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Dengue Virus- The Life Threatening Virus

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ABSTRACT

Dengue is an acute viral illness caused by RNA virus of the family Flaviviridae and spread by Aedes mosquitoes. The presenting features may range from asymptomatic fever to dreaded complications such as haemorrhagic fever and shock. Acute onset high fever, muscle and joint pain, myalgia, cutaneous rash, haemorrhagic episodes, and circulatory shock are the commonly seen symptoms. Early and accurate diagnosis is critical to reduce mortality. Although dengue virus infections are usually self-limiting, dengue infection has come up as a public health challenge in the tropical and subtropical nations. For the past ten years, the number of dengue cases has gradually increased in India. Dengue is driven by complex interactions among host, vector and virus that are influenced by climatic factors.

KEYWORDS: Break bone fever, Cutaneous Rash, Dengue virus.

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INTRODUCTION

The Dengue Virus, a member of the genus *Flavivirus* of the family *Flaviviridae*, is an arthropod-borne virus that includes four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4)^{1, 2}. The World Health Organization (WHO) considers dengue as a major global public health challenge in the tropic and subtropical nations. Dengue has seen a 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel, and lack of health care facilities^{3, 4, 5}. Two and a half billion people reside in dengue-endemic regions and roughly 400 million infections occurring per year, with a mortality rate surpassing 5–20% in some areas⁶. Dengue infection affects more than 100 countries, including Europe and the United States⁷. The first reported case of dengue like illness in India was in Madras in 1780, the first biologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963–1964⁸. Dengue virus infection presents with a diverse clinical picture that ranges from asymptomatic illness to DF to the severe illness of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). Oral mucosal involvement is seen in approximately 30% of patients, although oral features are more frequently associated with DHF than with DF⁹. Dengue virus infection exhibit varied clinical presentation hence accurate diagnosis is difficult and relies on laboratory confirmation. The condition is usually self-limiting and antiviral therapy is not currently available. Supportive care with analgesics, hydration with fluid replacement, and sufficient bed rest forms the preferred management strategy.

PATHOPHYSIOLOGY

DF is a severe flu-like infection that involves individuals of all age groups (infants, children, adolescents, and adults).⁹ Transmission among human beings occurs by the mosquito *Aedes aegypti* and chiefly occurs during the rainy season¹⁰.

The proposed aetiologies for Dengue Virus infection are

- Viral replication, primarily in macrophages¹¹
- Direct skin infection by the virus¹²
- Immunological and chemical-mediated mechanism induced by host–viral interaction¹².

Dengue virus gains entry into the host organism through the skin following an infected mosquito bite. Humoral, cellular, and innate host immune responses are implicated in the progression of the illness and the more severe clinical signs occur following the rapid clearance of the virus from the host organism. Hence, the most severe clinical presentation during the infection course does not correlate with a high viral load¹³. Alterations in endothelial microvascular permeability and thromboregulatory mechanisms lead to an increased loss of protein and plasma.

Proposed theories suggest that endothelial cell activation caused by monocytes, T-cells, the complement system, and various inflammatory molecules mediate plasma leakage. Thrombocytopenia may be related to alterations in megakaryocytopoiesis, manifested by infection of human hematopoietic cells and compromised progenitor cell growth. This may cause platelet dysfunction, damage, or depletion, leading to significant haemorrhages^{14, 15}.

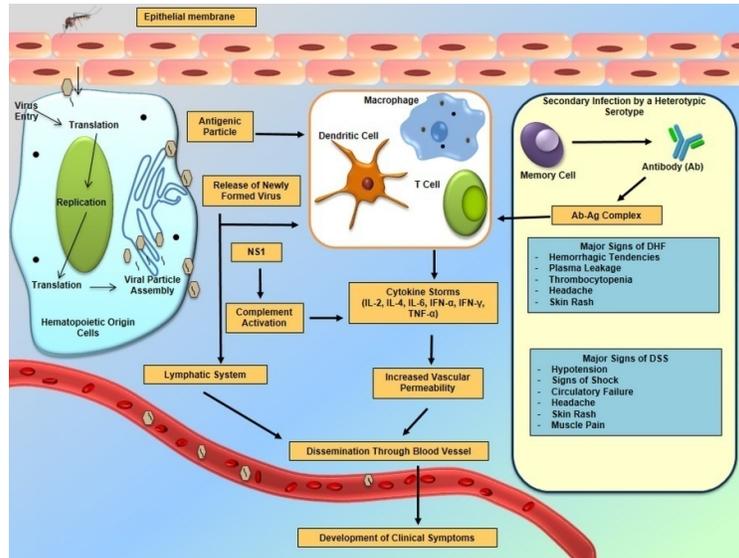


Fig. 1: Dengue virus replication and signs

Pathogenesis of dengue virus infection

Plasma leakage is specific to the pleural and peritoneal surfaces. In DHF there is no vasculitis and hence no injury to the vessel walls, and plasma leakage results from cytokine mediated increase in vascular permeability. The ensuing movement of albumin and the resultant reduction of intravascular oncotic pressure facilitate further loss of fluid from the intravascular compartment. The basic Starling principle still holds true in explaining microvascular ultrafiltration based on the balance of the oncotic and hydrostatic pressures. However the glycocalyx, which is a gelatinous layer lining the vascular endothelium is also implicated in controlling fluid movement by the adherence of albumin molecules in to its matrix, damage of which, leads to loss of albumin into the extravascular compartment¹⁶⁻¹⁹.

