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Review

Dengue and Soluble Mediators of the Innate Immune System

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Abstract: Huge emphasis has been placed on the role of the adaptive immune system in dengue pathogenesis. Yet there is increasing evidence for the importance of the innate immune system in regulating dengue infection and possibly influencing the disease. This review focuses on the interplay between the innate immune system and dengue and highlights the role of soluble immunological mediators. Type I and type II interferons of the innate immune system demonstrate non-overlapping roles in dengue infection. Furthermore, while some IFN responses to dengue are protective, others may exert disease-related effects on the host. But aside from interferons, a number of cytokines have also been implicated in dengue pathogenesis. Our expanding knowledge of cytokines indicates that these soluble mediators act upon a complicated network of events to provoke the disease. This cytokine storm is generally attributed to massive T cell activation as an outcome of secondary infection. However, there is reason to believe that innate immune response-derived cytokines also have contributory effects, especially in the context of severe cases of primary dengue infection. Another less popular but interesting perspective on dengue pathogenesis is the effect of mosquito feeding on host immune responses and viral infection. Various studies have shown that soluble factors from vector saliva have the capacity to alter immune reactions and thereby influence pathogen transmission and establishment. Hence, modulation of the innate immune system at various levels of infection is a critical component of dengue disease. In the absence of an approved drug or vaccine for dengue, soluble mediators of the innate immune system could be a strategic foothold for developing anti-viral therapeutics and improving clinical management.
Key words: dengue, innate immunity, cytokines

CONTENTS

1. INTRODUCTION

Dengue virus (DV) is transmitted to a human through the bite of an infected mosquito, particularly of the *Aedes* group. The period of incubation ranges from three to seven days [1]. Then viremia lasts for about one to seven days and can reach 10^7 to 10^9 mosquito infectious doses (MID_{50})/ml [2], which is more than sufficient to transmit the virus to another feeding mosquito.

After its injection into the skin, DV is presumed to undergo an initial round of replication in Langerhans dendritic cells (LDCs) [3, 4]. Infected LDCs migrate to draining lymph nodes [5], where infection spreads to monocytes and macrophages [6]. The lymphatic system may then play a key role in the ensuing viremia, through which the virus can be disseminated to other organs such as the spleen, liver and bone marrow [6].

The clinical manifestation of dengue ranges from mild febrile syndrome to fatal disease [1]. Dengue fever (DF) is an acute and self-limited illness manifested by fever, headache, myalgia and arthralgia, with physical evidence of rash. Laboratory tests reveal leukopenia, as well as varying degrees of thrombocytopenia and hemorrhage. The more severe dengue hemorrhagic fever (DHF) is complicated by

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plasma leakage that occurs around three to five days after the disease. A sudden and extensive plasma leakage may result in shock or death, a phenomenon called dengue shock syndrome (DSS). Typically, patients undergo a defervescence phase marked by an abrupt drop in body temperature, at which point the illness may either wane to recovery or proceed to serious complications [7].

Dengue is widely accepted to be an immunopathological disease. Compounding evidence also associates dengue severity with secondary infection of a heterologous serotype [8–11]. From this perspective, dengue pathogenesis can be explained through various hypotheses that implicate immune-related factors, including the: 1) enhancement of viral infection through cross-reactive antibodies, 2) activation of cross-reactive memory T cells, 3) cytokine storm, and 4) complement activation. Unfortunately, a huge part of dengue pathogenesis remains elusive despite years of arduous study. In particular, while the adaptive immune response has been central to dengue research, the initial immune events that lead to disease evolution also warrant consideration. Thus, we present a review of recent developments in the field of dengue and the innate immune system, with a focus on soluble mediators that might be involved in infection and pathogenesis.

