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Trends in Molecular Medicine

Review

Molecular mechanisms in the pathogenesis of dengue infections

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Dengue is the most rapidly emerging climate-sensitive infection, and morbidity/mortality and disease incidence are rising markedly, leading to healthcare systems being overwhelmed. There are currently no specific treatments for dengue or prognostic markers to identify those who will progress to severe disease. Owing to an increase in the burden of illness and a change in epidemiology, many patients experience severe disease. Our limited understanding of the complex mechanisms of disease pathogenesis has significantly hampered the development of safe and effective treatments, vaccines, and biomarkers. We discuss the molecular mechanisms of dengue pathogenesis, the gaps in our knowledge, and recent advances, as well as the most crucial questions to be answered to enable the development of therapeutics, biomarkers, and vaccines.

Highlights

Dengue is the most rapidly emerging climate-sensitive infection, and there has been a 10-fold rise in cases over the past 20 years.

Severe illness is characterized by vascular leakage, organ dysfunction, and severe bleeding which occur due to the direct effects of the viral non-structural protein NS1 and an aberrant host immune response.

Dengue NS1 antigen, cytokines such as IL-1β, TNF-α, and IL-6, lipid mediators such as platelet-activating factor (PAF), leukotrienes and prostaglandins, VEGF, chymase, tryptase, and MMP-9 are thought to contribute to endothelial dysfunction.

Many mechanisms contribute to liver dysfunction, including prolonged shock that causes hypoxic damage, direct liver cell death due to infection with the virus, and immune-mediated effects.

Bleeding following dengue virus infection occurs due to multiple mechanisms including platelet activation by NS1, serotonin, and PAF, accompanied by a wide range of other coagulation abnormalities.

Current dengue landscape and disease burden

Dengue is the most common mosquito-borne viral infection and was listed as one of the top 10 threats to global health by the World Health Organization (WHO) in 2019[i]. Half of the global population is at risk of infection with dengue virus (DENV), resulting in 390 million infections annually [1]. There has been a marked increase in dengue in recent years, and many dengue-endemic countries in the tropics and subtropics have reported a record number of cases with higher than usual mortality rates[ii] [2], and some countries in Europe have reported local transmission of dengue infections [3]. This rise in the burden of infection is thought to be due to multiple factors such as climate change (**climate-sensitive infections**, see Glossary), rapid and unplanned urbanization, improper waste management, and cocirculation of multiple DENV serotypes [4].

There are two licensed dengue vaccines, CYD-TDV (Dengvaxia) and TAK-003 (Qdenga). These two vaccines significantly reduce disease severity and hospitalizations in those previously infected with the virus (seropositive individuals). However, both vaccines demonstrated reduced efficacy in seronegative children[iii] [4], and an increased incidence of hospitalization and severe disease was observed in dengue-naïve children who received CYD-TDV. This increased disease severity in dengue-naïve children following CYD-TDV was attributed to potential antibody-dependent enhancement (ADE) [5]. TAK-003 showed significant efficacy against virologically confirmed dengue and hospitalization in seropositive individuals, but did not demonstrate efficacy against DENV3 in dengue-seronegative individuals, and there were inconclusive data for activity against DENV4[iii] [6].

The main challenge in identifying prognostic biomarkers, specific treatments, and safe and effective vaccines is the complex nature of disease pathogenesis and the comparatively fewer data on the pathogenesis of severe dengue compared to many other infections such as COVID-19 [7]. In this review we discuss the molecular mechanisms of dengue pathogenesis and highlight recent developments and gaps in our knowledge which are important for the identification of dengue biomarkers and drug targets. With the increase in burden of dengue, including the rise in cases of severe disease, there is an urgent need to understand the complex nature of disease pathogenesis

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to enable the development of effective therapeutics and prognostic markers, as well as to understand the correlates of protection for vaccine development.

The clinical features and different phases of acute dengue

Although infection with DENV results in a mild or asymptomatic infection in most individuals, some develop vascular leakage which can lead to severe disease [8]. Dengue typically manifests with high fever (**dengue fever**), headache, myalgia, and arthralgia, which coincide with the viremic phase [8]. After 3–6 days the patients may recover from dengue or progress to the critical phase which manifests by plasma leakage, bleeding, and organ dysfunction [8]. Endothelial dysfunction leads to leakage of plasma, which accumulates in pleural and peritoneal cavities leading to shock, metabolic acidosis, intravascular coagulation, and death. Plasma leakage is reported in overall 36.8% of patients who are admitted to hospital [9]. If the onset of this critical phase is detected in a timely manner, fluid replacement can help to prevent the development of shock and associated complications such as severe bleeding and organ dysfunction [8]. The critical phase typically lasts for 24–48 h, followed by a recovery phase. Endothelial dysfunction leading to vascular leakage is completely reversible within this time. Organ dysfunction may occur in the form of acute liver failure, renal failure, encephalitis, or myocarditis, and may occur in the absence of plasma leakage. Of these, liver dysfunction is the commonest organ dysfunction, and a rise in liver transaminase levels is a reliable marker of disease severity in many studies [10–12]. Details of clinical manifestations are given in Box 1.

Those with comorbidities such as diabetes, obesity, chronic kidney disease, or hypertension, as well as pregnancy, and those who experience a secondary dengue infection, are all more likely to develop severe disease [7,13,14]. Currently there is no specific treatment for dengue, and all patients with suspected dengue infection are therefore closely monitored for early detection of complications for timely intervention in the form of fluid replacement [4].

Mechanisms of dengue pathogenesis

DENV is a single-stranded positive-sense RNA virus with four antigenically different serotypes that share 75–80% amino acid sequence homology [15]. Each DENV serotype consists of multiple

Box 1. Clinical features and diagnosis of acute dengue

The onset of dengue fever is characterized by fever, headache, myalgia, and arthralgia which mimic many febrile illnesses such as COVID-19, leptospirosis, chikungunya, influenza, and typhus which are seen in dengue endemic countries [136]. A significant proportion of individuals develop gastrointestinal symptoms such as nausea, vomiting, loss of appetite, and diarrhea, and some individuals also develop a sore throat, cough (<20%), and rhinitis (<10%) [137]. Although there are point-of care diagnostic tests (NS1 antigen test), they become less sensitive, especially after day 3 of illness when the viremia declines and are also less sensitive in secondary dengue viral infections [4].

After 3–6 days, a proportion of patients may progress to the critical phase and develop dengue hemorrhagic fever (DHF) which is characterized by fluid leakage and bleeding tendencies. The onset of the critical phase can be identified by a rise in hematocrit caused by plasma leakage with a concurrent reduction in platelet counts. If the onset of this critical phase is detected in a timely manner, fluid replacement can help to prevent the development of shock and associated complications such as severe bleeding and organ dysfunction [8]. However, organ dysfunction (e.g., liver dysfunction and myocarditis) may occur in the absence of plasma leakage. Although it is important to determine when patients progress to the critical phase, it is also important to identify when fluid leakage is halted because fluid overload is one of the causes of fatalities. Typically, the critical phase lasts between 24 and 48 h.

