

Dengue virus life cycle: viral and host factors modulating infectivity

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Abstract Dengue virus (DENV 1–4) represents a major emerging arthropod-borne pathogen. All four DENV serotypes are prevalent in the (sub) tropical regions of the world and infect 50–100 million individuals annually. Whereas the majority of DENV infections proceed asymptomatically or result in self-limited dengue fever, an increasing number of patients present more severe manifestations, such as dengue hemorrhagic fever and dengue shock syndrome. In this review we will give an overview of the infectious life cycle of DENV and will discuss the viral and host factors that are important in controlling DENV infection.

Keywords Dengue · Flavivirus · Life cycle · Immune response · Pathogenesis · Host factors · Virulence · Antibody-dependent enhancement

Introduction

Dengue is the most common arthropod-borne viral infection in the world [1, 2]. The disease is endemic in more than 100 countries throughout Africa, the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific. There are four distinct serotypes of dengue virus (DENV) and each of these serotypes can cause disease symptoms ranging from self-limited febrile illness called

dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2–4]. Infection with one serotype confers protective immunity against that serotype but not against other serotypes. In fact, several retrospective and prospective studies have revealed that secondary infection with a heterologous serotype is a risk factor for developing DHF/DSS [4–8]. Also, infants born to dengue-immune mothers are at risk to develop more severe dengue during a primary infection [9, 10]. This suggests that antibodies play an important role in controlling the outcome of an infection. It is believed that antibodies specifically direct the virus particles to cells carrying Fc-receptors (FcR), such as monocytes, macrophages, and dendritic cells, which—as the natural targets for the virus—are permissive for DENV infection. This leads to enhanced infection of these cells and thus, to high viral loads, resulting in extensive T cell activation early in the infection process. As a consequence, high amounts of cytokines and chemical mediators are released, which may lead to endothelial cell damage and subsequent plasma leakage. Other factors that are implicated in disease pathogenesis include viral virulence, the ethnic background and age of the individual, and specific epidemiological conditions [11–15]. In this review we will give a general overview of the infectious life cycle of DENV, and describe the viral and host factors that may influence disease outcome.

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Dengue virus life cycle

Virion structure

DENV is an enveloped positive-strand RNA virus belonging to the *Flaviviridae* family [16, 17]. Mature

virions contain three structural proteins, the capsid protein C, membrane protein M, and the envelope protein E. Multiple copies of the C protein (11 kDa) encapsulate the RNA genome to form the viral nucleocapsid [18–20]. The nucleocapsid is surrounded by a host-cell-derived lipid bilayer, in which 180 copies of M and E are anchored. The M protein is a small (approx. 8 kDa) proteolytic fragment of its precursor form prM (approx. 21 kDa). The E protein is 53 kDa and has three distinct structural domains (Fig. 1) [21–24]. Domain I is structurally positioned between domain II, the homodimerization domain, and the immunoglobulin-like domain III.

Structural analysis of mature DENV virions revealed that the virus possesses an icosahedral envelope organization and a spherical nucleocapsid core [25]. In mature virions, E is organized as 90 head-to-tail orientated homodimers, which lie in sets of three nearly parallel to each other and to the viral surface, forming a smooth “herringbone” configuration. As a result, DENV virions lack a true $T = 3$ symmetry, which means that the three E monomers present in each icosahedral asymmetric unit exist in three chemically distinct environments and may therefore play a distinct role in different stages of the infection [25–27]. The infectious life cycle is depicted in Fig. 2 and will be discussed in detail below.

