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Review

Host-Virus Interactions in Dengue Infection indicate Targets for Detection and Therapeutic Interventions

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Abstract

Dengue is a mosquito-transmitted viral infectious disease that is endemic to 110 countries spanning tropical and subtropical regions. While infection is typically asymptomatic, symptoms of the estimated 50-100 million clinical cases are often debilitating and occasionally life-threatening, resulting in as many as 5 million hospitalisations annually. Consequently, the World Health Organization regards dengue as a significant global public health concern. Immense challenges exist in both formulating and constructing an efficacious vaccine for prophylaxis and in developing therapeutics for cure. Although there have been numerous molecular studies of the interaction between host and virus, and the metabolic pathways of several proteins are implicated in dengue virus replication, their biological significance remains unclear. It is important to consider clinical, immunopathological and epidemiological features to decipher the complexity of disease and to unravel the mechanisms attributed to its progression. This editorial emphasises the critical events causing vascular endothelial permeability, which underpins the manifestations in humans of dengue haemorrhagic fever and dengue shock syndrome.

Keywords

Dengue, host-virus interaction, vascular permeability, detection, vaccine, chemotherapy

Introduction

The dengue virus (DV) is a small, enveloped, positive sense, single-stranded RNA virus belonging to the family Flaviviridae and genus Flavivirus. It is an arthropod-borne virus and is transmitted by mosquitoes of the genus Aedes, principally A. aegypti and A. albopticus. Dengue virus is related to other flaviviruses that also cause acute haemorrhagic diseases globally, such as Japanese encephalitis virus, West Nile virus and Yellow fever virus. Based on neutralisation assays, there are five distinct serotypes, DENV-1 to DENV-5, the last of which was discovered only very recently [1]. The dengue virus genome contains about 11,000 nucleotide bases, which code for three structural proteins, capsid protein C, membrane protein M and envelope protein E, and seven other non-structural protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are found only in infected host cells and are required for replication of the virus.

Dengue infection is a major cause of disease in the tropics and subtropics with a reported incidence of more than 50 million clinical cases each year among 2.5 billion individuals who live in areas of transmission [2]. Infection with DENV serotypes causes a repertoire of manifestations from febrile illness, dengue fever (DF), to severe illness known as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [3,4]. Symptoms of mild DF include muscle pain (myalgia), headache, rash, swollen lymph nodes (lymphadenopathy) and a reduced leukocyte count (leukopenia) [5]. In addition to these, the characteristic risk features of acquiring DHF and DSS include haemorrhagic manifestations. Notably, a reduced platelet count – thrombocytopenia – is caused by a sudden increase in vascular leakage as a consequence of elevated capillary permeability [6,7].

The mechanisms involved in disease severity are still ambiguous. However, it has been noted that an antibody-dependent enhancement (ADE) of infection increases the likelihood of developing DHF and DSS [8,9]. This occurs when non-neutralising antiviral proteins facilitate virus entry into human endothelial cells (EC), thereby increasing infectivity of host cells. ADE may be observed when a person who previously has been infected with one serotype of DENV becomes infected with a different serotype months or even years later. The clinical course of disease is more severe, with higher viraemia compared to those in whom ADE has not developed [9]. This explains the observation that while primary infections cause mostly minor disease (typically DF) in children, secondary infection is more likely to be associated with severe disease, DHF and/or DSS, in individuals of all ages.

Factors involved in vascular permeability during infection

Recent research on vascular leakage as a consequence of severe dengue disease attributes the cause to a mechanism of molecular mimicry by cross-reaction of anti-DV NS1 antibodies with human EC and platelets which leads to their dysfunction

and thereby interference with haemostasis [10,11]. Protein disulfide isomerase (PDI), an endoplasmic reticulum/Golgi-bound chaperone which ordinarily plays a key role in protein folding in eukaryotes [12], has been demonstrated to interact with anti-DV-2 NS1, while PDI is also involved in platelet aggregation, consequently hypothesized to have implications for severe dengue pathogenesis [11]. A number of studies indicate that extracellular surface-expressed PDI is implicated in virus entry by modifying viral proteins [13-16]. It has also been reported that DENV-2 uses PDI as a receptor to gain entry into EC [17]. However, with the other four dengue serotypes the role of PDI is yet to be explored. The in vitro culture of EC may contribute to discovery of a novel early phase biological marker for severe dengue disease.

Levels of expression by EC and platelets of various cell adhesion molecules, including platelet endothelial cell adhesion molecule (PCAM), platelet (P)-selectin, endothelial (E)-selectin, vascular endothelial (VE)-cadherin, and of other factors such as matrix metalloproteinase (MMP) enzymes, are known to be altered by viral infection. Consequently, this leads to vascular leakage and causes impairment of haemostasis and coagulation of blood [7,18-21]. There are several soluble factors and mediators which are thought to alter expression in patients with severe dengue infections. These include vascular endothelial growth factor (VEGF), granulocyte-macrophage colony-stimulating factor (GMCSF), monocyte chemoattractant protein 1 (MCP-1), macrophage migration inhibitory factor (MIF), thrombopoietin, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), MMPs, thrombomodulin, endothelial (E)-selectin, tissue factor (TF), plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator (TPA) [18,20,22]. Potential biomarkers such as interleukin (IL)-10, fibrinogen, complement C4A, immunoglobulin subclasses, tropomyosin, and three isoforms of albumin are thought to be involved in DHF [23].

In Andes virus-induced hanta haemorrhagic fever, binding of VEGF to VEGF-receptor (R)2 may be blocked using an antibody to VEGF-R2 activation, resulting in inhibition of the Andes virus-induced VE-cadherin reduction [24]. A similar approach can be taken to examine factors that may inhibit or reduce EC permeability in DHF/DSS which could in turn serve as a potential immunotherapeutic target for the treatment of the disease.

