Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Dengue Virus- The Life Threatening Virus

CH.B. Praveena Devi*, D. Navitha and JVC Sharma

Department of Pharmacy, Joginpally BR Pharmacy College, Yenkapally (V), Moinabad (M), Hyderabad, India, 500075

ABSTRACT

Dengue is an acute viral illness caused by RNA virus of the family Flaviviridae and spreadby Aedes mosquitoes. The presenting features may range from asymptomatic fever to dreadedcomplications such as haemorrhagicfever and shock. Acute onset high fever, muscle and jointpain, myalgia, cutaneous rash, haemorrhagic episodes, and circulatory shock are the commonlyseen symptoms. Early and accurate diagnosis is criticalto reduce mortality. Although dengue virus infections are usually self-limiting, dengue infectionhas 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 byclimatic factors.

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

***Corresponding Author**

CH. B. Praveena Devi

Assistant Professor, Department of Pharmacy,

Joginpally B.R. Pharmacy College,

Hyderbad, Telangana,

India,500075.

E-mail: praveenadeviv@gmail.com

INTRODUCTION

The Dengue Virus, a member of the genus Flavivirus of the family Flaviviridae, is anarthropod-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 healthchallenge in the tropic and subtropical nations. Dengue has seen a 30-fold upsurge worldwidebetween 1960 and 2010, due to increased population growth rate, global warming, unplannedurbanization, inefficient mosquito control, frequent air travel, and lack of health carefacilities[3, 4, 5]. Two and a half billion people reside in dengue-endemic regions and roughly 400million infections occurring per year, with a mortality rate surpassing 5–20% in some areas[6].Dengue infection affects more than 100 countries, including Europe and the United States[7]. 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 in1963-1964[8].Dengue virus infection presents with a diverse clinical picture that ranges fromasymptomatic illness to DF to the severe illness of dengue haemorrhagic fever/dengue shocksyndrome (DHF/DSS). Oral mucosal involvement is seen in approximately 30% of patients,although oral features are more frequently associated with DHF than with DF[9].Dengue virusinfection exhibit varied clinical presentation hence accurate diagnosis is difficult and relies onlaboratory confirmation. The condition is usually self-limiting and antiviral therapy is notcurrently available. Supportive care with analgesics, hydration with fluid replacement, andsufficient 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 Aedesaegypti and chiefly occurs during the rainy season[10].

The proposed aetiologies for Dengue Virusinfection are

- Viral replication, primarily in macrophages[11]
- Direct skin infection by the virus[12]
- Immunological and chemical-mediated mechanism induced by host–viral interaction[12].

Dengue virus gains entry into the host organism through the skin following an infectedmosquito bite. Humoral, cellular, and innate host immune responses are implicated in theprogression of the illness and the more severe clinical signs occur following the rapid clearanceof the virus from the host organism. Hence, the most severe clinical presentation during theinfection course does not correlate with a high viral load[13]. Alterations in endothelialmicrovascular permeability and thromboregulatory mechanisms lead to an increased loss ofprotein and plasma.

Proposed theories suggest that endothelial cell activation caused bymonocytes, T-cells, the complement system, and various inflammatory molecules mediateplasma leakage. Thrombocytopenia may be related to alterations in megakaryocytopoiesis,manifested by infection of human hematopoietic cells and compromised progenitor cellgrowth. This may cause platelet dysfunction, damage, or depletion, leading to significanthaemorrhages[14, 15].

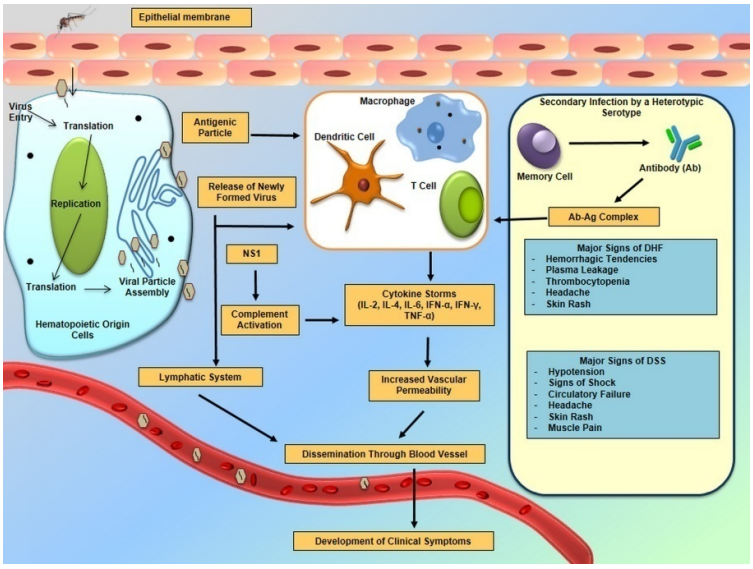

Fig. 1: Dengue virus replication and signs

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 compartment[16-19.]

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 permeability[20-22].

