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Understanding immunopathology of severe dengue: lessons learnt from sepsis

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Endothelial dysfunction leading to vascular permeability and plasma leakage are characteristic features of severe dengue and sepsis. However, the mechanisms underlying these immune-pathologies remain unclear. The risk of severe dengue and sepsis development depend on patient-related and pathogen-related factors. Additionally, comorbidities increase the risk of severe disease and their incidence hampers correct diagnosis and treatments. To date, there is no efficient therapy to combat severe dengue and sepsis. Here, we discuss the differences and similarities between the pathogenesis of severe dengue and that of bacterial sepsis. We identify gaps in knowledge that need to be better understood in order to move towards the rational development and/or usage of therapeutic strategies to ameliorate severe dengue disease.

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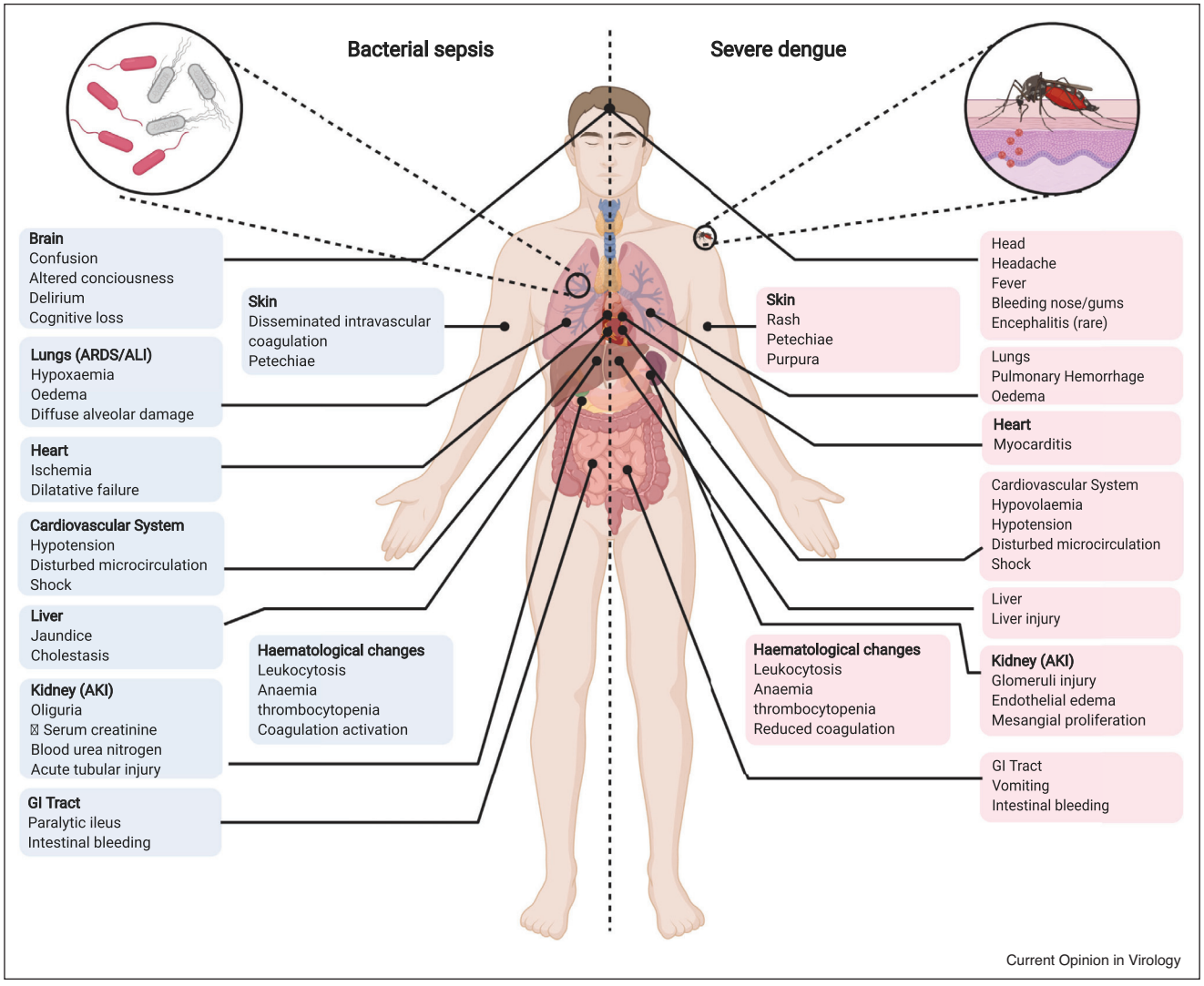
Introduction

