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Dengue Virus

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KEYWORDS

• Dengue virus • Flavivirus • Infection• Clinical diagnosis
• Vaccines

OVERVIEW

Dengue fever (DF), the most prevalent arthropod-borne viral illness in humans, is caused by the dengue virus (DENV). The 4 serotypes of DENV (DENV 1-4) are transmitted to humans primarily by the *Aedes aegypti* mosquito (**Fig. 1**).

DENV is a member of the Flaviviridae family and is related to the viruses that cause yellow fever and the Japanese, St. Louis, and West Nile encephalitides.[1] Infection by DENV causes a spectrum of clinical diseases that range from an acute debilitating, self-limited febrile illness, DF, to a life-threatening hemorrhagic and capillary leak syndrome of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). DENV causes an estimated 25 to 100 million cases of DF and 250,000 cases of DHF per year worldwide, with 2.5 billion people at risk of infection.[2,3] At present, no approved antiviral treatment or vaccine is in use, and therapy is supportive in nature (**Fig. 2**).

Epidemic DHF was first recognized in the 1950s in Southeast Asia, and by 1975 it had become a leading cause of hospitalization and death among children in many countries in that region. In the 1980s, DHF began a second expansion into Asia, and in countries where DHF is endemic, the epidemics have become progressively larger over the last 15 years (**Box 1**). In 1980, the first indigenous transmission of dengue in the United States in more than 40 years occurred. Later, infections also occurred in Texas. In 2001 to 2002, a dengue outbreak occurred in Hawaii spread by *Aedes albopictus* mosquitoes.

The Americas have seen the most dramatic rise in the emergence of dengue cases (**Fig. 3**). The mosquito vector for dengue was eradicated in most of the region as part of the Pan American Health Organization's yellow fever eradication campaign in the 1950s and 1960s. The *A aegypti* eradication program was officially discontinued in the United States and other Western Hemisphere regions, leading to reinfestation of the mosquito vector in most countries during the 1980s and 1990s. By 1997, the geographic distribution of *A aegypti* was wider than its distribution before the eradication program. Dengue is now endemic in much of the Western Hemisphere.

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Fig. 1. The *A aegypti* mosquito is the most common epidemic vector for spread of dengue virus. It can be identified by the white bands or scale patterns on its legs and thorax. (*Courtesy of* Centers for Disease Control and Prevention (CDC), http://www.cdc.gov/ncidod/dvbid/dengue.)

Hyperendemicity, the presence of multiple circulating serotypes, is widespread in most countries and epidemics caused by multiple serotypes are more frequent.

VIROLOGY

DENV is an enveloped virus with a single-stranded, positive-sense 10.7 kilobase RNA genome,[4] which is translated as a single polyprotein and then cleaved into 3 structural proteins (capsid [C], premembrane/membrane [prM/M], and envelope [E]) and 7 nonstructural (NS) proteins by virus- and host-encoded proteases. The 3 structural components are required for capsid formation (C) and assembly into viral particles (prM and E). The NS proteins contain a serine protease and ATP-dependent helicase (NS3), which is required for virus polyprotein processing, a methyltransferase and RNA-dependent RNA polymerase (NS5), and a cofactor for the NS3 protease (NS2B). NS4B has been implicated in blocking the interferon (IFN) response. NS1, NS2A, and NS4A have either unknown or incompletely understood functions. All the NS proteins appear to be necessary for efficient replication.

In primary DENV infection, the virus enters target cells after the E protein adheres to cell surface receptors, such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) on dendritic cells.[5] Viral uptake occurs by receptor-mediated endocytosis. Endosomal acidification induces a conformational change in the E protein, resulting in fusion of the viral and endosomal membranes and nucleocapsid release into the cytoplasm.[6,7] Virus genome replication occurs in discrete domains within the endoplasmic reticulum (ER). Virus assembly occurs at the ER, and virions are exocytosed via Golgi-derived secretory vesicles.[8]

EPIDEMIOLOGY

Following the bite of a mosquito, usually *A aegypti* or *A albopictus*,[2] DENV can cause a range of mild-to-severe illnesses. The mosquito eradication program, which was officially discontinued in the United States in 1970, gradually weakened elsewhere, and the mosquito began to reinfest countries from which it had been eradicated. Consequently, the geographic distribution of *A aegypti* in 2002 was much wider than that before the eradication program and there was a corresponding increase in dengue infections. There are 4 distinct serotypes of DENV. Primary infection with one DENV serotype provides lifelong immunity to that specific serotype. However, when an individual is infected with a different serotype of DENV, there is an increased

Fig. 2. World map indicating regions with known risks of dengue infection. (*Courtesy of* CDC, available at: http://www.cdc.gov/ncidod/dvbid/dengue.)