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-vascularpermeability. 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 surfaces [23-27]. 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 dys-regulation, 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 lymphocytes [28]. NS3 protein seems to be the major target for CD4+ and CD8+ T cells. Cytokines that may induce plasma leakage such as interferon g, 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 receptors [30]. 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 patients[31].

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 ones [32].

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 DHF [33-36]. Clearly immunopathogenicmechanisms 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-1β 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-1β, IFN-γ, IL-4, IL-6, IL-13, IL-7, and GM-CSF are associated with severe clinical manifestations while MIP-1β is elevated in patients with mild dengue. Marked thrombocytopenia is evident in patients with elevated IL-1β, IL-8, TNF-α, and MIP-1, while increased levels of MIP-1 and GM-CSF correlated with hypotension [32].

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 manifestations [39].

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 thrombocytopenia[42]. 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 APTT[43]. 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 DHF[44]. 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 DHF [39].

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 documented [45]. 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 micro vascular 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, withgeneral signs and symptoms consistent with dengue fever. When the fever declines, warningsigns may develop. This marks the beginning of a 24 to 48 hour period when the smallest bloodvessels (capillaries) become excessively permeable (“leaky”), allowing the fluid component toescape from the blood vessels into the peritoneum (causing ascites) and pleural cavity (leadingto pleural effusions). This may lead to failure of the circulatory system and shock, and possiblydeath without prompt, appropriate treatment. In addition, the patient with DHF has a lowplatelet count and haemorrhagic manifestations, tendency to bruise easily or have other types ofskin 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 toprevent bites by infected mosquitoes, particularly if you are living in or traveling to a tropicalarea. This involves protecting yourself and making efforts to keep the mosquito populationdown.

TREATMENT FOR DENGUE FEVER

There is no specific medicine to treat dengue infection. If you think you may havedengue fever, you should use pain relievers with acetaminophen and avoid medicineswith aspirin, which could worsen bleeding. You should also rest, drink plenty of fluids, and seeyour doctor. If you start to feel worse in the first 24 hours after your fever goes down, youshould get to a hospital immediately to be checked for complications[46].

Climatic parameters influence on Dengue Virus

Dengue is a major public health problem in India. Some studies have reported that anepidemiological shift in dengue viruses and climate change might be responsible for theobserved increase in dengue burden across India[47, 48].Studies have focused on severalepidemiological and entomological aspects of dengue and to a lesser extent on understandingthe relevance of climatic factors, but none have investigated the Extrinsic Incubation Period (EIP) ofthe dengue virus within the mosquito vector. This is the first study to estimate the extrinsicincubation period by using temperature data for different states of India. The EIP plays a majorrole in dengue-endemic regions, where the vectors ingest the virus through a blood meal, andthe virus escapes the mid gut, passes through the mosquito body and finally reaches thesalivary glands and can be transmitted to another susceptible host. Most modelling studies haveconsidered static EIP values rather than dynamic EIP estimates for a particular region.[49]Recent studies have shown that seasonal mean temperature in India hasincreased 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 thedengue risk by increasing the mosquito development rate and shortening the virus incubationtime, thereby increasing the rate of transmission. In India, temperature varies in differentclimatic zones at both temporal and spatial scales, and these variations influence the EIP. Thisinfluence is very pronounced in Punjab, Haryana and Rajasthan, where the EIP generallyexceeds 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 indengue virus transmission and vector–pathogen interactions.[53, 54]Similarly, understanding ofectotherm ecology has improved, thus providing novel ideas on how to quantify the impact ofanthropogenic climate change on pest and disease risk[55, 56]. In general, the EIP for dengueranges between 8 and 12

days.[57]Most studied states showed low EIP valuesduring the monsoon period, whereas other seasons had large spatial variation in the EIP. Thenorth-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, DENVcannot reproduce in mosquitoes, and transmission does not occur. An experimental study hasalso shown that below 18 °C, the virus cannot be found in the vector's salivary glands, whereasat 21 °C, the viral antigen is detectable in Ae. albopictus.[58]Similarly, above 20 °C, the dengueincidence 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 increasesfrom 26 to 30 °C, results similar to our findings. [60]In tropical countries, including India, thesetemperatures are generally experienced during monsoon or early post-monsoon periods.

Among the five states studied, Kerala experiences the highest number of dengue cases, possiblybecause 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 allseasons (9–14 days) and during the rainy season. These temperature ranges are mostly suitablefor mosquito development and virus transmission. Similar studies have also reported a highprevalence of dengue in Mexico during the rainy season, when temperatures typically rangebetween 17 and 30 °C. Temperatures in the lower range of dengue distribution (~17–18 °C) limit diseasetransmission through its effect on the EIP. High temperatures (~35 °C, depending on the vectorspecies) 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-bornediseases in India. In cooler areas, where temperature is a limiting factor, a slight increase intemperature might lead to disease transmission. As an example, dengue virus and its vectorshave rapidly expanded their range into Himalayan countries, such as Nepal and Bhutan, as wellas into northern states of India, such as Darjeeling, over the past 10 years. [61]

