

DENGUE IMMUNOPATHOGENESIS: A CROSSTALK BETWEEN HOST AND VIRAL FACTORS LEADING TO DISEASE. A CROSSTALK BETWEEN HOST AND VIRAL FACTORS LEADING TO DISEASE: PART II—DENV INFECTION, ADAPTIVE IMMUNE RESPONSES, AND NS1 PATHOGENESIS

;
;

© 2021, NA



This work is licensed under the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/legalcode>), which permits unrestricted use, distribution, and reproduction, provided the original work is properly credited.

IDRC Grant: 109071-002-Enabling Business and Technologies to Contribute to the Control of Mosquito-Borne Diseases in Latin America

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,000

Open access books available

125,000

International authors and editors

140M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Dengue Immunopathogenesis: A Crosstalk between Host and Viral Factors Leading to Disease: PART II—DENV Infection, Adaptive Immune Responses, and NS1 Pathogenesis

*Henry Puerta-Guardo, Scott B. Biering, Eva Harris,
Norma Pavia-Ruz, Gonzalo Vázquez-Prokopec,
Guadalupe Ayora-Talavera and Pablo Manrique-Saide*

Abstract

Severe disease is associated with serial infection with DENV of different serotypes. Thus, primary DENV infections normally cause asymptomatic infections, and secondary heterotypic infections with a new DENV serotype potentially increase the risks of developing severe disease. Despite many proposed hypotheses trying to explain it, the exact immunological mechanism leading to severe dengue disease is unknown. In turn, severe manifestations are believed to be a consequence of the combinations of many immunopathogenic mechanisms involving viral and host factors leading to increased pathogenesis and disease. Of these mechanisms, the adaptive immune response has been proposed to play a critical role in the development of severe dengue manifestations. This includes the effect of non-neutralizing but enhancing antibodies produced during primary infections, which results in enhanced-DENV infection of Fc- γ -receptor-expressing cells (e.g. monocytes and macrophages) during DENV heterotypic exposure in a phenomenon called *antibody-dependent enhancement* (ADE); the increased activation of memory T cells during secondary infections, which has low affinity for the current infecting serotype and high affinity for a past infection with a different serotype known as *the original antigenic sin*; the unbalanced production of pro-inflammatory cytokines that have a direct effect on vascular endothelial cells resulting in plasma leak in a phenomenon known as *cytokine storm*; and the excessive activation of the *complement system* that causes exacerbated inflammatory responses, increasing disease severity. In addition to the adaptive immune responses, a secreted viral factor known as the *nonstructural protein 1* (NS1) has been recently proposed as the missing corner piece of the DENV pathogenesis influencing disease. This *Part II* of the chapter will discuss the interplay between the distinct host adaptive immune responses and viral factors that together contribute to the development of DENV pathogenesis and severe disease.

Keywords: dengue, immunopathogenesis, dengue shock syndrome, severe dengue, adaptive immune response, antibody response, ADE, *cytokine storm*, T cells, complement system, *viral toxin*, NS1, endothelial dysfunction, vascular leak

1. Dengue immunopathogenesis and severe disease: host and viral factors

As discussed in *Part I* of this chapter, severe dengue is mainly characterized by the altered endothelial function in blood vessels and the disruption of the coagulation cascade that results in hypotension, shock, and severe hemorrhage manifestations [1, 2]. As the epidemiology of dengue indicates that appearance of severe manifestations occurs when the peak of viremia has passed, the key biological mechanisms leading to the pathogenesis of clinical complications during DENV infection, are believed to involve the activity of short-lived biological mediators closely linked to host innate and adaptive immune responses [3–6].

This *Part II* of the dengue immunopathogenesis section will address the multifactorial immunopathogenic process of DENV infection from the perspective of the pre-existing serotype cross-reactive antibodies, the hyperactivation of DENV-infected immune cells (e.g. monocytes, mast cells) leading to increased cytokine production, the role of T cell responses, the activation of complement pathways, and the new pathogenic roles of the secreted NS1 of DENV that may act together to occasionally cause severe dengue manifestations followed by shock and potentially death [1, 6–21].

1.1 Antibody response to DENV infection

The DENV complex refers to a group of four evolutionarily distinct, but antigenically and genetically related DENV serotypes (DENV-1 to DENV-4) [22]. During dengue disease, the humoral immune response is vital for controlling DENV virus infection and for the development of acquired immunity [10]. Neutralizing antibodies against the four serotypes are critical components of the protective immune response [23]. In this sense, during primary DENV infection, it is expected that a neutralizing type-specific antibody response should provide long-term protection against the primary DENV infecting serotype, but only transient protection against other DENV serotypes (cross-reactive antibodies) (See **Figure 1 Part I**). Infection with a primary serotype is thought to induce lifelong immunity that protects against re-infection with the same serotype (homotypic) [7, 23]. However, homotypic DENV infections have been found in symptomatic dengue cases in a community-based prospective cohort study suggesting that recurrent DENV infections, particularly in endemic areas can occur in patients over time [24]. After subsequent infection with a different serotype (secondary infection), the neutralizing antibody response becomes broadly neutralizing and is thought to reduce the incidence of severe disease [25] (See **Figure 1C, Part I**). In fact, individuals with higher cross-reactive neutralizing antibody titers originated from pre-infection correlates with reduced likelihood of symptomatic secondary infection [23]. However, numerous studies worldwide involving human infections during dengue epidemics (e.g. hospitalized cases) or multiple epidemiological studies from prospective cohorts strongly support the heterotypic secondary DENV infection, defined as two or more sequential infections by different serotypes, as the epidemiologic greatest risk factor for developing severe dengue disease [17, 26–29].

Antibody responses during DENV infection are mainly directed against the envelope protein (E), the major structural protein of the virion, and the dominant

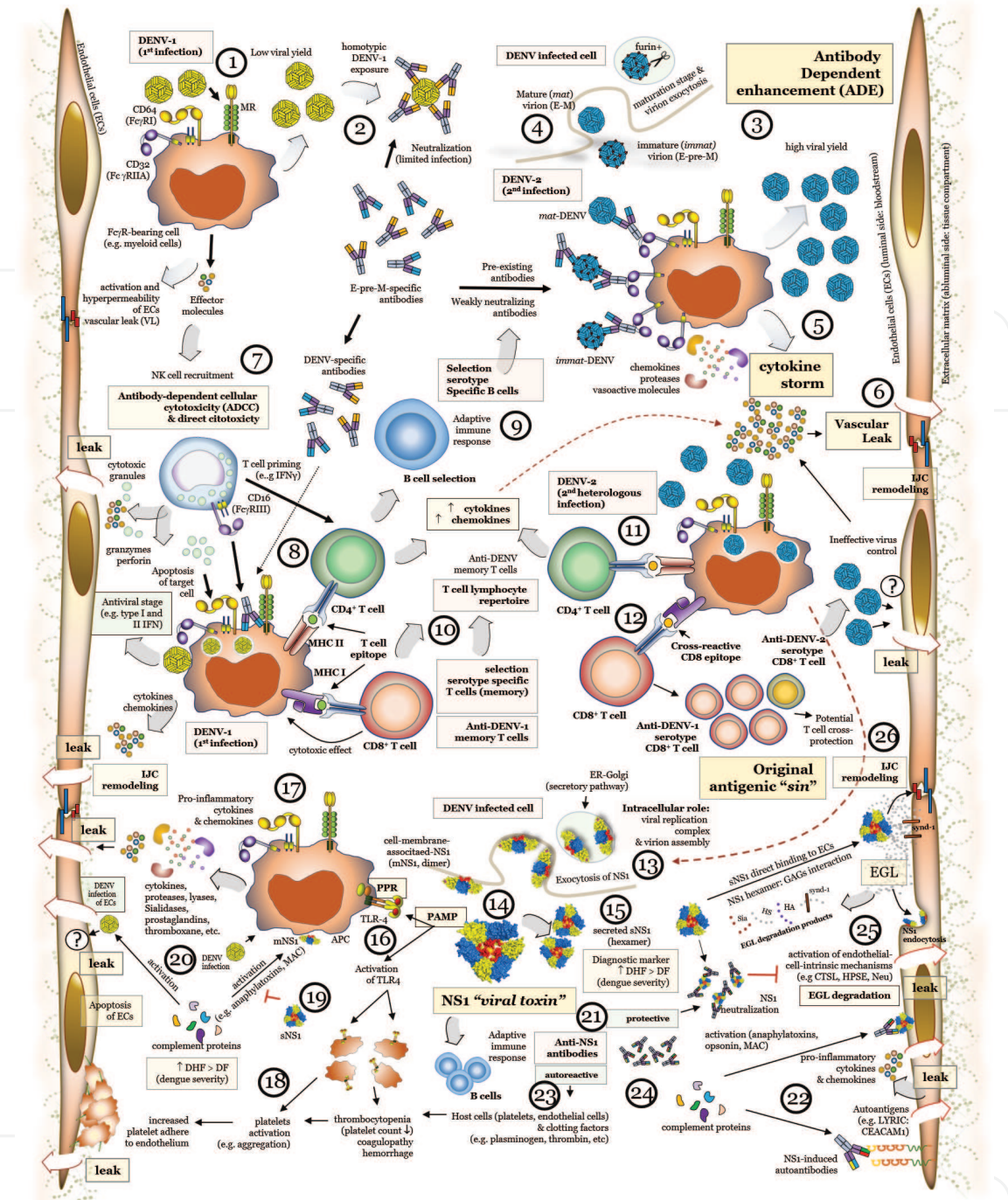


Figure 1. The supercomplex interplay of the immunopathogenic mechanisms triggered by systemic DENV infection leading to disease. The DENV complex is composed by four serotypes [1–4], and a primary infection with any of these serotypes triggers an adaptive immune response that results in the generation of neutralizing antibodies mainly directed against the infecting serotype, for instance, DENV-1 (here in yellow) [1, 2] (see also **Figure 1c**, Part I). When the host is re-exposed to the same DENV serotype as the one from the primary infection (homotypic infection), the neutralizing antibodies generated during the first encounter, prevent APCs to be infected [2]. However, during a sequential DENV infection with a different serotype, here DENV-2 (in blue) [3], the pre-existing antibody response (1st DENV infection) do not neutralize but increases the infection of APCs via a mechanism called antibody-dependent-enhancement (ADE). This ADE phenomenon relies on the cell surface expression of Fcγ-receptors (FcγRs) including FcγRI (CD64) and FcγRII (CD32) mainly found in monocytes/macrophages and dendritic cells [1, 3], also FcεR found in mast cells resident of the skin (see also **Figure 2**, Part I). Importantly, ADE can be also modulated by the structural heterogeneity of the DENV particles (maturation state) [4]. During the DENV replication cycle, right before virus exocytosis of the infected cell, the newly assembled virus particles suffer a final processing step known as the ‘maturation state’ of the virion, in which the pre-M protein is cleaved by the cellular protease, furin. Inefficient furin activity leads to the release of viral particles containing a wide variety of pre-M/E protein complex known as partially mature virions [4]. Although immature and partially mature DENV viral particles are unable to infect cells via its cognate receptor, they can use the ADE mechanisms as a way to infect FcR-bearing cells which results in increased viral production (high virus yield) and increased secretion of cytokines and chemokines and other soluble components with vasoactive and pro-inflammatory activities in a process known as the ‘cytokine storm’. [5] The term “cytokine storm” is referred to the exacerbated and unbalanced production of

cytokines and chemokines which exert many effector functions including antiviral response and inflammation. Many of these components pose some vasoactive activities which alter the homeostasis of mostly all biological barriers including the microvascular endothelium, leading to a phenomenon called vascular leak (hereafter, 'leak'), a consequence of the increased endothelial cell (EC) permeability, and a hallmark of severe dengue disease [6]. The homeostasis of the endothelial barrier is mainly maintained by two structures: a) the endothelial glycocalyx layer (EGL), a network of membrane-bound glycosaminoglycans (e.g. heparan sulfate [HS], hyaluronic acid [HA], chondroitin sulfate [CS]) and proteoglycans (e.g. syndecan-1, perlecan, glypicans), covering the endothelium lumenally and playing critical roles in vascular physiology and pathology, including mechanotransduction, hemostasis, signaling, and blood cell-vessel wall interactions; and b) the intercellular junction complex (IJC), mainly composed of the tight (e.g. ZO-1, occluding, claudins) and adherens (e.g. VE-cadherin, beta-catenin) junction proteins that maintain the cell-to-cell contacts to control fluids and small molecules exchange between the luminal side (bloodstream) and the abluminal side (tissues compartment) of blood vessels. The disruption of any of these two main components under pathological conditions leads to increased inflammatory responses with pathogenic consequences involving barrier dysfunction and vascular disorders that result in excessive extravasation of fluids and proteins into the tissues, hypotension, shock, and sometimes, death. In addition to modulating the endothelial barrier function, cytokine and chemokines secreted by DENV infected cells pose important effector functions that promote the recruitment and activation of other immune cells such as NK cells with the ability to induce apoptosis of susceptible target cells (e.g. DENV infected cells) via NK cell-mediated cytotoxicity, and antibody (Ab)-dependent NK cell-mediated cytotoxicity (ADCC) [7]. Activation of NK cells also lead to the secretion of an array of immunoregulatory cytokines which conditioned a bidirectional crosstalk between NK cells and other immune cells such as dendritic cells (DC), macrophages, mast cells, and T cells. The release of cytotoxic granules and cytokines by NK cells may also contribute to a cytokine storm, and the tissue damage associated with infection clearance may exacerbate the pro-inflammatory environment. On the other hand, during DENV infection of immune cells, viral clearance of DENV results from a coordinated action of multiple cell types and mechanisms including innate immune responses such as the production of type I and type II interferons, cell killing by cytotoxic lymphocytes and production of neutralizing antibodies by B cells [7, 8, 9]. Intracellular expression of newly synthesized DENV proteins (e.g. NS3, NS1, NS5 proteins) enter the MHC class I and II presentation pathways in which viral peptide epitopes are presented on the cell surface either through MHC class II molecules for CD4+ T cells, and MHC class I molecules for CD8+ T cells, which principally lyse infected cells but also produce cytokines with effector functions on the endothelial biology. Like with the antibody response, after primary DENV infection, T cell responses are mainly characterized by higher homotypic than heterotypic responses [8, 10]. However, during DENV secondary infections, this pattern breaks down as B and T cells induced by the prior exposure to a different DENV serotype rapidly turn into highly serotype cross-reactive responses mainly directed against the previously encountered DENV serotype [11]. This phenomenon results in the consistent preferential expansion of pre-existing cross-reactive heterologous memory T cells with higher avidity for the previous DENV serotype and weak-affinity for the new infecting DENV, that may lead to an inefficient in clearing DENV infection, while producing excessive cytokines, leading to the onset of immune pathology such as vascular leak which results in severe disease. This alteration in the T cell immune responses, skewed by the 'memory' of the previous infection, is referred to as 'original antigenic sin' [12]. In addition to these host factors, the non-structural protein 1 (NS1) of DENV is a multifaceted viral factor that has been also demonstrated to play many roles in DENV pathogenesis. In DENV infected cells, NS1 can be found intracellularly expressed as a monomer in the ER where it forms dimers that play critical roles in the viral replication cycle, particularly the assembly of new viral particles [13]. Secreted NS1 protein from DENV infected cells is a hexameric protein with a barrel-like shape that contains a hydrophobic lipid core (cholesterol and triglycerides), and three domains known as the "wing domain" (here in yellow), the β -ladder domain (in blue), and the β -roll domain (in red) ([14], see also **Figure 1B**). Plasma circulating levels of soluble NS1 (sNS1) have been described to correlate with the appearance of severe disease manifestations such as dengue hemorrhagic fever (DHF) in DENV-infected patients [15]. sNS1 from DENV has been shown to act as a potential pathogen associated-molecular pattern (PAMP) that on immune cells and platelets interacts with Toll-like receptor 4 (TLR4), a pattern-recognition-receptor (PPR) [16] resulting in the production of pro-inflammatory cytokines from human PBMCs and monocytes [17] and the activation of human platelets which results in their aggregation and increase adherent onto the ECs lining the blood vessels, which hampers the homeostasis of the EC-barrier and the microvasculature [18]. Due to its ability to trigger the production of cytokines via TLR4, the term 'viral toxin' is now used to describe this pathogenic function of sNS1 during systemic DENV infection [14]. Furthermore, sNS1 can activate and block the antiviral activity of the complement pathway, an important component of the innate immune response against many human pathogen infections [19]. NS1 can either lead to pathogenesis by increasing the deposition of active complement components on the surface of ECs and human tissues [20], or by directly interacting with the component itself blocking its function or getting rid of some of the critical components involved in complement activation cascade [19]. An additional route for NS1 to cause virus pathogenesis or protection is related to the adaptive immune response generated against the secreted NS1 protein during DENV infection [21]. First, NS1-induced autoantibodies (anti-NS1 antibodies that recognize antigens on the host components) increase the activation of ECs causing barrier dysfunction by increasing the production of proinflammatory cytokines and the action of the complement pathway or binds to autoantigens encoded in proteins of the clotting cascade such as plasminogen, thrombin, and fibrinogen inducing coagulation disorders that along with the thrombocytopenia leads to bleeding manifestations [22, 23]. Second, anti-NS1 antibodies have been reported to prevent the direct interaction of sNS1 with the surface of ECs which blocks the NS1-induced activation of endothelial-intrinsic pathways leading to the disruption of the EGL and the remodeling the cell-to-cell contacts which disrupts the integrity of the intercellular junction complex formed by tight and adherens junction proteins (TJ/AJ) [24–26]. Overall, this summary figure represents the complex crosstalk between distinct host and viral factors that together trigger a diverse cascade of effector functions leading to protection or pathogenesis, the latter sometimes associated to the development of life-threatening disease manifestations that can result in death.