Replication and assembly of dengue virus particles

A schematic representation of the DENV genomic RNA and the translation of the viral proteins are depicted in Fig. 3. After virus cell entry (see below) and uncoating of the nucleocapsid, the RNA molecule is translated as a single polyprotein (see [28], for a great review on this topic). During this process, the signal- and stop-transfer sequences of the polyprotein direct its back-and-forth translocation across the endoplasmic reticulum (ER) membrane. The polyprotein is processed co- and post-translationally by cellular and virus-derived proteases into three structural proteins (C, prM, and E) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The E protein is glycosylated at amino

acid residue Asn67 and Asn153 to assure proper folding of the protein [23, 29]. Other potential N-linked glycosylation sites are located in prM at position 7, 31, and 52 and within NS1 at position 130 and 207 [30, 31]. Upon protein translation and folding of the individual proteins, the NS proteins initiate replication of the viral genome [28]. The newly synthesized RNA is subsequently packaged by the C protein to form a nucleocapsid. The prM and E proteins form heterodimers that are oriented into the lumen of the ER. Then, the prM/E heterodimers associate into trimers and these oligomeric interactions are believed to induce a curved surface lattice, which guides virion budding [25, 26]. It is unclear how this is synchronized with the engulfment of the nucleocapsid since no specific interactions between C and prM/E proteins have been identified yet [32, 33]. Interestingly, encapsulation of the nucleocapsid during virus assembly is not crucial as the formation and release of capsidless subviral particles has been often documented [34–37].

Structural analysis of newly assembled immature virions revealed that a single virion contains 180 prM/E heterodimers that project vertically outward from the viral surface as 60 trimeric spikes [32, 33, 38]. The immature particles formed in the ER mature as they travel through the secretory pathway. The slightly acidic pH (~ 5.8 – 6.0) of the trans-Golgi network (TGN) triggers dissociation of the prM/E heterodimers, which leads to the formation of 90 dimers that lie flat on the surface of the particle, with prM capping the fusion peptide of the E protein. This global structural reorganization of the glycoproteins enables the cellular endoprotease furin to cleave prM [39–41]. Furin cleavage occurs at a Arg-X-(Lys/Arg)-Arg (where X is any amino acid) recognition sequence and leads to the generation of membrane-associated M and a “pr” peptide. A recent study has shown that the pr peptide remains associated with the virion until the virus is secreted to the extracellular milieu [40]. Both the prM protein as well as the pr peptide are believed to act as chaperones stabilizing the E protein during transit through the secretory pathway, thereby preventing premature conformational changes of the E protein that would lead to membrane fusion. Upon dissociation of the pr peptide, mature virions are formed that are able to infect new cells.

Receptor interaction and viral entry

During natural infection, cells of the mononuclear phagocyte lineage [monocytes (MO), macrophages (MØ), and dendritic cells (DCs)], including the skin-resident Langerhans cells, are primary targets for DENV infection [42, 43]. In insects, DENV was found to initially infect the midgut from where it spreads and replicates in many body compartments and organs [44–47]. Also, DENV has been

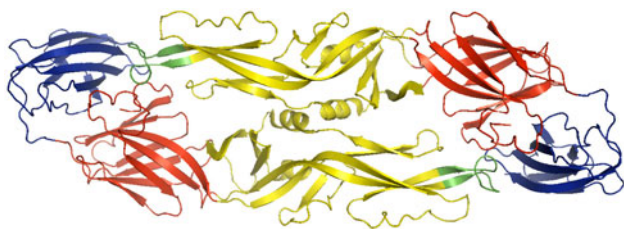


Fig. 1 Dengue E protein dimer with three defined domains within each monomer: domain I in red, domain II in yellow with fusion loop in green, and domain III in blue (23). The image was prepared using the program PyMol Molecular Graphics System

Fig. 2 Life cycle of Dengue virus. See text for details. Adapted from H. M. van der Schaar