A recent study has compiled all dengue outbreaks in India [62] showing that mostdengue outbreaks occurred in Punjab, Haryana, Rajasthan, Gujarat and Kerala states during themonsoon 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 transmissionof 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 $NS3_{133–142}$ 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 $NS3_{133–142}$-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 $NS3_{133–142}$-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 colleagues [73] also identified highly activated and proliferating $NS3_{133–142}$-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 Abtiter 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 TNFα-producing cells than IFNγ 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 cells [76]. 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 leakage [78]. 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 cases [81]. 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 ($PRNT_{50}$) 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 $PRNT_{50}$ 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 $PRNT_{50}$ 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 $PRNT_{50}$ 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 surface [85-87]. Some are serotype specific (e.g. 14C10 targeting DENV-1 [88] and 5J7 that targets DENV-3[89]) 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 $PRNT_{50}$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 replication [91]. 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 evasion [94-96]. 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 biosynthesis [105], IRE1 is involved in regulated RNA decay pathway promoting anti-viral defense [106]and ATF6 can regulate activation of innate immune responses and cell death[107]. 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 symptoms [108-112]. Recent studies have quantified the factors shaping transmission to either *Aedesaegypti* 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 *Aedesaegypti* 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 culture [115-117]. This SCF antigen was identified as a secreted non-structural viral protein [118]that was later designated NS1, following the sequencing of the

first flavivirus genome [119]. 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 lead 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, STAT3β, 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 engagement [130]. 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 hexamericlipoparticle 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 proteins [131]. 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 therapyfollowing 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 theinfection. 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 fluidadministration is mandatory in cases of shock, severe vomiting, and prostration (caseswhere 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 aserious concern for bleeding. Suspected cases of bleeding are best managed bytransfusion of fresh whole blood.[127]

CONCLUSION

Dengue has evolved as a global life-threatening public health concern, affecting around2.5 billion individuals in more than 100 countries. The physician should be aware about thevaried clinical manifestations of this condition and ensure an early and adequate treatmentplan. 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 byfocusing on understanding dengue pathogenesis, the virus itself, and vector biology.Researchers also aim to improve diagnostics for patients with dengue so that they can receiveeffective treatments sooner. In addition, by improving surveillance of dengue cases andmosquito vectors, researchers hope to reduce the effect of dengue epidemics.

REFERENCES

1. Halstead, S.B. Pathogenesis of dengue: Challenges to molecular biology. Science.,1988; 239:476–81.
2. Kurane, I.Dengue haemorrhagic fever with special emphasis on immunopathogenesis. CompImmunol Microbiol Infect Dis., 2007;30:329–40
3. Gubler, DJ. Dengue and dengue Hemorrhagic fever. Clin Microbiol Rev. 1998;11:480–96.

4. New ed. Geneva, Switzerland: World Health Organization; 2009. World Health Organization(WHO).Dengue- Guidelines for Diagnosis, Treatment, Prevention and Control.
5. Guzman, M.G., Halstead, S.B., Artsob, H., Buchy, P., Farrar J., Gubler, D.J. Dengue: Acontinuing global threat. Nat Rev Microbiol. 2010;8(Suppl):S7–16.
6. Linares, E.M., Pannuti, C.S., Kubota, L.T., Thalhammer, S.Immunospot assay based on fluorescentnanoparticles for dengue fever detection. BiosenseBioelectron. 2013;41:180–5.
7. San Martin, J.L., Brathwaite, O., Zanbrano, B., Solorzano, J.O., Bouckenooghe, A., Dayan, G.H.The epidemiology of dengue in the Americas over the last three decades: A worrisome reality.Am J Tropical Med Hyg.2010;82:128–35.
8. Gupta, N., Srivastava, S., Jain, A., Chaturvedi, U.C. Dengue in India. Indian J Med Res.2012;136:373–90.
9. Thomas,EA., John, M., Bhatia, A. Muco-Cutaneous manifstations of dengue viral infection inPunjab. Int J Dermatol. 2007;46:715–19.
10. Arshad, I., Malik, F.A., Hussain, A., Shah, SA. Dengue fever: Clinico-pathologic correlations andtheir association with poor outcome. Professional Med J. 2011;18:57–63.
11. Wu, S.J., Grouard, V.G., Sun, W., Mascola, J.R., Brachel, E., Putvatana, R. Human skin langerhans cells aretargets of dengue virus infection. Nat Med. 2000;6:816–20.
12. Bhamarapravati, N. Pathology and Pathogenesis of DHF. New Delhi: WHO Meeting; 1980.
13. Whitehorn, J., Simmons, C.P. The pathogenesis of dengue.Vaccine.2011;29:7221–8.
14. Guzman, M.G, Kouri, G. Dengue and dengue hemorrhagic fever in the Americas: Lessons andchallenges. J ClinVirol.2003;27:1–13.
15. Revised and Expanded ed. New Delhi: WHO; 2011. World Health Organization.Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever.
16. B. A. Wills, N. M. Dung, H. T. Loan et al., “Comparison of three fluid solutions for resuscitation in dengue shock syndrome,” The New England Journal of Medicine, vol. 2005; 353(9): 877–889,.
17. C. C. Michel and F. E. Curry, “Micro vascular permeability,” Physiological Reviews, 1999;79(3): 703–761,.
18. V. H. Huxley and F. E. Curry, “Differential actions of albumin and plasma on capillary solute permeability,” American Journal of Physiology, 1991; 260(5): H1645–H1654,.
19. B. A. Wills, E. E. Oragui, M. D. Nguyen et al., “Size and charge characteristics of the protein leak in dengue shock syndrome,” Journal of Infectious Diseases, 2004; 190(4): 810–818,.