Role of Non-Structural (NS) protein 1 in pathogenesis of disease

The complement system is an integral component of the innate immune response. During the defervescence phase of dengue infection increased leakage of plasma is associated with raised detection of activation products such as C3a and C5a in the plasma followed by increased usage and reduction of complement components in patients with DSS [25-27]. Complement components are also thought to be activated due to the circulating soluble form of dengue NS1 protein in the blood [28,29]. DV NS1 is reported to interact with complement regulatory factors, for example clusterin, and thereby increase vascular permeability [30]. Plasma levels of DV NS1 and terminal C5b-9 complement complexes are correlated with disease severity. Large quantities of NS1, complement anaphylatoxin C5a and the terminal complement complex SC5b-9 are present in pleural fluids from DSS patients [31]. Recent findings elucidate the function of the mannose-binding lectin (MBL) pathway for controlling DV infections [29,32]. This activation of complement may also contribute to vascular leakage and development of DHF and DSS. Identification of factors and mechanisms involved may provide opportunities for development of immunotherapeutics.

Host immune factors contribute to endothelial dysfunction

Vascular dysfunction is associated with DHF and DSS and is one of the hallmarks of disease progression. In addition to direct damage caused by the virus, immune responses of the host to DV infection may also contribute to the pathogenesis of disease. Our understanding of the important events and mediators involved in DV infection, such as antibody-dependent enhancement and inflammatory cytokines, has built progressively over many years but much of the molecular detail remains to be elucidated [33,34]. In patients with secondary DV infection with the same or a different pre-existing DENV serotype, neutralizing and non-neutralizing antibodies appear to enhance viral replication in Fc receptor (FcR)-bearing host cells, notably macrophages and monocytes [33]. Enhanced replication of virus particles in these target cells is probably responsible for the high levels of viraemia during the early stage of illness which have been correlated with DHF incidence [35,36]. Cross-reactive antibodies against DENV NS1 that cause EC damage have been attributed to nitric oxide-mediated apoptosis [37]. Studies also show that production of cytokines and chemokines such as IL-6, IL-8 and MCP-1 increase after treatment with anti-NS1 antibodies and by inhibition of the eukaryotic transcription factor NF-κB in EC [38]. Apart from auto-antibodies that are generated during DV infection, CD4+ and CD8+ T-cell activation is observed to be higher in patients with DHF compared to those with mild DF [39]. T cells that are sensitized during primary infection proliferate upon secondary exposure to release pro-inflammatory cytokines such as interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) [40], which can act directly on vascular EC to cause plasma leakage.

The current consensus of opinion is that activated T cells trigger a cascade of cytokines which target the vascular endothelium and that this may be a key factor responsible for vascular permeability [39]. Studies show that IFN-γ, TNF-α and IL-10 are elevated in the sera of patients with DHF/DSS [41-43]. It is observed that dengue-infected EC elicit high level induction of T cells, B cells and mast cells and secretion of cytokines B-cell activating factor (BAFF),

chemokine (C-X-C motif) ligand (CXCL) 9/10/11, chemokine (C-C motif) ligand 5 (CCL5) and IL-7, which leads to capillary permeability, viraemia and immune targeting of the endothelium [44].

T-cell responses during dengue virus infection

The hypothesis of 'original antigenic sin' of secondary infection postulates that prior exposure to an antigen leads to an alteration of immune response that is skewed by the 'memory' of the primary infection [45-47]. Several studies have indicated that both $CD4^+$ and $CD8^+$ T cells aid in controlling and resolving DV infection [47]. A recent cohort study performed in Sri Lanka by Weiskopf et al. has challenged this theory by demonstrating during primary dengue infection that $CD8^+$ T cells responses are skewed but not impaired qualitatively or quantitatively [48]; this also showed that the magnitude of T-cell response linked to human leukocyte antigen (HLA) alleles confers a reduction in susceptibility to disease severity [47,48]. However, the cohort considered for this study included adults with ages between 18-60 years. There is evidence to suggest that most children infected with DV progress to severe manifestations of DHF [49]. Hence, immune reactivity in juveniles may be expected to be different to that observed in adults. T-cell responses across the DV proteome are most frequent to NS3 and both the amplitude of response in DHF and disease severity are associated with high levels of pro-inflammatory cytokines and reduced expression by T cells of the marker of degranulation CD107a [50]. In contrast, T-cell responses in DV-infected Thai children showed low affinity for the current infecting serotype and higher affinity for an earlier infecting serotype, thereby supporting the hypothesis of 'original antigenic sin'[51]. Therefore, much remains to be unravelled about the role of T cells in DV infection. Although there is mounting evidence of HLA-linked $CD8^+$ T-cell protection in DHF and/or DSS it is arguably premature to challenge received wisdom on the subject.

Conclusion

In addressing the aetiology of dengue infection, it is important to understand what determines the threshold of activation for development of DHF/DSS. Establishing the role of PDI in dengue pathogenesis could serve as a marker for early detection of disease. Identifying and elucidating the role of host immune factors associated with endothelial cell permeability could open new windows to determining immunomodulatory drug targets and antiviral reagents for the successful management of patients with DF, thereby preventing progression to DHF/DSS. Characterization of the protective role of HLA-linked $CD8^+$ T-cell responses in both adults and children should enable a better understanding of immune responses to dengue infection. This may also accelerate the development of safe and effective vaccines as well as to facilitate the creation of humanised mouse models as a valuable resource for further study.

Competing Interests

The authors have declared no competing interests.

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