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REVIEW

Dengue virus: epidemiology, biology, and disease aetiology

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Abstract: Dengue is a vector-borne viral disease caused by the flavivirus dengue virus (DENV). Approximately 400 million cases and 22 000 deaths occur due to dengue worldwide each year. It has been reported in more than 100 countries in tropical and subtropical regions. A positive-stranded enveloped RNA virus (DENV) is principally transmitted by *Aedes* mosquitoes. It has four antigenically distinct serotypes, DENV-1 to DENV-4, with different genotypes and three structural proteins and seven non-structural proteins. Clinical symptoms of dengue range from mild fever to severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), with thrombocytopenia, leucopenia, and increased vascular permeability. Although primary infection causes activation of immune responses against DENV serotypes, the severity of the disease is enhanced via heterotypic infection by various serotypes as well as antibody-dependent enhancement (ADE). The first licensed DENV vaccine was tetravalent CYD Denvaxia, but it has not been approved in all countries. The lack of a suitable animal model, a proper mechanistic study in pathogenesis, and ADE are the main hindrances in vaccine development. This review summarizes the current knowledge on DENV epidemiology, biology, and disease aetiology in the context of prevention and protection from dengue virus disease.

Key words: dengue virus, DENV, dengue hemorrhagic fever, antibody-dependent enhancement, secondary infection.

Résumé : La dengue est une maladie virale à transmission vectorielle, causée par un flavivirus, le virus de la dengue (DENV). Environ 400 millions de cas et 22 000 décès sont dus à la dengue dans le monde chaque année. Elle est signalée dans plus de 100 pays des régions tropicales et subtropicales. Virus à ARN enveloppé à brin positif, le DENV est principalement transmis par les moustiques du genre *Aedes*. Il comporte quatre sérotypes antigéniquement distincts, DENV-1 à 4, avec des génotypes différents, ayant trois protéines structurelles et sept protéines non structurelles. Les symptômes cliniques de la dengue vont de la fièvre légère à la dengue hémorragique (DHF) grave ou au syndrome de choc de la dengue (DSS), avec thrombocytopénie, leucopénie et perméabilité vasculaire accrue. Bien que la primo-infection provoque l'activation de réponses immunitaires contre les sérotypes du DENV, la gravité de la maladie est accrue par l'infection hétérotypique par différents sérotypes et également par la facilitation dépendante des anticorps (ADE). Le premier vaccin homologué contre le DENV était le CYD Denvaxia tétravalent, mais il n'a pas été approuvé dans tous les pays. L'absence d'un modèle animal approprié, d'une étude adéquate du mécanisme de la pathogenèse et l'ADE, sont les principaux obstacles au développement de vaccins. Cette synthèse résume les connaissances actuelles sur l'épidémiologie, la biologie et l'étiologie du DENV dans le contexte de la prévention et de la protection contre la maladie associée au virus de la dengue. [Traduit par la Rédaction]

Mots-clés : virus de la dengue, DENV, DHF, ADE, infection secondaire.

1. Introduction

Dengue fever, caused by the dengue virus (DENV), has been a major public health concern during the last few decades (Bhatt et al. 2013). More importantly, it has been categorized as a "neglected tropical disease" (Hotez et al. 2009). Annually, approximately 400 million dengue cases and 22 000 deaths occur worldwide (Bhatt et al. 2013; Shepard et al. 2016). Dengue infection in humans is often inapparent and is globally established in both endemic and epidemic transmission cycles (Bhatt et al. 2013).

DENV, a flavivirus in the species *Dengue virus*, genus *Flavivirus* in the family *Flaviviridae*, is a positive (+) stranded RNA containing virus. Other viruses of this family include Japanese encephalitis virus (JEV), West Nile virus (WNV), and yellow fever virus (YFV). Four distinct serotypes (1, 2,

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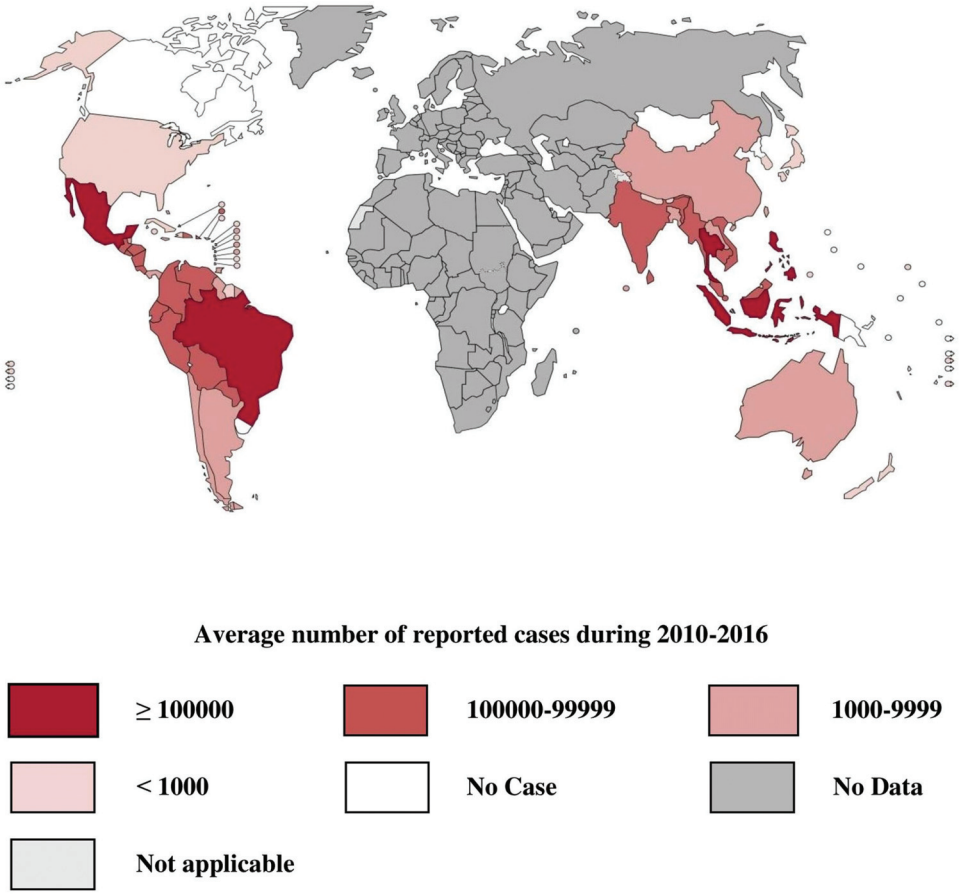
Fig. 1. Worldwide average number of suspected or confirmed dengue cases during 2010–2016 (Panacea Biotec. n.d.). Map created by Control of Neglected Tropical Disease (NTD). [Colour online.]



3, and 4) of DENV have been detected worldwide, which differ antigenically from each other. A newly discovered fifth serotype (DENV-5) was first detected in the blood of a patient in the Sarawak state of Malaysia in 2007 (Mustafa et al. 2015). All serotypes have several subtypes or genotypes based on several changes in the viral genome. The genotypes with their endemic regions are summarized in Supplementary Table S1[1]. All serotypes of dengue are detected throughout India. Dengue infection leads to a wide range of clinical manifestations, from mild fever to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In humans, one serotype of dengue produces lifelong immunity against re-infection but provides only temporary and partial immunity against other serotypes (Wahala and de Silva 2011). Antibody-dependent enhancement (ADE) also plays an important role in severe dengue pathogenesis (Rothman and Ennis 1999).