Box 1
Recent dengue virus infections in the United States

Texas:

- 1980: 23 cases, first locally acquired since 1945
- 1986: 9 cases
- 1995: 7 cases
- 1997: 3 cases
- 1998: 1 case
- 1999: 18 cases
- 2005: 25 cases

Hawaii:

- 2001 to 2002: 122 cases (first since 1944)

risk of severe dengue disease.[9] This can occur with all 4 serotypes; therefore, in regions with multiple endemic serotypes, the risk of severe disease is higher.

PATHOGENESIS

The pathogenesis of DHF/DSS, the most severe form of DENV infection, reflects a complex interplay of the host immune response and the viral determinants of virulence.[2,10,11] Epidemiologic studies have suggested an immune system linkage, because there is an increased risk of DHF with secondary DENV infection and in

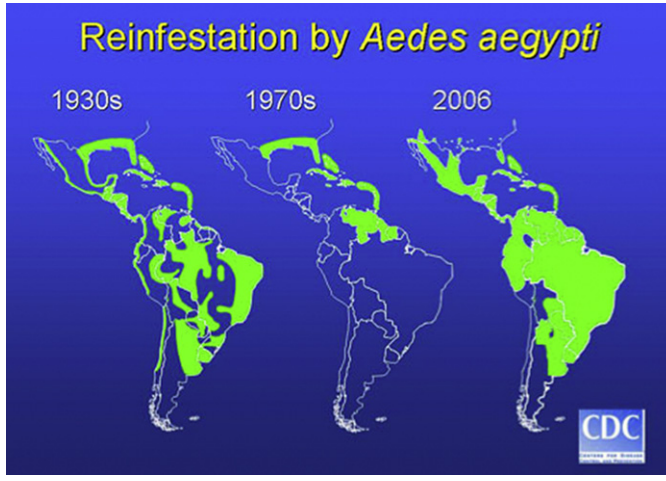

Fig. 3. Reinfestation of *A aegypti* in the Americas Unfortunately, the success of the eradication campaign was not sustained. Beginning in the early 1970s, it began to be disbanded, and many countries channeled their limited resources into other areas. Consequently, *A aegypti* began to reinfest the countries from which it had been eradicated. Comparing the 1970 and 2006 maps, the mosquito is seen reestablishing itself throughout Central America and most of South America. As the mosquito has spread, the number and frequency of dengue epidemics have increased, as has dengue hemorrhagic fever activity in the Americas. (*Courtesy of* CDC, available at: http://www.cdc.gov/ncidod/dvbid/dengue.)

children within the first year of life born to DENV-immune mothers.[12–15] From these observations, the hypothesis of antibody-dependent immune enhancement (ADE) of infection emerged. In support of the ADE pathogenesis concept, antibody enhancement of DENV infection in monocytes in vitro correlated with increased risk of DHF,[15,16] and peak viremia was increased in patients with severe secondary DENV infection.[17,18] Differences in specific genetic determinants among viral isolates[19–21] may also affect virulence, because some DENV strains fail to cause severe disease.[22,23] Finally, a pathologic cytokine response that occurs after extensive T-cell activation may contribute to the capillary leak syndrome associated with DHF.[11] Elevated levels of cytokines, including IFN-γ, tumor necrosis factor (TNF)-γ, and interleukin (IL)-10, to some extent correlate with severe disease[24–28]; and disease severity has been associated with activation of $CD8^+$ T cells and the expansion of serotype-reactive low-affinity DENV-specific T cells that produce high levels of vasoactive cytokines.[29–33]

CLINICAL PRESENTATIONS

Dengue fever may present in many forms: as an undifferentiated febrile illness with a maculopapular rash, particularly in children, as flulike symptoms, or as classic Dengue with 2 or more symptoms, such as fever, headache, bone or joint pain, muscular pain, rash, pain behind the eyes, and petechial hemorrhaging. Often, there is prolonged fatigue and depression. During dengue epidemics, hemorrhagic complications may also appear, such as bleeding from the gums, nosebleeds, and bruising. Case fatalities due to DF are low, whereas DHF mortality is fairly high. There is no specific treatment for dengue fever except for symptomatic treatment, rest, and rehydration. Recognizing the warning signs and symptoms of dengue infection are critical for appropriate diagnosis and treatment (**Fig. 4**).

DHF is characterized by spontaneous bleeding, plasma leakage, fever, and thrombocytopenia. Four clinical manifestations need to be observed to be classified as DHF. These include (1) fever; (2) hemorrhagic episodes with the presence of at least one of the following: a positive tourniquet test result (also called a capillary fragility test: a clinical diagnostic method to determine a patient's hemorrhagic tendency and assess fragility of capillary walls); petechiae, ecchymoses, or purpura; or bleeding from mucosa, gastrointestinal tract, injection sites, or others; (3) plasma leakage due to increased capillary permeability; and (4) thrombocytopenia (100,000/mm^3 or less).