antigenic target for DENV neutralizing antibodies during natural infection, thus the focus of vaccine candidates design [30–32]. Antibodies specific for DENV proteins can mediate a wide range of functions *in vitro* [10]. They can neutralize DENV infection by direct hindering of virus-receptor interactions, blocking viral fusion with the endosomal membrane within host cells, viral clearance in a Fc-receptor dependent manner, lysis of virus infected cells via complement activation, and antibody dependent cell cytotoxicity (ADCC) of infected cells [33] (**Figure 1**). The E glycoprotein is composed of three structural domains (DI, DII, DIII), and the most extensive characterization of B cell epitopes has been conducted against them [34]. Neutralizing antibodies against the E protein of DENV include antibodies to nearly all the epitopes [35]. However, during the natural course of infection, the serological response to the E glycoprotein is highly serotype cross-reactive and predominantly targets epitopes containing highly conserved residues, for instance, the fusion loop of the domain II [31]. In addition, high-avidity and highly neutralizing antibodies against DENV, bind to domain III (DIII) of the E glycoprotein, which is implicated in DENV binding to its cognate receptor [36, 37]. These antibodies appear to be most effective at providing protection from infection and/or disease [38–40]. However, with an ~60% amino acid divergence between the E glycoproteins of all four DENV serotypes, immunity to one serotype usually does not confer long-lasting cross-protective immunity to the other serotypes [41]. A mature DENV particle contains 180 copies of the E protein covering the surface of the virion in either dimeric or trimeric (pre-fusion) conformations [42]. Neutralization is estimated to require a minimum occupancy of ~30 epitope sites per virion [34]. This may be attributed to the dense arrangement of E glycoproteins on the virion surface which has shown to be important for antigenicity, as many potentially neutralizing human antibodies against flaviviruses that target either hidden cryptic or quaternary epitopes extent through multiple E proteins [43–47].

On the other hand, the adaptive immune response during DENV infection can also generate antibodies against pre-M proteins which are highly serotype cross-reactive [48]. Despite this high cross-reactivity, anti-pre-M antibodies rarely neutralize DENV infection even at high concentrations [48]. The pre-M protein forms a heterodimer with the E protein, and it gets cleaved by host cell-expressed furin during the final stage of virion maturation before egress [42]. The cleavage of pre-M is required for the activation of flavivirus infectivity, including DENV [49]. In the mature virion, the remaining M protein fragment is completely hidden by the E protein dimers which makes it inaccessible to antibody binding [34]. Therefore, maturation state of the virion matters and may influence the interaction between immature virus particles and anti-pre-M antibodies leading to neutralization or enhancement of the infection (**Figure 1**). The potential role of anti-E or pre-M antibodies in increasing DENV infection and pathogenesis will be further discussed later in this chapter.

Antibody responses in DENV infection can be also directed against several non-structural proteins such as NS1, NS3, and NS5 as found in sera collected from DENV infected patients [50, 51]. These antibody responses have been mainly detected during secondary cases which open the possibility of implementing it in early diagnostic assays of DENV infection [51, 52]. NS3 (viral helicase/protease) and NS5 (virus RNA dependent polymerase) localize exclusively within virus-infected cells, but cell lysis owing to viral cytopathic effect or immune cell-mediated lysis may make these proteins accessible for binding to B cell receptors, inducing an antibody response [40, 52]. In this same line of interest, T cells may play an important role in the immune response against DENV nonstructural proteins. This topic will be later discussed in this chapter. On the other hand, the NS1 protein is the only flavivirus glycoprotein secreted by infected cells [53]. NS1 forms a multimeric structures either expressed on the surface of infected cells (dimer) or released as a soluble

hexameric during DENV infection *in vitro* and *in vivo* (See **Figure 1B, Part I**) [12, 54–56]. Specific antibodies for NS1 proteins have been also found circulating in DENV infected patients, particularly in secondary cases and are highly serotype cross-reactive [57–59].

In vivo experiments using mouse models for DENV infection, the adoptive transfer of immune serum or monoclonal antibodies specific for pre-M, E or NS1 proteins prevented mortality from lethal challenge with DENV [9, 38, 60–63]. Similarly, passive transfer of anti-E antibodies can protect against infection with DENV in nonhuman primate models [64, 65]. Dengue virus-specific antibodies of the appropriate subclasses can also bind to complement proteins and promote their activation. Fixation of complement to virions by antibodies specific for the pre-M and/or E proteins can inhibit viral infection [66]. NS1-specific antibodies mediate complement-dependent lysis of infected cells; however, this may not fully explain their protective effects *in vivo* [67]. Additionally, NS1-specific antibodies may also contribute to antibody-dependent cellular cytotoxicity [67, 68]. Recently, the role of the antibody immune response against the soluble DENV NS1 has become more relevant in the development of future dengue vaccines [9, 62, 69] as NS1 was described to play a key role in the development of DENV pathogenesis. Further evidences from a candidate dengue vaccine has demonstrated the functionality of anti-NS1-specific IgG responses against NS1 pathogenesis *in vitro* [70]. The phenomenon of NS1 being directly involved in modulating DENV pathogenesis will be discussed in more detail in a different section of this chapter.

1.2 Antibody-dependent enhancement of DENV infection and the cytokine storm

DENV has four distinct serotypes, and infection with one serotype results in the development of homotypic immunity which has been suggested to confer a durable and possible life-long protection against the infecting DENV serotype, but only short-term cross-reactive protection against other serotypes (heterotypic immunity) [13]. This cross-serotype-reactive antibody response is thought to wane to subneutralizing levels, where antibodies still bind, but do not neutralize the infecting virion. In turn, these antibodies contribute to enhanced infection of Fc receptors (FcRs) bearing cells during heterologous DENV encounters (**Figure 1**) [71, 72]. This phenomenon called “*antibody dependent enhancement*” or ADE, potentially increases the risk of developing severe disease by virtue of increasing the number of virus infected cells and therefore the viral biomass *in vivo* accompanied by hyperactivation of infected-immune cells and increased release of vasoactive mediators that resembles some pathologic features of what occurs in patients suffering severe dengue disease including capillary permeability and vascular leak (**Figure 1**) [73–78].

Multiple prospective cohort studies in Asia and Latin America have identified secondary infection as an epidemiological risk factor for severe dengue [17, 28, 79, 80]. Classical epidemiologic and observational studies have suggested that pre-existing sub-neutralizing antibodies in closely association with immunologic markers and clinical events supports the hypothesis of ADE and the risk of severe dengue during secondary infections [16, 26, 27, 78, 81–83]. Maternally derived subneutralizing levels of DENV-reactive IgGs have been also postulated to be a critical risk factor for severe dengue during infancy [26, 27, 84–86]. Numerous studies performed in animal models have reiterated that ADE results in higher viral load in patients at specific concentration of antibodies (*a peak of enhancement*), especially at early stages of infection, thereby increasing the risk of developing DHF/DSS [14, 87]. Studies performed in rhesus monkeys confirmed that passive transfer of immune serum or monoclonal antibodies resulted in increased viremia; however, no apparent signs of

severe disease was observed, indicating that severe dengue manifestations may not only be a main consequence of increased viremia [65, 88].

Despite all these evidences, yet no conclusive evidence exist that a risk of severe dengue disease and ADE occur in humans. A recent study using samples from a well-characterized DENV cohort study in children showed that the risk of developing severe dengue disease during secondary dengue infections existed within a narrow range of preexisting anti-DENV antibody titers, *a peak of enhancement*, detected in humans [29]. Recently, a phase 3 clinical trial of the only dengue vaccine licensed, Dengvaxia [89] showed an increased risk of hospitalization for severe dengue in children not exposed to DENV before vaccination [90], raising concerns about the need to assess dengue vaccine safety at the earliest development stages prior to human vaccination, and confirming that vaccination of DENV-naïve individuals may induce poorly neutralizing anti-DENV antibodies that increase the risk of severe dengue disease [91]. All these observations indicate that ADE may occur in humans and should be an obligate consideration for future designing, implementation, and evaluation of vaccine trials, especially those in the flavivirus field.

DENV-ADE can be mediated by E protein-specific antibodies either at low antibody concentrations or low antibody avidity, when the number of antibody molecules bound per virion is below the threshold necessary for neutralization of the virus (*a peak of enhancement*) [92]. As DENV E protein binds to cellular receptors and mediates viral fusion during entry, it is thought to be the major target of neutralizing antibodies [33, 41, 93]. However, a substantial proportion of antibodies generated in response to natural DENV infection are directed toward the pre-M protein which represents an important part of the adaptive immune response in DENV infected patients [40, 94].

During DENV infection, the cleavage of pre-M protein represents a critical step for the virus maturation process [49, 95, 96]. As the cleavage of pre-M is not complete in all dengue virions, a proportion of secreted viral particles from infected cells are partially mature dengue virions that contain a varying amount of cleaved and uncleaved pre-M proteins [42, 96, 97]. Immature dengue viral particles contains regular trimeric E-pre-M protein complexes and are noninfectious [49, 98]. In contrast, some partially mature forms, containing some pre-M protein-E protein trimers, are partially infectious. In both cases, uncleaved pre-M protein on immature or partially mature virions can be targeted by the host anti-pre-M antibody response which despite being highly cross-reactive among all four DENV serotypes, can rarely neutralize virus infection even at high concentrations, but promote ADE (**Figure 1**) [48, 75, 76, 98, 99].

Historically, the DENV-ADE phenomenon has been recapitulated by numerous *in vitro* studies where FcR-bearing immune cells including human monocyte/macrophages-like cell lines [48, 73, 74, 77, 98–103], human pre-basophil-like and immature mast cell-like cells [104, 105], human primary derived-monocytes/macrophages, dendritic cells, mast cells [75, 76, 106–110], and human derived-PBMCs [111, 112] have been infected with distinct DENV serotype strains in the presence of monoclonal antibodies or human derived-serum/plasma obtained from DENV infected cases [59, 72, 75, 76, 104, 105, 113–115]. Controversially, DENV-ADE for other considered immune cells such as endothelial cells [116, 117] have been also described *in vitro* and *in vivo* [118–121]. However, histochemistry of autopsy samples from fatal dengue cases and *in vitro* assays using peripheral blood cells revealed that macrophages/monocytes are primary targets for DENV infection and not endothelial cells [122–127].

In vitro, the increased infection of these target cells results in augmented expression/production of cytokines and vasoactive mediators and their release into the extracellular milieu where provoke an exacerbated activation of neighboring

immune cells and endothelial cells which leads to another critical phenomenon that may frequently occur during severe dengue infections known as the “*cytokine storm*” [10, 11, 73, 107, 128–130]. This phenomenon has been described for several other virus infections (such as influenza viruses, hantaviruses, and potentially coronaviruses), where the scenario envisioned is that this excessive immune activation creates a cascade of cytokine production or “*cytokine storm*” resulting in increased vascular permeability [131–134]. *In vivo*, experimental DENV infection in AG129 [135], showed that sub-neutralizing concentrations of anti-E or anti-pre-M antibodies increased DENV pathogenesis and mortality in mice mainly associated with increased circulation of pro-inflammatory cytokines and increased vascular leakage [40, 48, 87, 118, 136].

Mechanistically, it is thought that ADE-DENV infection of Fc receptor-bearing cells, particularly through the Fc-gamma-receptor IIA (FcγRIIA or CD32) [75, 102, 137, 138], not only results in a large virus-infected cell mass, rather the activation of intracellular signaling pathways [109, 139–142] which leads to the increased secretion of vasoactive immune products by infected cells, such as TNF-α, a cytokine produced by activated monocytes, which in elevated levels has been found in serum of DHF/DSS patients [71, 86, 143–145]. In addition to TNF-α, an important number of immune modulators have been described to be implicated in DENV pathogenesis acting upon a complicated network of events to provoke the severe dengue outcomes mainly related to increased vascular leakage. In DENV-infected primary monocytes or monocytic cell lines, the production of IL-6, IL-8, and interferon gamma-induced protein 10 (IP-10), IL-12p70, IL-1β, IL-10 and the prostaglandin E2 (PGE2) have been found upregulated [73, 74, 76, 77, 109, 112, 137, 142, 146]. In DENV-infected dendritic cells, the production of inflammatory cytokines TNF-α, IFN-α, IL-6, IL-10; the chemokines IFN-γ-inducible chemokines CXCL9, CXCL10, and CXCL11, PGE2, and also matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 were found increasingly produced [75, 76, 147–149]. On the other hand, DENV infection of mast cells has showed to elicit the release of potent vasoactive cytokines such as IL-1β and IL-6; chemokines, such as CCL3, CCL4, CCL5 and CXCL10, and other mast cell-derived mediators including proteases such as chymase and tryptase, leukotrienes, prostaglandins, histamine, and vascular endothelial growth factor (VEGF) which shows the significant influence of mast cells in immunity and pathogenesis during DENV infection [104, 107, 150, 151].

In humans, increased levels of many of these soluble factors including IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-7, IL-10, IL-13, IL-15, IL-17, IL-18, macrophage migration inhibitory factor (MIF), chemokines such as IL-8, CCL2, CCL4, CCL5, CXCL10 (IP-10) and the monocyte chemoattractant protein-1 (MCP-1) have been reported in patients with DHF when compared to DF [1, 10, 152–154]. Studies show that elevated levels of IL-6, IL-10, IFN-γ, MIF, and CCL-4 could be used as potential biomarkers of severe dengue [155, 156]. Additional biological markers such as serum lipids [157, 158], prostaglandins, leukotrienes and thromboxane [151, 159], free radical compounds such as reactive oxygen and nitrogen oxide species [160, 161], MMPs [162–164], and several components of the endothelium in the microvasculature such as the vascular cell adhesion molecule 1 (VCAM-1), Angiopoietin-1 and -2 (Ang-1, -2), endothelial-1, [5, 163, 165–167] or carbohydrates including glycosaminoglycans (GAGs) and proteoglycans (e.g. HSPG) [168–171] have been suggested to play important roles in the pathogenesis of different viral infections, including DENV. Changes in the plasma or urine levels of these molecules have been shown to act as potential predictors for clinical outcome between patients with different stages of DHF disease severity and predictors of disease severity in animal models *in vivo* [168, 170–174].