2. THE INNATE IMMUNE RESPONSE TO DENGUE: TO DEFEND OR TO DESTROY

At the onset of skin infection, DV is immediately confronted with the host innate immune system. Whether infection becomes limited or progresses to disease depends on the balance between the defensive and destructive effect of the immune response. Here we explore various soluble mediators of the innate immune system and their role in dengue infection. Elucidating such early events will be valuable in shedding light on dengue pathogenesis and may impart prognostic utility for predicting disease.

2-1. Interferons

The initial immune response to viral infection is mediated by interferons (IFN). Most cells produce type I IFNs (IFNα/β) to inhibit viral translation and replication. On the other hand, type II IFNs (IFNγ) derived from NK cells and activated T cells modulate the production of proinflammatory and antiviral molecules. An early innate immune response accompanied by strong upregulation of IFN-related genes has been described for dengue [12–14]. Among the various pattern-recognition receptor (PRR) pathways utilized by the host for IFN activation, the IPS-1/Cardif system operated by retinoic acid-inducible gene I-like helicases (RLH) appears to be the most critical in initiating the innate immune response to dengue, as exhibited by delayed IFN production in lymphoid tissues of IPS-1-deficient mice, rendering them more susceptible to the virus [15]. RLH-dependent IFN activation also seems to be regulated by the intracellular localization of flavivirus double-stranded RNA (dsRNA). A recent study in our laboratory demonstrated dynamic changes in Japanese encephalitis virus dsRNA localization, which was initially concealed in intracellular membrane structures but eventually exposed to the cytosol [16]. This cytosolic exposure sets the stage for PRR recognition and IFN activation, and its timing occurs in a cell/species-specific manner, which ultimately determines cell permissiveness to infection [16]. We are currently exploring the applicability of this model to DV. Other PRRs such as toll-like receptors (TLRs) may also be involved, as TLR7 was demonstrated to be essential for DV-induced production of IFN in plasmacytoid dendritic cells [17].

Experiments using knock-out mice were conducted to examine the contribution of IFN to viral clearance and disease. Mice deficient in receptors for type I and type II IFN were extremely vulnerable to dengue infection, while B or T cell-deficient mice displayed a normal ability to resist infection, demonstrating the predominant role of IFN over the adaptive immune system in controlling primary infection by DV [18]. Although the protective effect of type I and type II IFNs is synergistic, their respective roles are non-overlapping. For instance, viral distribution was widespread in IFNα/β receptor-knockout mice, in contrast to a benign viral titer in IFNγ receptor-knockout mice, suggesting that type I IFNs are specifically responsible for limiting the initial replication and/or spread of dengue [18]. However, IFNγ receptor-knockout mice exhibited a lower survival rate than IFNα/β receptor-knockout mice, indicating the protective role of IFNγ, but not IFNα/β, against the disease [18].

Nevertheless, type I IFNs and other IFN-stimulated genes have been associated with disease severity in several clinical studies. These studies are characterized by the consistent theme of highly activated type I IFN response in uncomplicated cases of dengue, in contrast to a blunted IFN profile in severe cases [19–21]. Clearly, IFN serves as an early barrier against infection in mild cases of dengue, but is overridden or no longer functional in severe forms of the disease. A dampened IFN response could additionally cause the enhanced viremia associated with severe dengue [2]. This differential immune response can be accounted for by an altered profile of IFN-producing innate immune cells during disease progression. For example, expansion of TLR-expressing monocytes was observed in mild, but not severe forms of dengue [22], and plasmacytoid dendritic cells decreased at an accelerated rate in DHF compared

to DF patients [23]. It is not clear how these immune cells are differentially modulated, but it would certainly be an interesting aspect of dengue research. Suppressed IFN expression in severe dengue can also be explained by the antibody-dependent enhancement theory. According to Ubol *et al.* [24], DV-antibody complexes trigger negative regulators that disable IFN production in monocytes. Consistent with this finding is the observation that IFNβ, RLH and IPS-1 levels were downregulated in peripheral blood mononuclear cells of secondary DHF patients but not in secondary DF patients [24]. But since both groups have pre-existing antibodies to DV, other components such as antibody type may factor in on the repressed type I IFN profile of severe secondary dengue infections. For example, the overall effect on IFN response may vary from patient to patient, depending on the combination of enhancing and neutralizing antibodies present in the host.

