengue variant was able to elicit the highest response in all donors, regardless of infecting serotype, suggesting that certain epitopes may be immunodominant. A recently identified dengue HLA-A11-restricted NS3 T cell epitope has also been found to be presented by HLA A24, an unexpected finding because these alleles belong to different HLA superfamilies.48 Previous infection history to unrelated viruses can also lead to immunopathology, as shown in human and animal models.51

The studies performed so far appear to show a concomitant cellular activation as well as immune suppression during acute DV infection. T cell proliferation to mitogens is impaired during the acute infection, and this appears to be caused by a defect in the antigen-presenting cell.52 There is an impairment of the normal plasmacytoid dendritic cell response in children who subsequently develop DHF. This blunted response is thought to result from inadequate control of viremia with the subsequent enhanced activation of cross-reactive memory T lymphocytes resulting in DHF.53 Overall numbers of CD4 and CD8 T cells, natural killer cells, and γδ T cells have been found to be decreased in DHF compared with df, but despite their reduced numbers, CD8 and natural killer T cells expressed higher levels of the activation marker CD69 in patients with DHF compared with uncomplicated df. It has been shown that low affinity memory T cells occur at higher levels than cytotoxic T cells, the latter being lost through apoptosis,47 and DV epitope-specific T cell responses are associated with disease severity.47 Dengue-specific T cells are increased in DHF, but peripheral blood mononuclear cells are unable to produce IFN-γ in response to dengue epitope stimulation,48 reflecting the reduced ability of monocytes and dendritic cells to present antigen adequately. Alternatively, antigen-induced cell death may ensue after the in vitro stimulation of re-
cently activated CD8 T cells,\textsuperscript{52} a finding that has been directly demonstrated.\textsuperscript{48,54}

The short-lived nature of plasma leakage syndrome seen in DHF is another point to support a functional rather than destructive effect of DV infection on endothelial cells. In vitro studies of human endothelial cell lines infected with DVs can produce pro-inflammatory mediators such as IL-6 and IL-8 RANTES (regulated on activation; normal T cell expressed and secreted), alter intercellular cell adhesion molecule type 1 surface expression and actin cytoskeleton structure, and increase permeability to small molecules.\textsuperscript{55,56} These effects could be partially reversed by neutralizing antibodies directed against IL-8.\textsuperscript{55} Also, the infection of human umbilical cord endothelial cells with DV in the presence of antidengue immune serum induced the formation of activated complement via both classical and alternative pathways with complement activation appearing to be mediated by dengue NS1 protein.\textsuperscript{57} Serum from patients with acute dengue infection induced the activation and apoptosis of cultured endothelial cells that could be partially reversed by anti-TNF-\alpha monoclonal antibodies, further supporting the role of inflammatory mediators for plasma leakage.\textsuperscript{58} A recent murine model for DV-induced lethal vascular permeability has also demonstrated the importance of TNF-\alpha as a key mediator of DV induced in mice.\textsuperscript{59}

The presence of IgM, \(\beta\)-1-globulin, and fibrinogen has been demonstrated in the cutaneous vessels of patients with DHF with dengue antigen in perivascular mononuclear cells, indicating that the skin rashes associated with DHF are immune-mediated.\textsuperscript{60}

Cytokine cascades, complement, and other mediators

Although incomplete, the current evidence suggests that, after massive activation of memory T cells, a cytokine cascade that targets vascular endothelial cells is primarily responsible for the critical leakage of fluid and protein in DHF/DSS. Such cytokines, including IFN-\(\gamma\), TNF-\(\alpha\), IL-2, IL-6, IL1-\(\beta\), and IL-8, are released in high concentrations mostly by T cells, monocytes/macrophages, and endothelial cells and are demonstrable in the serum of patients with DHF/DSS. More recent studies have confirmed the presence of elevated levels of IFN-\(\gamma\), TNF-\(\alpha\), and IL-10 in patients from Vietnam, India, and Cuba.\textsuperscript{61-63} Such cytokines have the potential to induce the release and production of other