The immune system is implicated in the pathogenesis of DHF owing to the increased propensity to develop DHF with secondary dengue infection. The innate immune mechanisms comprising the complement pathway and NK cells as well as humoral and cell-mediated immune mechanisms launched in response to antigenic stimulation are involved in the clinical manifestations. Complement activation as well as vascular permeability may be influenced by viral products like NS1. Different immune mechanisms in the form of antibody enhanced viral replication leading to an exaggerated cytokine response impacts vascular permeability²⁰⁻²².

Infection with one dengue serotype elicits immunity to that serotype but does not provide long-term cross-protective immunity to the remaining serotypes. Subsequent infection with a different serotype results in the binding of the new virus to cross reactive non-neutralising antibody from the previous infection facilitating the uptake by mononuclear phagocytes enabling amplified viral replication. The resulting increase in viral load then drives an immune-pathogenic cascade and the resultant exaggerated cytokine response leads to a transient increase in micro-vascular permeability. The precise way in which micro-vascular permeability is altered is not clear but is more likely to be a functional change rather than structural damage, as dengue shock is rapidly recoverable, and no inflammation is evident in the leaking surfaces²³⁻²⁷. Adding to the complexity of the underlying immune-pathogenic mechanisms resulting in changes in vascular permeability is the proposal of an alternative mechanism whereby the rapid mobilisation of serotype cross-reactive memory T cells trigger the release of biological mediators. Some of the other factors implicated in this orchestration include viral virulence, molecular mimicry, and immune complex and/or complement mediated dysregulation, and genetic predisposition, all of which have been shown to correlate with disease severity. However, as yet no mechanism has been identified that links any of these established immunological derangements with a definitive effect on micro-vascular structure or function consistent with the observed alteration in permeability. In addition, most of the immunological abnormalities so far identified do not differ substantially from those seen in other infections without an apparent effect on permeability.

Neutralising antibodies are key factors in the aetiopathogenesis of the disease. However, the cellular immune response is also important. It has been demonstrated that memory dengue T lymphocyte response after a primary infection includes both serotype-specific and serotype-cross-reactive T lymphocytes²⁸. NS3 protein seems to be the major target for CD4+ and CD8+ T cells. Cytokines that may induce plasma leakage such as interferon γ , interleukin (IL) 2, and tumour necrosis factor (TNF) α are increased in DHF cases^{28,29}. Also, interferon γ enhances uptake of dengue particles by target cells through increasing Fc cell receptors³⁰. Other cytokines such as IL-6, IL-8, and IL-10 are also increased. A protein of 22–25 kDa has been associated with the pathogenesis of DHF. This cytotoxic factor able to induce increased capillary permeability in mice is capable of reproducing in mice all the pathological lesions that are seen in human beings, and has been detected in sera of DHF patients³¹.

A recent study has demonstrated the plasma cytokine profile in dengue fever from a Brazilian population which was detected by a multiplex bead immunoassay. MIP- β was indicated as a good prognostic marker which is in contrast to IFN- γ that was associated with severe disease. Both cytokines serve to discriminate mild from severe cases. It has also been shown that during the course

of dengue different cytokine profiles may be present and vary according to determined clinical manifestations. The cytokine profiles identified by bead array multiplex system may favour an early identification of patients with the worst prognosis and may contribute to the establishment of more directed therapeutic procedures than the present ones³².

Complement activation as a result of immune complexes (virus-antibody) or immune activation and cytokine production could also be involved in the mechanism of plasma leakage. Certain complement fragments such as C3a and C5a are known to enhance permeability. NS1 antigen in dengue virus has been shown to regulate complement activation and hence could play a role in the pathogenesis of DHF³³⁻³⁶. Clearly immunopathogenic mechanisms are involved in plasma leakage and coagulopathy. However alternate immune pathways are also implicated in a protective role adding to the complexity and intricacy of the pathogenesis of DHF. Activated NK cells release granzyme A, which has cytolytic functions. MIP-1 β produced by human monocytes and dendritic cells as well as activated NK cells and lymphocytes is chemoattractant for NK cells, recruiting them to inflammatory sites. These mechanisms could play a protective role in the immunopathology of DHF by the early and efficient clearance of DENV by direct or indirect NK functions thereby limiting viral replication and its attended cascading cytokine mediated plasma leakage. NK cells have been associated with mild dengue^{37, 38}.