For the type II IFN response, the correlation between IFNγ levels and disease severity is not so clear. Bozza *et al.* [7] observed higher IFNγ levels in severe versus mild dengue, and suggested its predictive utility for disease severity. Kadhiravan *et al.* [25] and Restrepo *et al.* [26] also observed higher IFNγ levels in DHF/DSS patients. However, in another study, IFNγ was higher in primary versus secondary infections but did not correlate with severity [27]. This inconsistency may be due to differences in study design, population sampling, and timing of cytokine measurements, which is difficult to standardize. Thus, sequential measurement of cytokine levels is a better option for a thorough analysis of IFN levels. Using this approach, Libraty *et al.* [28] and Priyadarshini *et al.* [29] observed earlier peak plasma IFNγ among DHF patients compared to DF patients. Furthermore, a significant number of DHF cases with plasma leakage had increased levels of IFNγ, indicating its role in dengue pathogenesis [29]. Possible sources of IFNγ are NK cells and T cells, which are highly activated in DHF versus DF patients [30, 31]. IFNγ modulates the microenvironment of immune cells by enhancing the activation of DV-infected dendritic cells (DC) and the release of IL-12, a T-cell activating cytokine [32]. IFNγ has also been shown to augment antibody-mediated dengue infection of monocytic cells and DCs [32–34]. Furthermore, IFNγ directly affects endothelial cell permeability [35, 36]. A combination of these effects is a fine recipe for a dysfunctional immune system. Hence, although IFNγ is originally designed to protect against disease [18], it may potentially inflict damage on the host, depending on the timing and level of production, and in concert with other pathogenic events of dengue infection.

A novel insight into the relationship between IFN and plasma leakage has been explored in an *in vitro* study on endothelial cells [37]. DV infection imparted a protective effect against TNFα-mediated hyperpermeability of endothelial cells at early periods of infection, but this protective effect diminished after several days of infection. The authors further proved that IFNβ mediates this protective activity against vascular permeability. But as IFNβ production wanes at later phases of infection, endothelial cells become more sensitized to TNFα-induced permeability. Indeed, the addition of recombinant IFNβ during late-stage infection restored the endothelial cells to normal permeability [37]. Type I IFNs have been shown to stabilize the vascular barrier in various studies [38–41]. Moreover, IFNα and IFNβ protect against IFNγ-induced endothelial permeability [42]. Based on these findings, it is tempting to speculate on a model for IFN-mediated pathogenesis of dengue. IFNγ, as well as other unknown factors, may be responsible for plasma leakage in dengue patients. However, a strong type I IFN response in mild dengue protects against vascular permeability, aside from controlling viral replication. On the other hand, a muted type I IFN response in severe dengue is unable to block either infection or plasma leakage. While this hypothesis sounds promising, it is challenged by the lack of an appropriate animal model for dengue disease [43].

2-2. Cytokines

The cytokine storm theory proposed for dengue claims that a dysregulated production of cytokines during DV infection contributes to the disease. These cytokines are believed to be a product of massive T cell activation [44, 45]. According to the "original antigenic sin" proposed by Mongkolsapaya *et al.* [46], preferential expansion of memory T cells from primary infection over high-affinity T cells for the current infection promotes cytokine responses that imperil rather than protect the host. However, this does not explain DHF/DSS in infants with primary infection. Upregulated levels of cytokines have also been reported for dengue patients at the age of less than 1 year [47–49], indicating that altered cytokine profiles are not solely directed by the cell-mediated immune response. It can be hypothesized that instead of T cells, maternal antibody-enhanced viremia is responsible for the increased cytokine response in infant cases of dengue [47, 48], most likely from cells of the innate immune system which are targets for initial replication of DV. For example, *in vitro* experiments show that monocytes and DCs could act as important sources of cytokines during DV infection [50–52]. Accordingly, Chen *et al.* [51] proposed a model for dengue pathogenesis in which cytokine production by monocytes during early DV infection triggers a cascade of events that eventually leads to an augmented cytokine response, thereby causing vascular permeability.