\begin{figure}[h]
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\includegraphics[width=\textwidth]{dengue_hemorrhagic_fever}
\caption{Organs from a 2-year-old Thai boy who died from dengue hemorrhagic fever. (A) The eviscerated intestines were markedly edematous and focally hemorrhagic, and there was a hemothorax of 300 mL. (B) The kidneys were edematous with focal hemorrhage and hemorrhage into the calyces and renal pelvis. (C) The liver was swollen with focal hemorrhage. (D) Both lungs were heavy and beefy in consistency and hemorrhagic.}
\end{figure}
cytokines so that a complex interactive network results in further increases in the levels of cytokines and other chemical mediators in a cascade, often with synergistic effects on vascular permeability. IFN-γ is also able to upgrade the expression of Fcγ receptors on monocytes and macrophages, further facilitating viral replication. Although this cascade of cytokine activation and production stimulated by memory T cells is an attractive proposition in adult DHF/DSS, it fails to explain the occurrence of this severe form of dengue infection in the primary infection of infants born of dengue-immune mothers.

Complement has been suggested to have a role in the immunopathogenesis of DHF/DSS, although its cause remains unknown. Large amounts of dengue NS1, complement anaphylatoxin C5a, and the terminal complement complex SC5b-9 were found in pleural fluids of patients with DHF/DSS. Other mediators, such as histamine, tissue plasminogen activator, and macrophage inhibitory factor, have also been found in this disease.

Together with the endothelial permeability seen in DHF, there is a marked thrombocytopenia. This has also been seen in dengue hemorrhagic fever, although the drop in platelet numbers is less marked. It has been suggested that there is transient suppression of hematopoiesis, although megakaryocytes have not been shown to be infected with the virus. Other suggestions for the thrombocytopenia include binding of the virus to platelets in the presence of virus-specific antibodies, antiviral antibodies that cross-react with human platelets, and antiplatelet antibodies, the latter in a mouse model.

The elevation of serum liver enzymes is a frequent finding in DHF infection, and infrequently midzonal necrosis is demonstrable in the liver and fulminant hepatitis may occur. Hepatocytes are therefore a likely target for the virus. In vitro infection of HepG2 cells results in apoptosis mediated by TNF-related apoptosis-inducing ligand and other chemokines, such as IL-8, RANTES, and monocyte chemoattractant proteins, with evidence that these actions may be mediated by NS5 protein.

Other mediators, such as histamine, tissue plasminogen activator, and macrophage migration inhibitory factor, have also been implicated in the pathogenesis of the severe forms of dengue infection.

Pathology

There are a limited number of morphological studies in DHF/DSS. In BALB/c mice inoculated with dengue-2 obtained from human serum, focal alterations were found in the liver, kidney, lung, and cerebellum. The presence of the virus in these organs was confirmed by ultrastructural and immunolocalization techniques in mosquito cell cultures of the infected tissues. Hepatocytes were ballooned; portal and centrilobular veins were congested; lungs were focally hemorrhagic with vascular congestion and focal alveolitis; cerebellar tissue displayed focal neuronal compaction and perivascular edema; and there was increased glomerular volume with augmented endocapillary and mesangial cellularity.

Children who die from DHF have extensive edema of viscera with leakage of blood and focal hemorrhage (Figure 1). In a study of five fatal cases of DHF in Vietnamese children, severe hepatitis was observed with midzonal necrosis and micro-vesicular steatosis, although in one patient the liver appeared normal. The necrotic areas showed apoptosis by the TUNEL technique, with destruction of both hepatocytes and Kupffer cells, and there was no recruitment of polymorphonuclear cells or lymphocytes. Another study of nine fatal cases with fulminant hepatitis confirmed the presence of DV cDNA by reverse transcriptase in situ PCR in more than 80% of the hepatocytes and in many Kupffer cells. Five livers showed massive hepatic necrosis or apoptosis with no accompanying involvement of bile ducts (Figure 2). There was rare bile canalicular cholestasis, and micro-vesicular steatosis was common. The pauci-cellular areas of massive hepatic necrosis showed only rare Kupffer cells to be positive for TNF-α and IL-2 compared with the upregulation of these and many other cytokines seen in the livers of fatal cases of hepatitis C. Thrombotic microangiopathy may be observed in the glomeruli (Figure 3), most likely the result of disseminated intravascular coagulopathy from hemconcentration.