2. History and epidemiology of dengue

Dengue virus infects humans in more than 100 countries each year, with roughly 3.6 billion people at risk (Diamond and Pierson 2015). During the last 50 years, the incidence of dengue has increased 30-fold (CDC 2014). DENV epidemics occur annually in the Americas, Asia, Africa, and Australia, and also affect travelers from endemic regions. Apart from the effects on public health, these epidemics have a massive economic impact in the affected countries, including India.

The first dengue outbreak was reported in 1779 in Jakarta, Indonesia and Cairo, Egypt (Wu et al. 2011). However, a confirmed outbreak in North America, by DENV, was the Philadelphia outbreak in 1780 (Rush 1951). The worldwide average number of suspected or confirmed dengue cases reported to the WHO (2010–2016) (see WHO 1997) is presented in Fig. 1. In 2010, more than 1.6 million dengue cases were reported all over North and South America, out of which 49 000 were severe cases. The largest outbreak of dengue was seen in 2016 in the US, with more than 2.38 million cases reported. In this outbreak, the highest contribution was in Brazil, with 1.5 million cases. Dengue cases have drastically increased in the US in 2019, with more than 3 million cases (PAHO 2019).

[1]Supplementary data are available with the article at https://doi.org/10.1139/cjm-2020-0572.

Dengue epidemics were reported in East, West, and South Africa from the beginning of the 19th century (Amarasinghe et al. 2011; Were 2012). From 1980 to 2000, several dengue outbreaks in both East and West African countries were caused by dengue virus serotypes 1, 2, and 3 (Sang 2007). Many dengue cases have been reported during five large epidemics in African countries: Seychelles (1977–1979), Réunion Island (1977–1978), Djibouti (1992–1993), Comoros (1992–1993), and Cape Verde (2009) (Cornet 1993; Sang 2007).

Dengue outbreaks became a burden in Southeast Asian countries after World War II, principally due to urbanization (Ooi and Gubler 2009). The first two outbreaks of dengue hemorrhagic fever were reported in the Philippines in 1953 and 1956, respectively (Gubler 1998). After 1950, dengue epidemics occurred cyclically every year in Southeast Asian countries, including the Philippines, Bangkok, Thailand, Bhutan, Brunei, Cambodia, East Timor, Indonesia, Laos, Malaysia, Myanmar, Singapore, and Vietnam (Ooi and Gubler 2009). Between 2004 and 2010, Indonesia had the second highest number of dengue cases after Brazil. During 2009–2010, most dengue cases were due to serotype-4 in Indonesia (Taslim et al. 2018). However, in 2013, severe dengue was reported due to infection with serotype-3 (Lardo et al. 2016). During 2007–2010, dengue virus serotype-1 was most prevalent in Indonesia (Sasmono et al. 2015).

Dengue virus was first isolated in Japan by inoculating an infected patient's serum in suckling mice in 1943 (Kimura 1944). From 1942 to 1945, three dengue strains were isolated by mice-brain passage experiments involving injection of dengue patient's blood into the brain of subsequent generations of white mice (Hotta 1952).

2.1. Dengue prevalence in India

The Indian subcontinent, owing to its suitable environment, has several reports of dengue outbreaks involving all serotypes but DENV-5 (Dar et al. 2006; Mustafa et al. 2015). In the 1996 epidemic, ~16 000 cases and 545 deaths occurred throughout India (Mutheneni et al. 2017). Since 2010, the incidence of dengue has increased to about 15 per million people annually in different states. Every year more than 100 000 infections and 200–400 deaths occur throughout India (NVBDCP 2021). A recent dengue epidemic was recorded in 2017, in which 188 401 infections and 325 deaths occurred (NVBDCP). Clinical dengue-like illness was first recorded in Madras (now Chennai) in 1780 (Gupta et al. 2012). However, dengue fever was first proven to be caused by a "virus" in 1946 (Gupta and Ballani 2014). After that, there was no significant dengue epidemic until 1963. During 2019–2020, the incidence of infection with all DENV serotypes was detected in the northern part of West Bengal, especially in the Siliguri, Darjeeling, Jalpaiguri, and Alipurduar regions. Co-infections with at least three serotypes have been recorded in different cities in India, including in our study areas. DENV infection occurrence by different serotypes in different cities of India is summarized in Table S2[1].

3. Structure of dengue virus

Electron micrographs revealed that dengue virions are spherical and characterized by a relatively smooth surface, with a diameter of approximately 50 nm, a well-organized outer protein layer on the surface of a lipid bilayer, and an inner nucleocapsid core (Kuhn et al. 2002) (Fig. 2A). DENV contains three structural proteins, namely, the capsid (C), membrane (M) (having a membrane precursor or PrM), envelope (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Perera and Kuhn 2008). Table 1 lists the structural and non-structural proteins of DENV and their descriptions. Image reconstructions from cryo-electron micrographs showed that the virion envelope has icosahedral symmetry, in which E protein dimers are organized in a herringbone-like arrangement (Fig. 2B). The spherical immature and mature particles have an outer membrane derived from the ER and contain E and M proteins, which form an outer glycoprotein surface in an icosahedral shape. There is an RNA–protein core consisting of a positive-sense ssRNA genome and capsid proteins (C) within the lipid bilayer. Infectious and non-infectious states of mature and immature DENV depend on the conformational changes of M and E proteins at different environmental pH levels (Perera and Kuhn 2008). The structural transition from immature (spiky) to mature (smooth) morphology occurs during transition through the trans-Golgi network (TGN) and is driven predominantly by conformational changes in the E protein (Modis et al. 2004). Before maturation of DENV, E proteins, bound to membrane proteins, change its conformation in the TGN (low pH) (Yu et al. 2008). After maturation, the Pr peptide is released from the E protein in the extracellular space (pH 7.0) with infectious properties (Figs. 3A–3D). There is relatively little information available regarding the molecular structures of the non-structural proteins NS1, NS2A, NS4A, and NS44B. NS1 helps in viral RNA replication (Lindenbach and Rice 1999) as well as in viral defense through the inhibition of complement activation (Chung et al. 2006). NS2A and NS4B are involved in the formation of a part of the replication complex (Chambers et al. 1989). The DENV genome is a positive (+) single-stranded RNA, approximately 11 kilobases in length (Miller et al. 2006). The RNA genome is divided into three parts: the 5′ UTR region (untranslated region), ORF (open reading frame), and the 3′ UTR region (Fig. 4). The genome has a type I cap (m7GpppAmp) at the 5′ end with a single ORF that encodes a polyprotein and lacks a poly (A) tail at the 3′ end.

4. Intracellular replication

After successful infection, DENV primarily attacks and replicates in dendritic cells and infects macrophages, monocytes, and lymphocytes (Wan et al. 2018). Entry to

Fig. 2. (A) Enveloped and spherical dengue virion with different structural proteins and (B) Cryo-electron Microscopic structure of the dengue virus (DENV-4). The black triangle shows the icosahedral asymmetric unit. E protein dimers are in blue. Three E proteins reside in one asymmetric unit. Scale bar = 100 Å (Kostyuchenko et al. 2014). [Colour online.]