In summary monocytes, macrophages, and dendritic cells are the major targets for DENV. During secondary infection with a different DENV serotype cross-reactive non-neutralising antibodies bind to DENV and facilitate uptake via Fc receptors resulting in enhanced viral replication. The resultant higher viral antigen load leads to an exaggerated activation of cross-reactive dengue specific T cells. Biological mediators released by the activated T cells as well as virus infected cells along with complement activation by viral proteins, and immune complexes are implicated in increasing vascular permeability and coagulopathy. These biological mediators influence clinical outcomes to a variable extent. Thus IL-1 β , IFN- γ , IL-4, IL-6, IL-13, IL-7, and GM-CSF are associated with severe clinical manifestations while MIP-1 β is elevated in patients with mild dengue. Marked thrombocytopenia is evident in patients with elevated IL-1 β , IL-8, TNF- α , and MIP-1, while increased levels of MIP-1 and GM-CSF correlated with hypotension³².

Haemorrhagic Manifestations in DHF

The pathogenesis of bleeding in DHF is unclear even though well-recognised coagulation disturbances do exist. The clinical haemorrhagic manifestations range from a mere positive tourniquet test, skin petechial and ecchymosis to epistaxis, and gum bleeding to severe gastrointestinal haemorrhages. Thrombocytopenia is a consistent finding, while prolonged partial thromboplastin time and reduced fibrinogen concentration are the other abnormal haemostatic

indices evident from early in the disease course. These haematological abnormalities seem to correlate better with the timing and severity of plasma leakage rather than the clinical haemorrhagic manifestations³⁹.

These recent findings raise the possibility for common pathogenic mechanisms responsible for both plasma leakage and abnormalities in the haemostatic indices. The true nature of the intrinsic coagulopathy evident early in the disease course and in mild forms of dengue can be confounded by the advent of hypovolemic shock and hypoxia in DHF with severe plasma leakage with less than optimal correction.

Thrombocytopenia is initially due to bone marrow suppression during the febrile viraemic phase of the illness. Progressive thrombocytopenia with defervescence results from immune mediated platelet destruction. Virus-antibody complexes have been detected on the platelet surface of DHF patients suggesting a role for immune-mediated destruction of platelets^{40, 41}. Augmented platelet adhesiveness to vascular endothelial cells resulting from the release of high levels of platelet-activating factor by monocytes with heterologous secondary infection also contributes to the thrombocytopenia⁴². Thrombocytopenia however correlates poorly with bleeding manifestations. It is strongly associated with the severity of vascular leakage. Counts below 100,000 cells/c.mm or a rapid drop in the platelet count was associated with severe disease.

The role of the glycocalyx rather than the endothelial cells in controlling ultrafiltration in the microvasculature is increasingly recognised and in vivo animal studies have shown the permeation of fibrinogen to the endothelial surface similar to albumin.

The low plasma fibrinogen detected in DHF could thus be a reflection of loss into the interstitial spaces in the setting of increased vascular permeability. Heparan sulphate forms an integral part of the glycocalyx which when damaged by the initial cytokine response in DHF gets liberated to the circulation and acts like an anticoagulant which could explain the prolonged APTT⁴³. The disturbance in both these important haemostatic indices is unlikely to cause spontaneous bleeding. Haemorrhages are triggered by trauma in this setting of coagulopathy.

Development of antibodies potentially cross-reactive to plasminogen could have a role in causing haemorrhage in DHF⁴⁴. However different studies have shown conflicting results as some have demonstrated an activation of fibrinolysis while others have shown an inhibition of the fibrinolytic pathway in DHF³⁹.

Endothelial Cells in DHF

Precise knowledge on the extent to which DENV infects endothelial cells is lacking as few studies have addressed the issue in the viraemic phase of the illness. Even though DENV has infected endothelial cells in vitro it is doubtful whether it reflects the effect in human infection as

limited human autopsy studies have detected only the dengue antigen but not the genome in various cell types ranging from monocytes, liver sinusoidal cells, alveolar macrophages, peripheral blood, and splenic lymphocytes. How important these findings are in the pathogenesis of clinical features are uncertain as some studies have shown swelling of endothelial cells but not cell death or vasculitis, while others have detected apoptosis of endothelial cells in lungs and intestinal mucosa in fatal DHF cases, but the extent of apoptosis has not been documented⁴⁵. DENV alters the endothelial cell surface protein production, its expression, and transcriptional activity.

Expression of ICAM-1 (intercellular adhesion molecule-1) and beta-integrin on microvascular endothelium by DENV has been reported. DENV also affects the expression of cytokine receptors. These may contribute to the mechanisms involved in plasma leakage in DHF. The role of DENV infected endothelial cells in the pathogenesis of coagulopathy in DHF is equally intriguing. There is up regulation of tissue plasminogen, thrombomodulin, protease activated receptor-1, and tissue factor receptor, while there is downregulation of tissue factor inhibitor and activated protein C.