1.3 T cell responses and “the antigenic sin” of DENV infection

During DENV replication cycle, the viral genomic RNA encodes for a single poly-protein that after being cleaved by cellular and viral proteases yield three structural proteins and seven nonstructural proteins [42, 175] (See **Figure 1A, Part I**). Numerous studies have demonstrated that DENV infection leads to potent T cell responses, and many DENV-T cell epitopes have been found throughout the DENV polyprotein *ex vivo* using human leukocytes and *in vivo* using murine models [176–179]. These T cell epitopes appear to follow the general principles of T cell epitope immunogenicity, as they show similar MHC molecule binding kinetics to those of other immunodominant viral epitopes. Several studies indicate that nonstructural proteins are more frequently recognized by CD8⁺ T cells, while structural proteins are better recognized by CD4⁺ T cells. [180, 181]. However, most of the identified CD8⁺ and/or CD4⁺ T cells epitopes predominantly reside in the nonstructural proteins 3 (NS3) suggesting that NS3 protein is immunodominantly recognized by T cell epitopes in humans infected with DENV [8, 177, 182–184]. On the other hand, CD8⁺ and CD4⁺ T cell responses have been also identified, to a lesser extent, against other viral proteins such as the viral capsid, Ns1, NS2A/B, NS4A/B, and NS5 proteins [177, 178, 185, 186]. The recognition pattern of T cell receptors (TCRs) to these proteins expressed in the context of MHC differs according to the type of HLA which confers either susceptibility or protection to severe dengue infections [187, 188]. HLA class I and class II alleles have been shown to be associated with the development of DHF/DSS in different populations [182, 187, 189–192]. However, some specific HLA alleles are found to be more significantly common among patients with dengue fever than those undergoing severe dengue manifestations suggesting a protective effect of DENV-specific T cells [191]. Overall, the T cell immunodominance of DENV is quite complex and widely focused on different epitopes identified across the whole virus proteome [177, 183, 192]. Given that around 70% of amino acid identity exist between all four DENV serotypes, T cell epitopes are also highly cross-reactive and this has been suggested to play important roles in protective immunity not only against DENV but also other related flavivirus such as Zika virus (ZIKV) [193–195].

The protective role of T cells in viral infections is well established [196]. Dengue virus-specific T cells recognize virus-infected cells and respond with a diverse set of effector functions, including proliferation, target cell lysis and the production of a range of cytokines [197]. *In vivo*, CD8⁺ T cells control viral infection via direct cytotoxicity, and production of pro-inflammatory cytokines such as IFN- γ and TNF- α ; in turn, CD4⁺ T cells induce enhancement of B and CD8⁺ T cell responses, production of inflammatory and anti-viral cytokines, cytotoxicity, and promotion of memory responses to defeat viral infections [178, 179, 198–200]. T cell activity requires the presentation of viral peptides on the surface of infected cells in the context of MHC molecules and, unlike B cells, T cells do not recognize intact virions [196]. *In vitro*, DENV specific CD8⁺ memory T-cells can lyse MHC-matched virus-infected cells as an antiviral mechanism leading to protection [199, 201]. Activation of cytotoxic CD8⁺ T cells after presentation of viral peptides by infected antigen presenting cells (APCs) can generate immediate effector functions by expressing cytotoxic molecules such as granzyme B and perforin to kill virus-infected cells via MHC I- and MHC II-dependent mechanisms [10]. *In vivo* studies have shown that adoptive transfer of DENV-specific CD8⁺ T cell can partially protect mice from lethal challenge with DENV [202]. Other studies involving immunization with antigens that induce DENV-specific T cells but not neutralizing antibodies, have also shown that T cells are enough to protect mice from lethal infection [179, 203, 204]. These studies suggest that CD4⁺ or CD8⁺ T cells may have beneficial roles in controlling virus replication during DENV infections.

Although T cells have important functions in combating viral pathogens, both pathological and protective effects of T cells have been reported in the context of DENV infection [177, 178]. The association of severe dengue symptoms with a rapid decline in viral loads and a peak of pro-inflammatory cytokine secretion have led to the proposal of a role for a T cell mediated immune response in driving immunopathology in severe dengue [205]. T cell responses after primary DENV infections are characterized by higher homotypic than heterotypic responses [194]. However, in secondary DENV infections, T cell responses are highly serotype cross-reactive [206] with higher responses maintained to the previously encountered DENV serotype [207]. This alteration in the T cell immune responses, skewed by the ‘memory’ of the previous infection, is referred to as ‘*original antigenic sin*’ [10, 207] (**Figure 1**). According to this hypothesis, secondary DENV infection is dominated by the expansion of pre-existing nonprotective, cross-reactive and low affinity T cells to the new infecting serotype that results in ineffective viral control and elicit an aberrant immune response that contribute to immunopathology and severe dengue disease through an excessive production of inflammatory cytokines [10, 177, 178, 205, 207, 208]. Distinct studies *in vitro* using tetramers containing peptides from either the primary or secondary infecting viruses, have provided evidences for the original antigenic sin occurring in secondary T cell responses to DENV [207, 209].

The magnitude of the T cell response positively correlates with disease severity [183, 207]. Studies of the function of dengue-specific T cells has revealed an interesting difference between mild and severe infections [183]. Profound activation of naïve T cells into effector T cells and increased cytokine production have been reported in patients with DHF during both primary and secondary DENV infections [1]. T cell responses in severe dengue patients mainly produce IFN- γ and TNF- α [183]. Additionally, a broad spectrum of cytokines has been shown to be produced by DENV-specific T cells in responses to the recognition of peptide-MHC complexes on target cells. This array of cytokines follows a T helper 1 (TH1)- or TH0-like profile including the production of IFN- γ , TNF- α , IL-2, and MIP1 β , also known as CCL4, and less commonly, the TH2-type cytokines, IL-4 [15, 193]. Many of these T cell-derived cytokines have pleiotropic effects, including the induction or enhancement of inflammation and the alteration of vascular permeability that may contribute to the systemic disturbances leading to DHF [1, 10, 208].

Despite all this evidence, the relative contribution of DENV activated T cells in dengue pathogenesis and control of viral infection is still controversial. Severe dengue can occur during a primary dengue infection in which cross-reactive T cells and original antigenic sin would not be operative [210]. In addition, the relatively low numbers of circulating T cells seen during acute DENV infection and the temporal mismatch between the appearance of DENV NS3-specific CD8 $^{+}$ T cells, and the appearance of vascular leakage manifestations, suggests that other mechanisms independent of CD8 $^{+}$ T cells are responsible for early triggering of capillary leakage in children with DHF [207, 211]. A recent study found that numbers of DENV-specific T cells were augmented in the skin of DENV-infected patients compared to those circulating in the peripheral blood, suggesting that during the acute phase, DENV-specific T cells may migrate to the skin and return the bloodstream upon viral clearance [212, 213]. During secondary infections, the expansion of the low-avidity T cells specific for the primary DENV infection may delay viral clearance and thereby lead to higher viral loads. These results may explain the asynchronies timing between T cells circulating in the blood and the beginning of capillary permeability that occurs in DENV infection. However, in the absence of a good animal model of disease, it remains controversial whether the expansion of low-avidity cross-reactive T cells in secondary dengue infection contributes to disease pathogenesis. In summary, during DENV infection, the generation of an early T cell response may be

protective, whereas the late generation of T cell populations that have a proportion of low-avidity T cells and are skewed to inflammatory cytokine production in the absence of degranulation may predispose to immunopathology in the presence of high viral antigen loads that contributes to the cytokine storm and vascular leak.

1.4 DENV infection, NS1 pathogenesis, and vascular leak

The hallmark and critical feature of severe dengue disease is the increased capillary permeability, causing plasma leakage, which can lead to hemodynamic compromise and dengue shock syndrome (DSS) [2–4]. The plasma leakage syndrome, is defined as the extensive extravasation and accumulation of fluids out of blood vessels into the surrounding tissues and serous cavities, causing serositis which includes pleural effusion, and pericardial and peritoneal ascites, leading to hemoconcentration, hypotension, organ dysfunction, and life-threatening shock [6, 214]. In DENV infection, the transient nature of plasma leakage, its association with the late febrile phase and the paucity of structural damage to the vasculature identified by autopsy studies initially suggested that circulating factors were primarily responsible for this phenomenon [5, 125, 126, 205].

Although a major risk factor for developing severe dengue disease is related to the DENV-ADE phenomenon that correlates with increased plasma levels of pro-inflammatory cytokines found in the acute phase of patients undergoing severe dengue manifestations [1, 83], ADE alone seems not to be sufficient to explain the vascular pathology associated with DHF since not all secondary heterotypic infections results in severe disease, and many individuals also experience DHF during primary infection [16]. The association of severe dengue symptoms with a rapid decline in viral loads and a peak of pro-inflammatory cytokine secretion suggests that although subneutralizing antibodies can increase dengue disease severity via ADE, other factors may also influence disease outcome, driving the immunopathology in severe dengue [10, 205].

Very recently, a new piece in DENV pathogenesis puzzle, known as the nonstructural protein 1 or NS1, was reported to directly be involved in inducing endothelial dysfunction *in vitro* and vascular leakage *in vivo* via alteration of the endothelial barrier function and activation of immune cells and platelets, the latter resulting in induction of pro-inflammatory signaling pathways leading to increased secretion of vasoactive molecules and vascular leakage [9, 215, 216]. Initially recognized as a soluble complement-fixing (SCF) antigen detected in the blood of DENV-infected patients [217], NS1 of the flavivirus genus including DENV, is the only flaviviral protein described to be secreted by infected mammalian and insect cells [218–221], which circulates in the bloodstream of DENV-infected patients for up to 14 days since the onset of symptoms [222]. This bioavailability feature of NS1 launched it as a diagnostic marker for acute primary and secondary DENV infections and potentially other flavivirus diseases [54, 223–225]. NS1 antigenemia can reach as high as 50 µg/mL during the acute phase of dengue illness correlating with the development of DHF and sometimes fatality cases [12, 14, 54, 170, 223, 226–228]. These observations suggest that circulating levels of NS1 in the bloodstream of patients during the clinical phase of the disease may contribute to DENV pathogenesis.

In infected cells, NS1 is found as a membrane-associated dimer in both cellular compartments and on the cell surface. NS1 is intracellularly generated as a monomeric glycoprotein in the ER of DENV infected cells, where it has been demonstrated to play essential roles as cofactor in virus replication and virus assembly by recruiting cellular proteins as well as viral proteins such as the envelope protein during virus morphogenesis which results in the biogenesis of the membranous DENV RC organelle [229–233]. NS1 is also secreted by infected cells and recent structural

analyses showed that secreted NS1 circulates as a soluble hexamer glycoprotein with an atypical open barrel-shape that contains a prominent central lipid-enriched core of triglycerides, cholesteryl esters, and phospholipids that evokes a plasma high-density lipoprotein [53, 234] (**Figure 1**). Elucidation of the crystal structures of the NS1 hexamers reveal an amphipathic molecule with a hydrophobic inner face and a hydrophilic outer face containing three structural domains known as the hydrophobic “ β -roll”, the “wing” domain, and the c-terminal “ β -ladder” domain that likely have distinct roles in membrane association, replication complex assembly, and interactions with the immune system and are the basis for elucidating the molecular mechanism of NS1 function [235, 236] (See **Figure 1B, Part I**). These same structural domains were also identified by cryo-EM reconstruction studies of other related flavivirus NS1 proteins such as West Nile virus (WNV), Zika virus (ZIKV), and yellow fever virus (YFV) [237–239].

In addition to the role played in viral replication, NS1 participates in dengue immunopathogenesis by inhibiting platelet aggregation and prothrombin activation, directing complement against endothelial cells, inducing endothelial cell apoptosis, and facilitating the evasion of DENV particles from complement system-dependent neutralization [229, 240]. Regarding the complement pathways, NS1 mediates complement inactivation through multiple interactions with the complement proteins including factor H, C1s and C4, and the C4 binding protein [241–244]. These interactions result in attenuation of complement classical, lectin, and alternative pathways suggesting that extracellular NS1 protein may function to minimize immune system responses by decreasing complement recognition of DENV infected cells [19]. However, in flavivirus infections, the complement system has been described to play an important role in protecting the host but also influencing disease pathogenesis [19]. In DENV infected patients with DSS, accelerated complement consumption and a marked reduction in plasma complement components has been observed, which led to the proposal that complement activation plays an important role in disease pathogenesis [20, 245]. Recent studies from human autopsies have identified more evidences of increased deposition of complement components from both classical and alternative pathways associated with increased liver damage [126].

In the context of DENV NS1, both soluble NS1 and cell membrane-associated NS1 have been identified to triggers complement activation and anaphylatoxin formation in the presence of polyclonal or monoclonal anti-NS1 antibodies [246] (**Figure 1**). *In vitro* and *in vivo* experiments using anti-NS1 specific antibodies as well as antiserum obtained from DENV immunized mice and rabbits have reported their cross-reactivity with various epitopes found on human plasma proteins involved in coagulation pathways such as fibrinogen, plasminogen, and thrombin as well as integrin/adhesion proteins, endothelial cells and platelets leading to inflammation, apoptosis, and dysfunction of endothelial cells and platelets which sometimes results in bleeding issues [247–252]. Based on these evidences, autoimmune mechanisms mediated by anti-NS1 antibodies have been also proposed to lead to symptoms of DHF related to increasing vascular permeability maybe in a complement dependent manner. However, numerous past and new growing evidence describing the role of anti-NS1 antibody responses in NS1-immunized mouse models, DENV infected patients or DENV vaccine trails suggest an important protective effect of anti-NS1 immune responses as prophylactic or therapeutic options against DENV infection and other related flavivirus infections [9, 57, 62, 63, 70, 186, 253–260]. Therefore, the dual role of anti-NS1 antibodies in protection and disease still represents a critical challenge that needs to be overcome to develop an effective and safe NS1-based vaccine against flavivirus infections.

As mentioned previously, endothelial barrier dysfunction leading to vascular leakage and shock are the major causes of death in patients with dengue

hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. The vascular alterations observed in dengue cases have been described to be a consequence of the imbalance of the host immune system, specially cytokine storm, cytotoxic T cell and complement activations [1, 10, 11], in addition to endothelium injuries caused by the direct infection of the virus of endothelial cells, as reported by several *in vitro* studies [261, 262]. Endothelial cells, lining the inner side of blood vessels, constitute a critical component of the vascular endothelium which are in direct contact with the plasma proteins and all cellular components circulating in the bloodstream [263]. Under homeostatic conditions, this layer of endothelial cells crucially conducts several essential processes such as maintenance of vessel integrity, supply of oxygen and nutrients to underlying tissues and patrolling immune cell trafficking [264, 265]. Thus, alterations on its integrity under pathologic circumstances result in malfunction which contributes to inflammation and disease [265] (**Figure 1**).