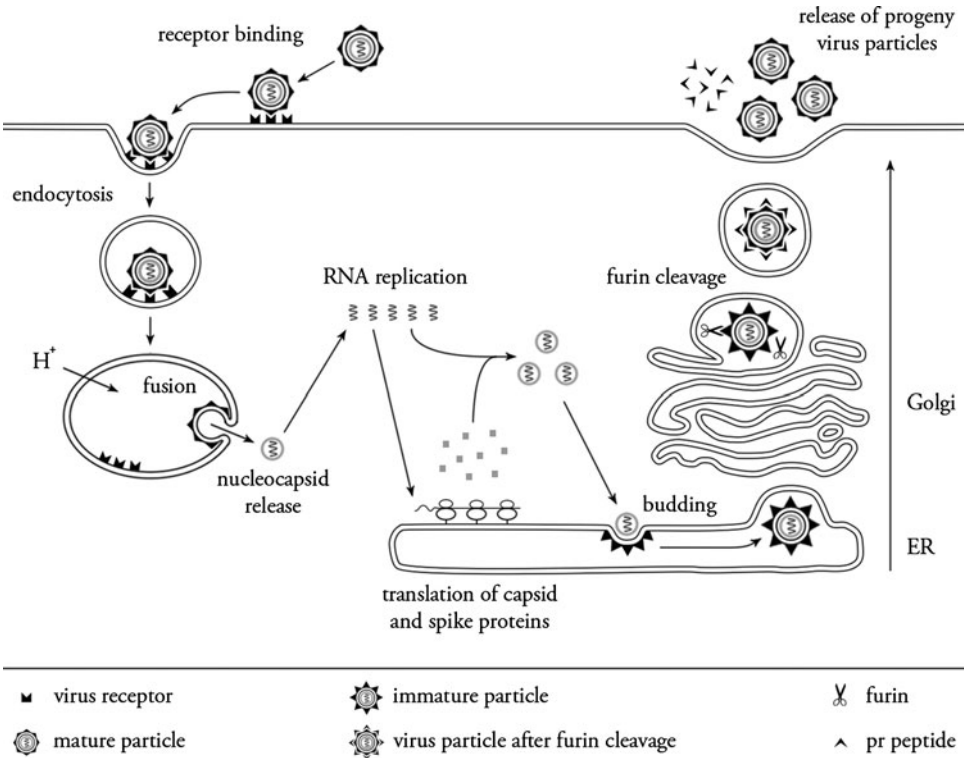
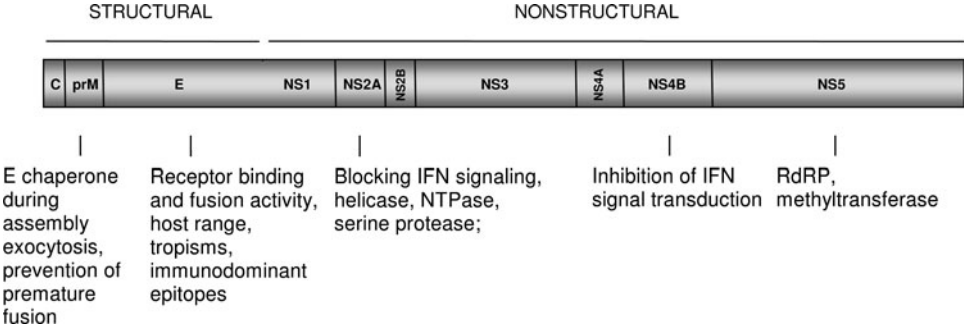


Fig. 3 Schematic representation of Dengue virus genome. See text for details



shown to infect numerous cell lines, including human (K562, U937, THP-1, HepG2, HUVEC, ECV304, Raji, HSB-2, Jurkat, LoVo, KU812), mosquito (C6/36), monkey (Vero, BS-C-1, CV-1, LLC-MK2), hamster (BHK), as well as murine MØ (Raw, P388D1, J774) cell lineages [39, 48–61]. The wide range of DENV-permissive cells indicates that the virus must bind to an ubiquitous cell-surface molecule, or exploit multiple receptors to mediate infection. Over the last decade, several candidate receptors and/or attachment factors have been identified, which suggests that DENV is capable of utilizing multiple molecules to enter the cell. In mosquito cells, DENV has been shown to interact with heat-shock protein 70 (Hsp70) [62], R80, R67 [63], and a 45-kDa protein [64]. Heparan sulfate [65–67], Hsp90 [62], CD14 [68], GRP78/BiP [69], and a 37/67-kDa high-affinity laminin receptor [70] have been identified as receptors on mammalian cells. C-type lectin receptors

