Immune responses and severe dengue: what have we learned?

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Purpose of review
With the marked rise in dengue globally, developing well tolerated and effective vaccines and therapeutics is becoming more important. Here we discuss the recent developments in the understanding of immune mechanisms that lead to severe dengue and the learnings from the past, that can help us to find therapeutic targets, prognostic markers, and vaccines to prevent development of severe disease.

Recent findings
The extent and duration of viraemia often appears to be associated with clinical disease severity but with some variability. However, there also appear to be significant differences in the kinetics of viraemia and nonstructural protein 1 (NS1) antigenemia and pathogenicity between different serotypes and genotypes of the DENV. These differences may have significant implications for development of treatments and in inducing robust immunity through dengue vaccines. Although generally higher levels of neutralizing antibodies are thought to protect against infection and severe disease, there have been exceptions and the specificity, breadth and functionality of the antibody responses are likely to be important.

Summary
Although there have been many advances in our understanding of dengue pathogenesis, viral and host factors associated with occurrence of severe dengue, vascular leak and the immune correlates of protection remain poorly understood.

Keywords
antibodies, dengue, nonstructural protein 1, pathogenesis, vaccine

INTRODUCTION

Dengue is the most rapidly emerging mosquito-borne virus infection globally with the incidence of dengue and associated morbidity and mortality markedly increased in recent years [1,2]. Although highly variable between regions and centres, it is estimated that 54% of infections with the dengue virus (DENV) lead to asymptomatic/inapparent illness [3] with 18% of those with symptomatic illness requiring hospitalization [4]. Of the hospitalized patients, 36.8% develop plasma leakage [5], which can lead to pleural effusions, ascites, shock, and poor organ perfusion, thereby contributing to organ dysfunction and severe bleeding.

During the early dengue outbreaks in Asia from 1950 to 1970, case fatality rates (CFRs) were between 10 and 20% in many countries in Southeast Asia [6,7]. The current CFR for Southeast Asia is approximately 0.2% [8], whereas the CFRs were reported to be higher in Bangladesh (0.52%), Philippines (0.34%), and in Indonesia (0.72%) in the recent outbreaks last year [1]. CFRs have declined over time, not because dengue has become less severe, but because plasma leakage and other complications are detected earlier, for initiation of timely and personalized fluid replacement. However, when healthcare systems become overwhelmed with many patients, careful monitoring and timely fluid replacement become difficult. This leads to more patients developing shock, organ dysfunction and severe bleeding, resulting in higher mortality rates. Being able to predict those who will progress to severe disease is an intense area of research activity.

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Curr Opin Infect Dis 2024, 37:349–356
DOI:10.1097/QCO.0000000000001040

KEY POINTS

- The extent and duration of viraemia appears to be often associated with clinical disease severity, but there is variation.
- Following a secondary dengue infection, most individuals develop broadly neutralizing antibodies, which protect against subsequent infection and severe disease, although some individuals may still develop infection and illness.
- DENV infects B cells, altering their receptor signalling, interferon responses and antigen processing, especially in those who progress to severe disease.
- An overall dysregulated IFN response, with an enhanced proinflammatory response in seen in individuals who progress to severe disease.
- Although many cytokines, chemokines and inflammatory lipid mediators have shown to be elevated during early illness in those who progress to develop plasma leakage, the nonredundant mediators and mechanisms responsible for endothelial dysfunction are not understood.

In this review, we discuss the recent developments in the understanding of immune mechanisms that lead to severe dengue and the learnings from the past that can help us to find therapeutic targets, prognostic markers and vaccines to prevent development of severe disease. We have focused primarily on recent advances based on publications within the last 18 months, as per journal guidelines.

CAN WE PREVENT SEVERE DISEASE BY TARGETING THE VIRUS?

Many clinical trials for antivirals for treatment of dengue have been carried out and so far, none of these have shown any substantial efficacy in phase 2 clinical trials [2]. Some of the challenges in assessing antivirals in acute dengue is the short viraemia, with peak viraemia coinciding with onset of symptoms. Data from controlled dengue human infection models (DHIMs) show that onset of viraemia occurs between 3 and 6 days from inoculation, with the onset of fever occurring 2.25 days, following detectable viraemia [9▪▪]. The duration of viraemia is often significantly shorter in those who experience a secondary dengue infection [10▪,11]. In dengue-naive DHIMs, the duration of viraemia is on average 7.3 days. In longitudinal studies, it is 4.9 days, possibly because of early clearance of the virus in those with preexisting antibodies to the DENV [10▪]. Indeed in, a DHIM following a prime-boost vaccination schedule where the individuals were challenged with DENV-1, the vaccinated individuals had earlier onset of viraemia, which cleared earlier than the control (vaccine-naive) individuals [12▪▪].

In DENV-3 DHIMs, both DENV viraemia and NS1 antigenemia correlated with duration of fever and rise in liver enzymes [9▪▪], whereas DENV-1 DHIMs failed to show such an association [13]. Higher viral loads during early illness have shown to associate with a modest but increased risk of progression to severe disease and thrombocytopenia [14], although some studies do not show any association [15▪▪,16]. Importantly, those who experience plasma leakage and severe dengue are likely to have delayed clearance of the virus, resulting in prolonged viraemia [11,14]. Progression to severe disease was associated with downregulation of type I and III interferon-signalling pathways and antigen presentation with an upregulation of pathways associated with proinflammatory immune responses (Fig. 1) [15▪▪]. Therefore, a dysfunctional antiviral response with an associated proinflammatory response could lead to prolonged viraemia, with a delay in clearance of the virus in patients with severe disease [17].