Secreted hexameric DENV NS1 protein has been described to attaches to the surface of uninfected cells, primarily human endothelial cells *in vitro* and *in vivo* via interactions with heparan sulfate and chondroitin sulfate E [266]. In 2015, a new role for soluble NS1 in eliciting direct pathogenic effects in DENV disease was described [9]. In this study, the barrier function of human pulmonary microvascular endothelial cells (HPMEC) cultured under polarized conditions on semipermeable membrane filters (*e.g.* Transwells inserts) was compromised after being exposed to physiological concentrations (0.5–5 ug/mL) of recombinant soluble and hexameric NS1 proteins from all four DENV serotypes (DENV 1–4) *in vitro*. Interestingly, this pathogenic effect was recapitulated *in vivo* when inoculation of mice (*e.g.* *Ifnar^{-/-}*) with only DENV NS1 in the absence of DENV infection results in increased mice morbidity. More interesting, a combination of DENV NS1 with a sublethal dose of DENV2 made mice succumbed. These NS1-induced morbidity/mortality effects *in vivo* was significantly related to the increased vascular leak observed in these mice, phenomenon that was shown to be prevented by NS1 immunization or prophylactic treatment of mice using NS1-derived mouse antiserum or anti-NS1 monoclonal antibodies that also blocked NS1-increased permeability of HPMEC cultures *in vitro*. This study demonstrate how NS1 alone was able to mediate DENV pathogenesis by triggering endothelial dysfunction *in vitro* which was linked to increased vascular leak and mortality *in vivo* [9]. Interestingly, an additional study showed that the secreted form of NS1 may act as a pathogen associated-molecular pattern (PAMP) as purified NS1 protein was able to directly activate mouse macrophages and human PBMCs via Toll-like receptor 4 (TLR4), which leads to the induction and release of pro-inflammatory cytokines and chemokines *in vitro* (*e.g.* TNF- α , IL-6, IFN- β , IL-1 β , and IL-12); later, this effect was prevented by TLR4 antagonists and anti-TLR4 antibody treatment in a mouse model of DENV infection [215]. These evidences strongly support the important contribution of NS1 in modulating the endothelial cell biology and the inflammatory responses of immune cells as two of the main mechanisms described to influence DENV pathogenesis and therefore severe disease.

On the endothelium, two main structures work together to maintain the homeostasis of the microvasculature: a network of glycosaminoglycans, glycoproteins, and proteoglycans known as the endothelial glycocalyx layer (EGL) and an array of protein-to-protein interactions that integrates the intercellular junction complex (IJC), mainly composed by tight and adherens junction proteins, and other structures such as gaps and desmosomes [257–259]. Based on the first set of evidence showing a direct role of NS1 on the endothelial cell barrier, subsequent studies have identified distinct mechanisms triggered by DENV NS1 to cause endothelial hyperpermeability and vascular leak such as disruption of EGL (*e.g.* sialic acid,

heparan sulfate, syndecan-1) expressed on the surface of HPMEC and the microvasculature *in vivo* via activation/expression of endothelial enzymes including sialidases, heparanase, and cathepsin L, a lysosomal cysteine proteinase, all of these occurring in a cytokine-independent manner [267, 268] (**Figure 1**). An additional study corroborates these findings showing that NS1 induces the increased secretion of vasoactive molecules such as the macrophage migration inhibitory factor (MIF) and the angiopoietin-1 and 2 (Ang-1/Ang-2) from human endothelial cells (e.g. HMEC-1 from dermis) and DENV infected patients. These molecules were shown to activate autophagy pathways, phosphorylation cascades, and actin cytoskeleton rearrangements leading to disarrangement and internalization of VE-cadherin, an adherens junction protein of endothelial cell-to-cell contacts, inflammation, and also secretion of heparanase, shedding of syndecan-1 (CD138), and expression of MMP-9 from immune cells, resulting in degradation of EGL and hyperpermeability *in vitro* [269–272]. Follow up studies in human primary monocytes, monocytic cell lines, and human platelets stimulated with exogenous NS1 *in vitro* have additionally demonstrated the NS1-mediated activation and stimulation of pro-inflammatory cytokines and proteases (e.g. MMPs) via TLR4 signaling supporting previous reports of NS1 protein acting as a PAMP leading to inflammation, thrombocytopenia, hemorrhage and disease in DENV infection [273–275].

Besides DENV, the flavivirus genus includes other human medically important mosquito-borne pathogens such as ZIKV, WNV, Japanese encephalitis virus (JEV), and YFV [276]. In humans, flaviviruses can cause a wide spectrum of systemic or neurotropic-encephalitic pathologies ranging from clinically inapparent infections to severe, sometimes fatal disease, characterized by hemorrhagic manifestations and vascular leakage with organ failure (DENV and YFV), encephalitic manifestations (JEV and WNV), and congenital Zika syndrome in pregnancy and Guillain-Barré syndrome in adults associated with ZIKV infection [277, 278]. In recent studies, NS1 proteins from other DENV-closely related flavivirus including ZIKV, WNV, JEV, and YFV also demonstrated to cause endothelial hyperpermeability and vascular leak [172, 173, 279]. Interestingly, NS1 proteins selectively bind to and alters permeability of human endothelial cells from distinct tissues including lung, dermis, umbilical vein, brain, and liver *in vitro* and causes tissue-specific vascular leakage in mice, reflecting the pathophysiology of each flavivirus. Mechanistically, flaviviruses NS1 trigger the disruption of EGL components to cause endothelial hyperpermeability [172, 173, 253, 279]. On the vascular endothelium, the EGL constitutes a network of GAGs such as heparan sulfate, chondroitin sulfate, and hyaluronic acid, and proteoglycans (e.g., syndecans, glypicans, and perlecan) that contributes to maintain the homeostasis of the endothelial barrier function [280]. Degradation of the EGL and the detection of its degradation products in cell supernatants and human plasma have been linked to virus pathogenesis and disease severity in several viral hemorrhagic fever diseases [281], including dengue, where increased levels of heparan sulfate, hyaluronic acid, sialic acid, and syndecan-1 have been found to correlate with severe dengue disease in humans and lethality in animal models [3, 168–170, 172].

This NS1 pathogenic effect on the endothelium requires the internalization of the soluble NS1 protein inside human endothelial cells [279] (**Figure 1**). This process occurs via clathrin-mediated endocytosis and relies on one of the glycosylation sites (Asparagine-207) located in the *Wing* domain of NS1 [279]. DENV NS1 contains two conserved N-linked glycans at the asparagine-130 (N130) and the asparagine-207 (N207) which have been implicated in NS1 hexamer secretion, stability, and function [234, 236, 282, 283]. Previous studies investigating the importance of the N-glycans on NS1 have found that deglycosylated flaviviral NS1 proteins at either site, exhibited significant attenuation of neurovirulence in mice

compared to the wild-type virus [284–286]. Additional *in vitro* studies have shown that endocytosis of DENV NS1 occur in human hepatocytes which may potentialize subsequent DENV infection [221].

Flavivirus infection has been shown to compromise the integrity of many biological barriers, including the lung microvascular endothelium and the blood–brain barrier, which are usually able to protect against virus infection [287, 288]. Numerous studies of flavivirus infection in different animal models as well as human autopsies have shown a selective tropism of distinct groups of flaviviruses that target different tissues, leading to systemic versus neurotropic-encephalitic pathology [277]. The fact that NS1 internalization is required to induce endothelial hyperpermeability and increased vascular leak through a flaviviral-conserved endothelial cell-intrinsic pathways, and the finding that the flaviviral virulence depends on the expression of N-glycans on soluble NS1, suggest the possibility that NS1 may favor virus propagation and pathogenesis *in vivo*. During the acute phase of DENV infection high NS1 antigenemia have been correlated with increased risk of developing severe dengue disease, including vascular leakage [12, 14]; however, little is known about circulating levels of NS1 from other flavivirus infections. Future studies intended to investigate the kinetics and dynamics of NS1 circulation in flavivirus-infected patients different than DENV, will help to better understand the role of NS1 in flavivirus pathogenesis and disease. NS1 is well conserved among flaviviruses (20–40% identity, 60–80% similarity) [289], therefore, these findings reveal the capacity of a secreted viral protein from related flaviviruses named as NS1 flaviviral toxin as critical for pan-flavivirus pathogenesis through modulation of the endothelial barrier function in a tissue-specific manner, potentially influencing virus dissemination and pathogenesis of target organs and representing a novel target for anti-flaviviral therapy and potential vaccine candidates against flavivirus infections.

2. Concluding remarks (PART II)

Systemic vascular leakage associated with DENV infection is the most serious complication and the most important contributor to severe clinical outcomes during severe dengue disease that result in life-threatening complications such as hypotension, organ failure, and shock. Epidemiological data strongly associate severe dengue disease with secondary heterotypic DENV infections occurring in the presence of pre-existing antibody responses, widely attributed to the phenomenon of *antibody-dependent enhancement* (ADE). Numerous studies *in vivo* and *in vitro* have tightened DENV-ADE to the increased activation of immune cells such as monocytes, macrophages, dendritic cells, and mast cells leading to the generation of the pro-inflammatory environment found in many patients undergoing severe dengue, known as “*cytokine storm*”. Along with DENV-ADE, heterotypic immunity originated during primary DENV infection may also lead to alterations in immune responses of T cells, skewed by the ‘memory’ of the previous infection, referred to as ‘*original antigenic sin*’. These DENV cross-reactive T cell responses produce only inflammatory cytokines and might be inherently inefficient in killing DENV-infected cells, resulting in enhanced infection, which may predispose to the immunopathology of DENV.

Along with this evidence, in the last decades, several other immunologic mechanisms such as activation of complement pathways and autoimmune responses (e.g. mimetic anti-DENV antibodies) have been also linked to ensure severe dengue manifestations leading to the activation and apoptosis of immune cells and endothelial cells, aggregation of platelets, and inactivation of plasma proteins involved in coagulation cascades. On the other hand, viral biomarkers such as NS1, which

high circulating levels correlated with the appearance of severe dengue disease, was reported to modulate complement pathways, facilitating virus infection via immune evasion strategies. NS1 has demonstrated to exert an amazing array of different functions. More recently, NS1 was demonstrated to be a multitasking protein of the flavivirus genus which could directly cause disruption of the EGL and endothelial cell-to-cell contacts, two main components of the homeostasis balance in the microvasculature, and to induce the production of soluble immunoregulators resulting in increased endothelial barrier dysfunction and vascular leakage. This evidence provides new insights into the biology of the multifaceted NS1 protein of flavivirus that may improve the understanding of the flavivirus pathogenesis, strongly supporting the inclusion of NS1 protein in flavivirus vaccine development and the generation of new targets for future therapies against flavivirus infections.

In conclusion, the immunopathogenesis of DENV infection represents an extraordinarily complex interplay between several viral and host factors that together contribute intimately to the activation of distinct immunopathological processes that although were intended to control the viral infection and replication, instead unleash an unbalanced host immune response leading to increased endothelial dysfunction and vascular leakage, reflected in the appearance of dengue severe manifestations. As no effective vaccine or antiviral therapy are available to treat either prophylactically or therapeutically the DENV infection, the incidence of dengue disease is expanding globally and continues to threaten the public health services worldwide, particularly in endemic areas. An increased understanding of DENV immunopathogenesis mechanisms involved in the development of severe disease, their components, biological triggers, and their potential connections will assist not only the development of potentially more effective novel therapeutic interventions but also the understanding of dengue vaccine efficacy or vaccine adverse events that can be considered during vaccine trial interventions.

Acknowledgements

To the Canadian Institutes of Health Research and the International Development Research Centre (Projects 108,412 & 109,071–002) and the Fondo Mixto CONACYT-Gobierno del Estado de Yucatan (Project YUC-2017-2103-01-556).

IntechOpen

Author details

Henry Puerta-Guardo^{1*}, Scott B. Biering², Eva Harris²,
Norma Pavia-Ruz³, Gonzalo Vázquez-Prokopec⁴, Guadalupe Ayora-Talavera³
and Pablo Manrique-Saide¹

1 Collaborative Unit for Entomological Bioassays and Laboratory for Biological Control of *Aedes aegypti*, Campus of Biological and Agricultural Sciences, Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico


2 Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, USA

3 Centro de Investigaciones Regionales, Dr. Hideyo Noguchi, Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

4 Department of Environmental Sciences, Emory University, Atlanta, Georgia, USA

*Address all correspondence to: hpuertaguardo@gmail.com

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. *Current Opinion in Infectious Diseases*. 2006;**19**(5):429-436
- [2] World Health O. Dengue Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. Geneva: World Health Organization; 2009
- [3] Trung DT, Wills B. Systemic vascular leakage associated with dengue infections—the clinical perspective. *Current Topics in Microbiology and Immunology*. 2010;**338**:57-66
- [4] Yacoub S, Wertheim H, Simmons CP, Screaton G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. *Nature Reviews. Cardiology*. 2014;**11**(6):335-345
- [5] Yacoub S, Lam PK, Vu le HM, Le TL, Ha NT, Toan TT, et al. Association of microvascular function and endothelial biomarkers with clinical outcome in dengue: An observational study. *The Journal of Infectious Diseases*. 2016;**214**(5):697-706
- [6] Srikiatkhachorn A. Plasma leakage in dengue haemorrhagic fever. *Thrombosis and Haemostasis*. 2009;**102**(6):1042-1049
- [7] OhAinle M, Balmaseda A, Macalalad AR, Tellez Y, Zody MC, Saborío S, et al. Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity. *Science Translational Medicine*. 2011;**3**(114):114ra28-114ra28
- [8] Malavige GN, Ogg GS. T cell responses in dengue viral infections. *Journal of Clinical Virology*. 2013;**58**(4):605-611
- [9] Beatty PR, Puerta-Guardo H, Killingbeck SS, Glasner DR, Hopkins K, Harris E. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. *Science Translational Medicine*. 2015;**7**(304):304ra141
- [10] Rothman AL. Immunity to dengue virus: A tale of original antigenic sin and tropical cytokine storms. *Nature Reviews. Immunology*. 2011;**11**(8):532-543
- [11] Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. *Seminars in Immunopathology*. 2017;**39**(5):563-574
- [12] Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *The Journal of Infectious Diseases*. 2002;**186**(8):1165-1168
- [13] Rothman AL. Dengue: Defining protective versus pathologic immunity. *The Journal of Clinical Investigation*. 2004;**113**(7):946-951
- [14] Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *The Journal of Infectious Diseases*. 2000;**181**(1):2-9
- [15] Mangada MM, Rothman AL. Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypes. *Journal of Immunology*. 2005;**175**(4):2676-2683
- [16] Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: An historical perspective and role of

antibody-dependent enhancement of infection. *Archives of Virology*. 2013;**158**(7):1445-1459

[17] Sangkawibha N, Rojanasuphot S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitul V, et al. Risk factors in dengue shock syndrome: A prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *American Journal of Epidemiology*. 1984;**120**(5):653-669

[18] Wang WH, Urbina AN, Chang MR, Assavalapsakul W, Lu PL, Chen YH, et al. Dengue hemorrhagic fever—a systemic literature review of current perspectives on pathogenesis, prevention and control. *Journal of Microbiology, Immunology, and Infection*. 2020;**20**:30067

[19] Conde JN, Silva EM, Barbosa AS, Mohana-Borges R. The complement system in Flavivirus infections. *Frontiers in Microbiology*. 2017;**8**:213

[20] Malasit P. Complement and dengue haemorrhagic fever/shock syndrome. *The Southeast Asian Journal of Tropical Medicine and Public Health*. 1987;**18**(3):316-320

[21] Rico-Hesse R. Dengue virus markers of virulence and pathogenicity. *Future Virology*. 2009;**4**(6):581

[22] Katzelnick LC, Fonville JM, Gromowski GD, Bustos Arriaga J, Green A, James SL, et al. Dengue viruses cluster antigenically but not as discrete serotypes. *Science*. 2015;**349**(6254):1338-1343

[23] Katzelnick LC, Montoya M, Gresh L, Balmaseda A, Harris E. Neutralizing antibody titers against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(3):728-733

[24] Waggoner JJ, Balmaseda A, Gresh L, Sahoo MK, Montoya M, Wang C, et al. Homotypic dengue virus reinfections in Nicaraguan children. *The Journal of Infectious Diseases*. 2016;**214**(7):986-993