(CLR) are involved in the interaction of DENV particles with human myeloid cells [71]. These include DC-specific intracellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN, CD209) [72–74], mannose receptor (MR) [75] and C-type lectin domain family 5, member A (CLEC5, MDL-1) [52]. DENV [76–78] as well as other flaviviruses [79, 80] use clathrin-mediated endocytosis for cell entry. Using a single-particle tracking approach, we have revealed that DENV-2 strain S1 particles land on the cell surface and migrate in a diffusive manner toward a pre-existing clathrin-coated pit [77]. This suggests that DENV particles move along the cell surface by rolling over different receptors, or migrate as virus–receptor complexes. Upon internalization, the particles are delivered to Rab5-positive early endosomes, which mature into Rab7-positive late endosomes, where membrane fusion primarily occurs [77].

A recent report also demonstrated that DENV, depending on the serotype and/or the target cell type used, is able to utilize an alternative internalization pathway, independent of clathrin, caveolae, and lipid rafts [81]. The subcellular organelle from which membrane fusion occurs is most likely dependent on the pH-dependent membrane fusion properties of the virus and may therefore vary between individual DENV strains [77, 78].

Numerous functional and structural studies have been undertaken to unravel the molecular mechanisms involved in the membrane fusion process of the virus [27, 82–85]. It is postulated that the acidic pH in endosomes triggers dissociation of E homodimers, which then leads to the outward projection of domain II and exposure of the hydrophobic fusion peptide to the target membrane [86]. Subsequently, the hydrophobic residues in the fusion loop would insert into the outer leaflet of the target membrane, triggering the assembly of E trimers. Next, domain III is assumed to shift and fold back toward the fusion peptide into a hairpin-like conformation. This folding-back mechanism would force the target membrane and the viral membrane to bend towards each other and eventually to fuse, releasing the nucleocapsid into the cell cytosol.

Immune response to DENV: a crucial determinant in the outcome of infection

Innate immunity

The first line of defense against invading DENV is the production of interferons (IFNs). Upon a mosquito bite, DENV first infects interstitial DCs which, within hours of infection, initiate production of type-I IFNs. Not only DCs but the majority of DENV-infected cells produce IFNs. Both type I (α , β) and type II (γ) IFNs have been found to be crucial for protection against DENV infection in vivo and in vitro [52, 87–90]. Furthermore, early activation of natural killer (NK) cells, the main producers of IFN γ , appears to be important in clearing DENV infection [91, 92].

The production of IFN is initiated upon virus interaction with pathogen-recognition receptors (PRRs), e.g., C-type lectins [71] and toll-like receptors (TLRs) that are expressed on the myeloid cells [93, 94]. C-type lectins such as DC-SIGN, MR, and CLEC5, but also TLR3 and TLR 7, have been reported to participate in the induction of an innate response upon DENV infection [52, 95, 96]. Activated PRRs convey their signal through several transcription factors, which ultimately induce the expression of IFN. Secreted IFN binds to IFN receptors present on the same cells as well as on neighboring cells. This activates the JAK/STAT pathway leading to the expression

of more than 100 effector proteins [97–100]. Both STAT-1-dependent and STAT-1-independent pathways have been implicated in the IFN-mediated response to DENV infection [51, 52, 101, 102]. IFN-mediated responses induce an antiviral state and initiate a variety of processes including metabolic control to limit virus infection. Moreover, these responses promote the adaptive immune response through stimulation of DC maturation and by direct activation of B and T cells [103].

While the importance of the innate immune response in controlling DENV infections is clear, several studies have demonstrated that DENV is able to inhibit the IFN α -mediated innate antiviral response [88, 104–106]. In particular, viral NS2A, NS4A, NS4B, and NS5 are thought to block IFN signaling by reducing STAT activation [88, 104, 106]. Interestingly, the ability of DENV to suppress type I IFN response has been shown to be strain-dependent, as within each serotype, both non-suppressive and suppressive strains that block STAT1/STAT2 pathways could be found [107]. Reduction of the IFN α -mediated response does not however correlate with disease progression to DHF, as no significant differences were found in IFN α levels between DENV isolates associated with DF and those associated with DHF [108].