20. P. Avirutnan, N. Punyadee, S. Noisakran et al., "Vascular leakage in severe dengue virus infections: a potential role for the non-structural viral protein NS1 and complement," Journal of Infectious Diseases, 2006;193(8): 1078–1088,.
21. P. Avirutnan, A. Fuchs, R. E. Hauhart et al., "Antagonism of the complement component C4 by flavivirus non structural protein NS1," The Journal of Experimental Medicine, 2010; 207(4): 793–806,.
22. P. Avirutnan, L. Zhang, N. Punyadee et al., "Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparansulfate and chondroitin sulfate E," PLoS Pathogens, 2007; 3(11): e183,.
23. S. Halstead, "Pathophysiology and pathogenesis of dengue hemorrhagic fever," in Monograph on Dengue/Dengue Haemorrhagic Fever, P. Thongcharoen, Ed., Regional Publication, SEARO 1993;22: 80–103, WHO,.
24. D. M. Morens, "Antibody-dependent enhancement of infection and the pathogenesis of viral disease," Clinical Infectious Diseases, 1994;19(3): 500–512,.
25. S. C. Kliks, A. Nisalak, W. E. Brandt, L. Wahl, and D. S. Burke, "Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever," American Journal of Tropical Medicine and Hygiene, 1989; 40(4): 444–451.
26. D. W. Vaughn, S. Green, S. Kalayanarooj et al., "Dengue viremiatiter, antibody response pattern, and virus serotype correlate with disease severity," Journal of Infectious Diseases, 2000;181(1): 2–9,.
27. D. H. Libraty, T. P. Endy, H. S. H. Houng et al., "Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections," Journal of Infectious Diseases, 2002; 185(9): 1213–1221,.
28. I. Kurane and F. A. Ennis, "Cytokines in dengue virus infections: role of cytokines in the pathogenesis of dengue hemorrhagic fever," Seminars in Virology, 1994; 5(6): 443–448,.
29. S. Green, S. Pichyangkul, D. W. Vaughn et al., "Early CD69 expression on peripheral blood lymphocytes from children with dengue hemorrhagic fever," Journal of Infectious Diseases, 1999; 180(5): 1429–1435,.
30. U. Kontny, I. Kurane, and F. A. Ennis, "Gamma interferon augments Fc(γ) receptor-mediated dengue virus infection of human monocytic cells," Journal of Virology, 1988; 62(11): 3928–3933.

31. R. Mukerjee, U. C. Chaturvedi, and R. Dhawan, "Dengue virus-induced human cytotoxic factor: production by peripheral blood leucocytes in vitro," Clinical and Experimental Immunology, 1995; 102(2); 262–267,. View at Google Scholar · View at Scopus
32. F. A. Bozza, O. G. Cruz, S. M. O. Zagne et al., "Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity," BMC Infectious Diseases, 2008; 8(86). I. Kurane and T. Takasaki, "Dengue fever and dengue haemorrhagic fever: challenges of controlling an enemy still at large," Reviews in Medical Virology, 2001; 11(5): 301–311,.
33. C. L. Medin, K. A. Fitzgerald, and A. L. Rothman, "Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion," Journal of Virology, 2005; 79(17): 11053–11061,.
34. P. Avirutnan, E. Mehlhop, and M. S. Diamond, "Complement and its role in protection and pathogenesis of flavivirus infections," Vaccine, 2008; 26(8); 100–I107,.
35. E. L. Azeredo, L. M. De Oliveira-Pinto, S. M. Zagne, D. I. S. Cerqueira, R. M. R. Nogueira, and C. F. Kubelka, "NK cells, displaying early activation, cytotoxicity and adhesion molecules, are associated with mild dengue disease," Clinical and Experimental Immunology, 2006; 143(2): 345–356,.
36. B. G. Dorner, H. R. C. Smith, A. R. French et al., "Coordinate expression of cytokines and chemokines by NK cells during murine cytomegalovirus infection," The Journal of Immunology, 2004;172(5): 3119–3131,.
37. B. Wills, V. N. Tran, N. T. H. Van et al., "Hemostatic changes in Vietnamese children with mild dengue correlate with the severity of vascular leakage rather than bleeding," American Journal of Tropical Medicine and Hygiene, 2009;81(4): 638–644,.
38. S. Wang, R. He, J. Patarapotikul, B. L. Innis, and R. Anderson, "Antibody-enhanced binding of dengue-2 virus to human platelets," Virology, 1995; 213(1):254–257,.
39. C.-F. Lin, H.-Y. Lei, C.-C. Liu et al., "Generation of IgM anti-platelet autoantibody in dengue patients," Journal of Medical Virology, 2001;63(2):143–149,.
40. K. D. Yang, C. L. Wang, and M. F. Shaio, "Production of cytokines and platelet activating factor in secondary dengue virus infections," Journal of Infectious Diseases, 1995;172(2): 604–605,.
41. R. N. Palmer, M. E. Rick, and P. D. Rick, "Circulating heparansulfate anticoagulant in a patient with fatal bleeding disorder," The New England Journal of Medicine, 1984;310(26): 1696–1699.