Localization of DV antigen and RNA by immunohistochemistry and in situ hybridization confirmed the presence of viral antigens in Kupffer cells, lymphoid cells in the splenic red and white pulp, renal tubular epithelium, vascular endothelium of the liver and lung, monocytes in the liver, spleen, and lung, and peripheral blood monocytes and lymphocytes (Figure 4 A and B), although high levels of

Figure 2 Liver biopsy from a 13-year-old Chinese boy from Singapore who presented with dengue hemorrhagic fever and fulminant hepatitis. The needle core biopsy showed extensive pauci-cellular necrosis concentrated in the midzone, but in areas tending to involve the entire lobule, the portal tract elements were not involved (arrows). The nuclear debris represented apoptotic bodies.
viral RNA were only found in the antigen-bearing cells of the spleen and peripheral blood supporting viral replication in these latter cells (Figure 4C and D). Viral antigen was present in the endothelium of the organs studied, but there was no evidence of viral replication. The presence of increased numbers of ultrastructural vacuoles and pinocytic vesicles in cutaneous vascular endothelium has suggested that the presence of viral antigen in these cells represents endocytosis and not infection.

Genetic predisposition and resistance

The patient’s genetic background appears to be a critical factor in determining progression to DHF/DSS. Outbreaks in Cuba have shown a reduced risk of people of Negroid race for DHF/DSS compared with those of Caucasoid race, an observation coinciding with the low reported incidence of dengue disease in African and Black Caribbean populations. Although dengue has been documented in 19 African nations and the virus repeatedly isolated, only sporadic cases of dengue have been reported, and mainly in nonindigenous populations. Even when outbreaks have been reported in Africa and the Seychelles, the clinical manifestations have been very mild. There is a clear absence of DHF/DSS despite hyperendemic dengue virus transmission in the Haiti, this despite the annual infection rate in Port-au-Prince being about 30% higher than that of Yangoon, Myanmar, where DHF/DSS rates and fatalities are high.

Although polymorphic genes have been suggested as possible contenders to account for the genetic susceptibility, the HLA region is an obvious candidate as it encodes several proteins involved in the immune response, including complement and TNF-α. Certain HLA types have been both
positively and negatively associated with DHF. For example, variations in HLA-A locus were significantly associated with susceptibility to DHF/DSS\(^7\); HLA-DR04 has the reverse association. Genetic background may also be related to the proliferation of low-affinity T cells, or the persistence of cytokine production by these cells, or both factors together.

**Conclusions**

Current information on the immunopathogenesis of DHF/DSS is still fragmentary. The severe forms of the DV infection occur as secondary infections. The target cells appear to be dendritic cells, monocytes, hepatocytes, T lymphocytes, and possibly vascular endothelial cells. The disease results from two major immune mechanisms that involve the production of nonneutralizing enhancing antibodies that cross-react between the serotypes of DV enhancing viral entry into dendritic cells and monocytes to increase the viral load and produce inefficient maturation of the infected cells. The other component of the immune response involves the massive activation of memory T cells sensitized in a previous infection. This activation results in the proliferation and release of pro-inflammatory cytokines and a cytokine cascade that targets the susceptible cells causing cell death through apoptosis and is responsible for the fluid and protein leakage and the liver damage characteristic of DHF/DSS. Research is hampered by the absence of a suitable animal model for DHF/DSS, and detailed pathological studies in humans are few. Current treatment is largely symptomatic, and prevention is through vector control, making it imperative that greater understanding of the pathogenesis be achieved with the view of developing novel molecules to inhibit viral replication and to stem the damaging effects of immune mediators. There is also impetus for vaccine development, and a greater understanding of the relation of resistance to the severe forms of the infection and genetics is needed.

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