Although all DENV serotypes can cause dengue haemorrhagic fever (DHF) or severe dengue, DENV-2 has shown to associate with an increased risk disease severity in many studies [16,18]. The kinetics of viraemia vary widely between different DENV serotypes, with the lowest viral loads seen in infection with DENV-2, although it is associated with a worse disease outcomes (Fig. 1) [16,19]. Furthermore, primary infections with DENV-3 were more likely to result in symptomatic infection and DHF [20]. This could potentially explain the association of viral loads with clinical disease severity in DENV-3 DHIMs, but not in DENV-1 [9▪▪,13]. Given that the four DENV serotypes share approximately 75% sequence homology and there is a further 6–8% nucleotide diversity between different genotypes within each DENV serotype [17], it is not surprising that significant variations between viral loads, disease severity and pathogenicity exist between serotypes and genotypes.

WHAT IS THE ROLE OF DENGUE NONSTRUCTURAL PROTEIN 1 IN THE PATHOGENESIS OF SEVERE DISEASE?

DENV NS1 is a viral particle and secretory protein and has shown to induce proinflammatory cytokine production, endothelial dysfunction and interfere with coagulation pathways based on in-vitro experiments and in mouse models [17]. In acute infection, many studies give contrasting results regarding NS1 antigenemia and disease severity. Although some

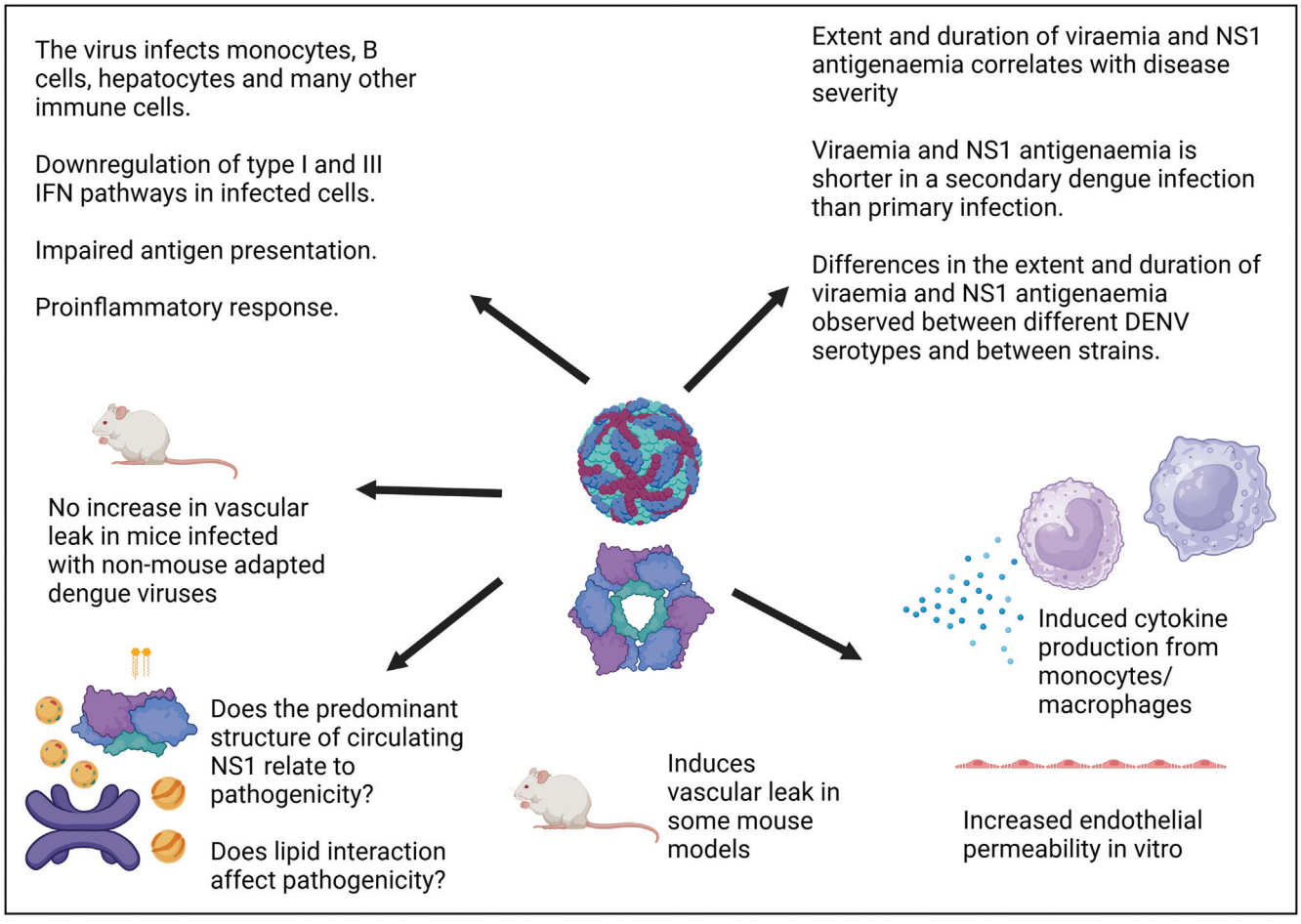

FIGURE 1. Contribution of the dengue virus and dengue NS1 in pathogenesis of severe disease. The extent and duration of viraemia appears to be often associated with clinical disease severity, but with notable variations. However, there appears to be significant differences in the kinetics of viraemia and NS1 antigenemia and pathogenicity between different serotypes and genotypes of the DENV. The existing data on NS1 causing disease pathogenesis are largely derived from in-vitro and mouse experiments, and there remain many unanswered questions. Figures were created using Biorender.com.