[25] Olkowski S, Forshey BM, Morrison AC, Rocha C, Vilcarromero S, Halsey ES, et al. Reduced risk of disease during postsecondary dengue virus infections. *The Journal of Infectious Diseases*. 2013;**208**(6):1026-1033

[26] Simmons CP, Chau TN, Thuy TT, Tuan NM, Hoang DM, Thien NT, et al. Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. *The Journal of Infectious Diseases*. 2007;**196**(3):416-424

[27] Kliks SC, Nimmanitya S, Nisalak A, Burke DS. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *The American Journal of Tropical Medicine and Hygiene*. 1988;**38**(2):411-419

[28] Kouri GP, Guzman MG, Bravo JR, Triana C. Dengue haemorrhagic fever/dengue shock syndrome: Lessons from the Cuban epidemic, 1981. *Bulletin of the World Health Organization*. 1989;**67**(4):375-380

[29] Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, et al. Antibody-dependent enhancement of severe dengue disease in humans. *Science*. 2017;**358**(6365):929-932

[30] Tsai WY, Chen HL, Tsai JJ, Dejnirattisai W, Jumnainsong A, Mongkolsapaya J, et al. Potent neutralizing human monoclonal antibodies preferentially target mature dengue virus particles: Implication for novel strategy for dengue vaccine. *Journal of Virology*. 2018;**92**(23)

[31] Lai CY, Tsai WY, Lin SR, Kao CL, Hu HP, King CC, et al. Antibodies to

envelope glycoprotein of dengue virus during the natural course of infection are predominantly cross-reactive and recognize epitopes containing highly conserved residues at the fusion loop of domain II. *Journal of Virology*. 2008;**82**(13):6631-6643

[32] Schieffelin JS, Costin JM, Nicholson CO, Orgeron NM, Fontaine KA, Isern S, et al. Neutralizing and non-neutralizing monoclonal antibodies against dengue virus E protein derived from a naturally infected patient. *Virology Journal*. 2010;**7**(1):28

[33] Pierson TC, Fremont DH, Kuhn RJ, Diamond MS. Structural insights into the mechanisms of antibody-mediated neutralization of flavivirus infection: Implications for vaccine development. *Cell Host & Microbe*. 2008;**4**(3):229-238

[34] Heinz FX, Stiasny K. Flaviviruses and their antigenic structure. *Journal of Clinical Virology*. 2012;**55**(4):289-295

[35] Sun H, Chen Q, Lai H. Development of antibody therapeutics against Flaviviruses. *International Journal of Molecular Sciences*. 2017;**19**(1):54

[36] Hu D, Zhu Z, Li S, Deng Y, Wu Y, Zhang N, et al. A broadly neutralizing germline-like human monoclonal antibody against dengue virus envelope domain III. *PLoS Pathogens*. 2019;**15**(6):e1007836

[37] Durham ND, Agrawal A, Waltari E, Croote D, Zanini F, Fouch M, et al. Broadly neutralizing human antibodies against dengue virus identified by single B cell transcriptomics. *eLife*. 2019;**8**:e52384

[38] Sukupolvi-Petty S, Austin SK, Engle M, Brien JD, Dowd KA, Williams KL, et al. Structure and function analysis of therapeutic monoclonal antibodies against dengue virus type 2. *Journal of Virology*. 2010;**84**(18):9227-9239

[39] Guzman MG, Hermida L, Bernardo L, Ramirez R, Guillen G. Domain III of the envelope protein as a dengue vaccine target. *Expert Review of Vaccines*. 2010;**9**(2):137-147

[40] Beltramello M, Williams KL, Simmons CP, Macagno A, Simonelli L, Quyen NT, et al. The human immune response to dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity. *Cell Host & Microbe*. 2010;**8**(3):271-283

[41] Heinz FX, Stiasny K. Flaviviruses and flavivirus vaccines. *Vaccine*. 2012;**30**(29):4301-4306

[42] Kuhn RJ, Zhang W, Rossmann MG, Pletnev SV, Corver J, Lenches E, et al. Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. *Cell*. 2002;**108**(5):717-725

[43] Gallichotte EN, Widman DG, Yount BL, Wahala WM, Durbin A, Whitehead S, et al. A new quaternary structure epitope on dengue virus serotype 2 is the target of durable type-specific neutralizing antibodies. *MBio*. 2015;**6**(5) e01461-15

[44] Widman DG, Young E, Nivarthi U, Swanstrom JA, Royal SR, Yount BL, et al. Transplantation of a quaternary structure neutralizing antibody epitope from dengue virus serotype 3 into serotype 4. *Scientific Reports*. 2017;**7**(1):17169

[45] de Alwis R, Smith SA, Olivarez NP, Messer WB, Huynh JP, Wahala WM, et al. Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**(19):7439-7444

[46] Dejnirattisai W, Wongwiwat W, Supasa S, Zhang X, Dai X, Rouvinsky A, et al. Corrigendum: A new class of highly potent, broadly neutralizing

antibodies isolated from viremic patients infected with dengue virus. *Nature Immunology*. 2015;**16**(7):785

[47] Li J, Watterson D, Chang CW, Che XY, Li XQ, Ericsson DJ, et al. Structural and functional characterization of a cross-reactive dengue virus neutralizing antibody that recognizes a cryptic epitope. *Structure*. 2018;**26**(1):51-9.e4

[48] Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S, Limpitikul W, et al. Cross-reacting antibodies enhance dengue virus infection in humans. *Science*. 2010;**328** (5979):745-748

[49] Zybert IA, van der Ende-Metselaar H, Wilschut J, Smit JM. Functional importance of dengue virus maturation: Infectious properties of immature virions. *The Journal of General Virology*. 2008;**89** (Pt 12):3047-3051

[50] Dos Santos Franco L, Gushi LT, Luiz WB, Amorim JH. Seeking Flavivirus cross-protective immunity. *Frontiers in Immunology*. 2019;**10**:2260

[51] Churdboonchart V, Bhamarapavati N, Peampramprecha S, Sirinavin S. Antibodies against dengue viral proteins in primary and secondary dengue hemorrhagic fever. *The American Journal of Tropical Medicine and Hygiene*. 1991;**44**(5):481-493

[52] Valdes K, Alvarez M, Pupo M, Vazquez S, Rodriguez R, Guzman MG. Human dengue antibodies against structural and nonstructural proteins. *Clinical and Diagnostic Laboratory Immunology*. 2000;**7**(5):856-857

[53] Gutsche I, Coulibaly F, Voss JE, Salmon J, d'Alayer J, Ermonval M, et al. Secreted dengue virus nonstructural protein NS1 is an atypical barrel-shaped high-density lipoprotein. *Proceedings of the National Academy of Sciences*

of the United States of America. 2011;**108**(19):8003-8008

[54] Young PR, Hilditch PA, Bletchly C, Halloran W. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *Journal of Clinical Microbiology*. 2000;**38**(3):1053-1057

[55] Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, et al. Relationship between nonstructural protein 1 detection and plasma virus load in dengue patients. *The American Journal of Tropical Medicine and Hygiene*. 2010;**83**(3):696-699

[56] Dussart P, Labeau B, Lagathu G, Louis P, Nunes MR, Rodrigues SG, et al. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. *Clinical and Vaccine Immunology*. 2006;**13**(11):1185-1189

[57] Jayathilaka D, Gomes L, Jeewandara C, Jayarathna GSB, Herath D, Perera PA, et al. Role of NS1 antibodies in the pathogenesis of acute secondary dengue infection. *Nature Communications*. 2018;**9**(1):5242

[58] Shu PY, Chen LK, Chang SF, Yueh YY, Chow L, Chien LJ, et al. Dengue NS1-specific antibody responses: Isotype distribution and serotyping in patients with dengue fever and dengue hemorrhagic fever. *Journal of Medical Virology*. 2000;**62**(2):224-232

[59] Shriver-Lake LC, Liu JL, Zabetakis D, Sugiharto VA, Lee CR, Defang GN, et al. Selection and characterization of anti-dengue NS1 single domain antibodies. *Scientific Reports*. 2018;**8**(1):18086

[60] Edeling MA, Austin SK, Shrestha B, Dowd KA, Mukherjee S, Nelson CA, et al. Potent dengue virus neutralization

by a therapeutic antibody with low monovalent affinity requires bivalent engagement. *PLoS Pathogens*. 2014;**10**(4):e1004072

[61] Kaufman BM, Summers PL, Dubois DR, Cohen WH, Gentry MK, Timchak RL, et al. Monoclonal antibodies for dengue virus prM glycoprotein protect mice against lethal dengue infection. *The American Journal of Tropical Medicine and Hygiene*. 1989;**41**(5):576-580

[62] Hertz T, Beatty PR, MacMillen Z, Killingbeck SS, Wang C, Harris E. Antibody epitopes identified in critical regions of dengue virus nonstructural 1 protein in mouse vaccination and natural human infections. *Journal of Immunology*. 2017;**198**(10):4025-4035

[63] Henchal EA, Henchal LS, Schlesinger JJ. Synergistic interactions of anti-NS1 monoclonal antibodies protect passively immunized mice from lethal challenge with dengue 2 virus. *The Journal of General Virology* 1988;69 (Pt 8):2101-2107

[64] Lai CJ, Goncalvez AP, Men R, Wernly C, Donau O, Engle RE, et al. Epitope determinants of a chimpanzee dengue virus type 4 (DENV-4)-neutralizing antibody and protection against DENV-4 challenge in mice and rhesus monkeys by passively transferred humanized antibody. *Journal of Virology*. 2007;**81**(23):12766-12774

[65] Goncalvez AP, Engle RE, St Claire M, Purcell RH, Lai CJ. Monoclonal antibody-mediated enhancement of dengue virus infection in vitro and in vivo and strategies for prevention. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**(22):9422-9427

[66] Mehlhop E, Ansarah-Sobrinho C, Johnson S, Engle M, Fremont DH, Pierson TC, et al. Complement protein

C1q inhibits antibody-dependent enhancement of flavivirus infection in an IgG subclass-specific manner. *Cell Host & Microbe*. 2007;**2**(6):417-426

[67] Falgout B, Bray M, Schlesinger JJ, Lai CJ. Immunization of mice with recombinant vaccinia virus expressing authentic dengue virus nonstructural protein NS1 protects against lethal dengue virus encephalitis. *Journal of Virology*. 1990;**64**(9):4356-4363

[68] Garcia G, Arango M, Perez AB, Fonte L, Sierra B, Rodriguez-Roche R, et al. Antibodies from patients with dengue viral infection mediate cellular cytotoxicity. *Journal of Clinical Virology*. 2006;**37**(1):53-57

[69] Reyes-Sandoval A, Ludert JE. The dual role of the antibody response against the Flavivirus non-structural protein 1 (NS1) in protection and Immuno-pathogenesis. *Frontiers in Immunology*. 2019;**10**:1651

[70] Sharma M, Glasner DR, Watkins H, Puerta-Guardo H, Kassa Y, Egan MA, et al. Magnitude and functionality of the NS1-specific antibody response elicited by a live-attenuated tetravalent dengue vaccine candidate. *The Journal of Infectious Diseases*. 2020;**221**(6):867-877

[71] Kliks SC, Nisalak A, Brandt WE, Wahl L, Burke DS. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *The American Journal of Tropical Medicine and Hygiene*. 1989;**40**(4):444-451

[72] Halstead SB, O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. *Nature*. 1977;**265**(5596):739-741

[73] Puerta-Guardo H, Raya-Sandino A, González-Mariscal L, Rosales VH, Ayala-Dávila J, Chávez-Mungía B, et al. The cytokine response of U937-derived

macrophages infected through antibody-dependent enhancement of dengue virus disrupts cell apical-junction complexes and increases vascular permeability. *Journal of Virology*. 2013;**87**(13):7486-7501

[74] Chareonsirisuthigul T, Kalayanarooj S, Ubol S. Dengue virus (DENV) antibody-dependent enhancement of infection upregulates the production of anti-inflammatory cytokines, but suppresses anti-DENV free radical and pro-inflammatory cytokine production, in THP-1 cells. *The Journal of General Virology* 2007;**88** (Pt 2):365-375

[75] Boonnak K, Slike BM, Burgess TH, Mason RM, Wu SJ, Sun P, et al. Role of dendritic cells in antibody-dependent enhancement of dengue virus infection. *Journal of Virology*. 2008;**82**(8):3939-3951

[76] Boonnak K, Dambach KM, Donofrio GC, Tassaneetrithep B, Marovich MA. Cell type specificity and host genetic polymorphisms influence antibody-dependent enhancement of dengue virus infection. *Journal of Virology*. 2011;**85**(4):1671-1683

[77] Ubol S, Phuklia W, Kalayanarooj S, Modhiran N. Mechanisms of immune evasion induced by a complex of dengue virus and preexisting enhancing antibodies. *The Journal of Infectious Diseases*. 2010;**201**(6):923-935

[78] Halstead SB, Nimmannitya S, Yamarat C, Russell PK. Hemorrhagic fever in Thailand; recent knowledge regarding etiology. *Japanese Journal of Medical Science & Biology*. 1967;**20** Suppl:96-103

[79] Endy TP, Nisalak A, Chunsuttiwat S, Libraty DH, Green S, Rothman AL, et al. Spatial and temporal circulation of dengue virus serotypes: A prospective study of primary school children in Kamphaeng Phet, Thailand.

American Journal of Epidemiology. 2002;**156**(1):52-59

[80] Gibbons RV, Kalanarooj S, Jarman RG, Nisalak A, Vaughn DW, Endy TP, et al. Analysis of repeat hospital admissions for dengue to estimate the frequency of third or fourth dengue infections resulting in admissions and dengue hemorrhagic fever, and serotype sequences. *The American Journal of Tropical Medicine and Hygiene*. 2007;**77**(5):910-913

[81] Gan ES, Ting DH, Chan KR. The mechanistic role of antibodies to dengue virus in protection and disease pathogenesis. *Expert Review of Anti-Infective Therapy*. 2017;**15**(2):111-119

[82] Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *The Yale Journal of Biology and Medicine*. 1970;**42**(5):350-362

[83] Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *The Yale Journal of Biology and Medicine*. 1970;**42**(5):311-328

[84] Chau TN, Hieu NT, Anders KL, Wolbers M, Lien le B, Hieu LT, et al. Dengue virus infections and maternal antibody decay in a prospective birth cohort study of Vietnamese infants. *The Journal of Infectious Diseases*. 2009;**200**(12):1893-1900

[85] Chau TN, Quyen NT, Thuy TT, Tuan NM, Hoang DM, Dung NT, et al. Dengue in Vietnamese infants—results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. *The Journal of Infectious Diseases*. 2008;**198**(4):516-524