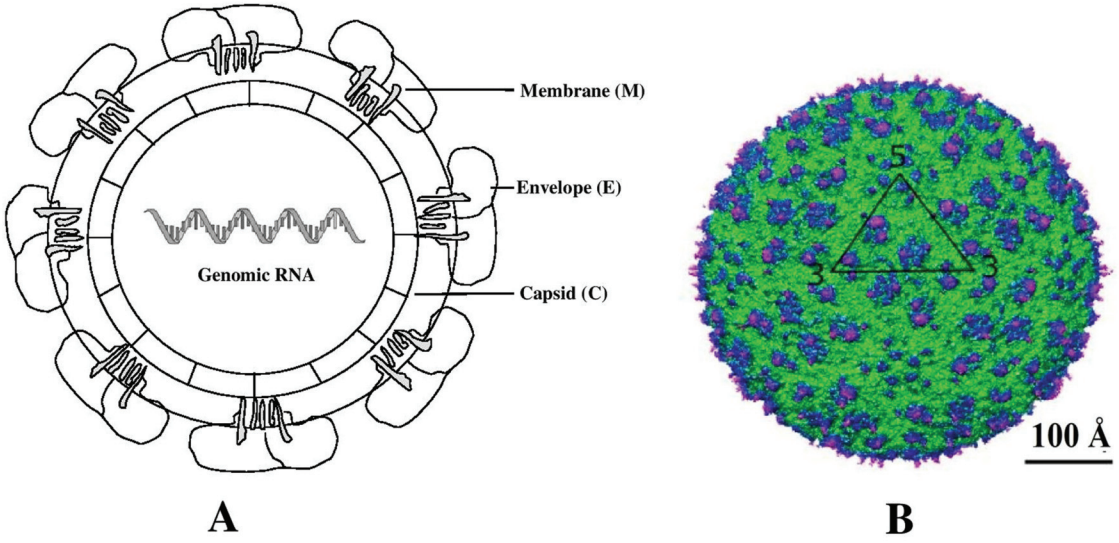
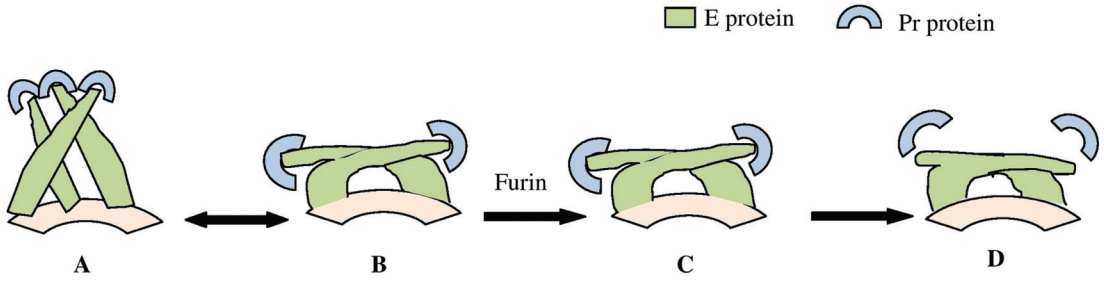


Table 1. Dengue virus structural and non-structural proteins with their molecular weight and amino acid residues.

Protein	No. of amino acids	Molecular weight (kDa)	References
Capsid (C)	100	12	Kuhn et al. 2002; Byk and Gamarnik 2016
Membrane (M)	75	8.2–8.5	Kuhn et al. 2002
Envelope (E)	495	—	Kuhn et al. 2002
NS1	350	45	Perera and Kuhn 2008; Meng et al. 2015
NS2A/B	218	22	Xie et al. 2013
NS3	618	—	Perera and Kuhn 2008
NS4A/B	127/248	16/27	Perera and Kuhn 2008; Zou et al. 2015
NS5	900	104	Perera and Kuhn 2008

Fig. 3. Changing infectious and non-infectious state of the dengue virus depends on conformations of the E protein in different pH. [Colour online.]



Immature and non-infectious DENV with bound PrM and E protein (in ER, pH 7.0)

Immature and non-infectious DENV with bound PrM and E protein (in TGN, pH 6.0)

Immature and non-infectious DENV with bound Pr, E and M protein (in TGN, pH 6.0)

Mature infectious DENV with released Pr protein and bound E and M protein (in Extracellular space, pH 7.0)

Fig. 4. Genomic structure with polyprotein sequence of the dengue virus.

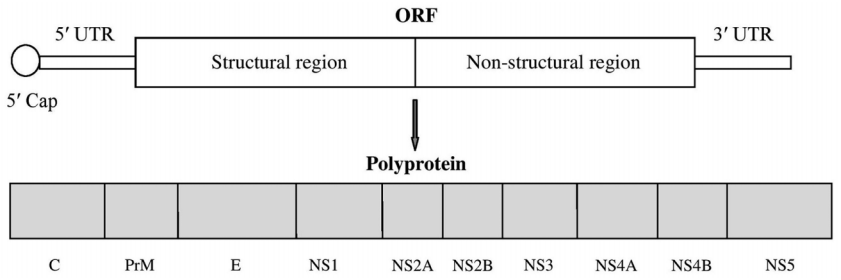
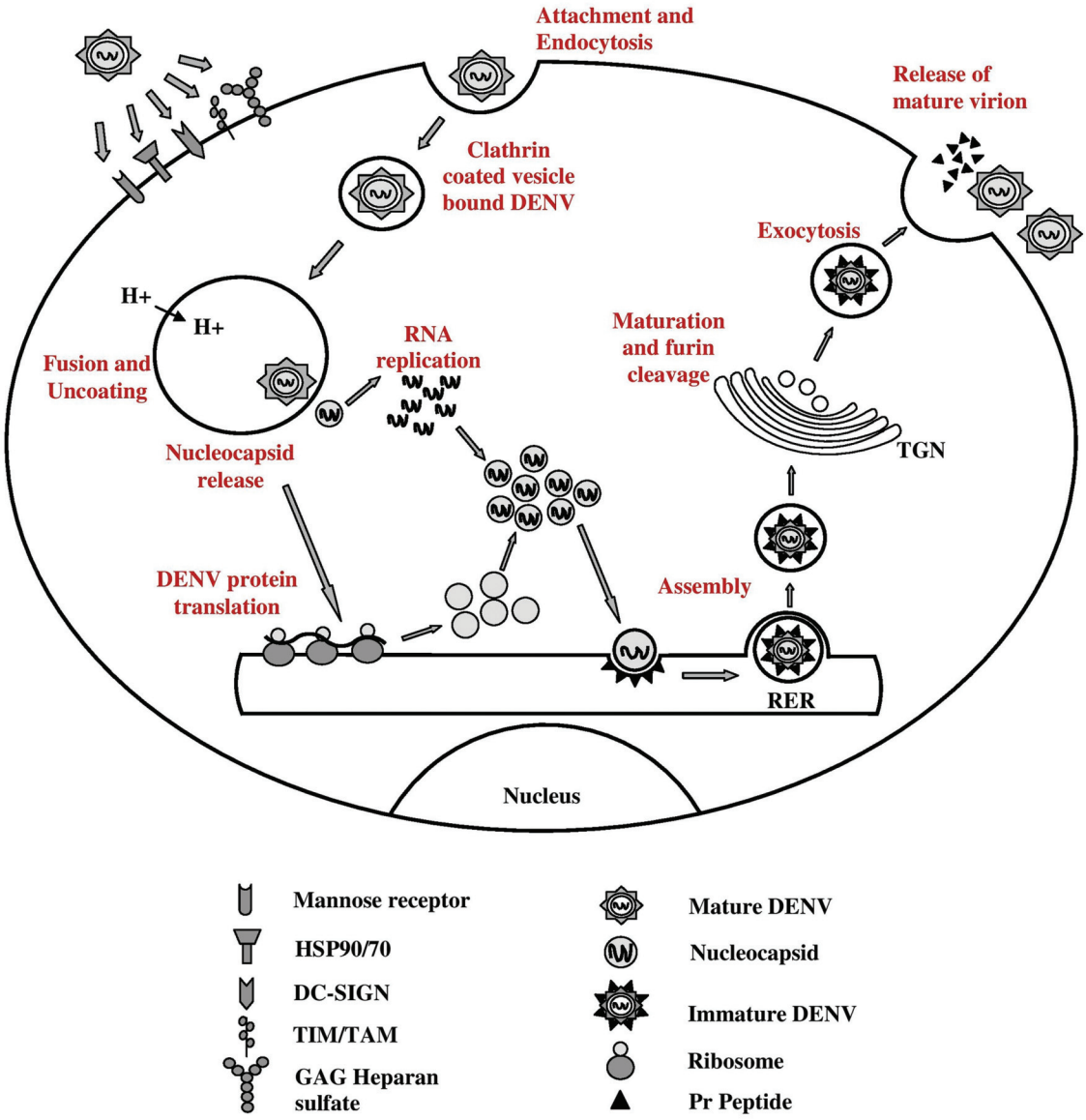