show earlier clearance of NS1 in those with DHF [11], some show that NS1 positivity is associated with severe disease in primary but not secondary dengue infections [21]. Furthermore, lower NS1 antigen levels are seen in DENV-2, which associates with higher risk of severe disease [22]. These contrasting findings may be because of differences in viraemia between DENV serotypes and genotypes, and possibly due to the structural differences of secretory NS1 (Fig. 1).

NS1 is essential in the formation of the virus replication complex and exists as a membrane associated form or a secretory form. The secretory form may exist as a dimer, tetramer or a hexamer and form complexes with high-density lipoprotein (HDL) (Chew, 2024). Although recent in-vitro data show NS1-HDL complexes induced proinflammatory cytokines from macrophages [23] and NS1 causes endothelial dysfunction *in vitro* and in mouse models [17,24], studies using nonmouse-adapted DENV strains show that exogenous administration of NS1 did not lead to worsening of vascular leak [25]. These studies using nonmouse-adapted DENVs in symptomatic mouse models showed that active immunization with NS1 or transfer of NS1-specific antibodies did not ameliorate DENV-induced pathogenesis [25]. Although NS1-HDL complexes were shown to induce proinflammatory cytokines *in vitro* [23], these studies do not explain the reasons for higher risk of severe disease in those with diabetes and obesity, which associate with lower HDL levels. It is possible that the interactions between NS1-HDL and other lipids could influence the pathogenicity of NS1, which warrants further investigations. Due to the limited understanding of NS1 in causing disease pathogenesis and given that the existing data are largely derived from *in vitro* and mouse experiments, it would be important to explore the

functional aspects of NS1, including structural interactions with different lipids and host molecules and more robust data from patients with varying severity of acute dengue.

WHAT TYPE OF DENGUE VIRUS-SPECIFIC ANTIBODIES PROTECT AGAINST SEVERE DISEASE?

Following the initial infection with the DENV, serotype-specific strongly neutralizing antibodies (Nabs) and cross-reactive antibodies are generated [26▪▪]. Although some of these cross-reactive antibodies neutralize all four DENV serotypes, the poorly neutralizing cross-reactive antibodies facilitate infection through binding to FcγRs on immune cells, which is known as antibody-dependent enhancement (ADE) [26▪▪]. ADE is thought to be responsible for increased disease severity during a secondary dengue infection with a different DENV serotype (Fig. 2) [17,18]. Although after a secondary dengue infection, most individuals develop broadly Nabs, which protect against subsequent infection and severe disease [27], symptomatic infection has still been reported in those with high Nabs following natural infection or vaccination [12▪▪,26▪▪]. Occasionally re-infection with the same serotype has also been reported in those with high Nabs to specific serotypes [28,29].

Previously it was shown that individuals who received a live-attenuated dengue vaccine (TV003), were completely protected from infection when re-challenged with DENV-2, 6 months following one dose of the vaccine [30]. However, recently, it was shown that those who received a prime-boost dengue vaccine schedule (tetravalent inactivated dengue vaccine followed by a tetravalent live-attenuated dengue vaccine), when challenged with an attenuated DENV-1 virus, 27–65 months following the booster dose were not protected against infection [12▪▪]. In fact, the onset of viraemia and peak viraemia occurred earlier in vaccinees, compared