Although case fatality rates (CFRs) due to dengue are <0.1% in many countries, CFRs rise during outbreaks because healthcare facilities are overwhelmed. The reduction in CFRs has only been achieved by extensive monitoring of all patients with a suspected dengue infection to determine who might develop plasma leakage and then to administer timely and personalized fluid replacement therapy to compensate for the fluid leakage. Because this is a very labor-intensive management strategy, during large outbreaks it becomes extremely difficult to monitor the large number of patients and deliver personalized fluid replacement therapy.

Glossary

Ascites: the accumulation of fluid within the peritoneal cavity.
Climate-sensitive infections: infections whose transmission potential is likely to change as a result of climate change (e.g., increased temperatures, erratic rainfall).
Dengue fever: a febrile illness that occurs following infection with dengue virus (DENV).
Dengue hemorrhagic fever (DHF): a fever caused by DENV which is accompanied by hemorrhagic manifestations and plasma leakage.
FcγR-expressing cells: FcγR is expressed on the surface of many myeloid and lymphoid cells and binds to the Fc (fragment crystallizable) portion of antibodies.
Hematemesis: vomiting of blood.
Melena: black, tarry stools that occur due to bleeding in the upper gastrointestinal tract.
Petechiae: reddish, round spots in the skin that occur due to bleeding into the skin.
Pleural effusion: the accumulation of fluid in the pleural space.
Primary dengue infection: the first infection that occurs due to infection with any DENV serotype.
Secondary dengue infection: any subsequent infection that occurs with a DENV serotype following the primary dengue infection.

lineages, and several distinct genotypes have been identified within each serotype that have a nucleotide sequence divergence of 6–8% [16]. Therefore, the four DENV serotypes are distinct from each other and associate with varying viral loads and NS1 antigen levels during acute illness [17,18]. Although all DENV serotypes have the potential to cause severe disease, some genotypes such as the cosmopolitan strain of DENV2 are associated with increased disease severity [19,20]. This genotype caused massive dengue outbreaks in many regions, with higher case fatality rates (CFRs) [19,21], but most infected individuals developed mild illness, suggesting that host–virus interactions are important in disease pathogenesis.

The DENV polyprotein comprises three structural proteins (envelope, prM, and capsid) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [16]. The non-structural proteins are essential for virus replication and are involved in immune evasion, while the envelope protein interacts with host cellular receptors resulting in cellular entry [16]. Of the non-structural proteins, NS1, which is a secretory protein, contributes to pathogenesis by multiple mechanisms independently of damage caused by virus replication. Many of the non-structural proteins including NS1, as well as antibodies to different DENV proteins, contribute to disease pathogenesis by multiple mechanisms, as described in the following sections.

Direct effects of the DENV in causing disease pathogenesis

Many of the non-structural proteins promote evasion of host immune responses by interfering with interferon (IFN) signaling pathways and detection by pattern recognition receptors (PRRs) [22–24]. The virus is recognized by many PRRs, including Toll-like receptor 3 (TLR3), retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5) and TLR2 [25–27]. The sensing of the DENV by RIG-I and MDA5 leads to subsequent phosphorylation of interferon regulatory factor 3 (IRF3) and IRF7, production of type I and III IFNs, activation of the JAK–STAT pathways, and upregulation of interferon-stimulated genes (ISGs) [28]. Immature DENV particles, which are seen in acute infection, are recognized by TLR2 and DC-SIGN expressed in monocytes and immature dendritic cells, resulting in release of many inflammatory mediators such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) which cause an increase in endothelial cell permeability [29,30].

DENV non-structural proteins interfere with these pathways, leading to enhanced viral replication and impaired antiviral responses. NS5 interacts with many host proteins such as STAT2 and possibly IFN receptors, thereby interfering with the production and action of IFNs (Figure 1) [31]. Furthermore, NS5 also binds to the *RANTES* promoter, increasing expression, and interacts with several proteins involved in lipid metabolism, facilitating DENV replication [31]. NS4A binds to mitochondrial antiviral signaling adaptor (MAVS), thereby preventing detection by RIG-I and leading to an impaired IFN response [32]. NS3, NS2A, and NS4B proteins also prevent RIG-I interacting with MAVS and lead to an impaired IFN response [24]. NS3 and NS2A affect RIG-I signaling pathways by degrading STING and IRF3 (Figure 1) [33]. Apart from the actions of the non-structural proteins, subgenomic RNA of the virus inhibits tripartite motif-containing protein 25 (TRIM25), leading to reduced activation of RIG-I and an impaired IFN response [34]. Although higher viral loads, prolonged viremia, and an impaired type I IFN response are seen in patients who develop **dengue hemorrhagic fever (DHF)** or severe dengue, some studies have shown that viral loads in fact do not correlate with disease severity, and viral loads were lowest in DENV2 infection which was associated with higher risk of severe disease [18,35–37].

Role of protective and disease-enhancing antibodies in dengue pathogenesis

The risk of DHF is higher during a secondary dengue infection [38], which is partly attributed to ADE. However, it was recently shown in a large study in India that the frequency of severe disease