Antibody response

Human humoral immunity develops approximately 6 days after a bite from a DENV-infected mosquito. The antibody response is mainly directed against the E and prM glycoproteins present on the surface of the virus [54, 109, 110]. NS1 antibodies are also generated, as this protein is expressed on the surface of infected cells and is secreted from these cells as a soluble factor [31, 110–112]. Antibodies against NS1 have been shown to activate complement-mediated lysis of DENV-infected cells and protect mice from DENV challenge [113–116]. Antibodies directed against E and prM antibodies were observed to directly influence the infectious properties of DENV particles. Antibodies can both neutralize and enhance DENV infectivity in vivo and in vitro and thus appear to play a dual role in controlling DENV virus infection [55, 56, 117–119].

Antibody-mediated neutralization of infection

Neutralization of infection occurs via a multiple ‘hit’ phenomenon. This means that virus inactivation occurs only when the number of antibodies docked on the virion exceeds a certain threshold [54, 120]. In case of strongly neutralizing antibodies, binding of only a small fraction of the accessible epitopes is required for neutralization. In contrast, weakly neutralizing antibodies that bind to poorly

accessible sites on the virion may require full occupation to achieve neutralization. For DENV as well as other flaviviruses, the most potent neutralizing antibodies are strain-specific and directed against domain III of the E protein [121–123]. Cross-reactive and weaker neutralizing antibodies were observed to predominantly localize to domain II near or within the fusion peptide [110, 124]. Notably, the human antibody response is dominated by antibodies directed against domains I and II.

In vitro studies have shown that neutralizing antibodies block attachment of the virus to its natural receptor on the cell surface and/or inhibit subsequent steps in the entry process of DENV [125–127]. However, inhibition of a virus binding to a cellular receptor will not result in neutralization of infection per se as virus-immune complexes can be internalized by cells carrying Fc receptors (FcR), including dendritic cells and macrophages. This process of FcR-mediated uptake may thus result in delivery of the virus into acidic compartments of the endosomal/phagosomal pathway, in a manner very similar to uptake of DENV after interaction with its natural receptor. Therefore, we believe that potent neutralizing antibodies should act downstream of virus–receptor interaction at the cell surface. A detailed structural and functional analysis of the potent neutralizing WNV antibody E16 has indeed shown that infection is blocked at the stage of membrane fusion presumably by inhibiting the conformational changes of the E protein required for membrane fusion [126].

Antibody-mediated enhancement of infection

Epidemiological studies have demonstrated that secondary infection with a heterologous serotype or primary infection of infants born to dengue immune mothers significantly increases the risk to develop severe disease. These clinical observations have led to the widely accepted hypothesis of antibody-dependent enhancement (ADE) of disease [4, 128, 129]. As indicated above, it is believed that antibodies specifically direct the virus particles to cells carrying FcR, such as monocytes, macrophages and dendritic cells, which—as the natural targets for the virus—are permissive for DENV infection, resulting in a higher virus burden and eventually enhancement of disease.

Studies with E-specific antibodies suggest that when virion opsonization occurs at the occupancy that does not exceed the threshold required for virus neutralization, these antibodies may enhance the efficiency of virus attachment to the cell surface and facilitate entry of virions via FcR-mediated endocytosis [120, 129]. The molecular mechanism underlying the ADE phenomenon remains to be identified [127]. Uptake of DENV-immune complexes via FcR-mediated entry may not only lead to a higher number of infected cells, it can also influence the number of virus

particles produced per infected cell. A recent study showed that infection of THP-1 cells with DENV-immune complexes resulted in downregulation of production of IL-12, IFN- γ , TNF- α , and NO, and enhanced expression of IL-6 and IL-10, indicating that FcR-mediated entry suppresses the antiviral immune response, thereby promoting virus particle production [51].