The four serotypes of mosquito-borne dengue virus (DENV1-4), members of the *Flaviviridae* family and genus Flavivirus, are responsible for an estimated of 390 million of infections per year, of which 96 million manifest clinically. Infections cause a broad range of clinical manifestations encompassing the self-limited dengue (previously dengue fever) to the potentially life-threatening severe dengue (previously known as dengue hemorrhagic fever and dengue shock syndrome) [1]. Severe dengue is hallmarked by severe bleeding, plasma leakage and/or organ impairment. Since severe symptoms develop only after the virus has been cleared from the blood, a dysregulated immune response to the infection often referred to as 'cytokine storm' leads to vascular permeability that is thought be a key contributor to disease pathogenesis [2,3]. Factors exacerbating inflammatory response to infection, such as inter alia, cross-reactive, non-neutralizing and/or infection-enhancing antibodies arose from previous heterologous infections, bacterial co-infections and/or microbial translocation increase the risk of severe disease development [4–8]. Yet, how and to which extent these factors contribute to the pathophysiology of severe dengue cases remains unclear. Consequently, there are no specific treatments available to alleviate severe disease pathogenesis.

Despite advances in the understanding it's pathophysiology, sepsis still remains one of the major causes of mortality and morbidity in critically ill patients [9]. For a long time, sepsis associated mortality was presumed to be a result of an uncontrolled immunological response to infection. Although this occurs, many other host responses are elicited simultaneously such as disrupted endothelial integrity. This has led to the new sepsis definition; a life-threatening organ dysfunction caused by a dysregulated host response to infection [10]. Risk factors associated with the development of severe sepsis as a result of infection are predominantly related to the patient's pre-sepsis health status. Age, chronic comorbid diseases, sex and ethnicity all influence the likelihood for the development of severe sepsis [9]. Although all pathogens can induce sepsis, bacteria are the leading cause of sepsis in critically ill patients [11–13]. Importantly, throughout the past years, it became evident that the identification of the etiologic agent is crucial to effectively treat sepsis. Patients presenting with sepsis caused by Gram-negative bacteria and LPS/endotoxin may not respond to the same treatment as those infected with Gram-positive bacterium or a virus [11,14,15]. Indeed, misdiagnosis of severe dengue patients with sepsis and septic shock, followed by an inappropriate treatment, has been associated with fatal outcome [11]. Thus, despite similar severe disease presentation (Figure 1) and laboratory characteristics, mechanisms driving pathophysiology of (bacterial) sepsis and that of severe dengue are fundamentally different. Understanding these differences is crucial since therapies aiming to alleviate the detrimental effects of infection could induce unwanted effects.

Figure 1



Clinical symptoms of bacterial sepsis and severe dengue.
Characteristic features of both sepsis and severe dengue is organ impairment due to disruption of the endothelium. These disturbances most likely reflect the clinical symptoms observed during bacterial sepsis and severe dengue. Severe sepsis and septic shock patients are often characterized by multiple organ failure. The precise pathophysiological mechanisms leading to organ failure are poorly understood. However, extensive evidence indicates that organ microcirculatory failure is a main feature of failing organs. Each organ fails in its own way and may be driven by organ-specific endothelial properties. In adults with severe dengue, minor plasma leak is observed in combination with skin and/or mucosal bleeding. However, in children, bleeding rarely occurs, and normally accompanied with profound shock. In severe dengue cases, liver failure, kidney damage, pulmonary oedema, myocardial impairment and encephalopathy have also been reported. Lung tissue from dengue patients is characterized by oedema and pneumonia accompanied by focal and local diffuse alveolar hemorrhage as well as an accumulation of immune cells similar to patients with sepsis. Interestingly, in contrast to acute kidney injury (AKI) in sepsis, kidney damage is associated with fatal cases of severe dengue characterized by glomeruli injury with endothelial edema, mesangial proliferation and infiltration of mononuclear cells.