Fig. 5. Step-by-step processes of dengue virus entry in the host cell and its life cycle (adapted from Urcuqui-Inchima et al. 2010; Rodenhuis-Zybert et al. 2010). [Colour online.]



the cells occurs through receptor-mediated endocytosis by using cell surface molecules such as Fc receptors, glycosaminoglycans (GAG), lipopolysaccharide-binding CD14 associated molecules, heparan sulfate, and lectin-like receptors, such as DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin). DENV enters the cell via clathrin-coated vesicles (Seema and Jain 2005). Acidification of the late-endosomes leads to structural rearrangements of the E protein, resulting in fusion of the viral and host cell membranes and subsequent release

of the nucleocapsid into the cytoplasm (Fig. 5). The fusion of the virus with the endosome membrane is facilitated by the acidic pH within the endosome. Thereafter, the nucleocapsid (NC) is released into the cytoplasm, the RNA genome is uncoated, and viral materials are transported to the ER by the cytoskeletal transport machinery (Cuartas-López et al. 2018). The positive-stranded RNA of DENV is first translated into a polyprotein in a cap-dependent manner. Replication of the RNA genome occurs at the ER membrane using positive-sense RNA as a template. DENV NS5 protein, which has RNA cap methylation and RNA-dependent RNA polymerase (RdRp) activities, is required for negative- and positive-strand viral RNA synthesis (El Sahili and Lescar 2017). Viral capsid proteins are translated in the cytoplasm, while viral E and M proteins are inserted into the ER membrane during translation. Once sufficient positive-sense RNA copies have been transcribed from negative anti-genome RNA, the genomic RNA is encapsidated by the capsid proteins in the cytoplasm and buds into the lumen of the ER, thereby acquiring the M (produced by the cleavage of PrM by host protease, furin, MW 57 kDa) and E containing envelope from the ER (Fischl and Bartenschlager 2011). The conformation and organization of the E protein on the mature virion surface changes, depending on pH (described in Fig. 3), during virion assembly and release. The enveloped virions pass through the ER and the trans-Golgi network remaining in the lumen. In the last step, the enveloped virions bud through the ER membrane into the cytoplasm, acquiring a second ER-derived outer membrane (Fig. 5). This ER membrane then fuses with the plasma membrane, releasing the enveloped mature progeny virions into the extracellular space where they can spread and infect adjacent cells (Diamond and Pierson 2015).

5. Transmission of DENV

The primary and secondary vectors of DENV are mosquito *Aedes aegypti* and *Aedes albopictus,* respectively (Carrington and Simmons 2014). *Aedes aegypti* are endophilic, occurring largely indoors (Carrington and Simmons 2014), container-breeder (i.e., breeding in water-filled containers), and day-biting mosquitoes that feed preferentially on human blood under field conditions and are found in tropical and subtropical areas, with their geographic range spanning almost all continents (Thavara et al. 2001). *Aedes albopictus*, a more aggressive day-time biter, is exophilic under natural field conditions, commonly living outdoors, but still feeds almost exclusively on humans (Ponlawat and Harrington 2005; Delatte et al. 2010). The transmission of all four DENV serotypes is maintained in two cycles: sylvatic (transmission in wild animals) and human (Chen and Vasilakis 2011). The sylvatic cycle is ecologically and evolutionarily distinct from the human transmission cycle. This is maintained by non-human primates or by a monkey-*Aedes*-monkey cycle in the sylvatic environments of Southeast Asia, West Africa, peninsular Malaysia, and eastern Senegal (Rudnick 1986).

6. Detection of dengue

Detection of dengue infection may be done in two ways: laboratory diagnosis from a culture or blood sample and detection of anti-dengue antibodies in serum/plasma. DENV is found in serum, plasma, or circulating blood cells or tissue during the first 1 to 7 days, most appropriately during the period of fever. Virus or viral RNA can be isolated for detection within that period by reverse transcriptase real-time PCR (RT-Q-PCR) amplification or by conventional PCR, using appropriate oligonucleotide primers. Quantitative PCR (Q-PCR) can be used to quantify the viral load in body fluids. Serological detection depends on the demonstration of anti-dengue immunoglobulin M (IgM) antibodies or by non-structural protein 1 (NS-1) antigen in the serum/plasma of patients using either enzyme-linked immunosorbent assay (ELISA) or immune chromatographic-based rapid card tests (Fig. 6). The five basic serological tests that are more accurate in diagnosing dengue infection are hemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), IgM capture enzyme-linked immunosorbent assay (MAC-ELISA), and indirect IgG ELISA. The NS1 and IgM diagnostics are not fully reliable because of cross-reactivity with other flaviviruses (e.g., Zika virus) (Wellekens et al. 2020).

7. Pathophysiology of dengue fever

Dengue virus infection presents with a broad spectrum of clinical symptoms ranging from mild fever to severe physiological conditions. Initial infection with a particular serotype of dengue is known as a primary infection, which is usually asymptomatic or results in mild disease manifestations, known as dengue fever (DF) (Mathew and Rothman 2008).

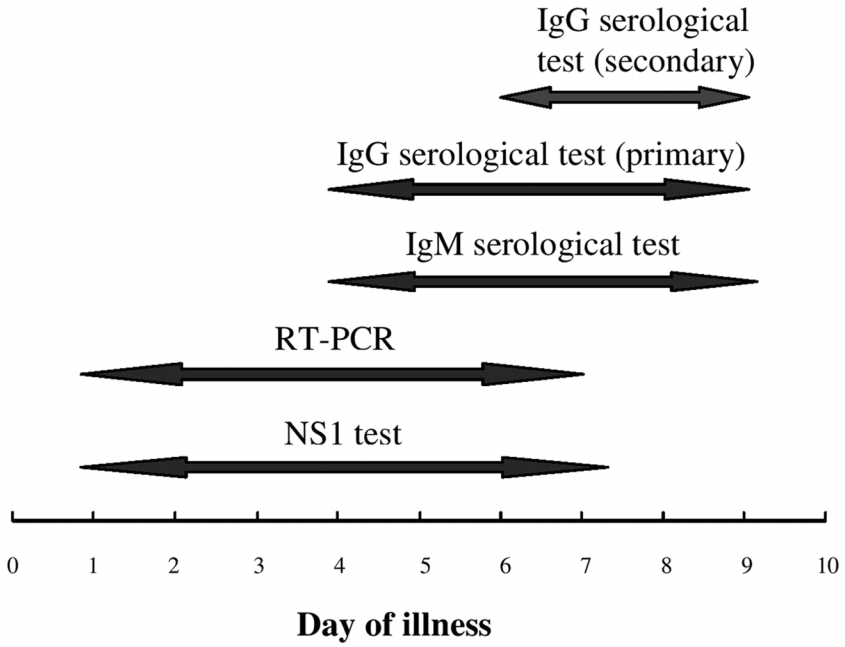
7.1. Dengue Fever (DF)

DF is an acute infectious disease symptom, characterized by biphasic fever, myalgia, headache, joint pain, retro-orbital pain, body rash, thrombocytopenia, lymphadenopathy, and leukopenia. Dengue fever has three distinct phases: febrile, critical, and convalescent. The febrile phase is characterized by a sudden high-grade fever and dehydration that can last for 2–7 days. The convalescence period is the recovery phase, when rash, itching, and increased appetite are observed.