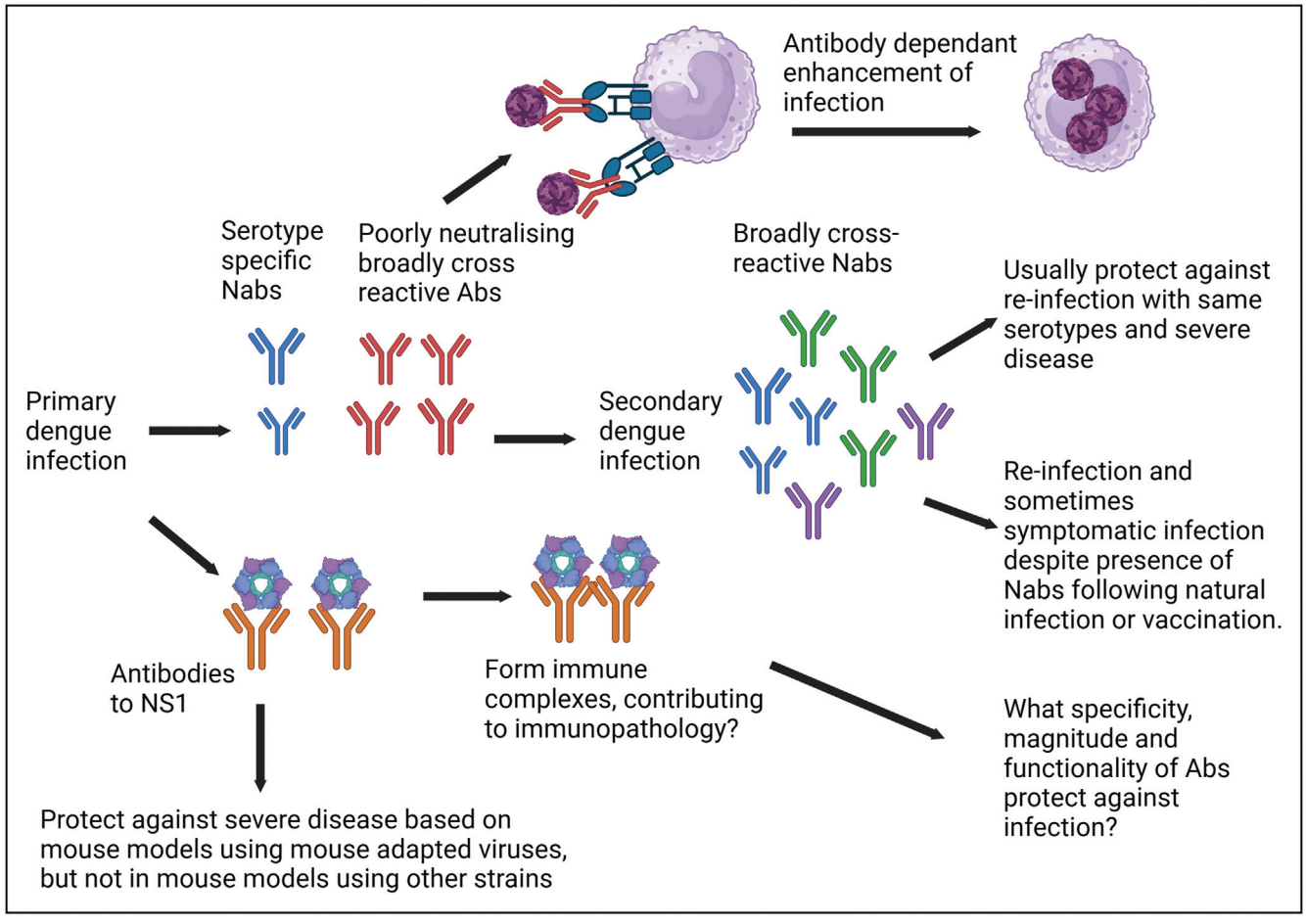

FIGURE 2. Contribution of the dengue virus-specific neutralizing antibodies and nonstructural protein 1 antibodies for protection against dengue and possible involvement in disease pathogenesis. Although generally higher levels of neutralizing antibodies are thought to protect against infection and severe disease, there have been exceptions and the specificity, breadth and functionality of the antibody responses are likely to be important. Figures were created using Biorender.com.

with dengue-naive controls, despite the presence of DENV-1-specific Nabs. Importantly, the vaccinees had an earlier disease onset and more severe symptoms compared with the controls [12▪▪]. A strong association was observed between in-vitro ADE assays and the onset of viraemia in the vaccinees, suggesting earlier viraemia and increased disease severity is likely to be due to ADE [12▪▪].

These findings have significant implications for dengue vaccination strategies. Firstly, different vaccines may elicit DENV-specific antibodies, targeting different epitopes resulting in broadly Nabs, while some vaccines may generate serotype-specific poorly neutralizing Nabs. Indeed, dengue vaccines may mimic a primary dengue infection and lead to more severe disease during subsequent natural infection, which was observed with the CYD-TDV vaccine [31]. Apart from neutralization, the functionality of the antibody response plays an important role in determining clinical outcomes [32,33]. Natural infection or dengue vaccines may induce antibodies, with varying affinity to different FcγRs, affecting their likelihood of causing ADE as shown in DHIMs in vaccinated individuals (those who received the prime-boost vaccination schedule) [12▪▪]. In acute dengue, higher levels of afucosylated DENV-specific IgG1 associated with increased dengue disease severity and not levels of Nabs [34]. Therefore, the functionality of DENV-specific antibodies does appear to play a significant role in subsequent disease severity or protection, which incompletely understood (Fig. 2).

Although a major portion of DENV-specific antibodies is directed against the virus envelope, a significant proportion of antibodies and memory B-cell responses are also directed against NS1, prM and the capsid protein [35,36]. Although previous studies have shown that NS1-specific antibodies rise in patients with DHF, it is not clear if this relates to higher NS1 antigen levels possibly seen in patients with DHF [37]. Although certain monoclonal antibodies targeting specific regions of NS1 were shown to protect against endothelial permeability *in vitro* and in dengue mouse models [38], there are limited data available on differences in the quantity, specificity and functionality of NS1-specific antibodies in preventing disease severity or causing immunopathogenesis in different settings, for example, when carrying different lipid payloads. In addition to NS1 antibodies, antibodies to precursor membrane (prM) can also contribute to disease pathogenesis by ADE. Although the immature virus with prM is noninfectious, these particles cause infection as anti-prM antibodies facilitate infection through binding to FcγRs causing ADE [36,39]. Therefore, the levels of antiprM antibodies, and their functionality could also affect disease severity, which has not been fully studied in acute dengue infection.