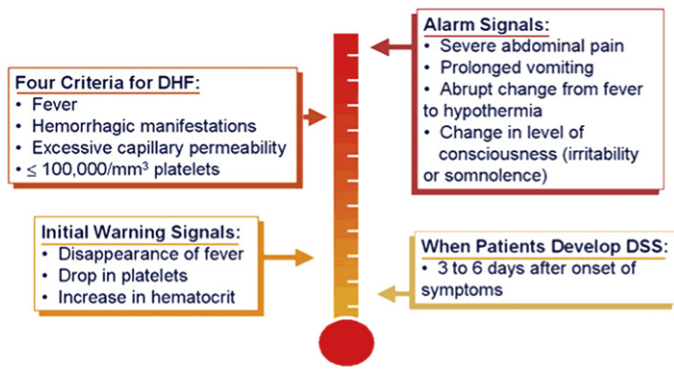

Fig. 4. Warning signs of dengue infection. (*Courtesy of* CDC, available at: http://www.cdc.gov/ncidod/dvbid/dengue.)

Moderate-to-marked thrombocytopenia with concurrent hemoconcentration is a distinctive clinical laboratory finding of DHF. However, to distinguish DHF from DF, an observation of plasma leakage manifested by a rising hematocrit value (ie, hemoconcentration) must be observed (**Fig. 5**).

The normal course of DHF lasts between 7 to 10 days, and with appropriate intensive maintenance of the circulating fluid volume, mortality may be reduced to less than 1%. Only severe DF and DHF cases should be hospitalized. Serologic tests are necessary to confirm cases of dengue. However, these tests may take several days.[34,35] Developing countries may not have the resources to perform these expensive confirmatory assays, and therefore, many suspected cases of dengue are not fully diagnosed. In severe cases of DHF, the patient's condition may suddenly deteriorate after a few days of fever; the temperature drops, followed by signs of circulatory failure; and the patient may rapidly go into a critical state of shock (dengue shock syndrome), dying within 12 to 24 hours or quickly recovering following appropriate volume replacement therapy **Box 2**.

DSS is the most severe form of DHF and is characterized by the presence of all 4 DHF clinical manifestations and circulatory failure. All 3 manifestations of circulatory failure must be present: rapid and weak pulse; narrow pulse pressure or hypotension for the patient's age; and cold, clammy skin and altered mental state.

DIAGNOSIS

Establishing a laboratory diagnosis of dengue infection is critical for diagnosis of dengue. A major challenge for disease surveillance and case diagnosis is that the dengue viruses produce asymptomatic infections and a spectrum of clinical illness ranging from a mild, nonspecific febrile illness to fatal hemorrhagic disease. Important risk factors of DHF include the strain and serotype of the infecting virus and the age, immune status, and genetic predisposition of the patient. The most common method of detecting the virus is to propagate virus from serum in cell culture or detect anti-dengue antibodies by serology. Virus can be cultured in vitro or by detection of viral RNA and specific dengue virus antigens. Countries that do not have access to sophisticated laboratory tests rely on identification of early clinical or simple laboratory indicators that can provide a reliable diagnosis of dengue before hospitalization. Early distinction between dengue and other febrile illnesses could help identify patients that should be monitored for signs of DHF.

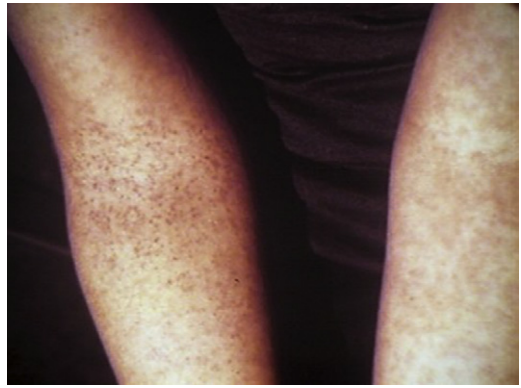

Fig. 5. Petechial hemorrhages from a dengue infected patient. (*Courtesy of* CDC, available at: http://www.cdc.gov/ncidod/dvbid/dengue.)

Box 2
Grades of DHF

All 4 grades must be met for a diagnosis of DHF.

Grade 1: Fever and nonspecific constitutional symptoms and positive tourniquet test result

Grade 2: Grade 1 manifestations plus spontaneous bleeding.

Grade 3[a]: Incipient shock with signs of circulatory failure.

Grade 4[a]: Profound shock with undetectable pulse and blood pressure.

[a] Grades 3 and 4 are Dengue Shock Syndrome.

DIFFERENTIAL DIAGNOSIS

Febrile illnesses, such as measles, typhoid fever, leptospirosis, and severe acute respiratory syndrome (SARS), can produce symptoms similar to DF.[36–41] At presentation, these illnesses may share similar clinical features, including headache, myalgia, and rash **Box 3**.