42. E. Chungue, L. Poli, C. Roche, P. Gestas, P. Glaziou, and L. J. Markoff, "Correlation between detection of plasminogen cross-reactive antibodies and hemorrhage in dengue virus infection," Journal of Infectious Diseases, 1994;170(5): 1304–1307,.
43. A. Srikiatkhachorn, "Plasma leakage in dengue haemorrhagic fever," Thrombosis and Haemostasis, 2009;102(6):1042–1049,.
44. https://www.webmd.com/a-to-z-guides/medical-reference/default.htm.
45. Halasa, Y.A., Dogra, V., Arora, N. Overcoming data limitations: design of a multi-component study for estimating the economic burden of dengue in India. Dengue Bull 2011; 35:1–14.
46. Dhiman, R.C, Pahwa, S., Dhillon, G.P. Climate change and threat of vector-borne diseasesin India: are we prepared? Parasitol Res 2010; 106: 763–773.
47. Luz, P.M., Codeço, C.T., Massad, E. Uncertainties regarding dengue modelling in Rio deJaneiro, Brazil. MemInstOswaldo Cruz 2003; 98: 871–878.
48. Arora, M., Goel, N.K., Singh, P. Evaluation of temperature trends over India. HydrolSci J 2005;50: 81–93.
49. Manorenjitha, M.S, Zairi, J. The adaptation of field collected Aedesaegypti (L.) andAedealbopictus (Skuse) in laboratory condition. Int J Life Sci Med Res 2015; 5: 25–30.
50. Patil, P.B., Reddy, B.P., Gorman, K. Mating competitiveness and life-table comparisonsbetween transgenic and Indian wild-type Aedesaegypti L. Pest ManagSci 2015; 71: 957–965.
51. Chan, T.C., Hu, T.H., Hwang, J.S. Daily forecast of dengue fever incidents for urban villages in acity. Int J Health Geogr 2015; 14: 9.
52. Lambrechts, L., Paaijmans, K.P, Fansiri, T. Impact of daily temperature fluctuations ondengue virus transmission by Aedesaegypti. Proc Natl Acad Sci 2011; 108: 7460–7465.
53. Rohr, J.R, Dobson, A.P., Johnson, P.T. Frontiers in climate change-disease research. Trends Ecol Evol 2011; 26: 270–277.
54. Dangles, O., Carpio, C., Barragan, A.R. Temperature as a key driver of ecological sortingamong invasive pest species in the tropical Andes. Ecol Appl 2008; 18: 1795.
55. World health organizationDengue: Guidelines for diagnosis. Treatment, prevention andcontrol: new edition. World health organization: Geneva. 2009.
56. Colon, F.J., Fezzi, C., Lake, I.R. The effects of weather and climate change ondengue. PLoSNegl Trop Dis 2013; 7: e2503.

57. Xiao, F.Z., Zhang, Y., Deng, Y.Q. The effect of temperature on the extrinsic incubation period andinfection rate of dengue virus serotype 2 infection in Aedesalbopictus. Arch Virol 2014; 159: 3053–3057.
58. Rohani, A., Wong, Y.C., Zamre, I. The effect of intrinsic incubation temperature ondevelopment of dengue serotype 2 and 4 virus in Aedesaegypti (L) South east asian J top medpublic Health 2009;40:942-950.
59. Dhimal, M., Ahrens, B., Kuch, U., Climate change and spatiotemporal distributions of vector-borne diseases in Nepal—a systematic synthesis of literature. PLoS ONE 2015; 10: e0129869.
60. Chakravarti, A., Arora, R., Luxemburger, C. Fifty years of dengue in India. Trans R Soc tropmed hyg 2102;106: 273-282.
61. Kraemer, M.U., Sinka, M.E., Duda, K.A. The global distribution of the arbovirus vectors Adesaegypti and Ae. albopictus. Elfie 2015; 4: e08347.
62. Musso, D., Nilles, E.J., Cao, V.M. Rapid spread of emerging zika virus in the pacificarea. ClinMicrobiol Infect 2014; 20: 595–596.
63. World health organization dengue: guidelines for diagnosis treatment and control.
64. Duangchinda, W. Dejnirattisai, S. Vasanawathana, W. Limpitikul, N. Tangthawornchaikul, P. Malasit, *et al.*Immunodominant T-cell responses to dengue virus NS3 are associated with DHFProcNatlAcadSci USA, 2010; 107:16922-16927
65. Mongkolsapaya, T. Duangchinda, W. Dejnirattisai, S. Vasanawathana, P. Avirutnan, A. Jairu ngsri, *et al.*T cell responses in dengue hemorrhagic fever: are cross-active T cells suboptimalJ Immunol, 2006; 176: 3821-3829
66. Zivna, S. Green, D.W. Vaughn, S. Kalayanarooj, H.A. Stephens, D. Chandanayingyong, *et al.*T cell responses to an HLA-B*07-restricted epitope on the dengue NS3 protein correlate with disease severityJ Immunol, 2002; 168 :5959-5965
67. L. Rivino, E.A. Kumaran, V. Jovanovic, K. Nadua, E.W. Teo, S.W. Pang, *et al.* Differential targeting of viral components by CD4+ versus CD8+ T lymphocytes in dengue virus infectionJ Virol, 2013; 87 : 2693-2706
68. D. Weiskopf, M.A. Angelo, E.L. dezeredo, J. Sidney, J.A. Greenbaum, A.N. Fernando, *et al.*Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells Proc Natl Acad Sci USA, 2013; 110 E2046-E2053.
69. 68. D. Weiskopf, M.A. Angelo, D.J. Bangs, J. Sidney, S. Paul, B. Peters, *et al.*The human CD8+ T cell responses induced by a live attenuated tetravalent dengue vaccine are directed against highly conserved epitopes J Virol, 2015; 89:120-128.