The current challenge of dengue research is to identify soluble factors that mediate pathogenesis, and a number of candidate cytokines have been identified. The reader is referred to other articles for a more comprehensive overview regarding these molecules [53–55]. Despite these leads, the definite cytokine determinant of dengue severity is still not established. Thus, an alternative approach is to identify the central regulator of cytokine expression during DV infection. So far, two such putative regulators have been identified. DV infection of macrophage inhibitory factor (MIF)-deficient mice produced a less severe clinical disease, with reduced proinflammatory cytokine levels [56]. Moreover, anti-MIF antibodies reduced cytokine expression in DV-infected macrophages [56]. These results suggest the involvement of MIF in the amplification of cytokine responses that inflict damage on the host. Elevated levels of MIF have been detected in severe dengue [56, 57], and macrophages and hepatocytes were identified as sources of this cytokine [56]. CLEC5A is another molecule implicated in dengue pathogenesis. CLEC5A is a C-type lectin expressed exclusively on monocytes and macrophages. Knockdown of CLEC5A suppressed monocyte production of proinflammatory cytokines without any effect on viral entry [58]. Furthermore, treatment of anti-CLEC5A antibodies in DV-infected STAT-deficient mice abrogated pro-inflammatory cytokine expression without affecting viral replication, prevented hemorrhage and vascular permeability, and reduced mortality [58]. Hence, CLEC5A may also act as a central regulator of pro-inflammatory cytokine responses during DV infection. However, this hypothesis contrasts with a recent study, which reports that CLEC5A expression is downregulated in dengue patients [59]. Hence, more extensive clinical data is required to confirm the importance of CLEC5A in dengue pathogenesis.

Another aspect of the cytokine storm theory that requires evaluation is how the cytokine storm develops and causes disease. One relevant hypothesis is that cytokines may not necessarily behave in a linear fashion but rather as a complex network of events. Chen *et al.* [51] proposed that cytokine production during DV infection occurs in a hierarchichal manner, progressing from a local gradient derived from monocytic cells and expanding further as other immune cells are recruited and activated. This complex nature of cytokines makes it difficult, if not impossible, to pinpoint the exact mediator of dengue severity.

To complicate things further, cytokines may not act alone but instead exert a synergistic effect in unison. For example, IL-4 exerts a synergistic effect on endothelial cell permeability when combined with either TNFα or IFNγ [60], and increased expression of all three cytokines has been reported in severe dengue [7, 25, 26, 52, 61, 62]. Hence, aside from increased cytokine levels, the cytokine profile of dengue patients should also be investigated. When patients with severe dengue were compared, the primary infection group (infants less than 1 year of age) and the secondary infection group (older children) similarly had elevated levels of IFNγ and IL-10, but IL-6 and TNFα were additionally upregulated in infants [48]. A specific combination of cytokines, instead of a solo cytokine, may be a prerequisite for disease development.

The timing of cytokine production also plays a role in the evolution of disease. To illustrate, IL-6 and IL-8 levels increased earlier in DHF compared to DF cases [29]. IL-6 levels were associated with the presence of pleural effusion/ascites in DHF, while IL-8 correlated with thrombocytopenia and increased serum alanine transaminase levels, an indicator of hepatic injury [29]. IL-8 has also been shown to mediate vascular permeability during DV infection [63]. The early appearance of these cytokines in DHF could enhance or hasten immunopathological events that lead to disease progression.