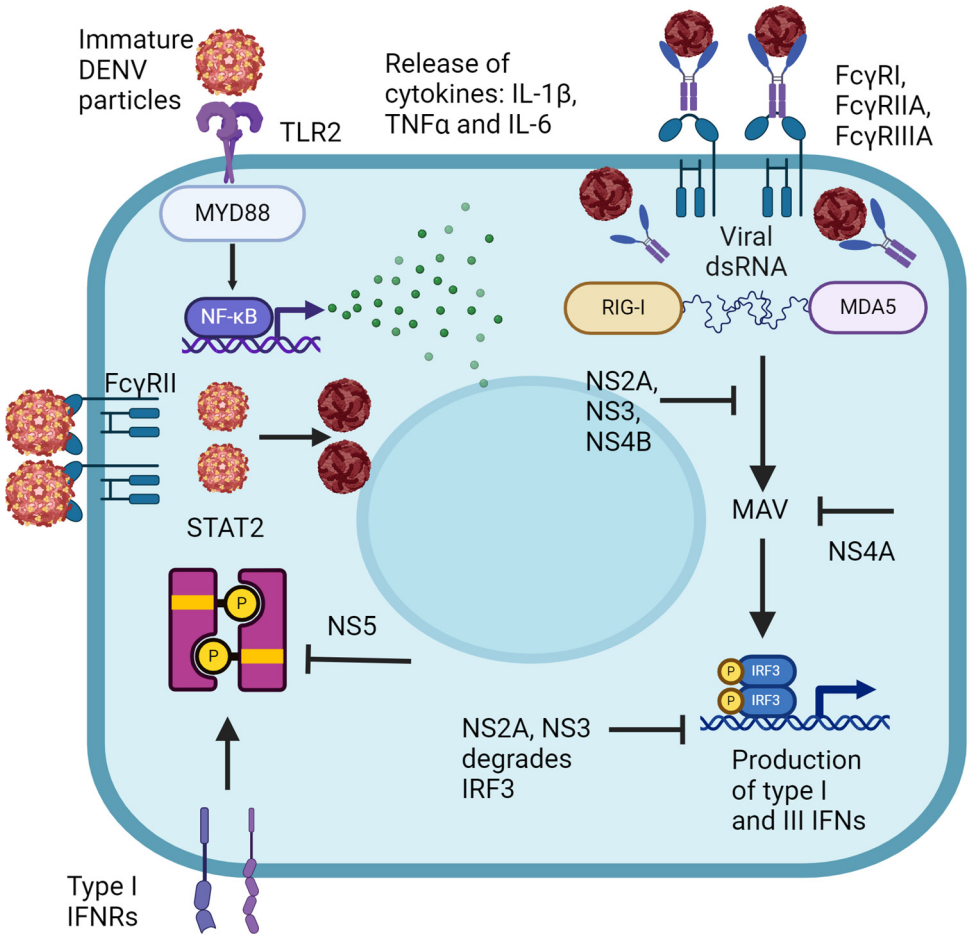

Figure 1. Possible direct effects of dengue virus (DENV) in causing disease pathogenesis. DENV is detected by retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5) that induce type I and III interferon (IFN) production. DENV non-structural proteins evade the host immune responses by interfering with IFN signaling pathways and detection by pattern recognition receptors (PRRs). Non-structural proteins NS2A, NS3, and NS4B prevent RIG-I interacting with the mitochondrial antiviral signaling protein (MAVS). NS3 and NS2A also affect the RIG-I signaling pathways by degrading interferon regulatory factor 3 (IRF3) and stimulator of interferon genes (STING) (not shown). NS4A binds to MAVS, thereby preventing detection. NS5 interacts with STAT2 and possibly IFN receptors, thereby interfering with the production and action of IFNs. Through engagement with different FcγR receptors, antibodies that poorly neutralize DENV facilitate infection of these cells (antibody-dependent enhancement, ADE), thereby increasing DENV infection. Antibodies to immature DENV particles (prM) facilitate entry of these immature virus particles, and they are rendered infectious within the cell by furin-cleavage of the protein. Immature viral particles seen in acute dengue are also detected by Toll-like receptor-2 (TLR2), leading to activation of NF-κB, which results in the production of proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 that stimulate the production of many other inflammatory mediators and vascular leakage. Abbreviations: dsRNA, double-stranded RNA; IFNRs, interferon receptors; P, phosphorylation. Figure created with BioRender.

was similar in children with **primary dengue infection** or **secondary dengue infection** [39]. Furthermore, a large longitudinal study in Nicaragua showed that both primary and secondary dengue infections were equally likely to cause an inapparent dengue infection [40]. Vaccine trials have shown that the specificity and crossreactivity of neutralizing antibodies determine subsequent protection from DENV [41]. Therefore, although ADE is important in dengue disease pathogenesis, its specificity and functionality of DENV-specific antibodies appear to play a role.

In ADE, poorly neutralizing and highly crossreactive antibodies enhance infection in **FcγR-expressing cells**, thereby increasing the intracellular viral loads (Figure 1) [42–44]. Even individuals with high neutralizing antibody levels are sometimes reinfected with the same virus serotype, suggesting that the functional characteristics of antibodies could also play a role in protection [45]. Those who had DHF and shock had higher levels of afucosylated IgG1 isoforms, which have an enhanced capacity to engage with the activating receptor FcγRIIIA [46,47]. The presence of higher levels of afucosylated IgG1 was a risk factor for thrombocytopenia and symptomatic primary dengue in infants [48]. In addition to the functionality and levels of neutralizing antibodies, the presence of mature versus immature viral particles contributes to disease pathogenesis. For instance, immature virus particles have higher surface expression of the prM and fusion loop proteins which are recognized by poorly neutralizing, crossreactive prM-specific antibodies that facilitate ADE [48]. Although these immature viral particles with prM are non-infectious, once they are taken into the cells, through binding to the FcγRIIA receptor, they can undergo furin-mediated cleavage, thereby rendering them infectious [49,50]. Internalization of antigen–antibody complexes through these mechanisms is likely to further suppress antiviral responses [51,52]. Although FcγRI has been shown to mediate ADE *in vitro* in monocytes (THP-1 cells), and both FcγRIIA and FcγRIIIA can mediate ADE in different monocyte cell lines (U937), the contribution of these receptors in facilitating ADE in acute infection in humans remains to be unequivocally defined. Therefore, although ADE does appear to play a role in dengue pathogenesis, many other factors such as the functionality, specificity, and quantity of DENV-specific antibodies are likely to play a role in determining disease outcome.

Apart from poorly neutralizing antibodies specific to the envelope protein leading to ADE, there are contrasting data suggesting that antibodies to NS1 may lead to disease pathogenesis or be required for protection. Antibodies to NS1 are higher in the critical phase in patients who develop DHF compared to those with dengue fever, although it is not evident whether this is due to higher antigen load or whether NS1-specific antibodies are themselves pathogenic [53]. Antibodies to NS1 are thought to lead to disease pathogenesis by binding to a wide array of host proteins, which has been shown *in vitro* [54,55]. Furthermore, these antibodies led to the destruction of platelets in dengue mouse models [56,57]. However, the significance of these antibodies in leading to disease pathogenesis in acute dengue has not been investigated. In longitudinal studies, children who developed an inapparent dengue infection were more likely to have higher levels of NS1 antibodies which are capable of mediating antibody-dependent cellular phagocytosis compared to children who had symptomatic infection, suggesting a protective role for NS1-specific antibodies [58]. The presence of sub-neutralizing levels of DENV-specific antibodies with altered functionality can increase intracellular viral loads and lead to increased production of inflammatory mediators.