Not only E antibodies but also prM antibodies have been implicated in the phenomenon of ADE [56, 119, 130, 131]. Remarkably, we recently observed that prM antibodies have the capacity to render essentially non-infectious fully immature particles nearly as infectious as wild-type virus [119]. We found that prM antibodies enhance the infectivity of immature virions but have no promoting effect on the number of progeny virions produced per infected cell. The potential role of immature DENV particles in disease pathogenesis is further discussed in the section about virus virulence.

T cell response

Little is known about the role of CD8+ T cells in protection or enhancement of disease. Both serotype-specific and cross-reactive CD8+ T cells that are cytolytic and produce IFN- γ and TNF- α have been detected in DENV-immune individuals [132–136]. Studies on the role of CD8+ T cells in protection against disease have been hampered by the lack of good animal models. However, a recent study revealed that CD8+ T cells are important in the host defense against DENV [137]. On the other hand, activation of memory CD8+ T cells during heterologous secondary DENV infection is believed to result in a massive production of cytokines and immune modulators characteristic of DHF/DSS [138].

Cytokine storm

The hallmark of the pathogenesis of DHF/DSS is the loss of endothelial integrity, which is assumed to be the result of an abnormal immune response against the virus. Clinical studies have shown that the levels of cytokines and immune mediators such as TNF- α , IL-1 β , IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, MCP-1, and IFN- γ and IFN- α are significantly increased in patients suffering from DHF/DSS [139–148]. The role of cytokines in increased vascular permeability has been substantiated in murine model systems [147, 149].

Many scientists believe that the cytokine storm is induced by the activation of a high number of cross-reactive low-avidity T cells through a process referred to as ‘original antigenic sin’. These low-avidity T cells have been shown to exhibit suboptimal degranulation, altered cytokine production and cytolytic activity, through which

not only are they unable to efficiently clear the infection but they cause a massive immune activation [132, 134, 150, 151]. However, aberrant T-cell responses during secondary infections cannot explain the observations of DHF/DSS in infants born to dengue-immune mothers [9, 10, 152, 153]. Interestingly, a recent study showed that antibody-mediated DENV infection of mature DCs also leads to increased levels of TNF- α and IL-6, indicating that ADE of infection may alter cytokine responses [154, 155]. The hypotheses are not mutually exclusive, and most probably both antibodies and T-cells play an important role in the progression of dengue disease to DHF during secondary infection. An integrated view of the immunological processes that contribute to DHF is shown in Fig. 4.

Other viral and host factors controlling DENV infection

Virus virulence

Genotype differences

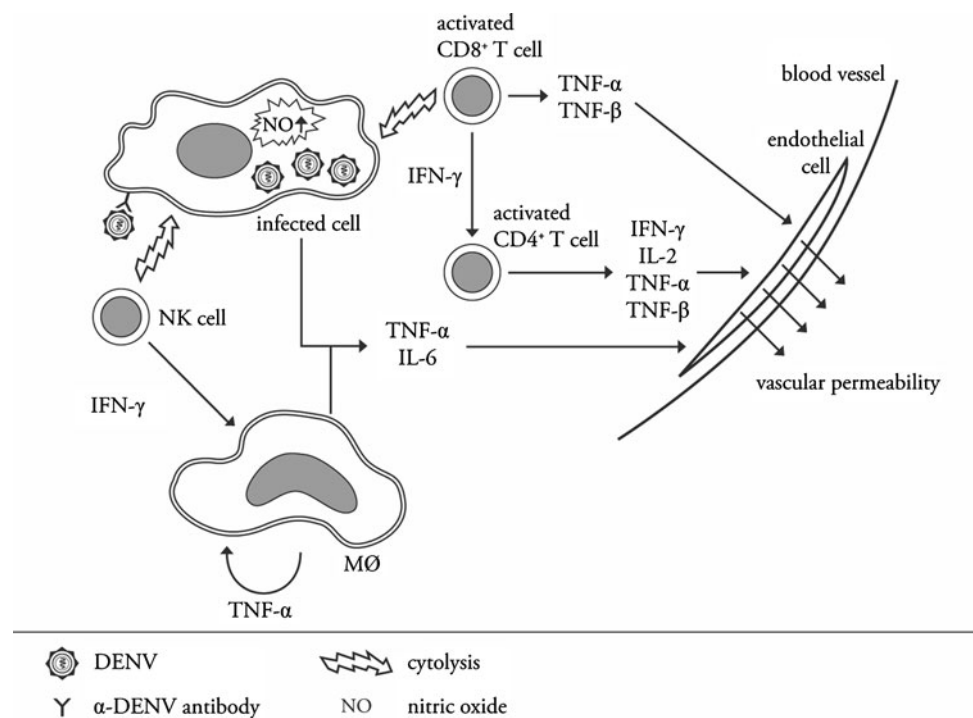
Low-fidelity replication together with natural-selection processes have led to the development of multiple genotypes within each DENV serotype (see [156] for a great review on this topic). Interestingly, several studies have indicated that certain DENV-2 and DENV-3 genotypes are more often associated with DHF [157–165]. For example, the first outbreak of DHF in the Americas coincided with the introduction of the DENV-2 Southeast Asia genotype.