SYMPTOMS

Dengue haemorrhagic fever (DHF) is characterized by a fever that lasts from 2 to 7 days, with general signs and symptoms consistent with dengue fever. When the fever declines, warning signs may develop. This marks the beginning of a 24 to 48 hour period when the smallest blood vessels (capillaries) become excessively permeable (“leaky”), allowing the fluid component to escape from the blood vessels into the peritoneum (causing ascites) and pleural cavity (leading to pleural effusions). This may lead to failure of the circulatory system and shock, and possibly death without prompt, appropriate treatment. In addition, the patient with DHF has a low platelet count and haemorrhagic manifestations, tendency to bruise easily or have other types of skin haemorrhages, bleeding nose or gums, and possibly internal bleeding.

The Principal symptoms of Dengue are

- High fever and at least two of the following-
- Severe headache
- Severe eye pain (behind eyes)
- Joint pain
- Muscle and/or bone pain
- Rash
- Mild bleeding manifestation (e.g., nose or gum bleed, petechiae, or easy bruising)
- Low white cell count

Preventing Dengue Fever

There is no vaccine to prevent dengue fever. The best way to prevent the disease is to prevent bites by infected mosquitoes, particularly if you are living in or traveling to a tropical area. This involves protecting yourself and making efforts to keep the mosquito population down.

TREATMENT FOR DENGUE FEVER

There is no specific medicine to treat dengue infection. If you think you may have dengue fever, you should use pain relievers with acetaminophen and avoid medicines with aspirin, which could worsen bleeding. You should also rest, drink plenty of fluids, and see your doctor. If you start to feel worse in the first 24 hours after your fever goes down, you should get to a hospital immediately to be checked for complications⁴⁶.

Climatic parameters influence on Dengue Virus

Dengue is a major public health problem in India. Some studies have reported that an epidemiological shift in dengue viruses and climate change might be responsible for the observed increase in dengue burden across India^{47, 48}. Studies have focused on several epidemiological and entomological aspects of dengue and to a lesser extent on understanding the relevance of climatic factors, but none have investigated the Extrinsic Incubation Period (EIP) of the dengue virus within the mosquito vector. This is the first study to estimate the extrinsic incubation period by using temperature data for different states of India. The EIP plays a major role in dengue-endemic regions, where the vectors ingest the virus through a blood meal, and the virus escapes the mid gut, passes through the mosquito body and finally reaches the salivary glands and can be transmitted to another susceptible host. Most modelling studies have considered static EIP values rather than dynamic EIP estimates for a particular region.⁴⁹ Recent studies have shown that seasonal mean temperature in India has increased significantly over the past 100 years, with an increase of 0.9 °C during the post-monsoon period and 1.1 °C during winter.⁵⁰ Slight increase in temperatures can increase the dengue risk by increasing the mosquito development rate and shortening the virus incubation time, thereby increasing the rate of transmission. In India, temperature varies in different climatic zones at both temporal and spatial scales, and these variations influence the EIP. This influence is very pronounced in Punjab, Haryana and Rajasthan, where the EIP generally exceeds the average life span (45–49 days) of both *Ae. aegypti* and *Ae. albopictus* mosquitoes, even during intense dengue transmission periods.^{51, 52}

Some studies have reported that daily temperature variation may play a major role in dengue virus transmission and vector–pathogen interactions.^{53, 54} Similarly, understanding of vector ecology has improved, thus providing novel ideas on how to quantify the impact of anthropogenic climate change on pest and disease risk^{55, 56}. In general, the EIP for dengue ranges between 8 and 12

days.⁵⁷ Most studied states showed low EIP values during the monsoon period, whereas other seasons had large spatial variation in the EIP. The north-western parts of India usually exhibited low-temperature conditions during the late post-monsoon or winter periods, owing to cold conditions. In cool temperature settings, DENV cannot reproduce in mosquitoes, and transmission does not occur. An experimental study has also shown that below 18 °C, the virus cannot be found in the vector's salivary glands, whereas at 21 °C, the viral antigen is detectable in *Ae. albopictus*.⁵⁸ Similarly, above 20 °C, the dengue incidence gradually increases and peaks at ~32 °C before declining at higher temperatures.⁵⁹ The EIP for dengue viruses has been found to decrease when the temperature increases from 26 to 30 °C, results similar to our findings.⁶⁰ In tropical countries, including India, these temperatures are generally experienced during monsoon or early post-monsoon periods.