Disease pathogenesis and vascular leak mediated by NS1

Dengue NS1 is an important component of the virus replication complex [59]. It can also be a secretory protein that circulates as a multimer carrying a lipid cargo that contributes to disease pathogenesis by multiple mechanisms (Figure 2) [60,61]. NS1 activates monocytes and macrophages through TLR4, and thereby induces cytokine production, which has shown to lead to vascular leakage *in vitro* and in mouse models [62,63]. Apart from inducing cytokine production, NS1 activates cathepsin L, sialidase, and heparinase, leading to sialic acid degradation and disruption of the endothelial glycocalyx *in vitro* and mouse models, resulting in endothelial dysfunction [64,65]. Furthermore, *in vitro* data show that NS1 activates inflammatory phospholipase A2 (PLA2) enzymes [66] which are responsible for generating many inflammatory lipid mediators such as PAF and leukotrienes [67]. Both PAF and leukotrienes mediate vascular leakage in dengue and many other diseases [68,69]. Also based on *in vitro* experiments, NS1 increases the expression of matrix metalloproteinase 9 (MMP-9), and subsequent NS1–MMP-9 interactions

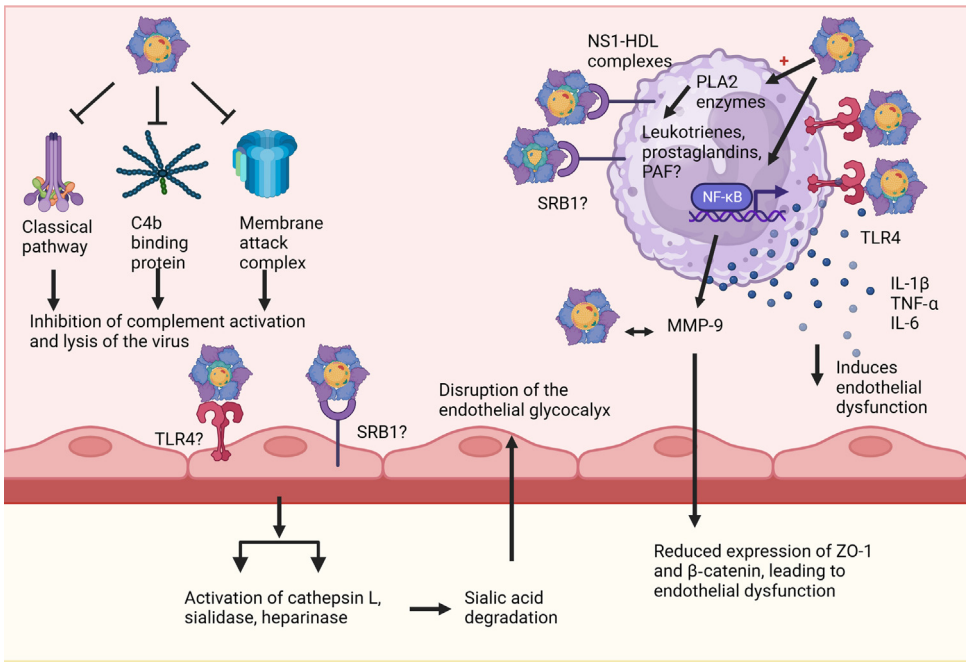

Figure 2. Hypothetical role of non-structural protein NS1 in the pathogenesis of dengue. NS1 causes disease pathogenesis by inducing cytokine production from monocytes/macrophages through Toll-like receptor 4 (TLR4) receptors leading to induction of phospholipase A2 (PLA2) enzymes. This induces the production of leukotrienes, prostaglandins, and possibly platelet-activating factor (PAF) and other mediators including matrix metalloproteinase 9 (MMP-9). NS1–high-density lipoprotein (HDL) complexes also stimulate cytokine production from monocytes/macrophages, possibly through SRB1 receptors or TLR4. Many cytokines cause endothelial dysfunction, and MMP-9–NS1 interactions also lead to endothelial dysfunction by downregulating junctional proteins such as zonula occludens 1 (ZO-1) and β-catenin. NS1 also activates cathepsin L, sialidase, and heparinase, leading to sialic acid degradation and disruption of the endothelial glycocalyx, which may occur through internalization by scavenger receptor class B type 1 (SRB1) receptors or by TLR4. NS1 also evades complement lysis by inhibiting complement activation by binding to C4b binding protein in the classical pathway and inhibiting membrane attack complex (MAC) formation. Figure created with BioRender.

result in increased endothelial dysfunction by reducing the expression of zonula occludens 1 (ZO-1) and β-catenin [70]. Therefore, NS1 appears to contribute to vascular leakage directly and indirectly in dengue infection through (i) direct damage to the endothelium, (ii) by inducing production of cytokines such as TNF-α, IL-1β, and MMP-9, and (iii) by activating PLA2 enzymes that generate mediators responsible for vascular leakage (Figure 2). However, whether these findings seen *in vitro* and in mouse models also translate to acute infection in humans is unknown. Given that many mediators are likely to lead to endothelial dysfunction, it would be difficult to dissect the contributions of each mediator or NS1 given the complex nature of the illness.

There have been many questions regarding the lipid cargo within the NS1 hexamer and whether the type of lipid cargo could lead to disease pathogenesis [71]. NS1 interacts with apolipoprotein A1 (APOA1) and high-density lipoprotein (HDL) in cell culture supernatants and in patient sera [72,73]. These NS1–HDL complexes induce proinflammatory cytokine production in monocytes *in vitro*, although the mechanisms have not been defined [73]. Both NS1 and the DENV use the HDL receptor scavenger receptor class B type 1 (SRB1) for internalization [74,75]. Again, it is unclear whether this is the main receptor used by NS1 to enter cells and whether events following such internalization contribute to disease pathogenesis. However, apart from being essential for replication of the virus, NS1 increases enrichment of lipid rafts, thereby enhancing DENV cell

entry, which is inhibited by APOA1 [76]. NS1 also increases DENV infection by directly interacting with complement component 4b (C4b) and inhibiting the membrane attack complex. In addition, NS1 inhibits complement activation by the classical and lectin pathways, and thereby reduces complement-mediated lysis of the virus [77–79].

However, severe dengue, that is characterized by plasma leakage and thrombocytopenia, can be seen in patients with a secondary dengue infection, where lower NS1 antigen levels may be seen compared to those with primary dengue [37,80]. Furthermore, lower NS1 levels are seen in patients infected with DENV2 which is associated with severe disease, and NS1 of some DENV2 strains (e.g., D2Y98P) did not cause disease pathogenesis in mouse models [81]. Therefore, high NS1 levels alone do not seem to cause disease pathogenesis, but NS1–HDL interactions or the type of structural interactions between the NS1–HDL complex with host molecules may affect the function of NS1. For instance, during early illness NS1 complexed with HDL associates with APOA1, while during the recovery phase the NS1–HDL complex was shown to associate with APOE [73]. It would therefore be important to answer these key questions regarding the mechanisms by which NS1–HDL complexes lead to disease pathogenesis to identify therapeutic targets.