Herewith, we discuss the current knowledge on the mechanisms of endothelial dysfunction and vascular leak in severe dengue and bacterial sepsis. We address the similarities and differences in their pathogenesis to provide new insights and future directions that will aid the development of strategies to prevent or alleviate severe disease.

Pathophysiology of sepsis

In sepsis, the endothelium becomes disrupted which is now known to play a major role in dysfunctional organs [16]. Additionally, the endothelium can become inflammation activated promoting leukocyte adhesion and infiltration into organs, as well as activating the coagulation cascade [17,18]. Changes in organ hemodynamics and

microvascular coagulation results in tissue hypoperfusion and ischemic injury. More recent studies have found that organs fail in different ways as a result of sepsis [19]. For instance, the microvasculature in the lungs becomes permeable which results in lungs drowning in fluids and a vast amount of immune cell infiltration. Alternatively, the kidney appears to shut down with minimal morphological alterations and very little immune cells infiltrating. The reason for these differences is likely a result of organ-specific heterogeneous endothelial responses. Around 50% of patients surviving sepsis suffer long-term morbidity with premature aging-like symptoms. Patients are often left with cognitive defects, chronic renal failure, cardiovascular disease, or have to be re-hospitalized due to a new septic insult, secondary infection, cardiovascular events, acute renal failure or acute respiratory dysfunction [20]. The reasons for the impaired recovery, the long-term effect of sepsis on immunity and vascular integrity resulting in organ dysfunction is currently unknown. Emerging evidence indicates that the pathogenesis of post-sepsis chronic disease involves chronic low-grade inflammation and suppressed host protective immunity as a result of epigenetic, metabolic reprogramming of immune cells induced by the original septic insult [21–23].

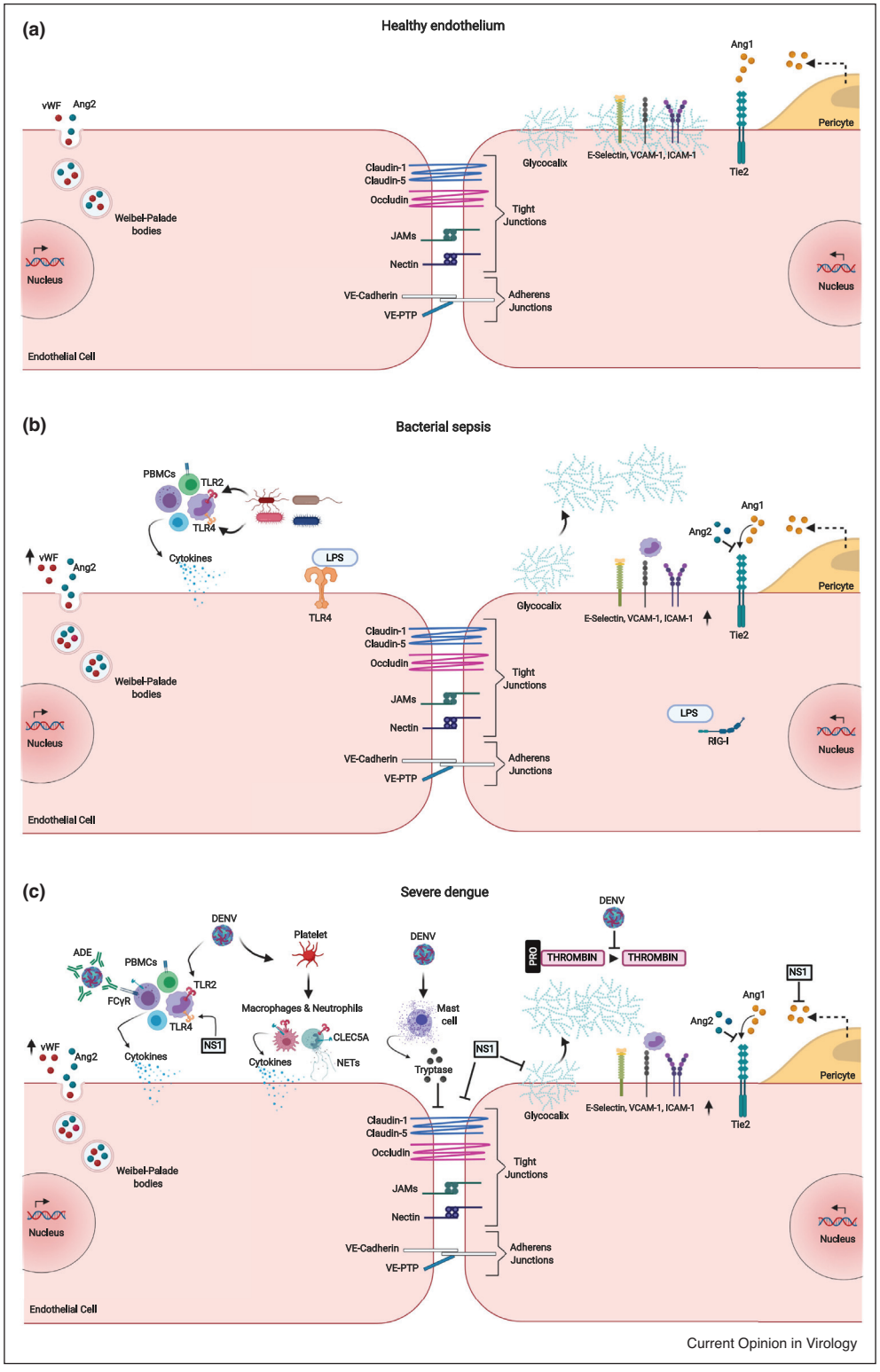
Pathophysiology of severe dengue versus sepsis