Finally, viral load also has an influence on the induction potential of cytokines. The minimum viral inoculation level required for cytokine expression in monocytes varies to a significant degree. While IL-8, MIP-1α and RANTES could be induced by a small viral input, TNFα and IL-1β demanded high doses of DV [51]. Thus, certain cytokines are readily inducible during the initial phases of DV infection, while others remain unexpressed or unaltered until high-titer viremia or tissue viral load is established. This probably explains the hierarchical expression of cytokines during DV infection, and thereby justifies the comprehensive analysis of cytokine profiles at different periods of illness to improve our understanding of dengue pathogenesis.

3. MOSQUITO-DERIVED IMMUNOMODULATORS IN FLAVIVIRUS INFECTION

When the host is bitten by a pathogen-transmitting arthropod, it also encounters saliva-associated factors. Arthropod saliva has been shown to manipulate host hemostasis in order to facilitate blood feeding. Almost all blood-feeding arthropods studied so far have at least one anti-clotting, one vasodilator, and one anti-platelet compound [64]. But research also shows that the saliva of ticks, blackflies, sand flies and mosquitoes have the capacity to regulate the host immune system [64–69], indicating that immunomodulation may be common among hematophagous arthropods. For arthropods that live in long-term, close association with their host (e.g. ticks), immune regulation serves the obvious purpose of averting host reactions that impede feeding and survival. But for rapid feeders like

mosquitoes, the arthropod has already terminated contact with the host by the time the immune defense reaches its climax. Thus, Schneider and Higgs [70] proposed two hypotheses to explain the immunomodulatory activity of mosquito saliva. Since the host is frequently exposed to mosquitoes, immune reactions to a previous exposure must be modeled to allow subsequent feeding. Alternatively, these inflammation and immune responses are by-products of mosquito anti-hemostatic activities, since these three physiological pathways are closely intertwined. In the context of arthropod-borne infections, vector saliva may alter immune reactions in a way that can influence pathogen transmission and establishment, as has been demonstrated for *Leishmania major*, *Plasmodium yoleii*, Cache Valley virus and vesicular stomatitis virus [71–74]. Thus, mosquito saliva has the potential to direct the course of flavivirus infections, specifically by interrupting the innate immune response. This section evaluates the impact of soluble mediators from mosquito saliva on the earliest process of dengue infection, as the virus initially establishes itself in the host.

Although research has been done on various mosquitoes, this review focuses on *Aedes aegypti*, the most important vector for dengue, with reference to other vectors. Mosquito feeding or saliva from *A. aegypti* creates a shift from Th1 cytokine to Th2 cytokine expression. Th1 cytokines such as IFNγ and IL-2 were downregulated [68, 75–78], while Th2 cytokines such as IL-4, IL-5 and IL-10 were either upregulated or unaffected [68, 75, 76, 78, 79], although in one case IL-10 was downregulated [77]. This is attributable to differences in preparation and/or dose of salivary gland extract (SGE) and the mice used. *Culex pipiens* feeding also favored Th2 cytokine responses [78]. A Th1-to-Th2 shift in cytokine response has been observed in severe dengue [25, 80], but there is no direct evidence so far linking this to mosquito bites. Th1 cytokines stimulate a proinflammatory response to kill intracellular parasites. On the other hand, Th2 cytokines have a counteractive effect on Th1 cytokines, and their function is targeted toward extracellular pathogens. Hence, a Th2-skewed cytokine response during mosquito feeding would benefit viral transmission and initial establishment in the host. In support of this, reconstitution of Th1 cytokines during vector feeding restored innate immunity and restricted infection by *Borrelia burgdorferi*, an intracellular spirochete, in mice [81]. Furthermore, other antiviral mediators such as IFNβ, iNOS and TLR3 were suppressed by mosquito feeding/saliva [75, 76, 79].