Among the five states studied, Kerala experiences the highest number of dengue cases, possibly because of the availability of breeding grounds, a higher percentage of infected mosquitoes, suitable temperature ranges (23.5–30 °C) and subsequent short incubation periods in all seasons (9–14 days) and during the rainy season. These temperature ranges are mostly suitable for mosquito development and virus transmission. Similar studies have also reported a high prevalence of dengue in Mexico during the rainy season, when temperatures typically range between 17 and 30 °C. Temperatures in the lower range of dengue distribution (~17–18 °C) limit disease transmission through its effect on the EIP. High temperatures (~35 °C, depending on the vector species) tend to decrease disease risk, because they can limit mosquito survival. Consequently, future climate change might further affect dengue burden and that of other vector-borne diseases in India. In cooler areas, where temperature is a limiting factor, a slight increase in temperature might lead to disease transmission. As an example, dengue virus and its vector have rapidly expanded their range into Himalayan countries, such as Nepal and Bhutan, as well as into northern states of India, such as Darjeeling, over the past 10 years.⁶¹

A recent study has compiled all dengue outbreaks in India⁶² showing that most dengue outbreaks occurred in Punjab, Haryana, Rajasthan, Gujarat and Kerala states during the monsoon or post-monsoon period. Thus, all study states are influenced by strong seasonality, underscoring the roles of both rainfall and ambient temperature in the potential transmission of dengue virus during monsoon and post-monsoon periods. During the past few decades, *Aedes* vectors have expanded their geographical range. Apart from dengue, *Aedes* vectors can also transmit other arboviruses, such as chikungunya and Zika virus.⁶³

Recent advances in understanding cellular immune responses to dengue virus infection

The role of T cells

T cells, and in particular cross-reactive memory T cells recalled during secondary heterotypic infections, have been nominated as contributing to the clinical pathogenesis of dengue. This hypothesis is based on the observation that T cells having surface and functional phenotypes indicative of antigen-driven activation are more abundant in early convalescence in children/adults with severe dengue versus those with milder disease.⁶⁴⁻⁶⁶ T cell responses cannot however explain the pathogenesis of severe dengue in infants with primary infection and thus there is particular context to the “T cell hypothesis”. Stronger evidence, for or against, a mechanistic role for DENV-reactive memory T cells in the severe clinical complications of dengue is needed to help guide therapeutic intervention strategies. Similarly, a better understanding of how T cells might contribute to protection from re-infection is needed for the advancement of vaccine development and identification of immune correlates. Recent advances in understanding the targets of DENV-specific T cell responses, their functional phenotypes and their tissue tropisms goes some way to providing the tools to acquire stronger mechanistic insights into their role in pathogenesis and immunity.

CD8+ T cell responses: their targets and phenotypes

Substantial new data has been acquired on the targets of T cell responses after natural infection. Rivinoet al.⁶⁷ confirmed and expanded upon previous work in determining that CD8(+) T cell epitopes are predominantly located in the nonstructural proteins NS3 and NS5. Similarly Weiskopf et al.⁶⁸ identified DENV-reactive CD8+ T cell responses in Sri Lankan blood donors; 408 immunoreactive peptides were identified, two thirds of which were located in NS3, NS5 or NS4b. Finally, following tetravalent vaccination with live attenuated DENV, Weiskopf et al.⁶⁹ demonstrated CD8+ T cell responses were universally (99.8%) against non-structural proteins, with 97% directed toward NS3 and NS5. Collectively, these data underscore the importance of DENV non-structural proteins as CD8+ T cell immunogens during natural infection; this remains an important consideration for vaccine strategies that, along with induction of neutralizing antibody, also seek to induce CD8+ T cell memory.

Whether DENV-reactive CD8+ T cell responses contribute mechanistically to vascular leakage, the hallmark of severe dengue, remains unresolved. Based on observations of HLA-A*11-restricted CD8+ T cell responses to the NS3₁₃₃₋₁₄₂ epitope, Mongkolsapaya et al.⁷⁰ have suggested original antigenic sin occurs amongst CD8+ T cell populations during secondary heterotypic infection. Yet these conclusions have been drawn from observations made in early convalescence,

when CD8+ T cell responses peak, and not during the febrile and critical phase, when vascular leakage commences and is most prominent. Indeed, Dung et al.⁷¹ reported that NS3₁₃₃₋₁₄₂-specific T cells were undetectable until after the development of plasma leakage among infected Vietnamese children. In contrast, Freiberg et al.⁷² detected very low frequencies of activated NS3₁₃₃₋₁₄₂-specific CD8+ T cells during the febrile phase in both primary and secondary dengue cases, but found no evidence that these responses correlated with immune status exposure or current disease severity. Although traditionally measured in the blood, Rivino and colleagues⁷³ also identified highly activated and proliferating NS3₁₃₃₋₁₄₂-specific CD8+ T cells in experimentally induced skin blisters on dengue cases. Whether these dermis-infiltrating CD8+ T cells are relevant to pathogenesis of the clinical and laboratory features of dengue is uncertain.

The next phase of research into CD8+ T cells and pathogenesis needs to take an expansive approach to measuring epitope-specific T cells (e.g. using panels of Class I tetramer reagents, targeting more than one specificity) and investigate responses longitudinally in the three phases of disease; early febrile, critical phase and convalescence, with sample sizes large enough to capture the spectrum of clinical outcomes, from the very mild to the severe. These are challenging studies to perform but are necessary if the field is to advance the understanding of CD8+ T cells in pathogenesis and immunity.