Role of inflammatory mediators in endothelial dysfunction leading to vascular leakage

Endothelial dysfunction is the hallmark of vascular leakage and is responsible for causing many complications in dengue such as **pleural effusions**, **ascites**, hypotension, and shock, and reduced organ perfusion leads to organ dysfunction [82]. As described in the preceding text, although the NS1 protein directly contributes to endothelial dysfunction, many inflammatory mediators released from monocytes, macrophages, dendritic cells, and mast cells are likely to lead to endothelial dysfunction [69,82]. Several cytokines, chemokines, inflammatory lipid mediators, and mast cell proteases are elevated during early illness before clinically apparent leakage is detected in patients with acute dengue infection [83–86]. Although many cytokines and chemokines such as IL-1RA, IL-6, IL-1β, IL-8, IL-10, IP-10, CRP, TNF-α, IFN-γ, IL-12p70, MIP-3α, IL-33, IL-17A, IL-18, MIF, and MMP-9 (matrix metalloproteinase 9) are elevated in early illness, only a few of these mediators cause endothelial dysfunction *in vitro* and in mouse models [10,84,87,88]. Of these cytokines, IL-1β, IL-6, and TNF-α directly cause endothelial dysfunction *in vitro*, and increased expression of MMP-9 is induced by NS1, leading to vascular leakage [70,89,90]. Some cytokines such as IL-10, which are significantly associated with clinical disease severity, reduce endothelial dysfunction [87,91,92]. Therefore, although elevation of some cytokines can directly result in endothelial dysfunction, other cytokines associated with severe dengue may be elevated because of the disease process or may have other detrimental effects on the immune response that are unrelated to endothelial dysfunction.

Many inflammatory lipid mediators such as PAF, leukotrienes, prostaglandins, PLA2 enzymes, and sphingosine-1-phosphatase (S1P) are altered in dengue [68,85,87,93–96]. PAF, leukotrienes, and prostaglandin metabolites cause endothelial dysfunction, and blocking the effects of some of these lipid mediators is associated with reduced vascular permeability *in vitro* and in dengue mouse models (Figure 3) [68,93,97]. Secretory PLA2 (sPLA2) levels, which are elevated in sepsis and severe COVID-19, are elevated very early in illness in those who subsequently progress to develop DHF [85,98]. sPLA2 hydrolyzes phospholipids to generate arachidonic acid and fatty acids, resulting in an increase in prostaglandins and leukotrienes which are involved in increased vascular leakage and inflammation. Cytokines such as IL-1β, IL-6, and TNF-α induce the activity of sPLA2, as do lipopolysaccharide (LPS) and NS1 [66,99]. Indeed, leukotrienes and prostaglandins are elevated in patients with DHF during early illness and in the critical

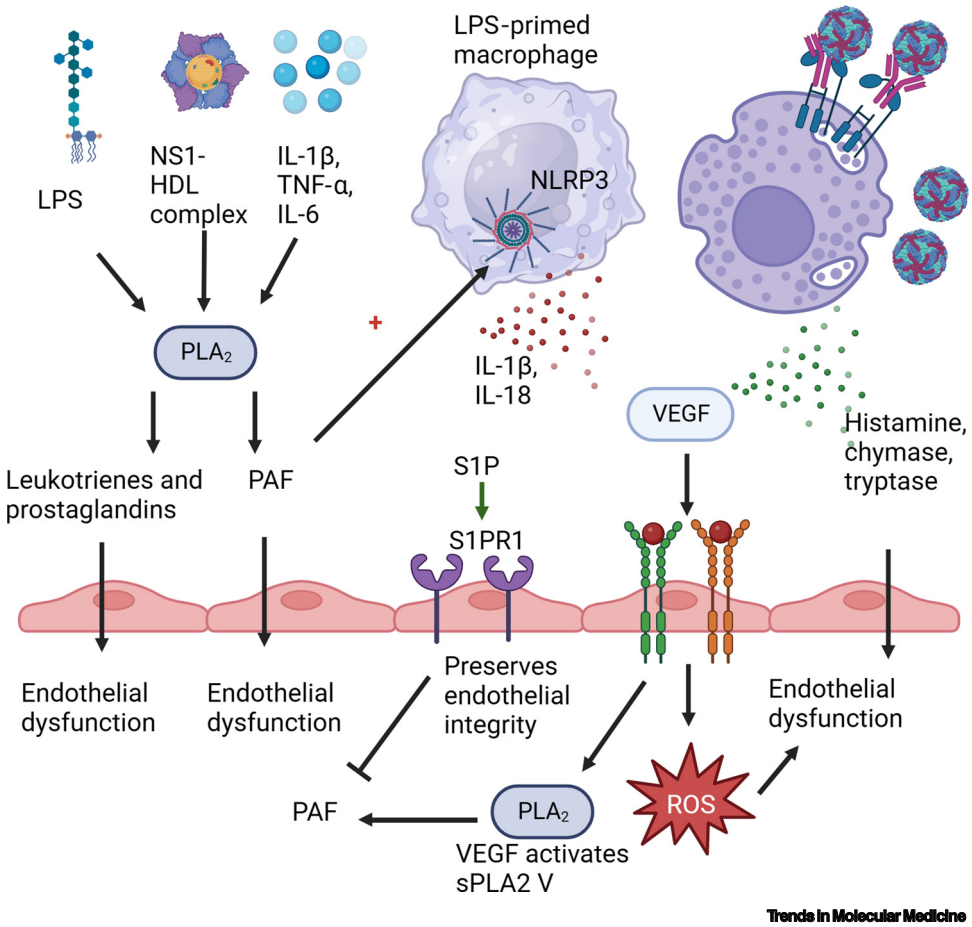

Figure 3. Potential mechanisms of endothelial dysfunction by lipid mediators and mast cell products. Phospholipase A2 (PLA2) enzymes are activated by many cytokines, lipopolysaccharide (LPS), and non-structural protein NS1, which leads to the generation of leukotrienes, prostaglandins, and platelet-activating factor (PAF), causing endothelial dysfunction. PAF also induces NLR family pyrin domain-containing 3 (NLRP3) activation in LPS-primed macrophages, leading to the production of IL-1β and IL-18. Mast cell degranulation may be triggered directly by dengue virus (DENV) itself, but takes place mainly through binding of DENV–IgG complexes to FcγRI receptors. This leads to the release of many inflammatory mediators such as chymase, tryptase, histamine, and leukotrienes, leading to endothelial dysfunction. Vascular endothelial growth factor (VEGF) is also produced by mast cells, and activates the endothelium leading to dysfunction via the generation of reactive oxygen species (ROS) and activation of sPLA2 enzymes to generate PAF. Sphingosine-1-phosphatase (S1P) produced by platelets and red blood cells can preserve endothelial integrity and inhibit the action of PAF. Figure created with BioRender.

phase, suggesting that they could contribute to vascular leakage [93,94]. In addition to inducing endothelial dysfunction, prostaglandin metabolites (PGD2) downregulate the type I IFN response, thereby facilitating infection of type 2 innate lymphoid cells (ILC2s) [93]. Infection of ILC2 cells has been observed in patients with acute dengue, and there was also an increase in ILC2 frequency [93].