TREATMENT AND LONG-TERM OUTCOMES

There are no specific antivirals that can eliminate the virus from an infected individual. However, supportive care and treatment can be effective in treating DF. Paracetamol and other antipyretics can be used to treat fever. Bone pain should be treated by analgesics or painkilling tablets. During episodes of DHF/DSS, the mortality rate in the absence of hospitalization can be as high as 50%. With proper treatment, such as intravenous fluid replacement, the mortality rate is greatly reduced.

VACCINES AND IMMUNITY

Multiple correlates of protection have been described for dengue. However, the primary correlate seems to be long-term homotypic protection.[42,43] Most protective antibodies are directed at the surface E glycoprotein.[44,45] However, antibodies to

Box 3
Differential diagnosis of dengue infection

Influenza

Measles

Rubella

Malaria

Typhoid fever

Leptospirosis

Meningococcemia

Rickettsial infections

Bacterial sepsis

Other viral hemorrhagic fevers

the M and NS1 proteins show some protective efficacy.[46] Passively transferring antibodies from seroconverted animals results in decreased infection and disease following challenge.[44,46] In addition, maternal antibodies decrease disease in infants.[15,47] Using in vitro neutralization assays, antibodies directed against the E protein prevent virus infection.[48] Antibodies that block viral attachment or prevent fusion to target cells neutralize virus infection.[49,50] In addition to neutralization, antibodies that mediate cell-mediated cytotoxicity reduce virus infection in complement-independent[51,52] and complement-dependent mechanisms.[53] Cellular immune responses are generally weakly protective.[54] However, these responses are critical for viral clearance.[55,56] Innate immune responses directed against NS proteins, such as NS4B (a putative IFN antagonist), seem to mediate viral escape.[57]

Currently, no DENV vaccine is approved by the US Food and Drug Administration (FDA). Four related but serologically distinct DENVs can cause disease. Non-neutralizing, cross-reactive antibodies may contribute to DHF pathogenesis via antibody-dependent enhancement. Therefore, an effective vaccine must induce high-titer neutralizing antibodies against all 4 strains[58,59]; failure to do so could increase the risk of severe disease on natural challenge. To circumvent this problem, tetravalent live-attenuated candidate vaccines are in varying stages of development.[60–64] In clinical trials, tetravalent serologic responses were observed in some individuals, but

Table 1
Experimental dengue virus vaccines

Type	Sponsor	Stage of Development
Live attenuated		
Tetravalent	Mahidol University/Sanofi Pasteur	Phase I
Tetravalent	WRAIR/GSK	Phase II
Chimeric		
ChimeriVax (17D YF)	Acambis/Sanofi Pasteur	Phase I
DENV-2/4d30 (all serotypes)	NIAID, NIH	Phase I/II
DENV-1	US FDA	Phase I
DENV-2 (16,681, PDK53)	CDC/Inviragen	Preclinical
DNA		
Several approaches	Various	
(ie, Domain III, prM/E, NS1)	NMRC/University of Pittsburgh	Phase I/Preclinical
Inactivated		
Several approaches	WRAIR	Preclinical
Subvirion particles/viruslike particles		
Drosophila cells	Hawaii Biotech	Phase I
Baculovirus (E, NS1)	Various	Preclinical
Replication-defective AV (E)	RepliVax-UTMB/Acambis	Preclinical
Yeast (C/prM/E, E-IIBsAg)	Various	Preclinical
Escherichia coli (E, E-NS1)	Various	Preclinical
DNA	University of Pittsburgh	Preclinical
Subunit/recombinant	Various	Preclinical

Abbreviations: AV, adenovirus; CDC, Centers for Disease Control and Prevention; GSK, GlaxoSmithKline; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; UTMB, University of Texas Medical Branch; WRAIR, Walter Reed Army Institute of Research; YF, yellow fever.

many do not develop high titer neutralizing antibodies despite multiple immunizations.[65,66] Additionally, each part of the tetravalent vaccine does not elicit high titer immune response leading to immunodominance. Subunit-based vaccines, as purified proteins or DNA plasmid, are alternative vaccine strategies. Repeated immunization of purified recombinant DENV domain III of the E protein (DIII) or DIII-encoding plasmids induced protective antibodies in mice, albeit at fairly low neutralizing titers.[67–71]

Live attenuated vaccines and nonreplicating vaccines, such as inactivated virus vaccines, viruslike particles, and DNA vaccines, have been developed for dengue (**Table 1**). These vaccines elicit protective neutralizing antibodies. These vaccines can elicit long-lasting immunity against the specific serotype of DENV. However, they are poorly cross-reactive against infection with another subtype of DENV.

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