Although the majority of the patients with mild dengue recover without further complications, 1 of 20 infected patients enter the critical phase and develop thrombocytopenia, vascular permeability and plasma leakage [24]. Severe disease symptoms have been observed in the course of infection with any of the 4 DENV serotypes. However, DENV2 is the most commonly associated with severe disease cases and despite being the most extensively studied, it is not yet clear why [25–27]. In adults, minor plasma leak is observed in combination with skin and/or mucosal bleeding. However, in children, bleeding rarely occurs and is normally accompanied with a profound shock [28]. In severe cases, liver failure, kidney damage, pulmonary oedema, myocardial impairment and encephalopathy have also been reported [3,11,28–30], however the mechanisms underlying pathology in different organs remain elusive. Lung tissue from dengue patients is characterized by oedema and pneumonia accompanied by focal and local diffuse alveolar hemorrhage as well as an accumulation of immune cells similar to the patients with sepsis [30,31]. Interestingly, in contrast to acute kidney injury (AKI) in sepsis, kidney damage associated with fatal cases of severe dengue is characterized by glomeruli injury with endothelial edema, mesangial proliferation and infiltration of mononuclear cells [29]. It is important to note that in contrast to sepsis, the mortality rate of hospitalized severe dengue cases is low (1–5%) [32]. The development of severe disease and poor prognosis are usually associated with pre-existing conditions that affect immune system [33]. The role of age and pre-existing conditions like diabetes, hypertension, obesity and chronic kidney disease [11,34,35] in pathogenesis of DENV infection has been reviewed elsewhere [8,34,36]. Severe dengue virus co-infections with several pathogens have also been reported. Yet the currently available data is insufficient to establish whether these co-infections contribute to disease severity [37–46]. Interestingly, a review on bacterial-co-infections in dengue patients reported that approximately 7% of dengue infections were accompanied with concurrent bacteremia, while 14–44% dengue-associated deaths could be related to bacterial co-infections [6]. The source of the bacterial infection, either external or internal, remains unclear. Indeed, both nosocomial infection as well as infection with own microbiota as a result of microbial translocation have been suggested to enhance immune activation and disease severity during DENV infection [4,5]. Importantly, despite the concomitant occurrence of dengue and bacterium, sepsis mortality differs from that of dengue where the majority of severe disease patients recover rapidly. The recovery phase starts when the endothelial disturbances return to homeostatic levels, which usually takes around 72 hour [28]. Also, unlike in the case of bacterial sepsis [23], post-recovery immunosuppression is not commonly reported in the recovery phase following severe dengue after dengue infection.