CD4+ T cells responses: Their targets and Phenotypes

In contrast to CD8+ T cell responses, DENV-specific CD4+ T cells have been less well characterized. In patients with secondary dengue, Rivino et al.⁷⁴ demonstrated that CD4+ T cell epitopes are predominantly located in structural proteins e.g. envelope, capsid, and NS1, which themselves are major targets of the B cell response. Circulating CD4+ T cells during early convalescence had the surface and functional phenotype of follicular helper T cells, suggesting that they are interacting with B cells *in vivo*, presumably to assist antibody production. Whether the size of the acute expansion of the follicular helper T population in blood is predictive of the anti-DENV neutralizing Ab titer deserves investigation as a possible immune correlate of vaccine or infection-driven humoral immune responses. Mangada et al.⁷⁵ examined CD4+ cells in 6 donors who had received monovalent live attenuated DENV vaccine 12 months prior. Stimulation with heterologous serotype peptide resulted in more TNF α -producing cells than IFN γ producers relative to stimulation with homologous peptides, suggesting differential functional phenotypes amongst these cell populations that are dependent on the type of antigenic stimulation. This repeats a theme also evident in studies of CD8+ T cells, that partial peptide agonists elicit a diverse range of functional phenotypes in cross-reactive T cells⁷⁶. In a mouse model, Yauch et al.⁷⁷ identified that DENV2-specific CD4+ T cells were of a Th1 phenotype and could mediate *in vivo* cytotoxicity and that

immunization with dominant CD4+ T cell epitopes led to enhance viral clearance. Whether results in murine models are informative for understanding human immunization or disease remains uncertain, but these results underscore the need for further investigations of CD4+ T cells in dengue pathogenesis.

Mast cells

Mast cells (MCs), whilst traditionally associated with hypersensitivity responses, express a wide range of Fc receptors and hence are candidates for involvement in dengue pathogenesis. St John et al. demonstrated in a mouse model that MCs are activated *in vivo* during experimental DENV infection and MC-deficient mice had greatly reduced vascular leakage compared to MC-sufficient controls. Treatment of experimentally infected animals with MC-stabilizing drugs also ameliorated vascular leakage⁷⁸. In humans, MC-derived vasoactive products such as chymase, a serine protease, are elevated in the peripheral blood of primary and secondary dengue cases^{79, 80}. As attractive as MCs might be as “actors” in the pathogenesis of severe dengue, there remain critical questions. For example, to explain the clinical observation that secondary heterotypic DENV infection is associated with greater risk of severe disease, the “MC hypothesis” requires some level of Ab-Ag, (e.g. viral particles or NS1) to trigger Fc receptor aggregation on MCs and their activation/degranulation; this has yet to be shown in a convincing fashion. Moreover, the timing of MC activation would need to occur in a manner temporally consistent with the evolution of vascular leakage, i.e. most pronounced leading up to the time of defervescence in severe cases⁸¹. Thus, whilst MCs are intriguing, further clinical studies, possibly even drug probe studies, are needed to better understand their contribution to human disease.

Humoral immunity

Antibodies are believed to be critical mediators of resolution of infection, immunity to reinfection and in some circumstances, are believed to be risk factors for severe disease, i.e. antibody dependent enhancement (ADE) of infection. Recent results of phase IIb and III clinical trials of Sanofi Pasteur's recombinant live attenuated tetravalent dengue vaccine (CYD-TDV) have questioned the decades-old assumption that plaque reduction neutralization assays (PRNT₅₀) are good predictors of immune status. Specifically, CYD-TDV elicited tetravalent virus neutralizing antibodies after three doses yet offered very low levels of efficacy against DENV-2 and modest efficacy against DENV-1^{82, 83}. That the relationship between PRNT₅₀ titer and immunity to DENV-2 is complex were reinforced by Buddhari et al.⁸⁴ who utilized data from geographic cluster studies in Thailand. They found an overall significant positive association between baseline PRNT₅₀ titers to DENV-1, -2 and DENV-4 and immunity from homotypic symptomatic infection during the follow-up period. However the threshold for defining immunity to DENV-2 was unclear and might be