70. D. Mongkolsapaya, X.N. Xu, S. Vasanawathana, N. Tangthawornchaikul, A. Chairunsri, *et al.*Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic feverNat Med, 2003; 9: 921-927
71. N.T. Dung, H.T. Duyen, N.T. Thuy, T.V. Ngoc, N.V. Chau, T.T. Hien, *et al.* Timing of CD8+ T cell responses in relation to commencement of capillary leakage in children with dengue J Immunol, 2010; 184 :7281-7287
72. H. Friberg, H. Bashyam, T. Toyosakiaeda, J.A. Potts, T. Greenough, S. Kalayanarooj, *etal.*Cr oss-reactivity and expansion of dengue-specific T cells during acute primary and secondary infections in humans Sci Rep, 2011; 1: 51
73. L. Rivino, E.A. Kumaran, T.L. Thein, C.T. Too, V.C. Gan, B.J. Hanson, *etal.*Virus-specific T lymphocytes home to the skin during natural dengue infection Sci Transl Med, 2015; 7: 278ra35
74. M.M. Mangada, A.L. Rothman. Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypesJ Immunol, 2005; 175 :2676-2683
75. H.S. Bashyam, S. Green, A.L. Rothman. Dengue virus-reactive CD8+ T cells display quantitative and qualitative differences in their response to variant epitopes of heterologous viral serotypes J Immunol, 2006; 176 :2817-2824
76. 75.L.E. Yauch, T.R. Prestwood, M.M. May, M.M. Morar, R.M. Zellweger, B. Peters, *et al.*CD4+ T cells are not required for the induction of dengue virus-specific CD8+ T cell or antibody responses but contribute to protection after vaccination J Immunol, 2010; 185: 5405-5416.
77. A.L. St John, A.P. Rathore, B. Raghavan, M.L. Ng, S.N. AbrahamContributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage Elife, 2 (2013), p. e00481.
78. T. Furuta, L.A. Murao, N.T. Lan, N.T. Huy, V.T. Huong, T.T. Thuy, *et al.* Association of mast cell-derived VEGF and proteases in Dengue shock syndrome PLo S Negl Trop Dis, 2012; 6: e1505.
79. 78.P.K. Lam, D.T. Tam, T.V. Diet, C.T. Tam, N.T. Tien, N.T. Kieu, *etal.* Clinical characteristics of Dengue shock syndrome in Vietnamese children: a 10-year prospective study in a single hospita l Clin Infect Dis, 2013; 57 :1577-1586
80. Sabchareon, D. Wallace, C. Sirivichayakul, K. Limkittikul, P. Chanthavanich, S. Suvannadab ba, *et al.* Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial Lancet, 2012; 380:1559-1567.