T cells are also largely affected by mosquito saliva. For example, *A. aegypti* SGE restricted splenocyte proliferation by inducing cell death and arresting cell division, with T lymphocytes as the most susceptible population [68, 77]. Dermal recruitment of T cells was also inhibited when mice were subjected to mosquito feeding prior to WNV infection [79]. Thus, mosquito bite-mediated T cell suppression can be exploited by DV for transmission and survival. In contrast, SGE from *Culex quinquefasciatus* did not affect T cell proliferation [77], although its effect on other immune cells was not investigated. This differential activity is probably due to biological differences between the two vectors, which may eventually influence host preference of the transmitted pathogen. For example, *Aedes*-transmitted viruses primarily cycle between mosquitoes and primates, and cause hemorrhagic disease. On the other hand, *Culex*-transmitted viruses usually cycle between mosquitoes and birds, and cause neurological disease in humans, who serve as dead-end hosts. Therefore, further studies are needed to look into the role of mosquito-dependent immunomodulation on host tropism and pathogenesis of arbovirus infections.

An increasing number of molecules responsible for blood feeding have already been identified in mosquito saliva, but the immunomodulatory components have yet to be determined. In the case of *Anopheles stephensi*, Owhashi and his group [82, 83] were able to isolate a neutrophil chemotactic factor and an eosinophil chemotactic factor. Zeidner *et al.* [78] attributed the cytokine-modulating activity of *A. aegypti* and *C. pipiens* saliva to sialokinins. Sialokinins are vasodilatory molecules released into the saliva. However, these molecules were also able to inhibit the production of IL-2 and IFNγ while enhancing IL-10 and IL-4 expression [78]. Thus, aside from promoting blood feeding, sialokinins may have a secondary function in immune modulation. Another mosquito salivary protein with immunomodulatory property was recently isolated from *A. aegypti* and identified as SAAG-4. SAAG-4 reduced IFNγ but enhanced IL-4 response in CD4+ T cells [84]. Interestingly, both sialokinin and SAAG-4 direct a Th2-skewed pattern of cytokine response. Hence, certain substances from mosquito saliva have the capacity for host immunoregulation.

Such immunological activity in mosquito saliva compels us to contemplate on its implications for pathogen infection and disease development. Scientific investigations along this line have been initiated for various arthropod-borne pathogens, including flaviviruses. Unfortunately, the majority of flavivirus work is conducted on West Nile Virus (WNV). Nevertheless, these findings should lay a foundation for our knowledge regarding mosquito-flavivirus-host interactions. Mosquito feeding/saliva-mediated enhancement of WNV infection has been demonstrated in mice using *Aedes aegypti* and *Culex tarsalis* [79, 85, 86], and in chicken using *C. pipiens* [87]. In the mouse studies, mosquito feeding increased viremia and accelerated neuroinvasion compared

to needle injection. When mosquito feeding was artificially mimicked in mice using SGE treatment prior to needle infection, the enhanced infection was similarly achieved [85, 86]. But when SGE was applied at a site distal from the infection, the enhancing effect was lost [86]. These results indicate that mosquito saliva is responsible for the augmented infection and that this activity is mediated locally. However, the effect of mosquito saliva on mortality is not clear. Schneider *et al.* [85] described a lower survival rate among mosquito fed-WNV infected mice, but Styer *et al.* [86] did not observe any differences in morbidity, mortality, onset of disease or survival time. This discrepancy may be due to differences in the mosquitoes or mice used or to the experimental methods employed. But it is also possible that mosquito feeding may enhance viral replication without affecting the outcome of disease. Further studies should clarify the role of mosquito-virus-host interactions on immunopathogenesis of flaviviral infections.