WHAT COULD AFFECT THE QUALITATIVE AND QUANTITATIVE DIFFERENCES IN DENGUE VIRUS-SPECIFIC ANTIBODIES?

Active replication of DENV within B cells in patients with acute dengue resulted in downregulation of genes involved in B-cell receptor signalling, interferon-stimulated genes and antigen processing, which was more pronounced in those who progressed to severe disease [15▪▪]. High frequencies of plasmablasts are seen in patients with acute dengue, especially in those with severe disease [40]. Significant expansion of the plasmablasts in patients with severe COVID-19, was shown to be due to extrafollicular B-cell activation [41]. Although this has not been investigated in acute dengue, it is possible that altered B–T-cell interactions lead to generation of extrafollicular plasmablasts, which potentially produce poor-quality, short-lasting antibodies with altered functionality. Unfortunately, there are very limited data available on the functionality and phenotype of B-cell responses in acute dengue and the specificity and functionality of antibody responses associated with protection in dengue.

WHICH TYPE OF T-CELL RESPONSES ARE ASSOCIATED WITH PROTECTION?

The debate whether DENV-specific T-cell responses are protective or pathogenic continues. Studies do show that early appearance of IFNγ-producing T cells to overlapping peptides of DENV NS3 and NS5 are associated with milder illness and early clearance of the virus [42]. Furthermore, a lower magnitude of DENV-specific T-cell responses were seen in those with HLA alleles associated with severe disease [43]. However, several earlier studies using T-cell clones have shown that both $CD4^+$ and $CD8^+$ T cells were highly activated, with magnitude of $CD8^+$ T cells specific to the primary infecting DENV serotype, correlating with disease severity [44,45▪].

More recent studies show that those who had mild illness had an expansion of memory $CD4^+$ and $CD8^+$ T cells, with an increased cell proliferation and enhanced cellular metabolism profile, whereas progression to DHF was associated with a predominantly type I innate immune response, with an inflammatory transcriptomic and inflammatory chemokine profile [45▪]. Another recent study showed that there was an expansion of regulatory T cells along with a higher frequency of $CD4^+$ and $CD8^+$ memory T-cell-expressing exhaustion markers in those who progress to severe disease [15▪▪]. $CD8^+$ T cells expressed

markers of T-cell dysfunction (CTLA-4, LAG3, TIGIT and PDCD1), with downregulation of interferon-stimulated genes in those who progressed to severe dengue [15▪▪]. Both these studies show a dysregulated IFN response with higher IFNγ levels (mainly derived from T cells and natural killer (NK) cells), and an increased expression of IFN-signalling genes was associated with progression to severe disease [15▪▪,45▪]. However, both these recent studies have focused on the overall T-cell compartment rather than DENV-specific T cells.

As it was shown that B cells and other antigen-presenting cells had impaired capacity for antigen presentation, this could lead to reduction in DENV-specific T cells in those with severe dengue [15▪▪]. Indeed, previous studies have shown a reduced frequency of DENV-specific T cells in those with severe dengue, for example, through suppression by IL-10 [42,46]. As regulatory T cells expressing many inhibitory co-stimulatory molecules were seen to expand during early illness in those who progress to severe disease, they could possibly be an important source of IL-10 leading to suppression of DENV-specific T-cell responses [15▪▪]. Given that Nabs alone do not confer protection against subsequent infection and severe disease, it would be crucial to further understand the role of DENV-specific T cells in protection and pathogenesis. This is an important gap in knowledge that needs to be addressed, by carefully conducted longitudinal studies in those with varying history of previous dengue or other flavivirus infection such as Zika, as these can modulate the subsequent T-cell responses upon natural infection or vaccination.

MECHANISMS OF VASCULAR LEAK AND SEVERE DENGUE

Endothelial dysfunction leading to increased capillary permeability is the hallmark of severe dengue [17]. Although many cytokines, chemokines and inflammatory lipid mediators have shown to be elevated during early illness in those who progress to develop plasma leakage [17,45▪,47], the exact mechanisms leading to endothelium dysfunction or the mediators responsible for these mechanisms are unknown. Although dengue NS1 has been shown to increase endothelial permeability *in vitro* and in mouse models using mouse-adapted strains [24,48], studies using nonmouse-adapted strains have not replicated these results [25]. Furthermore, increased capillary permeability occurs in acute dengue when NS1 antigen levels decline [22]. Therefore, the role of NS1 in causing vascular leakage in acute dengue and the potential structural differences in NS1 based on its interaction with different lipid mediators and DENV strain-dependent changes should be further investigated.

Mast cell proteases (chymase, tryptase) and inflammatory lipids, including platelet-activating factor (PAF), leukotrienes, prostaglandins, phospholipase A2 enzymes, Ang-2, VEGF, IL-6, IL-1β and TNFα, which lead to endothelial dysfunction and protection (sphingosine-1-phosphatase), are altered in those who developed plasma leakage [17,49,50]. However, the relative contribution of these different mediators in causing vascular leakage or the factors that lead to their increase are not fully understood. Given the crucial role that plasma leakage plays in dengue disease severity, it would be important for any potential treatment for dengue to reduce plasma leakage, in order to reduce associated disease severity.