81. Capeding, N.H. Tran, S.R.S. Hadinegoro, H.I.H.J.M. Ismail, T. Chotpitayasunondh, M.N. Ch ua, *et al.*Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trialLancet, 2014; 384: 1358-1365.
82. Buddhari, J. Aldstadt, T.P. Endy, A. Srikiatkhachorn, B. Thaisomboonsuk, C. Klungthong, *et al.*Dengue virus neutralizing antibody levels associated with protection from infection in thai cluster studies PLo S Negl Trop Dis, 2014; 8: e3230.
83. S.A. Smith, A.R. de Alwis, N. Kose, R.S. Jadi, A.M. de Silva, J.E. Crowe Jr.Isolation of dengue virus-specific memory B cells with live virus antigen from human subjects following natural infection reveals the presence of diverse novel functional groups of antibody clonesJ Virol, 2014; 88: 12233-12241
84. G. Fibriansah, J.L. Tan, S.A. Smith, R. de Alwis, T.S. Ng, V.A. Kostyuchenko, *et al.* A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins Nat Commun, 2015; 6:6341
85. 84. W. Dejnirattisai, W. Wongwiwat, S. Supasa, X. Zhang, X. Dai, A. Rouvinsky, *et al.*A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virusNat Immunol, 2015; 16: 170-177
86. 85. E.P. Teoh, P. Kukkaro, E.W. Teo, A.P. Lim, T.T. Tan, A. Yip, *et al.*The structural basis for serotype-specific neutralization of dengue virus by a human antibody Sci Transl Med, 2012; 4: 139ra83
87. A. Rouvinski, P. GuardadoCalvo, G. Barbapaeth, S. Duquerroy, M.C. Vaney, C.M. Kikuti, *et al.*Recognition determinants of broadly neutralizing human antibodies against dengue viruses Nature, 2015; 520: 109-113.
88. 87. A.M. Green, P.R. Beatty, A. Hadjilaou, E. Harris Innate immunity to dengue virus infection and subversion of antiviral responses J Mol Biol, 2014; 426: 1148-1160
89. M.M. Samsa, J.A. Mondotte, N.G. Iglesias, I. Assunção-Miranda, G. Barbosa-Lima, A.T. Da Poian, *et al.* Dengue virus capsid protein usurps lipid droplets for viral particle formation PLo S Pathog, 2009; 5: e1000632
90. R. Mateo, C.M. Nagamine, J. Spagnolo, E. Méndez, M. Rahe, M. Gale, *et al.* Inhibition of cellular autophagy deranges dengue virion maturationJ Virol, 2013; 87: 1312-1321[31]
91. J.M. Mackenzie, A.A. Khromykh, R.G. Parton Cholesterol manipulation by West Nile virus perturbs the cellular immune response Cell Host Microbe, 2007; 2: 229-239[32]
92. R. Soto-Acosta, C. Mosso, M. Cervantes-Salazar, H. Puertauardo, F. Medina, L. Favari, *et al.*The increase in cholesterol levels at early stages after dengue virus infection correlates

with an augment in LDL particle uptake and HMG-CoA reductase activityVirology, 2013; 442: 132-147.

93. C. Roth well, A. Lebreton, C. Young Ng, J.Y. Lim, W. Liu, S. Vasudevan, *et al*. Cholesterol biosynthesis modulation regulates dengue viral replication Virology, 2009; 389: 8-19
94. S. Miller, S. Kastner, J. Krijnse-Locker, S. Buhler, R. Bartenschlager.Non-structural protein 4A of Dengue virus is an integral membrane protein inducing membrane alterations in a 2K-regulated manner J Biol Chem, 2007; 282: 8873-8882[35]
95. J. Roosendaal, E.G. Westaway, A. Khromykh, J.M. MackenzieRegulated cleavages at the West Nile virus NS4A-2K-NS4B junctions play a major role in rearranging cytoplasmic membranes and Golgi trafficking of the NS4A protein J Virol, 2006; 80: 4623-4632[36]
96. J. Peña, E. Harris. Early dengue virus protein synthesis induces extensive rearrangement of the endoplasmic reticulum independent of the UPR and SREBP-2 pathway PLoS ONE, 2012; 7: e38202
97. R.L. Ambrose, J.M. MackenzieATF6 signalling is required for efficient West Nile virus replication by promoting cell survival and inhibition of innate immune responses J Virol, 2013; 87:2206-2214
98. R.L. Ambrose, J.M. Mackenzie West Nile virus differentially modulates the unfolded protein response to facilitate replication and immune evasionJ Virol, 2011; 85:2723-2732.
99. C.Y. Yu, Y.W. Hsu, C.L. Liao, Y.L. Lin. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stressJ Virol, 2006; 80 :11868-11880.
100. C. Yu, K. Achazi, M. Niedrig. Tick-borne encephalitis virus triggers inositol-requiring enzyme 1 (IRE1) and transcription factor 6 (ATF6) pathways of unfolded protein responseVirusRes, 2013; 178:471-477.
101. G.R. Medigeshi, A.M. Lancaster, A.J. Hirsch, T. Briese, W.I. Lipkin, V. Defilippis, *et al.*West Nile virus infection activates the unfolded protein response, leading to CHOP induction and apoptosisJ Virol, 2007; 81:10849-10860.
102. R. Sriburi, S. Jackowski, K. Mori, J.W. BrewerXBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulumJ Cell Biol, 2004; 167:35-41.
103. Cho Jin, A. H. Lee, B. Platzer, C.S. Cross Benedict, M. Gardner Brooke, H. De Luca, *et al.*The unfolded protein response element IRE1α senses bacterial proteins invading the ER to activate RIG-I and innate immune signalling Cell Host Microbe, 2013; 13: 558-569.

104. J. Wu, D.T. Rutkowski, M. Dubois, J. Swathirajan, T. Saunders, J. Wang, *et al.*ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stressDev Cell, 2007; 13: 351-364.