7.2. Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)

DHF is a severe febrile disease characterized by hemostasis malfunction, increased vascular permeability, and severe increased vascular leakage that may result in DSS with shock. DSS is a form of hypovolemic shock that causes reduced peripheral perfusion, which can lead to tissue injury and multi-organ failure. Critical and convalescent phases are observed in the DHF. The

Fig. 6. Laboratory diagnosis of dengue with respect to time of illness.



criteria for diagnosing DHF as prescribed by the WHO are acute and continuous fever of 2–7 days, hemorrhage associated with thrombocytopenia (100 000 cells/cu. mm or less), and hemoconcentration (hematocrit >20% from baseline of patients of the same age).

8. Pathogenesis of DENV infection

8.1. Host immune response against DENV

DENV takes over the host's cellular machinery to access cellular resources in various ways to accomplish its replication and further spread. DENV faces a series of challenges at each step of its lifecycle from its entry in the release of mature virions (Morrison et al. 2012). DENV not only indirectly or directly escapes immune surveillance, but also specifically targets immune mediators to prevent intracellular antiviral signal transduction (Green et al. 2014; Kao et al. 2018).

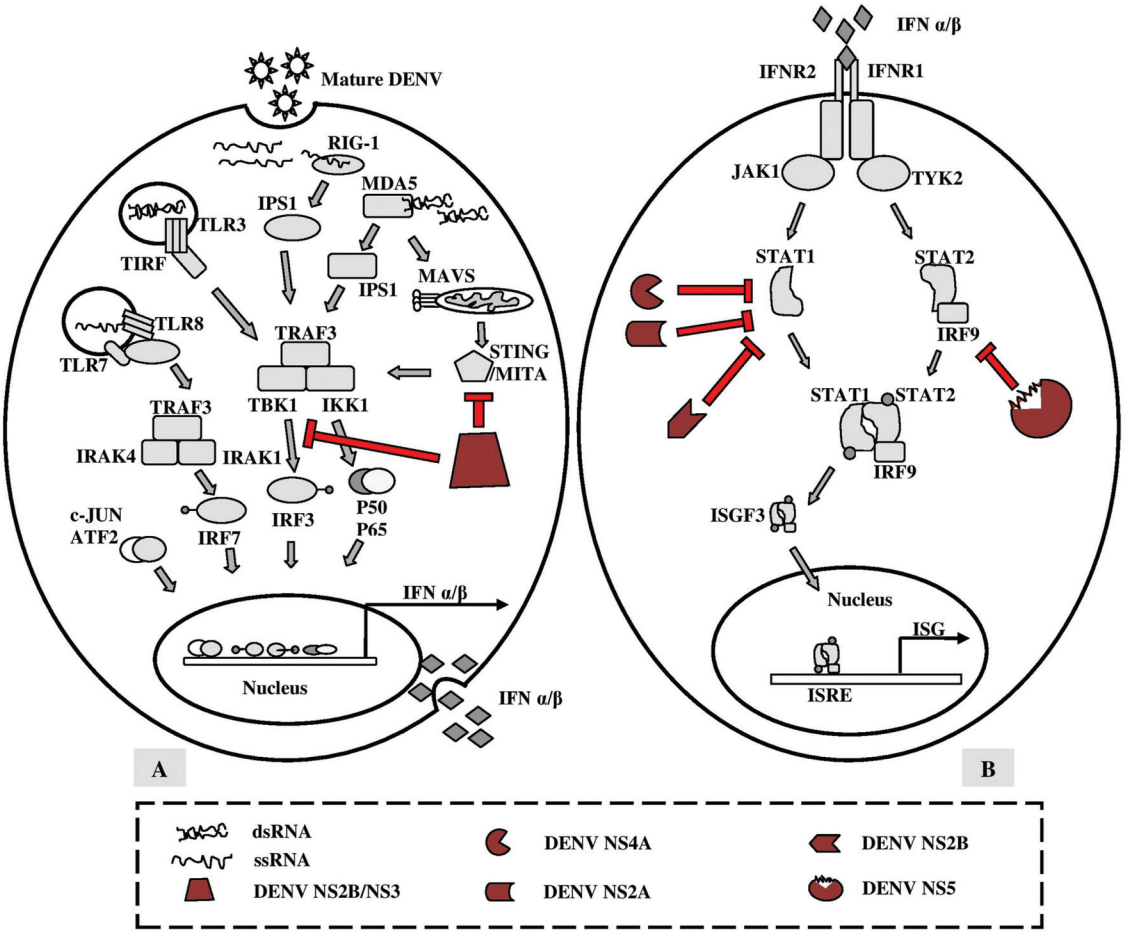
8.2. Innate immunity: first line of defense against DENV

Production of interferons (IFNs) is considered to be the first defense mechanism in DENV-infected cells (Rodenhuis-Zybert et al. 2010). DENV first infects interstitial dendritic cells (DCs) and activates the production of both type I and type II IFNs within hours (Shresta et al. 2004). IFNs are also produced by natural killer (NK) cells for virus clearance from the host body (Azeredo et al. 2006). Toll-like receptors (TLRs) also act as DENV sensors inside infected cells. Intracellular TLRs such as TLR3, TLR7, and TLR8 are mostly involved in the recognition of dengue viral RNA (Sariol et al. 2011). TLR3 recognizes DENV RNA after endosomal acidification and has been shown to induce strong interleukin-8 (IL-8) and interferon α/β (IFN-α/β) responses (Green et al. 2014). When DENV RNA is recognized by TLR3, TIR domain-containing adaptor-inducing interferon (TRIF) is phosphorylated and interacts with both TNF-receptor associated factor-3 (TRAF3) and TRAF6 (Fig. 7A). In association with TAK1, TRAF6 activates AP1 and dephosphorylates IKB, leading to the activation of NF-κB. On the other side, TRAF3 interacts with TANK-binding kinase 1 and IK kinase-1 (IKK1) resulting in the phosphorylation of IRF3. Finally, activated IRF3, AP-1, and NF-κB translocate to the nucleus and induce the transcription of IFN-α/β, interferon-stimulating genes (ISGs), and other cytokines (both interferons and chemokines) (Lee et al. 2012). Induction of IFN-α/β through TLR7/8/9 is also mediated by the adaptor molecule myeloid differentiation primary response protein 88 (MyD88) through the MAPK and NF-κB pathways (Kao et al. 2018).

Different cytoplasmic helicases, such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), recognize DENV RNA by their helicase domain to induce IFN-β production (Loo et al. 2008). After activation of RIG-I, it induces multiple ubiquitin E3 ligases such as TRAF3 and TRAF6 for translocation of transcription factors, such as IRF3, IRF7, and NF-κB, to activate the production of IFN-α/β (Liu et al. 2013).

IFN-α/β, secreted from DENV-infected cells, triggers a signal to nearby cells and inhibits DENV infection (Tremblay et al. 2019). IFN-α/β has a membrane-bound receptor (IFN-α/β receptor or IFNAR) that activates JAK-STAT signaling to stop DENV infection. Binding of IFN-α/β to its receptor phosphorylates adaptor molecules TYK2 and JAK1, which activate different signal transducers such as STAT1, STAT2, STAT3, and STAT5. These ultimately activate the interferon-stimulating gene factor 3 complex that translocate to the nucleus and binds to IFN-stimulated response elements located in the promoter region of IFN-stimulated

Fig. 7. (A) Dengue virus (DENV) entry and sensing of DENV by different PAMP and secretion of IFN from host DENV-infected cell. (B) Antiviral IFN response by host cell. DENV strategy against the host antiviral responses (A) and (B). [Colour online.]



genes (ISGs) (Fig. 7B). Thereafter, it results in the production of numerous antiviral proteins and pro-inflammatory cytokines (Green et al. 2014). It also induces alternative signaling cascades, including the mitogen-activated protein kinase p38 cascade and the phosphatidylinositol-3-kinase cascade that results in the production of pro-inflammatory cytokines and chemokines.