PAF is known to induce vascular leakage in many diseases such as sepsis and anaphylaxis by signaling through the PAF receptor (PAFR), resulting in activation of phospholipase C isozymes and rearrangement of the actin cytoskeleton (Figure 3) [100]. PAF levels are significantly higher in patients with DHF, and PAFR blockade was shown to reduce endothelial dysfunction *in vitro* and to reduce the rise in hematocrit in dengue mouse models [68,97]. PAF also activates NLR family pyrin domain-containing 3 (NLRP3) inflammasomes, and thereby induces IL-1β and

IL-18 in LPS-primed mice independently of PAFR [101]. Therefore, PAF potentially contributes to disease pathogenesis by multiple mechanisms. S1P protects the endothelial barrier integrity and inhibits the PAF-induced increase in endothelial permeability through S1PR1 receptors [102]. S1P levels have shown to be low in patients with DHF, especially in the critical phase, possibly due to the low HDL and serum albumin levels seen in such patients, which are the main carrier molecules of S1P [103].

Although PAF, leukotrienes, and prostaglandins are produced by many cells, mast cells are one of the most important sources [104]. Many mast cell products such as histamine, tryptase, chymase, vascular endothelial growth factor (VEGF), and serotonin are elevated in dengue and are associated with disease severity, leading to vascular leakage (Figure 3) [105–107]. Mast cell degranulation may be directly triggered by the virus or by DENV–IgG complexes formed by poorly neutralized antibodies binding to FcγRI receptors [108]. VEGF can directly induce an increase in vascular permeability through activation of reactive oxygen species (ROS) and by stimulating PAF production by endothelial cells by inducing sPLA2 enzymes [109,110]. Chymase and tryptase, which are mast cell proteases, and histamine all lead to breakdown of endothelial tight junctions, leading to vascular leakage [111,112]. Therefore, many inflammatory mediators lead to disease pathogenesis by inducing proinflammatory cytokines and directly causing vascular leakage, and blocking these mediators could potentially reduce or prevent vascular leakage in dengue.

Mechanisms of liver dysfunction

Varying degrees of liver involvement are seen in many patients who develop plasma leakage, although such involvement can be seen in the absence of any detectable fluid leakage [113]. Indeed, a rise in aspartate transaminase levels (AST) is one of the most important biomarkers of severe disease, as shown in several meta-analyses [10,12,114]. High AST levels were seen in >80% of patients with DHF or severe disease [113,115]. Varying degrees of hepatic necrosis, congestion, diffuse macrovascular and microvascular steatosis, Kupffer cell hyperplasia, and hemophagocytosis have been reported in postmortem studies of patients who succumbed to the illness, and widespread infection of hepatocytes was reported [116]. Although the exact mechanisms of liver cell damage in dengue are not understood, direct damage to the liver, poor organ perfusion, and immune-mediated damage could all play a role (Figure 4) [113]. Oxidative stress due to mitochondrial dysfunction is known to be an important factor contributing to non-alcoholic fatty liver disease (NAFLD) [117]. Oxidative stress resulting in ROS production is seen in dengue infection and is associated with disease severity [118]. The NS2B3 protease complex of the virus (a combination of NS2B and NS3) modulates nuclear factor erythroid 2-related factor 2 (NRF2) regulatory pathways, leading to increased oxidative stress, inflammation, and apoptosis of cells, which could lead to the pathological features seen in the liver [119].

Acute liver failure is an uncommon but serious complication that is seen in 0.31–1.1% of patients with dengue [120]. Prolonged shock and direct injury could contribute to acute liver failure. Importantly, secondary hemophagocytic lymphohistiocytosis (HLH) is being recognized as one of the important causes of severe liver dysfunction, often leading to fatalities in patients with dengue who may or may not have plasma leakage [121]. HLH is characterized by macrophage activation, natural killer (NK) cell dysfunction and depletion, and aberrant T cell activation leading to a cytokine storm [122]. HLH leading to severe dengue is one of the most important challenges in managing patients with dengue because it is associated with high mortality. Higher levels of ferritin and liver transaminases have been shown to associate with fatalities [121]. The pathogenesis of HLH in dengue is unclear, and many treatments such as corticosteroids (dexamethasone) and etoposide (an inhibitor of DNA topoisomerase) are currently being explored [121].