105. J.B. Cleland, B. Bradley Dengue fever in Australia: its history and clinical course, its experimental transmission by Stegomyiafasciata, and the results of Inoculation and other Experiments J Hyg (London), 1918; 16:317-418.

106. J.B. Cleland, B. Bradley, W. MacdonaldFurther experiments in the etiology of dengue feverJ Hyg (London), 1919; 18: 217-254.

107. L. Chandler. ACRO bservations on the etiology of dengue feverAm J Trop Med, 1923; 3:233-262.

108. J.F.H. Siler, M.W.A.P. HitchensResults obtained in the transmission of dengue feverJ Am Med Assoc, 1925; 84:1163-1172

109. J.F. Siler, M.W. Hall, A.P. HitchensDengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity and preventionPhilipp. J. Sci. 1926

110. M.N. Nguyet, T.H. Duong, V.T. Trung, T.H. Nguyen, C.N. Tran, V.T. Long, *et al.*Host and viral features of human dengue cases shape the population of infected and infectious *Aedesaegypti* mosquitoesProcNatlAcadSci USA, 2013; 110: 9072-9077.

111. J. Whitehorn, D.T. Kien, N.M. Nguyen, H.L. Nguyen, P.P. Kyrylos, L.B. Carrington, *et al.* Comparative susceptibility of *Aedesalbopictus* and *Aedesaegypti* to dengue virus infection after feeding on blood of viremic humans: implications for public healthJ Infect Dis 2015.

112. W.E. Brandt, D. Chiewslip, D.L. Harris, P.K. RussellPartial purification and characterization of a dengue virus soluble complement-fixing antigenJ Immunol, 1970;1565-1568.

113. P.K. Russell, D. Chiewsilp, W.E. BrandtImmunoprecipitation analysis of soluble complement-fixing antigens of dengue virusesJ Immunol, 1970; 105:838-845.

114. T.J. Smith, W.E. Brandt, J.L. Swanson, J.M. McCown, E.L. Buescher. Physical and biological properties of dengue-2 virus and associated antigensJ Virol, 1970; 5:524-532

115. D. Cardiff, W.E. Brandt, T.G. McCloud, D. Shapiro, P.K. Russell. Immunological and biophysical separation of dengue-2 antigensJ Virol, 1971; 7:15-23

116. C.M. Rice, E.M. Lenches, S.R. Eddy, S.J. Shin, R.L. Sheets, J.H. StraussNucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution Science, 1985; 229:726-733

117. P.K. Russell, A. Intavivat, S. KanchanapilantAnti-dengue immunoglobulins and serum beta 1 c-a globulin levels in dengue shock syndromeJ Immunol, 1969; 102:412-420

118. V.A. Bokisch, F.H. Top Jr., P.K. Russell, F.J. Dixon, H.J. Muller-Eberhard.The potential pathogenic role of complement in dengue hemorrhagic shock syndromeN Engl J Med, 1973; 289:996-1000.

119. W. Ruangjirachuporn, S. Boonpucknavig, S. Nimmanitya. Circulating immune complexes in serum from patients with dengue haemorrhagic fever Clin Exp Immunol, 1979; 36: 46-53.

120. A.T. Sobel, V.A. Bokisch, H.J. Muller-EberhardC1q deviation test for the detection of immune complexes, aggregates of IgG, and bacterial products in human serumJ Exp Med, 1975; 142:139-150.

121. A.N. Theofilopoulos, C.B. Wilson, F.J. Dixon. The Raji cell radioimmune assay for detecting immune complexes in human sera.J Clin Invest, 1976; 57 :169-182

122. P.R. Young, P.A. Hilditch, C. Bletchly, W. Halloran. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. J Clin Microbiol, 2000; 38:1053-1057

123. S. Alcon, A. Talarmin, M. Debruyne, A. Falconar, V. Deubel, M. Flamand Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections J Clin Microbiol, 2002; 40:376-381

124. D.H. Libraty, P.R. Young, D. Pickering, T.P. Endy, S. Kalayanarooj, S. Green, *et al.*High circulating levels of the dengue virus non-structural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic feverJ Infect Dis, 2002; 186: 1165-1168.

125. Avirutnan, N. Punyadee, S. Noisakran, C. Komoltri, S. Thiemmeca, K. Auethavornanan, *et al.*Vascular leakage in severe dengue virus infections: a potential role for the non-structural viral protein NS1 and complement J Infect Dis, 2006; 193:1078-1088.

126. D.A. Muller, P.R. Young. The flavivirus NS1 protein: molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker Antiviral Res, 2013; 98:192-208

127. D.L. Akey, W.C. Brown, S. Dutta, J. Konwerski, J. Jose, T.J. Jurkiw, *et al.* Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system Science, 2014; 343:881-885

128. D.L. Akey, W.C. Brown, J. Jose, R.J. Kuhn, J.L. Smith. Structure-guided insights on the role of NS1 in flavivirus infection Bioassays, 2015; 37:489-494