- [86] Nguyen TH, Lei HY, Nguyen TL, Lin YS, Huang KJ, Le BL, et al. Dengue hemorrhagic fever in infants: A study of clinical and cytokine profiles. *The Journal of Infectious Diseases*. 2004;**189**(2):221-232
- [87] Balsitis SJ, Williams KL, Lachica R, Flores D, Kyle JL, Mehlhop E, et al. Lethal antibody enhancement of dengue disease in mice is prevented by fc modification. *PLoS Pathogens*. 2010;**6**(2):e1000790
- [88] Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *The Journal of Infectious Diseases*. 1979;**140**(4):527-533
- [89] Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *The New England Journal of Medicine*. 2015;**373**(13):1195-1206
- [90] Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, et al. Effect of dengue Serostatus on dengue vaccine safety and efficacy. *The New England Journal of Medicine*. 2018;**379**(4):327-340
- [91] Ferguson NM, Rodriguez-Barraquer I, Dorigatti I, Mier YT-RL, Laydon DJ, Cummings DA. Benefits and risks of the Sanofi-Pasteur dengue vaccine: Modeling optimal deployment. *Science*. 2016;**353**(6303):1033-1036
- [92] Pierson TC, Xu Q, Nelson S, Oliphant T, Nybakken GE, Fremont DH, et al. The stoichiometry of antibody-mediated neutralization and enhancement of West Nile virus infection. *Cell Host & Microbe*. 2007;**1**(2):135-145
- [93] Roehrig JT. Antigenic structure of flavivirus proteins. *Advances in Virus Research*. 2003;**59**:141-175
- [94] Smith SA, Zhou Y, Olivarez NP, Broadwater AH, de Silva AM, Crowe JE Jr. Persistence of circulating memory B cell clones with potential for dengue virus disease enhancement for decades following infection. *Journal of Virology*. 2012;**86**(5):2665-2675
- [95] Kuhn RJ, Dowd KA, Beth Post C, Pierson TC. Shake, rattle, and roll: Impact of the dynamics of flavivirus particles on their interactions with the host. *Virology*. 2015;**479-480**:508-517
- [96] Pierson TC, Diamond MS. Degrees of maturity: The complex structure and biology of flaviviruses. *Current Opinion in Virology*. 2012;**2**(2):168-175
- [97] Junjhon J, Edwards TJ, Utaipat U, Bowman VD, Holdaway HA, Zhang W, et al. Influence of pr-M cleavage on the heterogeneity of extracellular dengue virus particles. *Journal of Virology*. 2010;**84**(16):8353-8358
- [98] Rodenhuis-Zybert IA, van der Schaar HM, da Silva Voorham JM, van der Ende-Metselaar H, Lei HY, Wilschut J, et al. Immature dengue virus: a veiled pathogen? *PLoS Pathogens*. 2010;**6**(1):e1000718
- [99] Rodenhuis-Zybert IA, Wilschut J, Smit JM. Partial maturation: An immune-evasion strategy of dengue virus? *Trends in Microbiology*. 2011;**19**(5):248-254
- [100] Smith SA, Nivarthi UK, de Alwis R, Kose N, Sapparapu G, Bombardi R, et al. Dengue virus prM-specific human monoclonal antibodies with virus replication-enhancing properties recognize a single Immunodominant antigenic site. *Journal of Virology*. 2016;**90**(2):780-789
- [101] Ayala-Nunez NV, Hoornweg TE, van de Pol DP, Sjollem KA, Flipse J, van der Schaar HM, et al. How antibodies alter the cell entry pathway of dengue virus particles in macrophages. *Scientific Reports*. 2016;**6**:28768

- [102] Kontny U, Kurane I, Ennis FA. Gamma interferon augments Fc gamma receptor-mediated dengue virus infection of human monocytic cells. *Journal of Virology*. 1988;**62**(11):3928-3933
- [103] Littaua R, Kurane I, Ennis FA. Human IgG fc receptor II mediates antibody-dependent enhancement of dengue virus infection. *Journal of Immunology*. 1990;**144**(8):3183-3186
- [104] Brown MG, McAlpine SM, Huang YY, Haidl ID, Al-Afif A, Marshall JS, et al. RNA sensors enable human mast cell anti-viral chemokine production and IFN-mediated protection in response to antibody-enhanced dengue virus infection. *PLoS One*. 2012;**7**(3):e34055-e
- [105] Brown MG, Huang YY, Marshall JS, King CA, Hoskin DW, Anderson R. Dramatic caspase-dependent apoptosis in antibody-enhanced dengue virus infection of human mast cells. *Journal of Leukocyte Biology*. 2009;**85**(1):71-80
- [106] Brown MG, King CA, Sherren C, Marshall JS, Anderson R. A dominant role for FcγRII in antibody-enhanced dengue virus infection of human mast cells and associated CCL5 release. *Journal of Leukocyte Biology*. 2006;**80**(6):1242-1250
- [107] King CA, Anderson R, Marshall JS. Dengue virus selectively induces human mast cell chemokine production. *Journal of Virology*. 2002;**76**(16):8408-8419
- [108] Diamond MS, Edgil D, Roberts TG, Lu B, Harris E. Infection of human cells by dengue virus is modulated by different cell types and viral strains. *Journal of Virology*. 2000;**74**(17):7814
- [109] Tsai T-T, Chuang Y-J, Lin Y-S, Chang C-P, Wan S-W, Lin S-H, et al. Antibody-dependent enhancement infection facilitates dengue virus-regulated signaling of IL-10 production in monocytes. *PLoS Neglected Tropical Diseases*. 2014;**8**(11):e3320
- [110] Flipse J, Diosa-Toro MA, Hoornweg TE, van de Pol DP, Urcuqui-Inchima S, Smit JM. Antibody-dependent enhancement of dengue virus infection in primary human macrophages; balancing higher fusion against antiviral responses. *Scientific Reports*. 2016;**6**:29201
- [111] Jiang L, Sun Q. The expression profile of human peripheral blood mononuclear cell miRNA is altered by antibody-dependent enhancement of infection with dengue virus serotype 3. *Virology Journal*. 2018;**15**(1):50
- [112] Sun P, Bauza K, Pal S, Liang Z, Wu SJ, Beckett C, et al. Infection and activation of human peripheral blood monocytes by dengue viruses through the mechanism of antibody-dependent enhancement. *Virology*. 2011;**421**(2):245-252
- [113] Morens DM, Halstead SB. Measurement of antibody-dependent infection enhancement of four dengue virus serotypes by monoclonal and polyclonal antibodies. *The Journal of General Virology* 1990;**71**(Pt 12): 2909-2914
- [114] Modhiran N, Kalayanarooj S, Ubol S. Subversion of innate defenses by the interplay between DENV and pre-existing enhancing antibodies: TLRs signaling collapse. *PLoS Neglected Tropical Diseases*. 2010;**4**(12):e924
- [115] Fang YT, Wan SW, Lu YT, Yao JH, Lin CF, Hsu LJ, et al. Autophagy facilitates antibody-enhanced dengue virus infection in human pre-basophil/mast cells. *PLoS One*. 2014;**9**(10):e110655
- [116] Al-Soudi A, Kaaij MH, Tas SW. Endothelial cells: From innocent bystanders to active participants in immune responses. *Autoimmunity Reviews*. 2017;**16**(9):951-962

- [117] Young MR. Endothelial cells in the eyes of an immunologist. *Cancer Immunology, Immunotherapy*. 2012;**61**(10):1609-1616
- [118] Zellweger RM, Prestwood TR, Shresta S. Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. *Cell Host & Microbe*. 2010;**7**(2):128-139
- [119] Arevalo MT, Simpson-Haidaris PJ, Kou Z, Schlesinger JJ, Jin X. Primary human endothelial cells support direct but not antibody-dependent enhancement of dengue viral infection. *Journal of Medical Virology*. 2009;**81**(3):519-528
- [120] Talavera D, Castillo AM, Dominguez MC, Gutierrez AE, Meza I. IL8 release, tight junction and cytoskeleton dynamic reorganization conducive to permeability increase are induced by dengue virus infection of microvascular endothelial monolayers. *The Journal of General Virology*. 2004;**85**(Pt 7):1801-1813
- [121] Pova TF, Alves AM, Oliveira CA, Nuovo GJ, Chagas VL, Paes MV. The pathology of severe dengue in multiple organs of human fatal cases: Histopathology, ultrastructure and virus replication. *PLoS One*. 2014;**9**(4):e83386
- [122] Bruggeman CW, Houtzager J, Dierdorp B, Kers J, Pals ST, Lutter R, et al. Tissue-specific expression of IgG receptors by human macrophages ex vivo. *PLoS One*. 2019;**14**(10):e0223264
- [123] Jessie K, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. *The Journal of Infectious Diseases*. 2004;**189**(8):1411-1418
- [124] Kangwanpong D, Bhamarapravati N, Lucia HL. Diagnosing dengue virus infection in archived autopsy tissues by means of the in situ PCR method: A case report. *Clinical and Diagnostic Virology*. 1995;**3**(2):165-172
- [125] Win MM, Charngkaew K, Punyadee N, Aye KS, Win N, Chaisri U, et al. Ultrastructural features of human liver specimens from patients who died of dengue hemorrhagic fever. *Tropical Medicine and Infectious Diseases*. 2019;**4**(2):63
- [126] Aye KS, Charngkaew K, Win N, Wai KZ, Moe K, Punyadee N, et al. Pathologic highlights of dengue hemorrhagic fever in 13 autopsy cases from Myanmar. *Human Pathology*. 2014;**45**(6):1221-1233
- [127] Begum F, Das S, Mukherjee D, Mal S, Ray U. Insight into the tropism of dengue virus in humans. *Viruses*. 2019;**11**(12):1136
- [128] Kuczera D, Assolini JP, Tomiotto-Pellissier F, Pavanelli WR, Silveira GF. Highlights for dengue Immunopathogenesis: Antibody-dependent enhancement, cytokine storm, and beyond. *Journal of Interferon & Cytokine Research*. 2018;**38**(2):69-80
- [129] Lin YW, Wang KJ, Lei HY, Lin YS, Yeh TM, Liu HS, et al. Virus replication and cytokine production in dengue virus-infected human B lymphocytes. *Journal of Virology*. 2002;**76**(23):12242-12249
- [130] Anderson R, Wang S, Osiowy C, Issekutz AC. Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. *Journal of Virology*. 1997;**71**(6):4226-4232
- [131] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. *Microbiology and Molecular Biology Reviews*. 2012;**76**(1):16-32

- [132] Khaiboullina SF, Levis S, Morzunov SP, Martynova EV, Anokhin VA, Gusev OA, et al. Serum cytokine profiles differentiating hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. *Frontiers in Immunology*. 2017;**8**:567
- [133] Guo X-ZJ, Thomas PG. New fronts emerge in the influenza cytokine storm. *Seminars in Immunopathology*. 2017;**39**(5):541-550
- [134] Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. *The Journal of Infection*. 2020;**S0163-4453**(20):30165-30161
- [135] Shrestha S, Kyle JL, Snider HM, Basavapatna M, Beatty PR, Harris E. Interferon-dependent immunity is essential for resistance to primary dengue virus infection in mice, whereas T- and B-cell-dependent immunity are less critical. *Journal of Virology*. 2004;**78**(6):2701-2710
- [136] Ng JKW, Zhang SL, Tan HC, Yan B, Maria Martinez Gomez J, Tan WY, et al. First experimental In vivo model of enhanced dengue disease severity through maternally acquired heterotypic dengue antibodies. *PLoS Pathogens*. 2014;**10**(4):e1004031
- [137] Moi ML, Lim CK, Takasaki T, Kurane I. Involvement of the fc gamma receptor IIA cytoplasmic domain in antibody-dependent enhancement of dengue virus infection. *The Journal of General Virology*. 2010;**91**(Pt 1):103-111
- [138] Rodrigo WW, Jin X, Blackley SD, Rose RC, Schlesinger JJ. Differential enhancement of dengue virus immune complex infectivity mediated by signaling-competent and signaling-incompetent human Fc gamma RIA (CD64) or Fc gamma RIIA (CD32). *Journal of Virology*. 2006;**80**(20):10128-10138
- [139] Flipse J, Wilschut J, Smit JM. Molecular mechanisms involved in antibody-dependent enhancement of dengue virus infection in humans. *Traffic*. 2013;**14**(1):25-35
- [140] Chan CYY, Low JZH, Gan ES, Ong EZ, Zhang SL, Tan HC, et al. Antibody-dependent dengue virus entry modulates cell intrinsic responses for enhanced infection. *mSphere*. 2019;**4**(5):e00528-19
- [141] Halstead SB, Mahalingam S, Marovich MA, Ubol S, Mosser DM. Intrinsic antibody-dependent enhancement of microbial infection in macrophages: Disease regulation by immune complexes. *The Lancet Infectious Diseases*. 2010;**10**(10):712-722
- [142] Huang X, Yue Y, Li D, Zhao Y, Qiu L, Chen J, et al. Antibody-dependent enhancement of dengue virus infection inhibits RLR-mediated type-I IFN-independent signalling through upregulation of cellular autophagy. *Scientific Reports*. 2016;**6**:22303
- [143] Hober D, Poli L, Roblin B, Gestas P, Chungue E, Granic G, et al. Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 beta) in dengue-infected patients. *The American Journal of Tropical Medicine and Hygiene*. 1993;**48**(3):324-331
- [144] Vitarana T, de Silva H, Withana N, Gunasekera C. Elevated tumour necrosis factor in dengue fever and dengue haemorrhagic fever. *The Ceylon Medical Journal*. 1991;**36**(2):63-65
- [145] Meena AA, Murugesan A, Sopnajothi S, Yong YK, Ganesh PS, Vimali JJ, et al. Increase of plasma TNF-alpha is associated with decreased levels of blood platelets in clinical dengue infection. *Viral Immunology*. 2020;**33**(1):54-60
- [146] Tan TY, Chu JJH. Dengue virus-infected human monocytes trigger late

activation of caspase-1, which mediates pro-inflammatory IL-1 β secretion and pyroptosis. *The Journal of General Virology*. 2013;**94**(Pt 10):2215-2220

[147] Libraty DH, Pichyangkul S, Ajariyakhajorn C, Endy TP, Ennis FA. Human dendritic cells are activated by dengue virus infection: Enhancement by gamma interferon and implications for disease pathogenesis. *Journal of Virology*. 2001;**75**(8):3501-3508

[148] Luplertlop N, Misse D, Bray D, Deleuze V, Gonzalez JP, Leardkamolkarn V, et al. Dengue-virus-infected dendritic cells trigger vascular leakage through metalloproteinase overproduction. *EMBO Reports*. 2006;**7**(11):1176-1181

[149] Wu WL, Ho LJ, Chang DM, Chen CH, Lai JH. Triggering of DC migration by dengue virus stimulation of COX-2-dependent signaling cascades in vitro highlights the significance of these cascades beyond inflammation. *European Journal of Immunology*. 2009;**39**(12):3413-3422

[150] King CA, Marshall JS, Alshurafa H, Anderson R. Release of vasoactive cytokines by antibody-enhanced dengue virus infection of a human mast cell/basophil line. *Journal of Virology*. 2000;**74**(15):7146-7150

[151] Avirutnan P, Matangkasombut P. Unmasking the role of mast cells in dengue. *eLife*. 2013;**2**:e00767

[152] Patro ARK, Mohanty S, Prusty BK, Singh DK, Gaikwad S, Saswat T, et al. Cytokine signature associated with disease severity in dengue. *Viruses*. 2019;**11**(1):34

[153] Espada-Murao LA, Morita K. Dengue and soluble mediators of the innate immune system. *Tropical Medicine and Health*. 2011;**39**(4 Suppl):53-62

[154] Srikiatkachorn A, Green S. Markers of dengue disease severity.

Current Topics in Microbiology and Immunology. 2010;**338**:67-82

[155] John DV, Lin YS, Perng GC. Biomarkers of severe dengue disease - a review. *Journal of Biomedical Science*. 2015;**22**:83

[156] Robinson M, Einav S. Towards predicting progression to severe dengue. *Trends in Microbiology*. 2020;**28**(6):478-486

[157] Lima WG, Souza NA, Fernandes SOA, Cardoso VN, Godoi IP. Serum lipid profile as a predictor of dengue severity: A systematic review and meta-analysis. *Reviews in Medical Virology*. 2019;**29**(5):e2056

[158] van Gorp EC, Suharti C, Mairuhu AT, Dolmans WM, van Der Ven J, Demacker PN, et al. Changes in the plasma lipid profile as a potential predictor of clinical outcome in dengue hemorrhagic fever. *Clinical Infectious Diseases*. 2002;**34**(8):1150-1153

[159] Malavige GN, Ogg GS. Pathogenesis of vascular leak in dengue virus infection. *Immunology*. 2017;**151**(3):261-269

[160] Chaturvedi UC, Nagar R. Nitric oxide in dengue and dengue haemorrhagic fever: Necessity or nuisance? *FEMS Immunology and Medical Microbiology*. 2009;**56**(1):9-24

[161] Cheng YL, Lin YS, Chen CL, Wan SW, Ou YD, Yu CY, et al. Dengue virus infection causes the activation of distinct NF- κ B pathways for inducible nitric oxide synthase and TNF- α expression in RAW264.7 cells. *Mediators of Inflammation*. 2015;**2015**:274025

[162] Voraphani N, Khongphatthanayothin A, Srikaew K, Tontulawat P, Poovorawan Y. Matrix metalloproteinase-9 (mmp-9) in children with dengue virus infection.