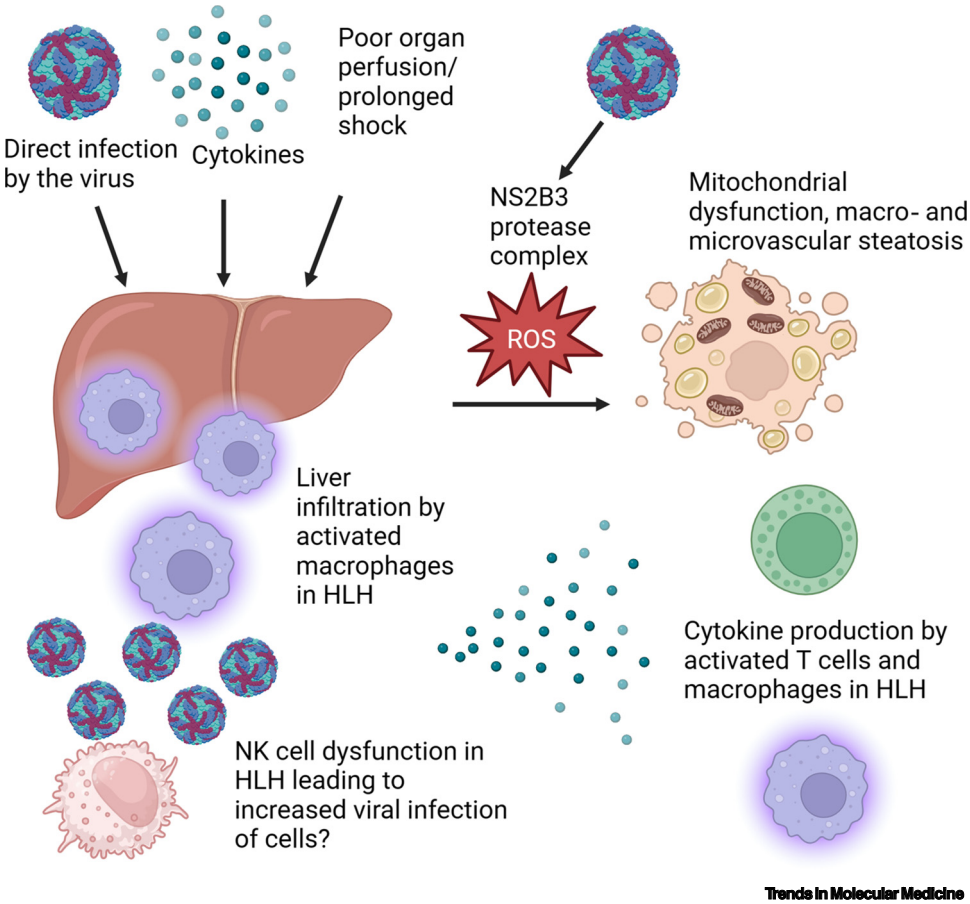

Figure 4. Potential mechanisms of liver dysfunction in dengue. Liver dysfunction may occur due to prolonged shock leading to poor organ perfusion and subsequent hypoxic damage, as well as via direct liver cell death due to virus infection and cytokines. Dengue virus (DENV) can lead to mitochondrial dysfunction owing to excessive oxidative stress, and the NS2B3 protease complex of the virus modulates regulatory pathways leading to a further increase in oxidative stress. As a result, varying degrees of hepatic necrosis, congestion, diffuse macrovascular and microvascular steatosis, and Kupffer cell hyperplasia are the predominant liver features seen in acute dengue. Hemophagocytic lymphohistiocytosis (HLH) is an important cause of acute liver failure in dengue, which could be due to invasion of the liver by activated macrophages, damage induced by cytokines released from activated T cells and macrophages, and possibly by dysfunctional natural killer (NK) cells, leading to increased viral infection in the liver. Abbreviation: ROS, reactive oxygen species. Figure created with BioRender.

Mechanisms of bleeding in dengue

Patients with dengue may present with a wide range of bleeding manifestations such as **petechiae**, gum bleeding, increased bleeding from sites of venipuncture to **hematemesis**, **melena**, hematuria, epistaxis, and per vaginal bleeding [97,123]. The mechanisms leading to bleeding include thrombocytopenia, dysfunctional platelets, abnormalities of the coagulation pathways, and prolonged shock [124,125]. Although thrombocytopenia (<50 000 cells/mm^3) is seen in >50% of hospitalized patients, timely fluid management has reduced the proportion of patients who develop bleeding manifestations from 38.9% in 2004 to 8.9% in 2015 in Sri Lanka [97,126].

Although severe thrombocytopenia is commonly seen in patients with acute dengue, there is not typically a strong correlation between the extent of thrombocytopenia and bleeding [125]. Platelets can be infected by DENV and the extent of platelet activation correlates with disease

severity [127]. Platelet activation is likely to be due to multiple factors such as serotonin released from mast cells, cell-free histone H2A, and direct activation by the virus and NS1 (Figure 5) [106,127,128]. NS1 has many detrimental effects on coagulation pathways. It binds to thrombin, preventing prothrombin activation and leading to a prolonged activated partial thromboplastin time (aPTT) [129]. In addition, NS1 also activates platelets by upregulating P-selectin and causing apoptosis of platelets through TLR4 [130]. It was shown that NS1 failed to induce thrombocytopenia and bleeding in *Tlr4*$^{-/-}$, suggesting that NS1/TLR4 pathways are important for platelet activation, aggregation, and apoptosis, and are likely to contribute to the thrombocytopenia and bleeding seen in dengue patients [130]. Serotonin derived from mast cells activates platelets, increasing platelet aggregation and phagocytosis through 5HT2A receptors (a subtype of the serotonin receptor family) [106]. PAF is known to cause platelet activation and aggregation and also the release of serotonin from platelets, which could further contribute to the altered platelet function and coagulation seen in dengue (Figure 5) [131]. Indeed, two clinical trials using a drug that blocks PAFRs (rupatadine) in patients with acute dengue found that this significantly reduced the extent of thrombocytopenia [97,123].

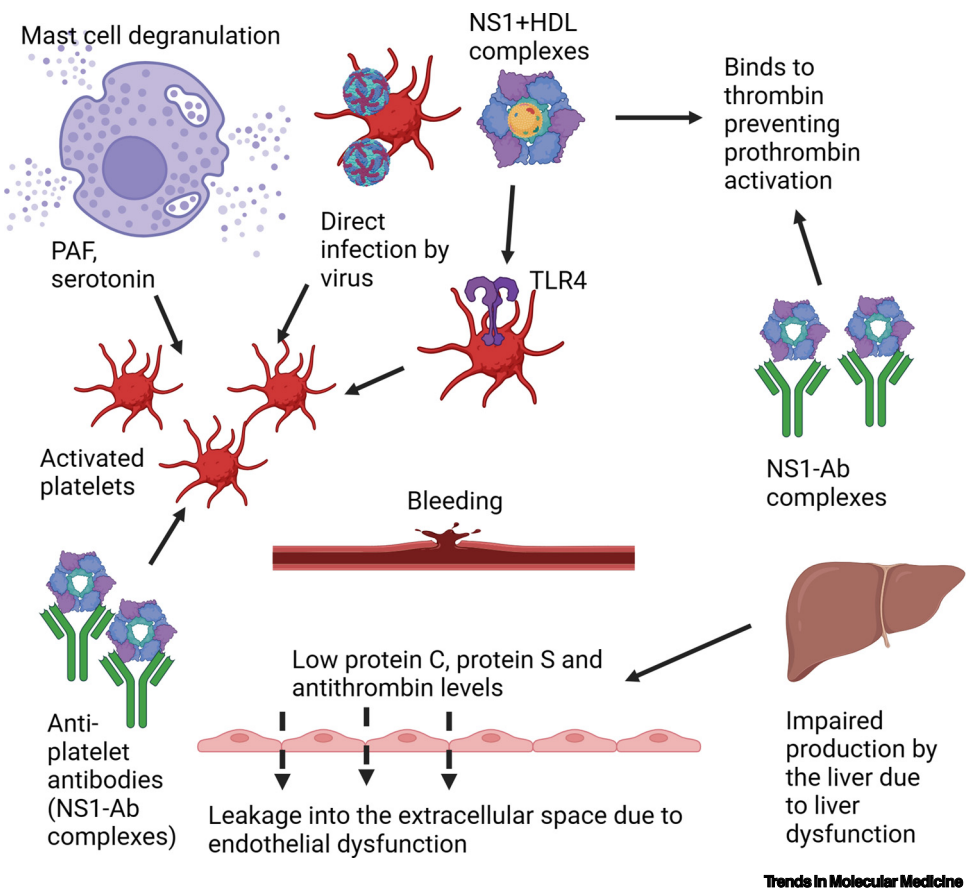

Figure 5. Possible mechanisms of bleeding in dengue. Direct infection and activation of platelets by dengue virus (DENV), by non-structural protein NS1 and high-density lipoprotein (HDL) complexes through TLR4, and by serotonin and platelet-activating factor (PAF) generated by mast cell degranulation, together lead to platelet activation, aggregation, and bleeding. NS1 also interacts with thrombin, preventing prothrombin activation, and generates NS1–antibody (Ab) complexes which are also directed at thrombin and plasminogen. NS1–Ab complexes bind to platelets and further activate them, contributing to bleeding. Increased vascular permeability possibly leads to extravasation of many proteins in the coagulation pathways, including protein C, protein S, and antithrombin. Liver dysfunction is also likely to result in lower production of coagulation pathway components. Figure created with BioRender.