WHY DO ONLY SOME INDIVIDUALS DEVELOP VASCULAR LEAK AND ORGAN DYSFUNCTION?

Diabetes, cardiovascular disease, hypertension, obesity and asthma are associated with an increased risk of developing severe dengue [18]; and obesity has shown to associate with an increased risk of hospitalization [51]. The risk of severe dengue associated with these comorbidities is greater than the risk with a secondary dengue infection [18]. Obesity, diabetes and the other comorbidities are also a risk factor for severe disease with SARS-CoV-2 and other viral infections [52]. Obesity and diabetes are linked with dysfunctional adipose tissue, increased infiltration of activated classical macrophages, increase in mast cells with an altered phenotype, altered adipokine profile leading to inflammation, increased intestinal permeability and endotoxaemia, chronic low-grade inflammation, dysfunctional NK and T-cell responses and waning of Nab responses [53–55]. Diabetes also has shown to lead to concurrent bacterial infection in those with dengue contributing to increased disease severity and mortality [56]. Given that diabetes, obesity and other related comorbidities are rapidly increasing globally, it would be important to identify the mechanisms that lead to severe dengue in these individuals for better management and development of therapeutic targets.

CONCLUSION

In summary, as the extent of viraemia and more importantly the duration of viraemia often appears to associate with clinical disease severity, antivirals would perhaps be anticipated to reduce disease severity. However, given the very short duration of viraemia, it is challenging to initiate treatment early enough to achieve a therapeutic benefit.

Furthermore, there appears to be significant differences in the kinetics of viraemia and pathogenicity between different genotypes within the serotypes of the DENV. These differences may have significant implications for development of treatments and in inducing robust immunity through dengue vaccines. Although dengue NS1 has been shown to contribute to disease pathogenesis *in vitro* and in mouse models, many studies have shown conflicting results with limited data in individuals with acute dengue. Again, the pathogenicity may vary in different DENV strains as well as different interactions between NS1 and host lipids and structural differences.

Although generally higher levels of Nabs are thought to protect against infection and severe disease, this may not always be the case and there are many unknowns regarding the specificity, magnitude, breadth and functionality of antibody responses that associate with protection. Similarly, although recent studies highlight important changes in the T-cell compartment that are associated with progression to severe disease, there are limited data on the specificity and functionality of T-cell responses that are associated with protection. Most importantly, there are again limited data on how the past infection history with previous DENV serotype or other flavi-viruses affect subsequent immune responses to dengue. Therefore, although there has been many advanced in our understanding of dengue pathogenesis, there are still many unknowns regarding the most important questions in immune responses that associate with severe dengue such as the mediators that lead to vascular leak, the viral factors that associate with disease pathogenesis and the correlates of protection against dengue disease.

Acknowledgements

None.

Financial support and sponsorship

We are grateful for support from the MRC.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. WHO: Dengue - global situation. In: WHO, editor. WHO; 2023.
2. Malavige GN, Sjo P, Singh K, *et al.* Facing the escalating burden of dengue: Challenges and perspectives. PLOS Glob Public Health 2023; 3:e0002598.
3. De Santis O, Bouscaren N, Flahault A. Asymptomatic dengue infection rate: a systematic literature review. Heliyon 2023; 9:e20069.
4. Shepard DS, Undurraga EA, Halasa YA, Stanaway JD. The global economic burden of dengue: a systematic analysis. Lancet Infect Dis 2016; 16:935–941.
5. Rodrigo C, Sigera C, Fernando D, Rajapakse S. Plasma leakage in dengue: a systematic review of prospective observational studies. BMC Infect Dis 2021; 21:1082.
6. Harapan H, Michie A, Mudatsir M, *et al.* Epidemiology of dengue hemorrhagic fever in Indonesia: analysis of five decades data from the National Disease Surveillance. BMC Res Notes 2019; 12:350.
7. Halstead SB, Cohen SN. Dengue hemorrhagic fever at 60 years: early evolution of concepts of causation and treatment. Microbiol Mol Biol Rev 2015; 79:281–291.
8. Togami E, Chiew M, Lowbridge C, *et al.* Epidemiology of dengue reported in the World Health Organization's Western Pacific Region, 2013–2019. Western Pac Surveill Response J 2023; 14:1–16.
9. ■■ Waickman AT, Newell K, Lu JQ, *et al.* Low-dose dengue virus 3 human challenge model: a phase 1 open-label study. Nat Microbiol 2024; 9:1356–1367.

This study shows the kinetics of viraemia and NS1 antigenaemia in relation to onset of symptoms and clinical disease severity in DENV-3 human challenge models.

10. ■ Riswari SF, Velies DS, Lukman N, *et al.* Dengue incidence and length of viremia by RT-PCR in a prospective observational community contact cluster study from 2005–2009 in Indonesia. PLoS Negl Trop Dis 2023; 17: e0011104.

This study shows the kinetics of viraemia following natural infection in naive individuals and in those with preexisting immunity to flaviviruses, who develop symptomatic and asymptomatic infections living in dengue endemic areas.