This suggested that the Southeast Asian genotype is more virulent than the co-circulating American DENV-2 genotype in that region, which almost exclusively caused DF [164]. Detailed genomic sequence analysis of both genotypes have revealed nucleotide differences in prM, E, NS4B, and NS5 genes as well as in the 5' and 3' UTRs [166]. Nucleotide variation at amino acid position 390 of the E protein may be of major significance, as this region has been linked to host-range specificity and virulence [166]. Furthermore, it was shown that the Asian DENV-2 genotype replicates to higher titers in human monocyte-derived macrophages and DCs compared to the American genotype [167, 168]. In addition, analysis of the American and Asian lineages for their ability to infect several populations of *A. aegypti* demonstrated that the overall infection rates were higher for the Southeast Asian genotypes.

Glycosylation of E and NS1 proteins

The addition of carbohydrates to viral proteins is important for viral virulence [29, 169–171]. For example, glycosylation at position E Asn153 has been suggested to play a role in stabilizing dimer interactions between E monomers by partially occluding the fusion peptide [21]. Furthermore, the addition of carbohydrates to E Asn67 have been observed to be critical for virus particle production in mammalian and mosquito cell lines [29]. Also, this carbohydrate group has been implicated to mediate binding of the virus particle to DC-SIGN on DCs [172].

Fig. 4 Immunopathogenesis of severe dengue—an integrated model



In addition to the E protein, several studies have suggested that glycosylation of the NS1 protein is important for viral virulence [30, 170, 173]. While complete ablation of DENV-2 16681 NS1 glycosylation results in a genetically unstable virus, removal of only one glycosylation site allows replication of the mutated virus albeit with a less virulent phenotype [30]. Furthermore, it was shown that NS1 protein glycosylation is required for efficient secretion of the protein from infected cells [31, 169]. This, combined with the observation that patients experiencing DHF have high levels of NS1 protein in the blood, suggests that NS1 protein glycosylation is important in disease pathogenesis [112, 174].

Maturation state of the virus

Dengue virus maturation appears to be inefficient as dengue-infected mosquito and mammalian cells have been shown to secrete large numbers (up to 30%) of prM-containing particles [39, 48, 130, 175–184]. Moreover, the presence of prM-specific antibodies in sera of DENV-positive patients suggests that immature particles are also formed during a natural infection [109, 110, 185–187]. Incomplete cleavage of DENV has been linked to the existence of an acidic residue at position P3 within the 13-amino-acid sequence proximal to the prM cleavage site [188, 189].

Numerous studies have demonstrated that fully immature particles lack the ability to infect cells and therefore these particles are generally believed to be of minor importance in DENV pathogenesis [39–41, 190]. Remarkably, however, we recently showed that prM antibodies render essentially non-infectious fully immature particles nearly as infectious as wild-type virus [119]. We observed that prM antibodies facilitate efficient binding and internalization of virus-immune complexes in cells after which furin within the target cell cleaves prM to M thereby activating the membrane fusion potential of the E protein. Enhancement of infection was also observed using DENV-immune sera, which indicates that prM-containing particles may be important in disease pathogenesis. This notion is supported by a recent report which showed that the rates of prM antibody responses are higher in patients experiencing a secondary infection [110].