Microvascular endothelial disturbances in severe dengue versus sepsis

The macrovasculature and microvasculature are lined by a semi-permeable layer of cells called the endothelium [47]. The integrity of endothelium is crucial for the maintenance of organ homeostasis (Figure 2a). In the course of inflammation, the permeability of endothelium is transiently compromised, allowing infiltration of immune cells and soluble modulators. Importantly, the responses and regulatory functions of the endothelium differ per organ and even between microvascular compartments within the same organ [48]. Adjacent endothelial cells (ECs) are sealed by tight junctions such as the claudins and occludin, as well as adherens junction, VE-Cadherin [49]. In sepsis, expression of these junctions differs between organs [19], highlighting that different endothelial barrier regulatory mechanisms exist in different organs. How these heterogeneous endothelial responses are regulated and what distinguishes the 'bad and potentially pathological' responses from the 'good' ones is currently not well understood. For instance, the angiopoietin/Tie2 system is involved in barrier maintenance. In quiescent conditions, pericytes, cells associated with the stabilization and hemodynamic processes of blood vessels [50], release Angiopoietin 1, which bind to the Tie2 receptor ensuring integrity [51]. In sepsis conditions, Angiopoietin 2 is produced by ECs and competitively binds to the Tie2 receptor leading to vascular

Figure 2



Mechanisms leading EC dysfunction in both sepsis and dengue.
(a) The endothelium is a semi-permeable layer of cells that lines the macro and microvasculature of all blood vessels. Its integrity is crucial for the maintenance of organ homeostasis. Several molecules and cells play a vital role keeping this homeostasis. For instance, tight junctions such as the claudins and occludin, as well as adherens junctions and VE-Cadherin seal adjacent endothelial cells (ECs). Additionally, the glycocalyx barrier exert a protective role to the vasculature. Pericytes, cells associated with the stabilization and hemodynamic processes of blood vessels, release

leakage and immune cells such as influx into organs (Figure 2b). Also, high levels of angiopoietin 2 released from ECs are indicative of severe disease development in dengue [2,52,53] (Figure 2c). Yet, as described above, only in the case of severe dengue endothelium seem to return to homeostasis without further complications, suggesting that Angiopoietin/Tie2 system does not play a key role in severe dengue. Furthermore, during sepsis, inflammation mediates glycocalyx degradation which can facilitate leukocyte adhesion to the endothelium as well as promoting vascular permeability and thrombosis [54]. Similarly, in dengue, glycocalyx degradation is associated with plasma leakage [55], again suggesting that this is not the pathway discerning between the diseases. The clue may however lie in the coagulation system. Namely, in sepsis, coagulation plays an important role in organ dysfunction. Inflammation can activate the coagulant system and down regulate anticoagulant pathways frequently leading to disseminated intravascular coagulation [56]. The role of the coagulant system and the factors involved has recently been reviewed in detail [57]. In contrast, DENV decreases coagulation activation by binding directly prothrombin and inhibiting its conversion to thrombin [58], which may contribute to the internal bleeding associated with severe disease. Understanding the differences and commonalities in the endothelial disturbances in dengue and sepsis will aid diagnostics strategies for severe dengue and sepsis. Consequently, the mechanisms underlying directly and indirectly interactions between the pathogens and endothelial cells need to be completely understood.

Host and pathogen-specific factors leading to vascular dysfunction

Host cells sense invading pathogens by recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The recognition occurs by binding of PAMPs and DAMPS to pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), C-type lectins and Retinoic acid-inducible gene I (RIG-I)- like receptors expressed on and in these cells. Depending on the combination and dose of PAMP/ DAMPs, activation of PRRs initiates downstream signaling promoting the production of various pro- and anti-inflammatory mediators [59,60]. These in turn can lead to EC activation characterized by upregulation of endothelial adhesion molecules such as E-selectin, VCAM-1 and ICAM-1, which promote leukocyte rolling, adhesion to the endothelium and paracellular or transcellular transmigration [48,61]. In addition, inflammatory mediators can lead to the activation the complement system, disruption of and coagulation cascade and an array of other dysfunctional processes leading to organ injury [56]. Importantly, the mechanisms leading to endothelium activation and/or dysfunction are complex and not necessarily overlapping. Indeed, it's now known that microvascular inflammation and permeability are 2 independent pathways rather than being dependant on one another [62]. These changes may explain why despite seemingly identical initial hyperinflammatory response in both severe dengue and sepsis patients, only the latter find themselves become immunosuppressed for a few days up to months after the initial sepsis episode [23].