8.3. DENV strategy to interfere with host antiviral response

DENV infection causes extensive rearrangement of cellular membranes (e.g., unfolded protein response causes ER membrane expansion). DENV manipulates the cell to maintain host metabolism and protein production, while sequestering itself in vesicles that are not degraded by host lysosomes. This complex process involves a delicate balance of activating cellular pathways for ER expansion and inducing lipid metabolism while preventing ER stress-induced death. DENV uses autophagy to facilitate viral replication. Finally, DENV non-structural proteins act directly on components of the innate immune response signaling cascade, inhibiting the RNAi pathway and IFN-α/β induction/signaling. DENV interferes with RNAi pathways via two mechanisms that involve non-structural protein 4B (NS4B) and subgenomic flavivirus RNA (sfRNA). NS4B hampers Dicer activity, resulting in the inhibition of RNAi pathways. DENV sfRNAs inhibit the cleavage of dsRNA by Dicer by NS4B binding with RNase. DENV non-structural proteins interfere with host antiviral responses via different mechanisms. Viral NS5 protein forms 2′-*O*-methylation on the 5′-cap structure of the viral RNA, mimicking the host cellular mRNAs to evade the innate immune system (Tremblay et al. 2019). This mimicry also helps DENV to evade melanoma differentiation-associated gene 5 (MDA5), thus disrupting IFN induction (Dong et al. 2012). DENV NS4A can bind to MAVS CARD domains and effectively prevent host immune responses by interfering with the RIG-I/MAVS interaction (He et al. 2016). Suppression of the host antiviral IFN (type I) pathway can be achieved directly by DENV with the recruitment of viral NS2B-NS3 protease (Aguirre et al. 2012). DENV NS2A and NS4B inhibit TBK1/IKK-directed downstream signaling to regulate the innate immune response (Dalrymple et al. 2015). Viral NS2A, NS4A, and NS4B proteins together block STAT1 phosphorylation, nuclear translocation, and

transcriptional activation of ISGs (Fig. 7B). DENV NS5 protein binds to the STAT protein and acts as a bridge between UBR-4 and STAT2. This bridge directs the STAT2 protein for ubiquitination and ultimately proteasome-mediated degradation of STAT (Ashour et al. 2009). Finally, the ability of DENV to perturb the host's innate immune response may impact the adaptive immune response and modulate disease outcomes.

8.4. Adaptive immune response against DENV

In the case of the adaptive immune response, both cellular and humoral immunity develops after approximately 6 days of infection. After the recognition of DENV antigens, with the help of CD4+ T lymphocytes, antibodies are generated against DENV envelope (E) (domain III) and PrM glycoproteins of the surface of the virus (Gromowski and Barrett 2007; Lai et al. 2008). Experimental evidence indicates that, while non-structural proteins are recognized by CD8+ T cells, the structural proteins are preferentially recognized by CD4+ T cells (Rivino et al. 2013). DENV infection causes an adaptive immune response by activating naive CD4+ and CD8+ T cells to differentiate into effector T cells for the lysis of virus-infected cells or by the production of cytokines (Rothman 2011). Activated CD4+ cells respond to viral infection in two directions with the help of helper T cells: Th1 cell-mediated and Th2 cell-mediated. Th1 cells secrete mostly IL-2, IFN-γ, and TNF-β, which directly potentiate cell-mediated inflammatory responses and tissue injury by disrupting intracellular DENV through cell-death pathways. However, Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13, which help in specific T-cell proliferation and activation (Sun and Kochel 2013). As the NS1 protein is very important for DENV biology and present in patient's sera at high levels, the antibody against NS1 is also generated by B-cells. These NS1 soluble antibodies mediate the activation of the complement system to lyse DENV-infected cells. DENV NS1 protein activates macrophages and PBMCs by interacting with TLR4, leading to disruption of endothelial cell monolayer integrity in blood vessels (Modhiran et al. 2015). NS1 proteins from DENV-infected cells interact with TLR4 on the plasma membrane of platelets. This results in the increased expression of P-selectin (e.g., CD62P or platelet activation-dependent granule membrane protein) that induces apoptotic pathways and the destruction of platelets (Chao et al. 2019). DENV NS1 antibodies can stimulate the release of multiple inflammatory factors in an NF-κB-dependent manner (Lin et al. 2005). The unbalanced release of different cytokines is considered a major factor underlying the pathogenesis of DHF. Viral epitopes expressed on the surface of infected cells interact with memory T cells and induce the production of pro-inflammatory cytokines that affect the vascular endothelium, resulting in plasma leakage. Elevated levels of IL-2R, soluble CD4, and soluble CD8 lead to disease severity in DHF and DSS (Kurane et al. 1991).

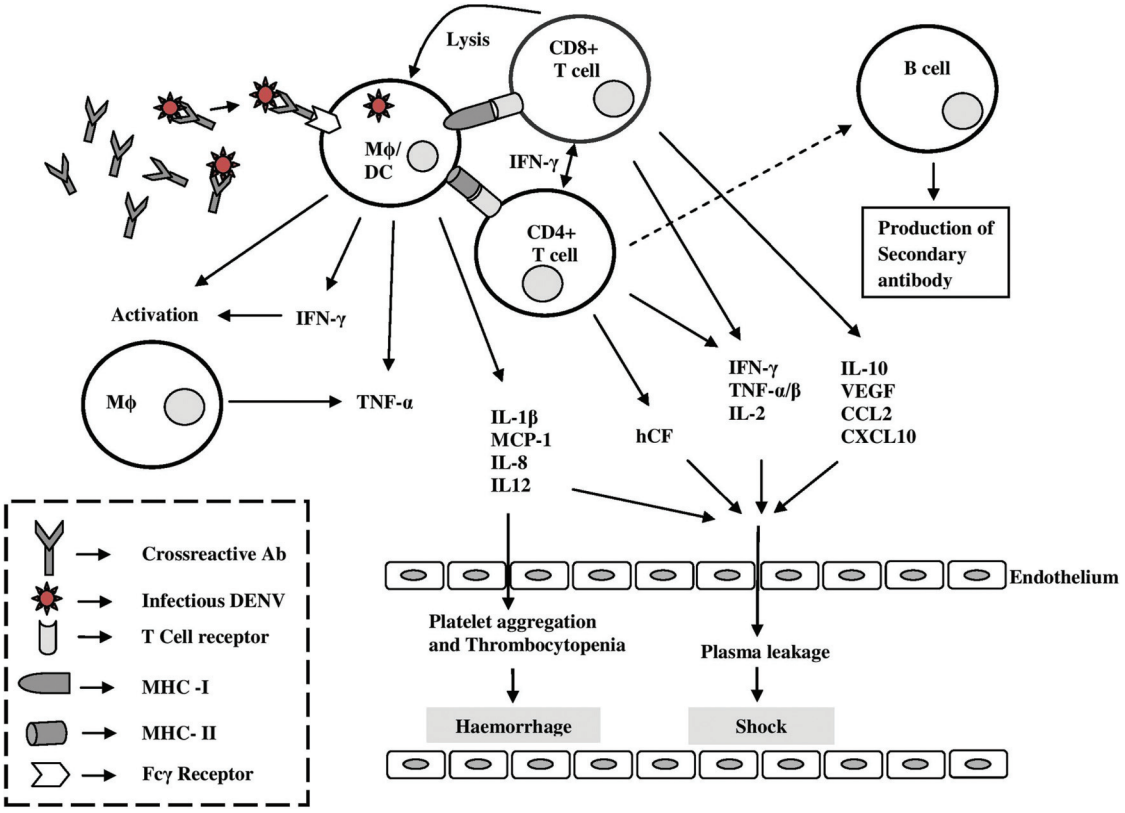
8.5. Antibody-dependent enhancement (ADE)-induced pathogenesis