Japanese Journal of Infectious Diseases. 2010;**63**(5):346-348

[163] Her Z, Kam YW, Gan VC, Lee B, Thein TL, Tan JJ, et al. Severity of plasma leakage is associated with high levels of interferon gamma-inducible protein 10, hepatocyte growth factor, matrix metalloproteinase 2 (MMP-2), and MMP-9 during dengue virus infection. *The Journal of Infectious Diseases*. 2017;**215**(1):42-51

[164] Kubelka CF, Azeredo EL, Gandini M, Oliveira-Pinto LM, Barbosa LS, Damasco PV, et al. Metalloproteinases are produced during dengue fever and MMP9 is associated with severity. *The Journal of Infection*. 2010;**61**(6):501-505

[165] Figueroa CL, Gelvez M, Niederbacher J. Regulators of endothelial integrity as severity predictors in dengue. *Biomédica*. 2016;**36**(0):148-155

[166] van de Weg CA, Pannuti CS, van den Ham HJ, de Araujo ES, Boas LS, Felix AC, et al. Serum angiopoietin-2 and soluble VEGF receptor 2 are surrogate markers for plasma leakage in patients with acute dengue virus infection. *Journal of Clinical Virology*. 2014;**60**(4):328-335

[167] Mariko R, Darwin E, Yanwirasti Y, Hadinegoro SR. The difference of Angiopoietin-2 levels between dengue hemorrhagic fever patients with shock and without shock. *Open Access Macedonian Journal of Medical Sciences*. 2019;**7**(13):2119-2122

[168] Tang TH, Alonso S, Ng LF, Thein TL, Pang VJ, Leo YS, et al. Increased serum hyaluronic acid and Heparan sulfate in dengue fever: Association with plasma leakage and disease severity. *Scientific Reports*. 2017;**7**:46191

[169] Suwanto S, Sasmono RT, Sinto R, Ibrahim E, Suryamin M. Association

of Endothelial Glycocalyx and Tight and Adherens junctions with severity of plasma leakage in dengue infection. *The Journal of Infectious Diseases*. 2017;**215**(6):992-999

[170] Lin CY, Kolliopoulos C, Huang CH, Tenhunen J, Heldin CH, Chen YH, et al. High levels of serum hyaluronan is an early predictor of dengue warning signs and perturbs vascular integrity. *eBioMedicine*. 2019;**48**:425-441

[171] Espinosa DA, Beatty PR, Puerta-Guardo H, Islam MN, Belisle JT, Perera R, et al. Increased serum sialic acid is associated with morbidity and mortality in a murine model of dengue disease. *The Journal of General Virology*. 2019;**100**(11):1515-1522

[172] Puerta-Guardo H, Tabata T, Pettitt M, Dimitrova M, Glasner DR, Pereira L, et al. Zika virus nonstructural protein 1 disrupts Glycosaminoglycans and causes permeability in developing human placentas. *The Journal of Infectious Diseases*. 2020;**221**(2):313-324

[173] Puerta-Guardo H, Glasner DR, Espinosa DA, Biering SB, Patana M, Ratnasiri K, et al. Flavivirus NS1 triggers tissue-specific vascular endothelial dysfunction reflecting disease tropism. *Cell Reports*. 2019;**26**(6) 1598-613.e8

[174] Wills BA, Oragui EE, Dung NM, Loan HT, Chau NV, Farrar JJ, et al. Size and charge characteristics of the protein leak in dengue shock syndrome. *The Journal of Infectious Diseases*. 2004;**190**(4):810-818

[175] Apte-Sengupta S, Sirohi D, Kuhn RJ. Coupling of replication and assembly in flaviviruses. *Current Opinion in Virology*. 2014;**9**:134-142

[176] Vaughan K, Greenbaum J, Blythe M, Peters B, Sette A. Meta-analysis of all immune epitope data

in the Flavivirus genus: Inventory of current immune epitope data status in the context of virus immunity and immunopathology. *Viral Immunology*. 2010;**23**(3):259-284

[177] Weiskopf D, Sette A. T-cell immunity to infection with dengue virus in humans. *Frontiers in Immunology*. 2014;**5**:93

[178] Tian Y, Grifoni A, Sette A, Weiskopf D. Human T cell response to dengue virus infection. *Frontiers in Immunology*. 2019;**10**:2125

[179] Yauch LE, Zellweger RM, Kotturi MF, Qutubuddin A, Sidney J, Peters B, et al. A protective role for dengue virus-specific CD8⁺ T cells. *Journal of Immunology (Baltimore, Md.: 1950)*. 2009;**182**(8):4865-4873

[180] Rivino L, Kumaran EA, Jovanovic V, Nadua K, Teo EW, Pang SW, et al. Differential targeting of viral components by CD4⁺ versus CD8⁺ T lymphocytes in dengue virus infection. *Journal of Virology*. 2013;**87**(5):2693-2706

[181] Weiskopf D, Cerpas C, Angelo MA, Bangs DJ, Sidney J, Paul S, et al. Human CD8⁺ T-cell responses against the 4 dengue virus serotypes are associated with distinct patterns of protein targets. *The Journal of Infectious Diseases*. 2015;**212**(11):1743-1751

[182] Appanna R, Huat TL, See LL, Tan PL, Vadivelu J, Devi S. Cross-reactive T-cell responses to the nonstructural regions of dengue viruses among dengue fever and dengue hemorrhagic fever patients in Malaysia. *Clinical and Vaccine Immunology*. 2007;**14**(8):969-977

[183] Duangchinda T, Dejnirattisai W, Vasanawathana S, Limpitikul W, Tangthawornchaikul N, Malasit P, et al. Immunodominant T-cell responses to dengue virus NS3 are associated with DHF. *Proceedings*

of the National Academy of Sciences of the United States of America. 2010;**107**(39):16922-16927

[184] Simmons CP, Dong T, Chau NV, Dung NT, Chau TN, Thao le TT, et al. Early T-cell responses to dengue virus epitopes in Vietnamese adults with secondary dengue virus infections. *Journal of Virology*. 2005;**79**(9):5665-5675

[185] Green S, Kurane I, Pincus S, Paoletti E, Ennis FA. Recognition of dengue virus NS1-NS2a proteins by human CD4⁺ cytotoxic T lymphocyte clones. *Virology*. 1997;**234**(2):383-386

[186] Espinosa DA, Beatty PR, Reiner GL, Sivick KE, Hix Glickman L, Dubensky TW Jr, et al. Cyclic dinucleotide-Adjuvanted dengue virus nonstructural protein 1 induces protective antibody and T cell responses. *Journal of Immunology*. 2019;**202**(4):1153-1162

[187] Loke H, Bethell DB, Phuong CX, Dung M, Schneider J, White NJ, et al. Strong HLA class I--restricted T cell responses in dengue hemorrhagic fever: A double-edged sword? *The Journal of Infectious Diseases*. 2001;**184**(11):1369-1373

[188] Xavier-Carvalho C, Cardoso CC, de Souza Kehdy F, Pacheco AG, Moraes MO. Host genetics and dengue fever. *Infection, Genetics and Evolution*. 2017;**56**:99-110

[189] Malavige GN, Rostron T, Rohanachandra LT, Jayaratne SD, Fernando N, De Silva AD, et al. HLA class I and class II associations in dengue viral infections in a Sri Lankan population. *PLoS One*. 2011;**6**(6):e20581

[190] Nguyen TP, Kikuchi M, Vu TQ, Do QH, Tran TT, Vo DT, et al. Protective and enhancing HLA alleles, HLA-DRB1*0901 and HLA-A*24, for severe

forms of dengue virus infection, dengue hemorrhagic fever and dengue shock syndrome. *PLoS Neglected Tropical Diseases*. 2008;**2**(10):e304

[191] Stephens HA. HLA and other gene associations with dengue disease severity. *Current Topics in Microbiology and Immunology*. 2010;**338**:99-114

[192] Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, et al. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8⁺ T cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(22):E2046-E2053

[193] Bashyam HS, Green S, Rothman AL. Dengue virus-reactive CD8⁺ T cells display quantitative and qualitative differences in their response to variant epitopes of heterologous viral serotypes. *Journal of Immunology*. 2006;**176**(5):2817-2824

[194] Imrie A, Meeks J, Gurary A, Sukhbataar M, Kitsutani P, Effler P, et al. Differential functional avidity of dengue virus-specific T-cell clones for variant peptides representing heterologous and previously encountered serotypes. *Journal of Virology*. 2007;**81**(18):10081-10091

[195] Rivino L, Lim MQ. CD4(+) and CD8(+) T-cell immunity to dengue—lessons for the study of Zika virus. *Immunology*. 2017;**150**(2):146-154

[196] Rosendahl Huber S, van Beek J, de Jonge J, Luytjes W, van Baarle D. T cell responses to viral infections—opportunities for peptide vaccination. *Frontiers in Immunology*. 2014;**5**:171

[197] Kurane I, Matsutani T, Suzuki R, Takasaki T, Kalayanaroj S, Green S, et al. T-cell responses to dengue virus in humans. *Tropical Medicine and Health*. 2011;**39**(4 Suppl):45-51

[198] Gagnon SJ, Ennis FA, Rothman AL. Bystander target cell lysis and cytokine production by dengue virus-specific human CD4(+) cytotoxic T-lymphocyte clones. *Journal of Virology*. 1999;**73**(5):3623-3629

[199] Kurane I, Meager A, Ennis FA. Dengue virus-specific human T cell clones. Serotype crossreactive proliferation, interferon gamma production, and cytotoxic activity. *The Journal of Experimental Medicine*. 1989;**170**(3):763-775

[200] Aberle JH, Koblishke M, Stiasny K. CD4 T cell responses to flaviviruses. *Journal of Clinical Virology*. 2018;**108**:126-131

[201] Bukowski JF, Kurane I, Lai CJ, Bray M, Falgout B, Ennis FA. Dengue virus-specific cross-reactive CD8⁺ human cytotoxic T lymphocytes. *Journal of Virology*. 1989;**63**(12):5086-5091

[202] An J, Zhou DS, Zhang JL, Morida H, Wang JL, Yasui K. Dengue-specific CD8⁺ T cells have both protective and pathogenic roles in dengue virus infection. *Immunology Letters*. 2004;**95**(2):167-174

[203] Elong Ngono A, Chen HW, Tang WW, Joo Y, King K, Weiskopf D, et al. Protective role of cross-reactive CD8 T cells against dengue virus infection. *eBioMedicine*. 2016;**13**:284-293

[204] Zellweger RM, Tang WW, Eddy WE, King K, Sanchez MC, Shresta S. CD8⁺ T cells can mediate short-term protection against heterotypic dengue virus reinfection in mice. *Journal of Virology*. 2015;**89**(12):6494-6505

[205] Screaton G, Mongkolsapaya J, Yacoub S, Roberts C. New insights into the immunopathology and control of dengue virus infection. *Nature Reviews. Immunology*. 2015;**15**(12):745-759

- [206] Mathew A, Kurane I, Green S, Stephens HA, Vaughn DW, Kalayanaroj S, et al. Predominance of HLA-restricted cytotoxic T-lymphocyte responses to serotype-cross-reactive epitopes on nonstructural proteins following natural secondary dengue virus infection. *Journal of Virology*. 1998;**72**(5):3999-4004
- [207] Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nature Medicine*. 2003;**9**(7):921-927
- [208] Dong T, Moran E, Vinh Chau N, Simmons C, Luhn K, Peng Y, et al. High pro-inflammatory cytokine secretion and loss of high avidity cross-reactive cytotoxic T-cells during the course of secondary dengue virus infection. *PLoS One*. 2007;**2**(12):e1192
- [209] Friberg H, Beaumier CM, Park S, Pazoles P, Endy TP, Mathew A, et al. Protective versus pathologic pre-exposure cytokine profiles in dengue virus infection. *PLoS Neglected Tropical Diseases*. 2018;**12**(12):e0006975
- [210] Halstead SB. Controversies in dengue pathogenesis. *Paediatrics and International Child Health*. 2012;**32**(Suppl 1):5-9
- [211] Dung NT, Duyen HT, Thuy NT, Ngoc TV, Chau NV, Hien TT, et al. Timing of CD8⁺ T cell responses in relation to commencement of capillary leakage in children with dengue. *Journal of Immunology*. 2010;**184**(12):7281-7287
- [212] Rivino L, Kumaran EA, Thein TL, Too CT, Gan VC, Hanson BJ, et al. Virus-specific T lymphocytes home to the skin during natural dengue infection. *Science Translational Medicine*. 2015;**7**(278):278ra35
- [213] Rivino L. Understanding the human T cell response to dengue virus. *Advances in Experimental Medicine and Biology*. 2018;**1062**:241-250
- [214] Karkhanis VS, Joshi JM. Pleural effusion: diagnosis, treatment, and management. *Open Access Emergency Medicine*. 2012;**4**:31-52
- [215] Modhiran N, Watterson D, Muller DA, Panetta AK, Sester DP, Liu L, et al. Dengue virus NS1 protein activates cells via toll-like receptor 4 and disrupts endothelial cell monolayer integrity. *Science Translational Medicine*. 2015;**7**(304):304ra142
- [216] Thomas SJ. NS1: A corner piece in the dengue pathogenesis puzzle? *Science Translational Medicine*. 2015;**7**(304):304fs37
- [217] Smith TJ, Brandt WE, Swanson JL, McCown JM, Buescher EL. Physical and biological properties of dengue-2 virus and associated antigens. *Journal of Virology*. 1970;**5**(4):524-532
- [218] Alcala AC, Hernandez-Bravo R, Medina F, Coll DS, Zambrano JL, Del Angel RM, et al. The dengue virus non-structural protein 1 (NS1) is secreted from infected mosquito cells via a non-classical caveolin-1-dependent pathway. *The Journal of General Virology*. 2017;**98**(8):2088-2099
- [219] Alcala AC, Medina F, Gonzalez-Robles A, Salazar-Villatoro L, Fragoso-Soriano RJ, Vasquez C, et al. The dengue virus non-structural protein 1 (NS1) is secreted efficiently from infected mosquito cells. *Virology*. 2016;**488**:278-287
- [220] Ludert JE, Mosso C, Ceballos-Olvera I, del Angel RM. Use of a commercial enzyme immunoassay to monitor dengue virus replication in cultured cells. *Virology Journal*. 2008;**5**:51