Clinician's corner

Although most patients with acute dengue have a mild illness, some individuals develop vascular leakage and progress to severe illness. Unfortunately, because there are no robust prognostic markers, all individuals with an acute dengue infection need to be monitored for early detection of complications to implement timely interventions.

Vascular leakage occurs due to an increase in endothelial permeability, which typically occurs at around day 3–6 of illness and lasts for 24–48 h. Owing to the short-lived and reversible nature of endothelial dysfunction, it is likely to be secondary to many inflammatory mediators that cause an increase in endothelial permeability.

The extent of vascular leakage varies from person to person, and some people may develop profound leakage within a few hours. If undetected, this leads to hypotension, poor organ perfusion, multi-organ dysfunction, and severe bleeding due to disseminated intravascular coagulation. Early detection of vascular leakage and timely fluid replacement helps to prevent shock and associated complications including organ dysfunction and bleeding. Therefore, careful monitoring of patients and personalized fluid replacement therapy reduces the incidence of severe dengue.

At the end of the critical phase the fluid extravasated from the capillaries reenters the vasculature. The end of the fluid leakage phase should be monitored carefully to stop replacement therapy and prevent fluid overload.

Although fluid leakage is associated with the development of many of the complications due to dengue, liver dysfunction and bleeding may occur in the absence of fluid leakage and are due to multiple mechanisms. Secondary hemophagocytic lymphohistiocytosis (HLH) is one of the important causes of severe liver dysfunction in the absence of leakage and is associated with high mortality.

The commonest bleeding manifestation in dengue in the absence of leakage is per vaginal bleeding in women, which can be very severe.

have been observed in dengue, including low levels of protein C, protein S, and antithrombin II, high levels of thrombomodulin, tissue factor, and plasminogen activator inhibitor type 1 (PAI-1), and prolonged prothrombin time, prolonged activated partial thromboplastin time (aPTT), and prolonged thrombin time [124,125]. Alterations in these parameters are likely to be due to multiple mechanisms, including fluid leakage, where many of these proteins involved in the coagulation pathways leak out of the capillaries, as well as to impaired production secondary to liver dysfunction [124]. Furthermore, DENV infection induces the generation of antibodies targeting thrombin and plasminogen, thereby altering their function and enhancing fibrinolysis [132,133]. Some of these antibodies were found to be generated against the C-terminal region of NS1, which were subsequently crossreactive with platelets, thrombin, and plasminogen [55]. However, because these host protein-crossreactive antibodies directed at NS1 do not seem to cause pathogenesis in the absence of dengue infection, it is possible that NS1–antibody complexes contribute to disease pathogenesis. Overall, there are very limited data concerning the mechanisms of altered coagulation in dengue and bleeding because early detection of fluid leakage and timely fluid replacement appear to reduce the incidence of severe bleeding in dengue.

Concluding remarks

Recurrent dengue epidemics have been fuelled by the growth of cities and unplanned urbanization which are set to be worsened by climate change. Despite having a huge impact on healthcare systems and the economies of low- and low-middle income countries, there are no specific treatments or robust prognostic markers. The available data on dengue pathogenesis are very limited compared to infections such as COVID-19, which has greatly hindered the development of safe and effective treatments and vaccines (see Outstanding questions) [7]. Given the direct effects of the DENV in causing disease pathogenesis, which are enhanced through altered immune responses, antivirals are likely to have a beneficial role in the treatment of dengue. So far the few antiviral trials conducted for dengue have not met their primary endpoint – typically an accelerated reduction of viremia [134]. Serum viremia declines rapidly during early infection, and therefore assessment of the efficacy of antivirals based on an accelerated reduction of viremia has been challenging. However, serum viremia might not reflect viral loads within FcγR-expressing cells, hepatocytes, or many other cells infected by DENV [93,116]. It would therefore be important to consider clinical parameters, and importantly their efficacy in reducing the extent of liver damage in dengue, when evaluating antivirals instead of evaluating accelerated reduction in viremia (the main challenges in management of patients with dengue are covered in the Clinician's corner).

In addition to antivirals, clinical trials of drugs targeting inflammatory lipid mediators such as PAFR blockers (rupatadine), cysteinyl leukotriene receptor type 1 antagonists (montelukast), mast cell stabilizers (ketotifen), and immunomodulators (steroids) have reported varied effects [97,123,135]. Given that many inflammatory mediators are elevated in dengue and contribute to disease pathogenesis, and because drugs targeting inflammatory lipid mediators have a very good safety profile and are currently used in combination for treatment for many diseases, it would be important to evaluate the efficacy of combination treatments targeting these mediators for the prevention of vascular leakage in dengue. Because the incidence of dengue is rapidly increasing in many countries, including associated complications, there is an urgent need to address many unresolved issues (see Outstanding questions). It would be prudent for the scientific community to focus on this global threat, and to identify safe and effective therapeutics, biomarkers, and effective vaccines, rather than solely relying on vector control measures to reduce the burden, and on fluid replacement as the main management strategy for the treatment of acute dengue.

Outstanding questions

What are the main mediators that lead to endothelial dysfunction? Which cells produce them, and what triggers their production?

Although secondary dengue is a risk factor for severe illness, ascribed in part to ADE, why does this only occur in some individuals? What are the characteristics of antibodies that lead to severe disease?

Why is severe disease less common in a primary dengue infection, despite sometimes higher NS1 antigen levels and viral loads, compared to secondary infections?

Are higher viral loads alone associated with a delayed IFN response, or does an impaired IFN response lead to higher viral loads and a delay in clearance? Are some individuals more prone to severe disease owing to dysfunctional IFN responses related to genetic factors, the presence of anti-IFN antibodies, or altered transcription of genes that regulate the IFN response?

Many inflammatory mediators are high in the early phase of illness in those who subsequently progress to severe disease. What stimulates their increase in early illness? Can any of these be used as prognostic markers? If immune modulators are a strategy to treat severe disease, which non-redundant inflammatory mediators or pathways should they target?

Why are obesity, diabetes, and pregnancy risk factors for severe illness?

Do infection rates and viral loads in cells such as hepatocytes, keratinocytes, and innate immune cells correlate with viral loads in serum? If not, would measuring serum viral clearance be useful for evaluating the efficacy of antivirals?

What are the roles of T cells in disease protection or pathogenesis?

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Declaration of interests

G.N.M. is a consultant to the Drugs for Neglected Diseases Initiative and heads the dengue program (until June 2024), and is also a member of the Expert Committee for the Control of Dengue in Sri Lanka.

Resources

[i]https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

[ii]https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON448

[iii]https://www.who.int/publications/m/item/highlights-from-the-meeting-of-the-strategic-advisory-group-ofexperts-(sage)-on-immunization25-29-september-2023

[iv]https://ctv.veeva.com/study/ketotifen-as-a-treatment-for-vascular-leakage-during-dengue-fever

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