DENV infection by one serotype provides long-lasting protection against that specific serotype (homotypic immunity) and short-lived protection against other serotypes (heterotypic immunity). Virus-neutralizing antibodies are believed to be the basis for homotypic immunity. However, over time, these antibody concentrations decline, and the individual is then susceptible to infection with other DENV serotypes. Cross-reactive antibodies, present at the time of secondary dengue virus infection, bind to virions without neutralization and then enhance the entry of the virus into monocytic cells (Mϕ) that express immunoglobulin receptors (FcγR) on its membrane. This phenomenon is termed antibody-dependent enhancement (ADE) of infection that occurs during secondary dengue virus infections (Rothman and Ennis 1999).

Serotype cross-reactive memory CD4+ and CD8+ T lymphocytes recognize viral antigens in the context of class I and class II HLA molecules. These activated dengue-specific cross-reactive T lymphocytes (both CD4+ and CD8+) produce high levels of IFN-γ, IL2, and TNFα, which induce capillary leakage through multiple direct and indirect effects on the vascular endothelium. IFN-γ also induces viral antigen presentation to T lymphocytes by enhancing HLA class II expression, which increases antibody-enhanced uptake of DENV by monocytes. Activated DENV-specific T lymphocytes also target virus-infected cells for lysis by perforin and granzyme pathways (Rothman and Ennis 1999). Infection of THP-1 cells with DENV-immune complexes results in the downregulation of IL-12, IFN, and NO production, and enhanced expression of IL-6 and IL-10, indicating that FcR-mediated entry suppresses the antiviral immune response, thereby promoting virus particle production (Chareonsirisuthigul et al. 2007).

During DHF or DSS, cytokines and immune mediators such as TNFα (Hober et al. 1993), IL-1 and IL-2 (Kurane et al. 1991), IL-10 (Green et al. 1999), vascular endothelial growth factor/VEGF, CCL2, and CXCL10 (Srikiatkhachorn et al. 2017), MCP-1 (Bozza et al. 2008), and IFN-γ and IFN-α (Chakravarti and Kumaria 2006) are elevated compared to mild DF (Suharti et al. 2003), a condition referred to as cytokine storm, caused mainly by hyperactivation of Th2 cell response compared to Th1 cell responses. The hyperactivation of Th2 cell responses induces unbalanced production of cytokines and chemical mediators by activated immune cells. Overproduction of IL-10 can suppress IFN signaling to increase DENV replication, resulting in an increase in viral titers in severe dengue cases (DHF) (Tsai et al. 2013). IL-10 also has a role in inhibiting pro-inflammatory cytokines to damage the endothelium for increased plasma leakage (Abhishek et al. 2017) (Fig. 8). Activated helper T cells (CD4+) secrete human cytotoxic factors (hCFs) that stimulate immune cells such as macrophages to generate free radicals (reactive nitrogen) that activate Th2 cell responses and cause

Fig. 8. Antibody-dependent enhancement (ADE) causes severe plasma leakage. [Colour online.]



increased vascular permeability and severe plasma leakage (Chaturvedi et al. 2000). The complement cascade might be activated in DHF by immune complexes formed by circulating DENV and DENV-specific antibodies. Antibody levels rise more rapidly in secondary dengue virus infections; thus, high antibody titers are reached before viremia disappears, increasing the potential for immune complex formation. All these result in the disruption of endothelial cells, which leads to increased plasma leakage and severe disease symptoms (Fig. 8).

9. Treatment and management

The treatment for dengue virus infection mostly involves the use of tepid sponging for fever and antipyretics for pain or fever management. No specific antiviral drug is available against dengue, but several sulfated polysaccharides extracted from seaweeds have been studied, and high antiviral activity against DENV has been observed (Damonte et al. 2004). Two polysaccharide compounds from two different seaweeds, kappa/iota/nu carrageenan G3d and the dL-galactan hybrid C2S-3, showed antiviral activity against all serotypes of DENV by interfering with virus internalization inside the host cell by inhibiting host cell receptors (heparan sulfate) (Talarico et al. 2005). Curdlan, a sulfated polysaccharide, also showed an inhibitory effect on DENV by interacting directly with the viral E protein and altering E protein structure to restrict ADE and pathogenicity of the virus in the host body (Ichiyama et al. 2013). A polysulfated fraction from the coenocytic green seaweed *Caulerpa cupressoides* can also inhibit DENV-1 infection pathogenicity in vitro (Rodrigues et al. 2017). Ribavirin (a guanosine analog) with a combination of other nucleotide analogs (brequinar, INX-08189) has been shown to inhibit nucleoside biosynthesis, thus reducing DENV activity in the host cell (Patkar and Kuhn 2006; Yeo et al. 2015). Glycyrrhizin and its derivatives or modified products induce antiviral activity by affecting the secretion of interferons against DENV and inhibiting DENV protein transport and post-translational modifications (Baltina et al. 2019). The uridine analogue 6-azauridine inhibits de novo pyrimidine synthesis and DNA synthesis and is converted intracellularly into mono-, di-, and triphosphate derivatives, which are incorporated into RNA and inhibit protein synthesis (NCBI PubChem Database 2021). One analog of nucleoside adenosine, NITD008, has shown antiviral effects against DENV as well as against all other flaviviruses both in vitro and in vivo (Yin et al. 2009). Recently, an experimental antiviral drug, curcumin (from turmeric) and its derivatives, was assessed to determine its anti-dengue activity. Curcumin {1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione} and its analogs, such as bis-demethoxy curcumin (CC2), acyclic analog (CC3), and cyclohexanone analog (CC5) showed efficacy in inhibiting DENV replication to prevent severe

infection (Balasubramanian et al. 2019). A few other experimental antiviral treatments using CP26, CDDO-me, UV-4B, ivermectin, and ketotifen are in trial and are also facing great challenges in controlling dengue (Wellekens et al. 2020).

10. Development of vaccine

The development of a DENV vaccine has become a priority in the absence of effective and sustained control of the vectors. The complex pathogenesis and ADE effect of DENV along with genomic alterations over time are the main obstacles for the development of a vaccine. DENV has a high average mutation rate of 10^{-3} to 10^{-5} substitution/nucleotide/round of replication, which might result in the emergence of a new lineage of viruses over time (Dolan et al. 2021). For example, the emergence of a new lineage of DENV-3 during 2006–2008 and the cosmopolitan genotype of DENV-2 in India in 2011 have been reported (Harapan et al. 2020). This type of reemergence and replacement of new genotypes of DENV is responsible for severe outbreaks of dengue, which might become a great challenge for vaccine development.

The most effective and trustworthy way to evaluate the basic immunology for the development of vaccines against DENV infections is the use of animal models (Shresta et al. 2006). Mice are the most commonly used animal model before testing in non-human primates. It was recently reported that Asian rhesus macaques (Indian origin) might be a good animal model for dengue hemorrhagic fever (Onlamoon et al. 2010). One mouse model (AG129) has been established, showing both mild and severe symptoms and clinical features of dengue (Sarathy et al. 2018), which can be used both in the trial of a candidate vaccine and in the discovery of antiviral drugs. Some genetically engineered or transgenic mouse dengue models have also been established for research purposes (C57BL/6J hTNF+++, IFN-α/βR −/− Tg, Tg HLA-A*02:01, and B10.Tg HLA-DR3) (reviewed in Coronel-Ruiz et al. 2020). *Tupaia belangeri* (northern treeshrew) fibroblast cells have been reported to show permissibility and susceptibility to DENV infection and replication (Bustos-Arriaga et al. 2011).