- [221] Alcon-LePoder S, Drouet M-T, Roux P, Frenkiel M-P, Arborio M, Durand-Schneider A-M, et al. The secreted form of dengue virus nonstructural protein NS1 is endocytosed by hepatocytes and accumulates in late endosomes: Implications for viral infectivity. *Journal of Virology*. 2005;**79**(17):11403-11411
- [222] Hu D, Di B, Ding X, Wang Y, Chen Y, Pan Y, et al. Kinetics of non-structural protein 1, IgM and IgG antibodies in dengue type 1 primary infection. *Virology Journal*. 2011;**8**:47
- [223] Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. 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. *Journal of Clinical Microbiology*. 2002;**40**(2):376
- [224] Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardoso MJ, Devi S, et al. Evaluation of diagnostic tests: Dengue. *Nature Reviews. Microbiology*. 2010;**8**(12 Suppl): S30-S38
- [225] Casenghi M, Kosack C, Li R, Bastard M, Ford N. NS1 antigen detecting assays for diagnosing acute dengue infection in people living in or returning from endemic countries. *Cochrane Database of Systematic Reviews*. 2018;**2018**(5):CD011155
- [226] Libraty DH, Endy TP, Houn H, Green S, Kalayanarooj S, Suntayakorn S, et al. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *The Journal of Infectious Diseases*. 2002;**185**(9):1213-1221
- [227] Nunes PCG, Nogueira RMR, Heringer M, Chouin-Carneiro T, Damasceno Dos Santos Rodrigues C, de Filippis AMB, et al. NS1 Antigenemia and Viraemia load: Potential markers of progression to dengue fatal outcome? *Viruses*. 2018;**10**(6)
- [228] Martinez-Cuellar C, Lovera D, Galeano F, Gatti L, Arbo A. Non-structural protein 1 (NS1) of dengue virus detection correlates with severity in primary but not in secondary dengue infection. *Journal of Clinical Virology*. 2020;**124**:104259
- [229] Muller DA, Young PR. The flavivirus NS1 protein: Molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker. *Antiviral Research*. 2013;**98**(2):192-208
- [230] Mackenzie JM, Jones MK, Young PR. Immunolocalization of the dengue virus nonstructural glycoprotein NS1 suggests a role in viral RNA replication. *Virology*. 1996;**220**(1):232-240
- [231] Scaturro P, Cortese M, Chatel-Chaix L, Fischl W, Bartenschlager R. Dengue virus non-structural protein 1 modulates infectious particle production via interaction with the structural proteins. *PLoS Pathogens*. 2015;**11**(11):e1005277
- [232] Plaszczyca A, Scaturro P, Neufeldt CJ, Cortese M, Cerikan B, Ferla S, et al. A novel interaction between dengue virus nonstructural protein 1 and the NS4A-2K-4B precursor is required for viral RNA replication but not for formation of the membranous replication organelle. *PLoS Pathogens*. 2019;**15**(5):e1007736
- [233] Cervantes-Salazar M, Angel-Ambrocio AH, Soto-Acosta R, Bautista-Carbajal P, Hurtado-Monzon AM, Alcaraz-Estrada SL, et al. Dengue virus NS1 protein interacts with the ribosomal protein RPL18: This interaction is required for viral translation and

replication in Huh-7 cells. *Virology*. 2015;**484**:113-126

[234] Flamand M, Megret F, Mathieu M, Lepault J, Rey FA, Deubel V. Dengue virus type 1 nonstructural glycoprotein NS1 is secreted from mammalian cells as a soluble Hexamer in a glycosylation-dependent fashion. *Journal of Virology*. 1999;**73**(7):6104

[235] Akey DL, Brown WC, Dutta S, Konwerski J, Jose J, Jurkiw TJ, et al. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. *Science*. 2014;**343**(6173):881-885

[236] Akey DL, Brown WC, Jose J, Kuhn RJ, Smith JL. Structure-guided insights on the role of NS1 in flavivirus infection. *BioEssays*. 2015;**37**(5):489-494

[237] Edeling MA, Diamond MS, Fremont DH. Structural basis of Flavivirus NS1 assembly and antibody recognition. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**(11):4285-4290

[238] Brown WC, Akey DL, Konwerski JR, Tarrasch JT, Skinotis G, Kuhn RJ, et al. Extended surface for membrane association in Zika virus NS1 structure. *Nature Structural & Molecular Biology*. 2016;**23**(9):865-867

[239] Wang H, Han M, Qi J, Hilgenfeld R, Luo T, Shi Y, et al. Crystal structure of the C-terminal fragment of NS1 protein from yellow fever virus. *Science China. Life Sciences*. 2017;**60**(12):1403-1406

[240] Amorim JH, Alves RP, Boscardin SB, Ferreira LC. The dengue virus non-structural 1 protein: Risks and benefits. *Virus Research*. 2014;**181**:53-60

[241] Avirutnan P, Fuchs A, Hauhart RE, Somnuk P, Youn S, Diamond MS, et al. Antagonism of the complement

component C4 by flavivirus nonstructural protein NS1. *The Journal of Experimental Medicine*. 2010;**207**(4):793-806

[242] Avirutnan P, Hauhart RE, Somnuk P, Blom AM, Diamond MS, Atkinson JP. Binding of flavivirus nonstructural protein NS1 to C4b binding protein modulates complement activation. *Journal of Immunology*. 2011;**187**(1):424-433

[243] Chung KM, Liszewski MK, Nybakken G, Davis AE, Townsend RR, Fremont DH, et al. West Nile virus nonstructural protein NS1 inhibits complement activation by binding the regulatory protein factor H. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**(50):19111-19116

[244] Conde JN, da Silva EM, Allonso D, Coelho DR, Andrade IDS, de Medeiros LN, et al. Inhibition of the membrane attack complex by dengue virus NS1 through interaction with Vitronectin and terminal complement proteins. *Journal of Virology*. 2016;**90**(21):9570-9581

[245] Bokisch VA, Top FH Jr, Russell PK, Dixon FJ, Muller-Eberhard HJ. The potential pathogenic role of complement in dengue hemorrhagic shock syndrome. *The New England Journal of Medicine*. 1973;**289**(19):996-1000

[246] Avirutnan P, Punyadee N, Noisakran S, Komoltri C, Thiemmecca S, Auethavornanan K, et al. Vascular leakage in severe dengue virus infections: A potential role for the nonstructural viral protein NS1 and complement. *The Journal of Infectious Diseases*. 2006;**193**(8):1078-1088

[247] Falconar AK. The dengue virus nonstructural-1 protein (NS1) generates antibodies to common epitopes on human blood clotting, integrin/adhesin proteins and binds to human endothelial cells: Potential

implications in haemorrhagic fever pathogenesis. *Archives of Virology*. 1997;**142**(5):897-916

[248] Chuang YC, Lin YS, Liu HS, Yeh TM. Molecular mimicry between dengue virus and coagulation factors induces antibodies to inhibit thrombin activity and enhance fibrinolysis. *Journal of Virology*. 2014;**88**(23):13759-13768

[249] Lin CF, Lei HY, Shiao AL, Liu CC, Liu HS, Yeh TM, et al. Antibodies from dengue patient sera cross-react with endothelial cells and induce damage. *Journal of Medical Virology*. 2003;**69**(1):82-90

[250] Lin CF, Lei HY, Liu CC, Liu HS, Yeh TM, Wang ST, et al. Generation of IgM anti-platelet autoantibody in dengue patients. *Journal of Medical Virology*. 2001;**63**(2):143-149

[251] Lin CF, Lei HY, Shiao AL, Liu HS, Yeh TM, Chen SH, et al. Endothelial cell apoptosis induced by antibodies against dengue virus nonstructural protein 1 via production of nitric oxide. *Journal of Immunology*. 2002;**169**(2):657-664

[252] Chen MC, Lin CF, Lei HY, Lin SC, Liu HS, Yeh TM, et al. Deletion of the C-terminal region of dengue virus nonstructural protein 1 (NS1) abolishes anti-NS1-mediated platelet dysfunction and bleeding tendency. *Journal of Immunology*. 2009;**183**(3):1797-1803

[253] Glasner DR, Puerta-Guardo H, Beatty PR, Harris E. The good, the bad, and the shocking: The multiple roles of dengue virus nonstructural protein 1 in protection and pathogenesis. *Annual Review of Virology*. 2018;**5**(1):227-253

[254] Wan SW, Chen PW, Chen CY, Lai YC, Chu YT, Hung CY, et al. Therapeutic effects of monoclonal antibody against dengue virus NS1 in a STAT1 knockout mouse model

of dengue infection. *Journal of Immunology*. 2017;**199**(8):2834-2844

[255] Wan SW, Lu YT, Huang CH, Lin CF, Anderson R, Liu HS, et al. Protection against dengue virus infection in mice by administration of antibodies against modified nonstructural protein 1. *PLoS One*. 2014;**9**(3):e92495

[256] Lin YL, Chen LK, Liao CL, Yeh CT, Ma SH, Chen JL, et al. DNA immunization with Japanese encephalitis virus nonstructural protein NS1 elicits protective immunity in mice. *Journal of Virology*. 1998;**72**(1):191-200

[257] Amorim JH, Diniz MO, Cariri FA, Rodrigues JF, Bizerra RS, Goncalves AJ, et al. Protective immunity to DENV2 after immunization with a recombinant NS1 protein using a genetically detoxified heat-labile toxin as an adjuvant. *Vaccine*. 2012;**30**(5):837-845

[258] Bailey MJ, Broecker F, Duehr J, Arumemi F, Krammer F, Palese P, et al. Antibodies elicited by an NS1-based vaccine protect mice against Zika virus. *MBio*. 2019;**10**(2):e02861-18

[259] Schlesinger JJ, Brandriss MW, Cropp CB, Monath TP. Protection against yellow fever in monkeys by immunization with yellow fever virus nonstructural protein NS1. *Journal of Virology*. 1986;**60**(3):1153-1155

[260] Li A, Yu J, Lu M, Ma Y, Attia Z, Shan C, et al. A Zika virus vaccine expressing premembrane-envelope-NS1 polyprotein. *Nature Communications*. 2018;**9**(1):3067

[261] Dalrymple NA, Mackow ER. Roles for endothelial cells in dengue virus infection. *Advances in Virology*. 2012;**2012**:840654

[262] Avirutnan P, Malasit P, Seliger B, Bhakdi S, Husmann M. Dengue virus infection of human endothelial cells leads to chemokine production,

- complement activation, and apoptosis. *The Journal of Immunology*. 1998;**161**(11):6338
- [263] Félétou M. The endothelium: Part 1: Multiple Functions of the Endothelial Cells—Focus on Endothelium-Derived Vasoactive Mediators. San Rafael (CA): Morgan & Claypool Life Sciences; 2011. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK57148/>
- [264] Yuan SYR, Robert R Rigor. Regulation of Endothelial Barrier Function. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK54117/>
- [265] Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. *Nature Reviews. Immunology*. 2007;**7**(10):803-815
- [266] Avirutnan P, Zhang L, Punyadee N, Manuyakorn A, Puttikhunt C, Kasinrerak W, et al. Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparan sulfate and chondroitin sulfate E. *PLoS Pathogens*. 2007;**3**(11):e183
- [267] Puerta-Guardo H, Glasner DR, Harris E. Dengue virus NS1 disrupts the endothelial Glycocalyx, leading to Hyperpermeability. *PLoS Pathogens*. 2016;**12**(7):e1005738
- [268] Glasner DR, Ratnasiri K, Puerta-Guardo H, Espinosa DA, Beatty PR, Harris E. Dengue virus NS1 cytokine-independent vascular leak is dependent on endothelial glycocalyx components. *PLoS Pathogens*. 2017;**13**(11):e1006673
- [269] Chen HR, Chuang YC, Lin YS, Liu HS, Liu CC, Perng GC, et al. Dengue virus nonstructural protein 1 induces vascular leakage through macrophage migration inhibitory factor and autophagy. *PLoS Neglected Tropical Diseases*. 2016;**10**(7):e0004828
- [270] Chen HR, Chao CH, Liu CC, Ho TS, Tsai HP, Perng GC, et al. Macrophage migration inhibitory factor is critical for dengue NS1-induced endothelial glycocalyx degradation and hyperpermeability. *PLoS Pathogens*. 2018;**14**(4):e1007033
- [271] Singh S, Anupriya MG, Modak A, Sreekumar E. Dengue virus or NS1 protein induces trans-endothelial cell permeability associated with VE-Cadherin and RhoA phosphorylation in HMEC-1 cells preventable by Angiopoietin-1. *The Journal of General Virology*. 2018;**99**(12):1658-1670
- [272] Barbachano-Guerrero A, Endy TP, King CA. Dengue virus non-structural protein 1 activates the p38 MAPK pathway to decrease barrier integrity in primary human endothelial cells. *The Journal of General Virology*. 2020;**101**(5):484-496
- [273] Niranjana R, Sumitha MK, Sankari T, Muthukumaravel S, Jambulingam P. Nonstructural protein-1 (NS1) of dengue virus type-2 differentially stimulate expressions of matrix metalloproteinases in monocytes: Protective effect of paracetamol. *International Immunopharmacology*. 2019;**73**:270-279
- [274] Adikari TN, Gomes L, Wickramasinghe N, Salimi M, Wijesiriwardana N, Kamaladasa A, et al. Dengue NS1 antigen contributes to disease severity by inducing interleukin (IL)-10 by monocytes. *Clinical and Experimental Immunology*. 2016;**184**(1):90-100
- [275] Chao CH, Wu WC, Lai YC, Tsai PJ, Perng GC, Lin YS, et al. Dengue virus nonstructural protein 1 activates platelets via toll-like receptor 4, leading to thrombocytopenia and hemorrhage. *PLoS Pathogens*. 2019;**15**(4):e1007625
- [276] Calisher CH, Gould EA. Taxonomy of the virus family Flaviviridae. *Advances in Virus Research*. 2003;**59**:1-19

- [277] Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet*. 2008;**371**(9611):500-509
- [278] Counotte MJ, Meili KW, Taghavi K, Calvet G, Sejvar J, Low N. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barre syndrome: A living systematic review. *F1000Res*. 2019;**8**:1433
- [279] Wang C, Puerta-Guardo H, Biering SB, Glasner DR, Tran EB, Patana M, et al. Endocytosis of flavivirus NS1 is required for NS1-mediated endothelial hyperpermeability and is abolished by a single N-glycosylation site mutation. *PLoS Pathogens*. 2019;**15**(7):e1007938
- [280] Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. *Annual Review of Biomedical Engineering*. 2007;**9**:121-167
- [281] Connolly-Andersen AM, Thunberg T, Ahlm C. Endothelial activation and repair during hantavirus infection: Association with disease outcome. *Open Forum Infectious Diseases*. 2014;**1**(1):ofu027
- [282] Somnuk P, Hauhart RE, Atkinson JP, Diamond MS, Avirutnan P. N-linked glycosylation of dengue virus NS1 protein modulates secretion, cell-surface expression, hexamer stability, and interactions with human complement. *Virology*. 2011;**413**(2):253-264
- [283] Pryor MJ, Wright PJ. Glycosylation mutants of dengue virus NS1 protein. *The Journal of General Virology*. 1994;**75**(Pt. 5):1183-1187
- [284] Crabtree MB, Kinney RM, Miller BR. Deglycosylation of the NS1 protein of dengue 2 virus, strain 16681: Construction and characterization of mutant viruses. *Archives of Virology*. 2005;**150**(4):771-786
- [285] Annamalai AS, Pattnaik A, Sahoo BR, Guinn ZP, Bullard BL, Weaver EA, et al. An attenuated Zika virus encoding non-glycosylated envelope (E) and non-structural protein 1 (NS1) confers complete protection against lethal challenge in a mouse model. *Vaccines (Basel)*. 2019;**7**(3):112
- [286] Whiteman MC, Wicker JA, Kinney RM, Huang CYH, Solomon T, Barrett ADT. Multiple amino acid changes at the first glycosylation motif in NS1 protein of West Nile virus are necessary for complete attenuation for mouse neuroinvasiveness. *Vaccine*. 2011;**29**(52):9702-9710
- [287] Li F, Wang Y, Yu L, Cao S, Wang K, Yuan J, et al. Viral infection of the central nervous system and Neuroinflammation precede blood-brain barrier disruption during Japanese encephalitis virus infection. *Journal of Virology*. 2015;**89**(10):5602-5614
- [288] Basu A, Chaturvedi UC. Vascular endothelium: The battlefield of dengue viruses. *FEMS Immunology and Medical Microbiology*. 2008;**53**(3):287-299
- [289] Xu X, Vaughan K, Weiskopf D, Grifoni A, Diamond MS, Sette A, et al. Identifying candidate targets of immune responses in Zika virus based on homology to epitopes in other *Flavivirus* species. *PLoS Currents*. 2016;**8**:1-38