Other findings do not support this mosquito saliva-mediated enhancement theory of flavivirus infection. Mosquito feeding did not alter WNV infection in hamsters [88], nor did it affect the viremia and antibody response of St. Louis encephailitis virus-infected chicken and house finches [89]. These findings can be easily explained by differences in experimental methodology. Alternatively, saliva-mediated enhancement may be determined by the combination of mosquito, virus and host species. Hence, saliva-mediated enhancement may be or not be a universal feature of flavivirus infections. In the case of DV, only one study on mosquito saliva has been conducted. *A. aegypti* saliva inhibited DV infection of human myeloid DCs, and this effect was augmented when cells were pre-sensitized with saliva, leading the authors to conclude that mosquito saliva has instead an antiviral function [90]. At this point, it is difficult to generalize since the effect of mosquito saliva on DV infection in other susceptible cells or *in vivo* has not yet been investigated. To resolve this, more extensive research on mosquito saliva and its role in DV infection and pathogenesis is recommended. Nevertheless, mosquito saliva has immunomodulatory effects that can be exploited by DV to enhance transmission and infection. Although there is no compelling evidence for this as yet, the findings based on WNV emphasize the impact of vector feeding on early events of flavivirus infection.

4. PROSPECTS FOR DENGUE MANAGEMENT AND THERAPY

The identification of soluble factors required for dengue infection and pathogenesis would facilitate the development of anti-viral and disease management strategies. Until now, no therapeutic drug has been developed for dengue, and the success of hospital treatment relies heavily on careful observation and supportive care. With up to 50 million DV infections every year [91], the race is on to find an improved intervention method for dengue.

The success of IFN in hepatitis B and C virus therapy prompted some researchers to look into its utility for dengue. IFNα has been shown to inhibit DV replication *in vitro* using various cell lines [92–94]. To assess its effectiveness *in vivo*, two types of rIFN-α-2a (nonpegylated and pegylated) were administered in DV-infected rhesus monkeys one day after the onset of viremia [92]. The pegylated form has a covalently conjugated 40kD branched methoxy-polyethylene glycol (PEG) molecule, which prolongs the systemic prevalence of rIFN-α-2a. A single administration of either preparations inhibited DV replication to some extent, but not completely [92]. The nonpegylated form delayed viremia, while the pegylated form slightly reduced viral titers. In future studies, a combination of the two forms is recommended to potentiate absolute clearance of the virus. rIFN-α-2a surpasses other antiviral drugs for its clinical safety and tolerability, especially among children [95]. Furthermore, if a single dose administration is proven to be effective, IFN treatment would be simple and affordable. A combination therapy with other antiviral drugs may also increase its potency. However, since dengue viremia peaks within the first 72 hours of illness [2, 32], early intervention is an important prerequisite for the success of this strategy.

Another therapeutic approach is to neutralize cytokines/cytokine mediators that promote disease. For example, antibody treatment against MIF and CLEC5a has been suggested as a method of intervention. Using this strategy in mice, cytokine production was reduced, and the clinical outcome of DV infection was improved, suggesting its potential for application in human dengue patients [56, 58]. Other soluble factors are also being considered for similar purposes, but the complex nature of cytokines precludes the identification of a single target that can generate promising results. Moreover, the intricate networks that link cytokines raise the possibility of undesirable physiological effects on the human body. Finally, whether the host is still capable of viral clearance when certain cytokines are neutralized is a risk that should be given proper consideration. Alternatively, identification of soluble factors that mediate dengue severity could be applied as clinical and laboratory tools for predicting disease, which is integral to dengue management strategies, especially in endemic areas.

5. CONCLUSION

Dengue pathogenesis is a multifaceted phenomenon,

initially dictated by complex interactions between the virus, vector and host, thereby resulting in a modulated immune response. Although the adaptive immune response is usually associated with dengue pathogenesis, the innate immune system has much to contribute as well, especially in early events of infection. The innate immune reaction to dengue is characterized by the production of soluble factors that shape the early events of infection to favor or counter the virus. Soluble mediators may also influence disease evolution, most likely by operating on an intricate network of reactions. Since the host operates on a critical balance of immune responses, a disrupted equilibrium mounted by such soluble mediators could facilitate in disease development. The current challenge is to advance the conventional simplistic approach of dengue immunopathogenesis research towards a holistic strategy, which could better assist in the development of rational, practical and effective methods for dengue diagnosis and intervention.

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