The tetravalent vaccine formulation, which is the most developed attenuated vaccine candidate, has undergone repeated phase I trials in the United States. Recent molecular technologies for the development of an alternative vaccine for DENV include the use of inactivated whole-virion vaccines, synthetic peptides, subunit vaccines, vector expression, recombinant live vector systems, infectious cDNA clone-derived vaccines, and naked DNA (Gubler 1998; Blaney et al. 2004). Three live-attenuated tetravalent DENV vaccine candidates currently being evaluated in large clinical trials are the Sanofi Pasteur CYD-TDV (Dengvaxia) candidate, DENVax, and TV005 vaccines (Diamond and Pierson 2015). The latter two are currently being tested in phase III trials (Prompetchara et al. 2019).

The tetravalent chimeric yellow fever virus-DENV (CYD) vaccine is the first licensed dengue vaccine that has recently been approved for clinical use in Mexico, Thailand, Brazil, El Salvador, and Costa Rica (Aguiar et al. 2016; Prompetchara et al. 2019). It was developed by Sanofi Pasteur (Mexico) based on a yellow fever (YF) 17D vaccine virus backbone, chimerized with prM and E proteins from DENV1-4 replacing the YF prM and E (Guy et al. 2015), and is currently registered in the European Union, United States, and 20 other dengue-endemic countries (Thomas and Yoon 2019). However, Brazil and the Philippines are the only two countries where vaccination implementation occurs against dengue (Thomas and Yoon 2019). It was first licensed in specific doses only for 9–45 years or 9–60 years aged people, living in dengue-endemic areas. CYD-TDV also had a favorable safety profile and elicited antibody responses against all four dengue serotypes in 9–16-year-olds in Latin America (Villar et al. 2013). In 2016, the WHO recommended the implementation of Dengvaxia under specific medical supervision, only for patients aged 9 years and over, but not for seronegative patients. In 2019, the US FDA approved Dengvaxia in the US territories of American Samoa, Guam, Puerto Rico, and the US Virgin Islands as the first vaccine approved for the prevention of dengue disease caused by all dengue virus serotypes (1, 2, 3, and 4) only for people from the age of 9 to 16 and those living in dengue-endemic areas and having confirmed dengue infection (Thomas and Yoon 2019).

The other promising tetravalent recombinant live-attenuated dengue vaccine is DENVax, originally developed by the Centers for Disease Control and Prevention of the USA (CDC, USA) in cooperation with Inviragen, now licensed to Takeda (Osaka, Japan). TAK-003 showed antibody responses against all four serotypes, up to 48 months after vaccination, without any risk of severity in the case of baseline seropositive or seronegative individuals (Tricou et al. 2020). In a phase III trial, when assessed in children aged 4–16 years, TAK-003 showed high efficacy in cases of severe, hospitalized patients and seropositive and seronegative individuals (Biswal et al. 2020). However, it showed different immunogenicity depending on the infection by serotypes of DENV, although the reason for this is not yet apparent. TAK-003 worked (80.6% efficacy) against DENV-1, DENV-2 (most efficient), and DENV-3 in participants with both previously seropositive and seronegative status (Biswal et al. 2019). However, there have not yet been conclusive results regarding its effectiveness against DENV-4 infections.

Another tetravalent vaccine candidate (TV005) (NIAID, USA) contains a mixture of modified full-length and chimeric DENV strains and is based on directed mutagenesis, inducing attenuation without losing immunogenicity (Prompetchara et al. 2019). Mutations at the 3′ end were induced in DENV-1, DENV-3, and DENV-4 strains, and a DENV-2/4 chimera was made using the DENV-4 backbone

with DENV-2 prM and E, replacing the DENV-4 prM and E (Whitehead 2016). Before TV005, TV003 was prepared by combining four vaccine candidates: rDEN1D30, rDEN2/4D30, rDEN3D30/31, and rDEN4D30. A single dose of TV005 has been shown to be sufficient to elicit a neutralizing antibody response against all four DENV serotypes in 90% of recipients (Kirkpatrick et al. 2015). There are a few other vaccines, such as the V180 vaccine (DEN1-80, Hawaii Biotech) and D1ME100 DNA vaccine, which are currently under investigation (Wellekens et al. 2020).

In India, the live-attenuated tetravalent vaccine TV003/TV005 has been licensed for clinical development and commercialization by the three well-established vaccine manufacturers Panacea Biotec, Serum Institute, and Biological E (Swaminathan and Khanna 2019). Pancacea Biotech has received approval from the Indian National Regulatory Authority for conducting human trials on monovalent vaccines. Phase I and II trials have been planned to evaluate the safety, reactogenicity, and immunogenicity of Tetra-Vax-DV (TV-003/TV-005) in northern and southern India (Swaminathan and Khanna 2019). After the trial of the vaccine, Panacea Biotech has tentatively scheduled its commercial launch in 2020. The Serum Institute of India is currently conducting preclinical toxicity studies for the development of TetraVax-DV. Recently, the Department of Biotechnology (DBT) under the Indian Ministry of Science and Technology, the Department of Health Research/Indian Council of Medical Research (DHR/ICMR) of the Indian Ministry of Health and Family Welfare, and the National Institute of Allergy and Infectious Diseases (NIAID) of the U.S. National Institutes of Health, Department of Health and Human Services, decided to prioritize collaborative research on promising dengue vaccine candidates.

11. Conclusion

Dengue has become a serious life-threatening burden for humans, and its incidence has been increasing day-by-day as there is presently no highly effective vaccine to control the severity of dengue by all serotypes. There is no consensus on how an individual is protected from the dengue virus. The pathogenesis of DHF and DSS is complicated and multifactorial, involving both viral and host factors. Vaccines against dengue virus infection should be inexpensive, as the majority of countries experiencing outbreaks are economically challenged. The unique complexity of DENV pathogenesis and its relationship to immune enhancement are the main hindrances for the development of an efficient dengue vaccine, although some live-attenuated tetravalent vaccines are licensed for dengue and some are in the trial stage. This calls for further research toward understanding host genetics and soluble proteins or factors such as cytokines, including chemokines, which affect the susceptibility or extent of protection against dengue virus infection. The Pediatric Dengue Vaccine Initiative (PDVI) and the WHO have collaborated to characterize antibody responses to distinguish between neutralizing and potential viral-enhancing features. Current research is focused on the development of live-attenuated, DNA, viral vector-based tetravalent vaccines. Another important part of the research should be the development of a suitable animal model for dengue infection. We should also focus on the management of vector control strategies, as there are a limited number of therapeutic drugs and effective vaccines against dengue. Dengue also spreads drastically because of anthropogenic activities, such as water retention in plastic, metal drums, and cement tanks, which increases the breeding of dengue infectious mosquitoes. Dengue vector mosquito control management should be performed through environmental, chemical, and biological management approaches. These approaches should target areas of high human–vector contact to minimize the transmission of the dengue virus. Insecticide-treated curtains and new mosquito traps have also shown promise for the reduction of dengue virus infections. Another component for dengue prevention is surveillance, which provides the necessary information for risk assessment and program guidance. Data collection regarding the infected DENV serotypes or genetic sequences and the correlation of mild/severe illness due to primary or secondary infection with the circulating serotype in epidemic areas is required to predict the epidemiology.

Conflict of interest statement

Authors declare there is no conflict of interest.

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