higher than for other serotypes. Thus, traditionally accepted PRNT₅₀ values (i.e. titer>10) regarded as denoting “immunity” to DENV may not be accurate, especially so for DENV-2. This has provided motivation to the field to explore alternative correlative assays. This is being enabled in part by productive basic research that has identified new, highly potent virus-neutralizing human mAbs elicited by natural infection. Mapping the epitope targets of these mAbs has revealed many target quaternary epitopes on the virus surface⁸⁵⁻⁸⁷. Some are serotype specific (e.g. 14C10 targeting DENV-1⁸⁸ and 5J7 that targets DENV-3⁸⁹) whilst others are cross-reactive (e.g. the so-called Envelope Dimer Epitope mAbs B7 and C10)⁹⁰. The existence of rare but potently neutralizing, cross-reactive human mAbs elicited by natural infection is provocative. Potentially, such Abs, and their epitope targets, could be deployed for pan-serotype therapeutic indications or vaccine development efforts. Additionally, these mAbs could be used for development and/or validation of 2nd generation virus neutralization assays that replace or augment the PRNT₅₀ measurement. For example, 2nd generation assays might compete individual or panels of highly potent virus neutralizing mAbs with polyclonal sera from vaccinees for binding to viral particles or recombinant proteins and thus determine a titer of antibodies in polyclonal sera specific for the critical epitope regions on the virus. Finally, for vaccine development, it is likely that strategies employing DENV envelope protein subunits as immunogens will fail to elicit some of these quaternary epitope binding immune responses; whether this significantly reduces the probability of success of this vaccine strategy is unknown.

Immune subversion

A recent expert review has reported the latest mechanistic understanding of how DENV manipulates intracellular antiviral responses and directly inhibits cellular signaling cascades in order to favor its own replication⁹¹. Here, we will focus on the role of lipids, and induction of the autophagy and endoplasmic reticulum (ER) stress pathways during DENV infection. Intracellular replication of DENV occurs on the cytoplasmic side of remodeled ER membranes and requires sufficient lipid resources from the host cell to enable efficient encapsidation and assembly of virions. Recent evidence suggests lipid droplets and the autophagy pathway positively contribute to the pool of lipid resources that enables DENV genome encapsidation and assembly^{92, 93}. Hence autophagy, sometimes regarded as a biochemical arm of the innate anti-viral immune response, actually benefits DENV replication. Additionally, cholesterol has been shown to play a pivotal role in the replication cycle of DENV and other flaviviruses, contributing not only to efficient replication but also to immune evasion⁹⁴⁻⁹⁶. Although, the mechanism by which dengue regulates lipid homeostasis and recruitment for these purposes is not currently understood, the membrane remodelling protein NS4A appears to be a likely candidate^{97, 98}. In addition to autophagy it is observed that all flavi-viruses

modulate the ER stress/unfolded protein response. DENV, JEV and WNV all activate Xbp-1, ATF6 and IRE1 regulatory factors to promote cell survival and immune evasion.⁹⁹⁻¹⁰⁴ Although the exact underlying molecular and functional aspects of this induction are not currently understood it is pertinent to highlight that Xbp-1 can promote lipid biosynthesis¹⁰⁵, IRE1 is involved in regulated RNA decay pathway promoting anti-viral defense¹⁰⁶ and ATF6 can regulate activation of innate immune responses and cell death¹⁰⁷. Thus there appears to be an integrated involvement of autophagy and ER stress/UPR modulation to promote lipid balance, intracellular replication and survival. Given the seemingly central role for these cellular responses in DENV replication key enzymes or by-products within these pathways are attractive candidates for antiviral therapeutics.

Recent advances in understanding virological determinants of DENV transmission

Whilst humans are dead-end hosts for many *Flaviviruses*, the dynamics of human-to-mosquito transmission of DENV has been central to its successful emergence. Accumulated data from empirical infection studies on human subjects conducted in the first half of the twentieth century showed that humans can be infectious to mosquitoes from 1.5 days prior to the onset of symptoms to around 5 days after the commencement of symptoms¹⁰⁸⁻¹¹². Recent studies have quantified the factors shaping transmission to either *Aedes aegypti* or *Ae. albopictus*. In infected humans, the concentration of virus circulating in the blood, and the duration that it circulates, influences the likelihood of a permissive *Aedes* mosquito becoming infected after imbibing a blood meal^{113,114}. Nguyet et al.¹¹³ and separately Whitehorn et al.¹¹⁴ experimentally measured the plasma viremia characteristics in Vietnamese adult dengue cases that led to DENV infection of directly blood-fed *Aedes aegypti* or *Ae. albopictus*. For *Ae. aegypti*, the plasma viremia required to infect 50% of mosquitoes differed between serotypes and was ~10-fold lower for DENV-1 and DENV-2 (6.29 or 6.51 log₁₀ RNA copies/ml) than for DENV-3 and DENV-4 (7.49 or 7.52 log₁₀ RNA copies/ml). For *Ae. albopictus* the 50% mosquito infectious dose was highly similar to that in parallel-fed *Ae. aegypti*, suggesting equal permissiveness between these species for initial infection of midgut tissues. In addition, Nguyet et al.¹¹⁴ demonstrated that patients with a high early viremia have a longer window of infectiousness to *Ae. aegypti*. Collectively, these findings define the viremia level that interventions such as vaccines and antivirals must target for prevention or amelioration to reduce DENV transmission.