Activation and dysfunction of the endothelium can occur (1) directly via recognition of PAMPs and DAMPs by EC-associated PRRs and (2) indirectly via a secondary innate immune response. For instance, in the model of sepsis caused by Gram-negative bacteria, direct recognition of endotoxin by RIG-I and TLR4 but also TLR2 was shown to mediate endothelial activation [63,64]. Interestingly, endothelial responses initiated indirectly by proinflammatory mediators released from PBMCs stimulated with bacteria and fungi have been shown to be largely similar and predominantly driven by TNFα and IL-1β [65]. Also, for DENV endothelial activation can occur either by

(Figure 2 Legend Continued) Angiopoietin 1 which subsequently bind the Tie2 receptor promoting endothelial integrity.
(b) During sepsis, the expression of tight and adherens junctions differs between organs, highlighting that different endothelial barrier regulatory mechanisms exist in different organs. In addition, activated endothelial cells shed the glycocalyx facilitating enhanced leukocyte adhesion. Coagulation plays an important role in organ dysfunction. Inflammation can activate the coagulant system and down regulate anticoagulant pathways frequently leading to disseminated intravascular coagulation Also, in sepsis, Angiopoietin 2 is produced by ECs and competitively binds to the Tie2 receptor leading to vascular leakage and immune cells such as influx into organs. Moreover, bacterial LPS/Endotoxin can induce the activation of ECs directly or indirectly. The direct interaction of LPS/endotoxin with Toll-like receptor (TLR) 4 on the surface of ECs and to intracellular RIG-I, induces the production of pro-inflammatory cytokines and enhanced expression of the adhesion molecules E-selectin, VCAM-1 and ICAM-1 allowing leukocyte adhesion and extravasation. Indirectly, binding of LPS/endotoxin to TLR4 on human peripheral blood mononuclear cells (PBMCs) promotes cytokine production resulting in ECs activation. **(c)** Similar to (bacterial) sepsis, during dengue, high levels of Angiopoietin 2 released from ECs are indicative of severe disease development. DENV can also inhibit the paracrine signaling between ECs and pericytes. Moreover, DENV decreases coagulation activation by binding directly prothrombin and inhibiting its conversion to thrombin. DENV NS1 can directly and indirectly affect endothelial function. On one hand, NS1 which is released from infected cells, contribute to vascular permeability and endothelial dysfunction *in vitro* and *in vivo* by directly interacting with the endothelial glycocalyx components. Indirectly, TLR4-mediated recognition of NS1 by immune cells such as monocytes underlie EC activation. Additionally, TLR2, and its coreceptors TLR6 and CD14-mediated sensing of DENV infection induce the production of soluble inflammatory mediators and the subsequent activation of the endothelial cells. Also, TLR2 and the C-type lectin domain family 5 member A (CLEC5A)-mediated sensing of extracellular vesicles (EVs) produced by DENV-infected platelets, induce neutrophil extracellular trap (NET) formation and cytokine production promoting systemic vascular permeability. Mast cells sensing DENV particles, induce the production of tryptase, which subsequently mediates the breakdown of tight junctions. Lastly, the phenomenon of antibody-dependent enhancement (ADE) mediates the suppression of TLR- signaling pathways on immune cells, leading to aberrant immune responses.

direct interaction with the virus and viral components, and/or by soluble inflammatory mediators released from DENV-infected cells (Figure 2c). The identity of soluble mediators correlating with severe disease development is not unanimous between the studies [66–69].