Antibodies may react differently to immature, partially mature and fully mature particles given the large structural differences between these particles [124, 191]. Antibodies specifically recognizing (partially) immature virions may be detrimental for disease protection. For example, it is tempting to speculate that prM antibodies may not be able to neutralize viral infection of partially immature particles as the mature aspect of the virion would be able to mediate infection. Indeed, Nelson and colleagues showed that

neutralization of WNV infection by E antibodies is significantly influenced by the maturation state of the virus. Increased virus maturation resulted in the reduction of neutralizing potency of many E antibodies that bind to epitopes which are predicted to be poorly accessible in mature virions [124]. Taken together, the maturation status of the virus particle may be important in determining the ability of an antibody to neutralize or enhance viral infection.

Host genetic factors

Differences in disease symptoms are seen at the individual level but also within certain human populations. For example, no apparent DHF/DSS was documented in a Haitian population while there was hyperendemic transmission of several DENV serotypes. This study, together with the observation that black people less frequently develop severe dengue than whites has led to the notion that gene polymorphisms and gene mutations may contribute to variable susceptibility among humans [192–194]. A number of studies have identified candidate genes variants that may predispose or protect an individual to develop DHF/DSS. These include specific human leukocyte antigens (HLAs) alleles and non-HLA gene polymorphisms. Several human HLA class I alleles (A*01, A*0207, A*24, B*07, B*46, B*51) and class II alleles (DQ*1, DR*1, DR*4) were found to be associated with severe disease susceptibility whereas individuals expressing HLA-B*13, HLA-B*14, and HLA-*29 were found to be protected [195–200]. Out of the non-HLA polymorphic alleles, the FcRII, vitamin D receptor [201], tumor necrosis factor alpha (TNF- α) [202], CTLA-4 [203], and transforming growth factor β (TGF- β) [203] have been linked to the development of more severe dengue. Moreover, analysis of three independent cohorts from Thai hospitals by the group of Sakuntabhai revealed that a promoter variant in the DC-SIGN1-336 gene is involved in disease progression to DHF [204]. Other host factors like glucose 6-phosphate dehydrogenase (G6PD) deficiency may also predispose individuals to develop DHF, as DENV has been shown to replicate to high titers in monocytes derived from these individuals [205]. Furthermore, individuals with chronic diseases such as diabetes mellitus (DM) are more susceptible to develop severe dengue [206–209]. It has been speculated that increased production of cytokines in type 2 DM patients might predispose them to vascular leakage, a hallmark of DHF.

Concluding remarks and future directions

Significant progress has been made in recent years with regard to our understanding of the structure of DENV

particles, life cycle, and disease pathogenesis. However, despite the discovery of many host and viral factors that predispose or protect to severe disease, the complex nature of their mutual interactions during natural infection as well as the lack of a suitable animal model makes it difficult to fully explain the pathogenesis of DHF/DSS. The low percentage of DHF/DSS cases during secondary DENV infections suggests that host factors are critical determinants of disease progression. Therefore, more research is required to probe the role of host gene polymorphism (for example immunological pathways) in predisposition to DHF. Furthermore, it will be important to further investigate the role of genetic variations between circulating DENV strains in predisposition to DHF. It will be of interest to determine whether virus virulence is correlated with ADE of infection and aberrant T-cell responses. The observation that infants born to dengue-immune mothers develop DHF during primary infections demonstrates that antibodies play an important role in disease pathogenesis. Future research should investigate by which pathway DENV-immune complexes are internalized in cells, and whether this entry mechanism activates signaling pathways that may prime aberrant immune responses. Given our recent results on the infectious properties of immature DENV-immune complexes, it will be of particular interest to determine if antibody-dependent enhancement of infection by immature DENV particles correlates with disease presentation.

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