The evolving story of NS1

More than 40 years ago, a series of papers described a soluble complement fixing (SCF) antigen in dengue virus infected mice and cell culture¹¹⁵⁻¹¹⁷. This SCF antigen was identified as a secreted non-structural viral protein¹¹⁸ that was later designated NS1, following the sequencing of the

first flavivirus genome¹¹⁹. It was immediately seen as a potential player in the pathogenesis of severe disease primarily because of the reported association between high levels of complement consumption and dengue shock syndrome (DSS)¹²⁰. These studies lead to an expansion of interest in the role of complement pathway engagement by dengue viruses in infected patients and in particular, the role of immune complexes in potentiating the severity of disease.^{116,121-124} However, the underlying *in vivo* mechanism of complement activation and the role of secreted NS1 has remained a matter of conjecture ever since. The development of NS1 capture assays^{125, 126} and the discovery that high levels of circulating NS1 in patient sera early during the course of infection correlate with progression to severe disease has provided further impetus to research in NS1 as a mediator of disease. These studies have led to a greater understanding of the structure and trafficking of this protein within and from infected cells, its proposed role in viral replication, potential as a vaccine candidate, value in diagnostic applications and its role in pathogenesis *in vivo* through its interaction with an ever increasing number of host cell targets. Not surprisingly, these host cell binding partners have been shown to comprise a number of different complement pathway components in addition to other host cell regulatory proteins. These include the complement regulation protein factor H (fH), complement inhibitory factor clusterin, complement proteins C4 and proC1s/C1s, hnRNP C1/C2, STAT3 β , thrombin/prothrombin and has been shown to trigger the generation of C5b-9 and SC5b-9 complexes. The recent publication of the crystal structure of both dengue and WNV NS1 has provided some clues into the structural basis of NS1-host cell protein engagement¹³⁰. Both dimer and hexamer forms of NS1 reveal three distinct structural domains, a hydrophobic β -roll (residues 1–29), an α/β -wing (residues 38–151; comprising a RIG-I like fold) and a central β -ladder (residues 181–352). These are all connected via a 3-stranded β -sheet (residues 30–37 and 152–180). The hydrophobic N-terminal β -roll, along with a hydrophobic loop extension from the connector (residues 159–162) provide a hydrophobic face to the dimeric form, thereby explaining membrane association of this otherwise hydrophilic protein as well as its ability to assemble as a hexameric lipoparticle that carries a lipid cargo. The RIG-I like wing domain is intriguing as it suggests that NS1 may act as an RNA sensor, interacting with dsRNA recognition systems of the innate immune response. However RIG-I is located in the cytoplasm of cells, on the opposite side of the ER membrane to NS1 and so how it could operate in this way is unknown. Perhaps the most revealing structural insight has come from comparisons with crystal structures of complement components bound to other pathogen proteins¹³¹. A common feature of these structures is the association of anti-parallel β -sheets from the pathogen proteins with the conserved complement control protein (CCP) domain of their complement partners. The CCP domain is found in factor H, C1s, C4 and C4 binding proteins, all demonstrated binding partners of NS1. The β -roll and β -ladder

are clearly candidates for the NS1 domains responsible for complement protein binding. Mutagenesis and binding studies should quickly reveal the potential *in vivo* role for these interactions.

Management of Dengue Infection

Fluid replacement and anti-pyretic therapy with paracetamol is the preferred therapy following the febrile phase. Care should be taken not to use other non-steroidal anti-inflammatory drugs. Judicious fluid administration forms the mainstay of treatment during the critical phase of the infection. Normal saline, Ringer, Lactate, and 5% glucose diluted 1:2 or 1:1 in normal saline, plasma, plasma substitutes, or 5% albumin are the routinely administered fluids. WHO guidelines summarize the following principles of fluid therapy. Oral fluid supplementation must be as plentiful as possible. However, intravenous fluid administration is mandatory in cases of shock, severe vomiting, and prostration (cases where the patient is unable to take fluids orally)

- Crystalloids form the first-line choice of intravenous fluid (0.9% saline)
- Hypotensive states that are unresponsive to boluses of intravenous crystalloids, colloids (e.g., dextran) form the second-line measures
- If the patient remains in the critical phase with low platelet counts, there should be a serious concern for bleeding. Suspected cases of bleeding are best managed by transfusion of fresh whole blood.¹²⁷

CONCLUSION

Dengue has evolved as a global life-threatening public health concern, affecting around 2.5 billion individuals in more than 100 countries. The physician should be aware about the varied clinical manifestations of this condition and ensure an early and adequate treatment plan. Future directions to combat this dreadful disease aim at methods of mosquito control, development of vaccine, and antiviral drug regimen. Scientists are investigating the mechanisms by which the dengue virus causes disease by focusing on understanding dengue pathogenesis, the virus itself, and vector biology. Researchers also aim to improve diagnostics for patients with dengue so that they can receive effective treatments sooner. In addition, by improving surveillance of dengue cases and mosquito vectors, researchers hope to reduce the effect of dengue epidemics.

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