11. Nainggolan L, Dewi BE, Hakiki A, *et al.* Association of viral kinetics, infection history, NS1 protein with plasma leakage among Indonesian dengue infected patients. PLoS One 2023; 18:e0285087.
12. ■■ Lyke KE, Chua JV, Koren M, *et al.* Efficacy and immunogenicity following dengue virus-1 human challenge after a tetravalent prime-boost dengue vaccine regimen: an open-label, phase 1 trial. Lancet Infect Dis 2024. S1473-3099(24)00100-2. doi: 10.1016/S1473-3099(24)00100-2.

This human challenge model study shows that individuals with preexisting immunity to dengue following immunization with an inactivated vaccine prime and live-attenuated vaccine boost, were unprotected from a DENV-1 challenge and in fact developed more symptomatic and severe infection than naive individuals.

13. Endy TP, Wang D, Polhemus ME, *et al.* A phase 1, open-label assessment of a dengue virus-1 live virus human challenge strain. J Infect Dis 2021; 223:258–267.
14. Vuong NL, Quyen NTH, Tien NTH, *et al.* Dengue viremia kinetics and effects on platelet count and clinical outcomes: an analysis of 2340 patients from Vietnam. medRxiv 2024; 13:RP92606.
15. ■■ Ghita L, Yao Z, Xie Y, *et al.* Global and cell type-specific immunological hallmarks of severe dengue progression identified via a systems immunology approach. Nat Immunol 2023; 24:2150–2163.

This study shows that progression to severe disease was associated with down-regulation of type I and III interferon-signalling pathways and antigen presentation with and upregulation of pathways associated with proinflammatory immune responses. It is also the first study showing conclusive evidence of infection of B cells during acute dengue.

16. Dayarathna S, Kuruppu H, Silva T, *et al.* Are viral loads in the febrile phase a predictive factor of dengue disease severity? medRxiv 2023.
17. Malavige GN, Ogg GS. Molecular mechanisms in the pathogenesis of dengue infections. Trends Mol Med 2024; 30:484–498.
18. Sangkaew S, Ming D, Boonyasiri A, *et al.* Risk predictors of progression to severe disease during the febrile phase of dengue: a systematic review and meta-analysis. Lancet Infect Dis 2021; 21:1014–1026.
19. Vuong NL, Quyen NTH, Tien NTH, *et al.* Higher plasma viremia in the febrile phase is associated with adverse dengue outcomes irrespective of infecting serotype or host immune status: an analysis of 5642 Vietnamese cases. Clin Infect Dis 2021; 72:e1074–e1083.
20. Bos S, Zambrana JV, Duarte EM, *et al.* Serotype-Specific Epidemiological Patterns of Inapparent versus Symptomatic Primary Dengue Virus Infections: A 17-year cohort study in Nicaragua. medRxiv [Preprint] 2024; 2024.04.05.24305281. doi: 10.1101/2024.04.05.24305281.
21. Martinez-Cuellar C, Lovera D, Galeano F, *et al.* Nonstructural protein 1 (NS1) of dengue virus detection correlates with severity in primary but not in secondary dengue infection. J Clin Virol 2020; 124:104259.
22. Duyen HT, Ngoc TV, Ha do T, *et al.* Kinetics of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: differential effects according to serotype and immune status. J Infect Dis 2011; 203:1292–1300.
23. Benfrid S, Park KH, Dellarole M, *et al.* Dengue virus NS1 protein conveys pro-inflammatory signals by docking onto high-density lipoproteins. EMBO Rep 2022; 23:e53600.
24. Puerta-Guardo H, Glasner DR, Espinosa DA, *et al.* Flavivirus NS1 triggers tissue-specific vascular endothelial dysfunction reflecting disease tropism. Cell Rep 2019; 26:1598.e8–1613.e8.
25. Lee PX, Ting DHR, Boey CPH, *et al.* Relative contribution of nonstructural protein 1 in dengue pathogenesis. J Exp Med 2020; 217.
26. ■■ Bos S, Graber AL, Cardona-Ospina JA, *et al.* Protection against symptomatic dengue infection by neutralizing antibodies varies by infection history and infecting serotype. Nat Commun 2024; 15:382.

This study shows the ability of Nabs to neutralize the virus depends on the state of maturation of the virions, and the prior infection history with different DENV serotypes. The neutralizing antibody repertoire and cross reactivity depends on previous dengue infection history.