Although many PRRs have been shown to sense DENV [70], limited number of studies associated the triggered immune response to EC activation and/or dysfunction [71–74]. Out of these, the most evidence for the role in the endothelial dysfunction is attributed to the function of DENV nonstructural protein 1 (NS1), which depending on the experimental model has been shown to directly and indirectly affect endothelial function. Indeed, NS1 which is released from infected cells was shown to contribute to vascular permeability and endothelial dysfunction *in vitro* and *in vivo* by directly interact with the endothelial glycocalyx components [75–78]. In other studies however, TLR4-mediated recognition of NS1 by immune cells such as monocytes was shown to underlie EC activation [73,79]. Additionally, we have recently reported that TLR2, and its coreceptors TLR6 and CD14-mediated sensing of DENV infection induces the production of soluble inflammatory mediators and the subsequent activation of the endothelial cells *in vitro* [74]. TLR2 expression on classical monocytes correlated with severe disease development. Importantly, how monocyte-derived TLR2-mediated responses affected EC permeability is currently investigated. However, TLR2 is not only expressed by monocytes. In fact. concurrently to our study, TLR2 together with the C-type lectin domain family 5 member A (CLEC5A) where shown to sense extracellular vesicles (EVs) produced by DENV-infected platelets. The TLR2/CLEC5-mediated sensing induces neutrophil extracellular trap (NET) formation and cytokine production promoting systemic vascular permeability *in vivo*, indicating the pleiotropic function of TLR2 depending on the cells type [71]. Indeed, CLEC5A-mediated inflammatory responses triggered following sensing of DENV infection were also shown to contribute to severe disease in the *in vivo* model of antibody-mediated enhancement of infection (ADE) [80]. It is however difficult to assess whether these mechanisms play a role in all severe dengue cases. Firstly, *in vivo* models used in majority of studies include mice deficient in IFN type 1 signaling [81,82], which plays not only an important antiviral but also an immunomodulatory role [83]. Moreover, while heterotypic infections increase the risk of severe disease, the phenomenon of ADE was shown to suppress TLR-dependent signaling pathways [84], suggesting that mechanisms leading to severe disease may be different during infection in the presence or absence of DENV-specific infection-enhancing antibodies. Of note, the presence of DENV cross-reactive and non-neutralizing/non-enhancing antibodies also alter innate immune responses of DENV-infected dendritic cells *in vitro* [85]. Yet, how non-neutralizing/non-enhancing antibody titers alter TLR-mediated sensing and whether it contributes to endothelial dysfunction remains to be evaluated. Lastly, mast cells, which recently were shown to sense DENV particles *in vivo* can promote vascular leakage by releasing tryptase, which subsequently induce breakdown of endothelial cell tight junctions [72]. The mechanisms underlying the sensing of DENV by mast cells and whether the mediated responses contribute to severe disease remain however, unknown. Taken together, we now know of several mechanisms triggering inflammatory responses upon DENV infection, however it is not clear if these responses underlie endothelial dysfunction that underlie in severe disease.

Conclusions

The correct diagnosis of the etiological agent(s) of septic or DENV patients is vital for a favorable outcome. Understanding the underlying mechanisms is thus important to achieve a correct diagnosis and identify effective treatment. Although we begin to identify pathways leading to endothelial activation, little is known about the kinetics and crosstalk between different pathways leading to severe dengue. Moreover, while some microvascular inflammation and permeability may overlap between severe dengue and sepsis, the upstream regulatory mechanisms likely differ not only in quality and quantity but also in kinetics. Importantly, thirty years of clinical trials have shown that single target therapies does not work in patients with sepsis and severe dengue [86–88]. Thus, multiple mechanisms associated with the host response in sepsis/ severe dengue pathogenesis should be targeted and/or focus should be made on normalizing aberrant endothelial responses associated with severe disease. One of the caveats that limits our understanding of severe dengue and sepsis is the lack of mouse models that fully recapitulate the immunopathogenesis of these diseases. This, together with the limited access to human material other than peripheral blood, hampers the elucidation of mechanisms associated with endothelial permeability. Finally, the heterogenic clinical and pathophysiological characteristics between patients needs to be better understood in order to identify patients that might benefit from specific therapies rather than treating all patients with a one-size-fits-all therapy.

Conflict of interest statement

Nothing declared.

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