27. Gallichotte EN, Baric RS, de Silva AM. The molecular specificity of the human antibody response to dengue virus infections. Adv Exp Med Biol 2018; 1062:63–76.
28. Waggoner JJ, Balmaseda A, Gresh L, *et al.* Homotypic dengue virus reinfections in Nicaraguan children. J Infect Dis 2016; 214:986–993.
29. Saez-Llorens X, Biswal S, Borja-Tabora C, *et al.,* TIDES Study Group. Effect of the tetravalent dengue vaccine TAK-003 on sequential episodes of symptomatic dengue. Am J Trop Med Hyg 2023; 108:722–726.
30. Kirkpatrick BD, Whitehead SS, Pierce KK, *et al.* The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. Sci Transl Med 2016; 8:330–336.
31. Sridhar S, Luedtke A, Langevin E, *et al.* Effect of dengue serostatus on dengue vaccine safety and efficacy. N Engl J Med 2018; 379:327–340.
32. Zhang A, Stacey HD, D'Agostino MR, *et al.* Beyond neutralization: Fc-dependent antibody effector functions in SARS-CoV-2 infection. Nat Rev Immunol 2023; 23:381–396.
33. Paquin-Proulx D, Gunn BM, Alrubayyi A, *et al.* Associations between antibody Fc-mediated effector functions and long-term sequelae in Ebola virus survivors. Front Immunol 2021; 12:682120.
34. Bournazos S, Vo HTM, Duong V, *et al.* Antibody fucosylation predicts disease severity in secondary dengue infection. Science 2021; 372:1102–1105.
35. Ramu ST, Dissanayake M, Jeewandara C, *et al.* Antibody and memory B cell responses to the dengue virus NS1 antigen in individuals with varying severity of past infection. Immunology 2023; 170:47–59.
36. Dowd KA, Sirohi D, Speer SD, *et al.* prM-reactive antibodies reveal a role for partially mature virions in dengue virus pathogenesis. Proc Natl Acad Sci U S A 2023; 120:e2218899120.
37. Jayathilaka D, Gomes L, Jeewandara C, *et al.* Role of NS1 antibodies in the pathogenesis of acute secondary dengue infection. Nat Commun 2018; 9:5242.
38. Biering SB, Akey DL, Wong MP, *et al.* Structural basis for antibody inhibition of flavivirus NS1-triggered endothelial dysfunction. Science 2021; 371:194–200.
39. Wirawan M, Fibriansah G, Marzinek JK, *et al.* Mechanism of enhanced immature dengue virus attachment to endosomal membrane induced by prM antibody. Structure 2019; 27:253.e8–267.e8.
40. Wijesinghe A, Gamage J, Goonewardena H, *et al.* Phenotype and functionality of follicular helper T cells in patients with acute dengue infection. J Biomed Sci 2020; 27:50.
41. Woodruff MC, Ramonell RP, Haddad NS, *et al.* Dysregulated naive B cells and de novo autoreactivity in severe COVID-19. Nature 2022; 611:139–147.
42. Wijeratne DT, Fernando S, Gomes L, *et al.* Quantification of dengue virus specific T cell responses and correlation with viral load and clinical disease severity in acute dengue infection. PLoS Negl Trop Dis 2018; 12:e0006540.
43. Weiskopf D, Angelo MA, de Azeredo EL, *et al.* Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. Proc Natl Acad Sci U S A 2013; 110:E2046–E2053.
44. Culshaw A, Mongkolsapaya J, Screaton GR. The immunopathology of dengue and Zika virus infections. Curr Opin Immunol 2017; 48:1–6.
45. ■ Ioannidis LJ, Studniberg SI, Eriksson EM, *et al.* Integrated systems immunology approach identifies impaired effector T cell memory responses as a feature of progression to severe dengue fever. J Biomed Sci 2023; 30:24.

This study shows that those who had mild illness had an expansion of memory $CD4^+$ and $CD8^+$ T cells, with an increased cell proliferation and enhanced cellular metabolism profile, while progression to DHF was associated with a predominantly type I innate immune response, with an inflammatory transcriptomic and inflammatory chemokine profile.

46. Malavige GN, Jeewandara C, Alles KM, *et al.* Suppression of virus specific immune responses by IL-10 in acute dengue infection. PLoS Negl Trop Dis 2013; 7:e2409.
47. Vuong NL, Lam PK, Ming DKY, *et al.* Combination of inflammatory and vascular markers in the febrile phase of dengue is associated with more severe outcomes. Elife 2021; 10:e67460.
48. Beatty PR, Puerta-Guardo H, Killingbeck SS, *et al.* Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. Sci Transl Med 2015; 7:304–141.
49. Rathore AP, Mantri CK, Aman SA, *et al.* Dengue virus-elicited tryptase induces endothelial permeability and shock. J Clin Invest 2019; 130:4180–4193.
50. Rathore APS, Senanayake M, Athapathu AS, *et al.* Serum chymase levels correlate with severe dengue warning signs and clinical fluid accumulation in hospitalized pediatric patients. Sci Rep 2020; 10:11856.
51. Jeewandara C, Karunananda MV, Fernando S, *et al.,* Seroprevalence study group. Is the rise in childhood obesity rates leading to an increase in hospitalizations due to dengue? PLoS Negl Trop Dis 2024; 18:e0012248.
52. Sattar N, Valabhji J. Obesity as a risk factor for severe COVID-19: summary of the best evidence and implications for healthcare. Curr Obes Rep 2021; 10:282–289.
53. Divoux A, Moutel S, Poitou C, *et al.* Mast cells in human adipose tissue: link with morbid obesity, inflammatory status, and diabetes. J Clin Endocrinol Metab 2012; 97:E1677–E1685.
54. Andrade FB, Gualberto A, Rezende C, *et al.* The Weight of Obesity in Immunity from Influenza to COVID-19. Front Cell Infect Microbiol 2021; 11:638852.
55. van der Klaauw AA, Horner EC, Pereyra-Gerber P, *et al.* Accelerated waning of the humoral response to COVID-19 vaccines in obesity. Nat Med 2023; 29:1146–1154.
56. Chen CM, Chan KS, Cheng KC, *et al.* The exploration of risk factors of concurrent bacteraemia in patients critically ill with severe dengue. J Med Microbiol 2016; 65:1505–1511.