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Continued overleaf

Pathogenesis of severe dengue infection

Dengue viral infections have increased dramatically over past three decades, and are currently the mosquito borne infections with the highest mortality in Sri Lanka. As a result of the high disease burden, dengue has been declared a priority infection by the WHO, UNICEF and World Bank [1]. Although the Sri Lankan population has been exposed to the virus for decades, severe forms of dengue infection [dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS)] were rare until 1989. Since then Sri Lanka has been experiencing yearly epidemics of DHF, with the number of cases rising each year. However, case fatality has remained <1% in recent years, both in Sri Lanka and the region as a whole, probably due to better management practices [1].

Dengue can occur due to infection with any of the four dengue virus (DV) serotypes (DEN-1-4), which are closely related. Infection can result in asymptomatic infection, undifferentiated fever, dengue fever, DHF/DSS or dengue infection complicated with organ failure [1, 2]. The key question is why only some individuals develop severe forms of the infection, and others have asymptomatic or mild clinical disease. Although severe manifestations are thought to result from a complex interplay between the virus, host genetic makeup and host immune factors, many questions regarding factors that lead to severe disease and the pathophysiology of dengue infection itself remain unanswered. In addition, the clinical severity of dengue infections appears to differ in adults and children and also in altered physiological states such as in pregnancy [3-5]. For instance, children have been shown to be more predisposed to develop shock during dengue infections, while other complications such as bleeding and liver involvement have been shown to be commoner in adults [4, 5].

Initial infection with a particular serotype is known as a primary infection, which is usually asymptomatic or results in mild disease manifestations [6]. Subsequent infection with other serotypes (secondary dengue infections) may lead to severe disease [6]. Most people who are infected have silent infections, but 0.18-1% of primary infections and 2-9% of secondary infections manifest as DHF/DSS [6]. Certain DV strains are more virulent than others, and apart from host factors, frequency of severe infections may vary depending on the circulating virus genotype or serotype [7, 8].

Pathogenesis of severe disease

Following the bite of an infected mosquito the DV initially infects langerhans cells in the epidermis and keratinocytes. Subsequently it infects many other cells, such as, monocytes, dendritic cells (DCs), macrophages, endothelial cells (EC) and hepatocytes [9, 10]. Infected monocytes and DCs produce massive amounts of proinflammatory cytokines and chemokines

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which, along with activated T cells, are thought to cause endothelial dysfunction [11,12]. Endothelial dysfunction leads to increased vascular permeability which is the hallmark of DHF; it causes vascular leak, collection of fluid in pleural and peritoneal cavities and shock [13]. ECs are activated during dengue infections, and also contribute to the immune responses that induce vascular permeability [13]. As increased capillary permeability and vascular leakage are reversible, they were thought to result from EC dysfunction rather than EC injury [10]. However, autopsy studies on victims of fatal dengue shock syndrome have shown selective apoptosis in ECs of the intestinal and pulmonary vasculature [14]. Although the mechanisms for apoptosis are not clearly understood, DV-NS1 protein specific antibodies have been shown to induce EC apoptosis [15]. Therefore, increased vascular permeability and fluid leakage may not be solely due to EC dysfunction in all patients. In some it could be the result of EC damage leading to more severe disease.

Organ involvement

The liver is commonly involved in dengue infections, and acute liver failure (ALF) has also been reported [16-18]. Autopsy studies carried out in patients with fatal hepatitis associated with dengue infection have shown midzonal hepatocyte necrosis, macrovascular steatosis and councilman bodies, with little inflammation [10,19]. Although most patients with ALF associated with dengue infection also have other complications, such as, fluid leakage and shock, ALF can occur in the absence of shock [20]. Dengue associated ALF has a high mortality due to complications such as encephalopathy, severe bleeding, renal failure and metabolic acidosis [18, 20]. Direct hepatocyte damage by the virus [9,10], immune factors and apoptosis of cells due to oxidative stress, have all been suggested as possible mechanisms for liver cell damage [21].

Varying forms of central nervous system (CNS) involvement have been reported in dengue infections; they include encephalopathy, encephalitis and meningoencephalitis [22]. Fluid extravasation, cerebral oedema, ALF, renal failure and electrolyte imbalance are thought to contribute to encephalopathy associated with dengue [18, 22]. The DV can be neurovirulent, and has been isolated from the CSF in patients with encephalitis, in the absence of fluid leakage and other clinical features of severe dengue [23,24].

Myocarditis during dengue infections is associated with cardiac arrhythmias, reduced left ventricular ejection fraction, T wave inversions, and elevated troponin I and CPK-MB levels reflecting myocardial damage [25-27]. Cardiac involvement is thought to be either immune mediated or due to direct infection of myocytes [26]. A few autopsy studies in patients with myocarditis have demonstrated presence of virus antigen within cells of the myocardium [27] associated with a mild inflammatory cell infiltrate [25, 27].

Immunopathogenesis

Host genetic factors play a role in the development of severe manifestations in dengue infections. Certain HLA- class I such as HLA-A*24, HLA-A*53, HLA-A*203, HLA-B*51 and class II alleles [28, 29], polymorphisms in the tumour necrosis factor alpha (TNF- α), Vitamin D receptor [30], CTLA-4, and transforming growth factor β (TGF- β) [31] have been shown to be associated with development of DHF/DSS [10]. In Sri Lankan patients, HLA-A*31 and DRB1*08 are significantly associated with susceptibility to DSS [32]. However, careful assessment of other host genetic factors that may predispose individuals to severe dengue is necessary to further our understanding of the pathogenesis of dengue infections.

There is evidence that both cross reactive T cells and antibodies contribute to pathogenesis in secondary dengue infections. Poorly neutralizing and disease enhancing antibodies are thought to increase infection of cells such as macrophages and DCs [10]. As more and more cells are infected larger quantities of proinflammatory cytokines are secreted resulting in increasing endothelial dysfunction. Although it is widely believed that immune factors are solely responsible for severe clinical disease, patients with severe forms of dengue have higher viral loads and prolonged viraemia [33]. Therefore, it appears that severe dengue is associated with impaired viral control, and indeed, immunosuppressive cytokines such as IL-10 are significantly elevated in patients with DSS compared to those who do not develop shock [34]. T cell responses generated during acute DV infection are highly cross reactive. Cross reactive DV-specific T cells have suboptimal degranulation capacity, but secrete high levels of cytokines [35]. Therefore, reduced or suboptimal antiviral responses may also contribute to disease pathogenesis [36].

Treatment and prevention

Currently, there are no specific drugs for treatment of dengue infection. Management of DHF/DSS is currently based on careful monitoring and early detection of complications, and appropriate intravenous fluid therapy [1]. In addition, organ failure should be treated on its own merits. There are ongoing efforts to develop specific antiviral drugs and other treatment modalities that may improve the outcome of dengue infection [37] and its associated organ specific complications, such as, the use of N-acetylcysteine in ALF [38, 39]. More research, including randomised clinical trials, is required to replace much of the dogma with evidence based management practices.

The ultimate solution would be to develop a safe and effective vaccine to prevent dengue. However, development of a vaccine has been extremely challenging. The main obstacle is the lack of data regarding correlates of a protective immune response [40]. A dengue vaccine should provide lifelong immunity for all four DV serotypes. Before vaccine programmes can be initiated critical issues, such as, the impact of poor antibody responses to the vaccine in causing disease enhancement, effect of possible waning of antibody responses with time, possible induction of disease enhancing and poorly neutralising antibody responses, induction of cross reactive T cell responses with the vaccine, and finally, the impact of large scale vaccination on evolution of the wild type DV, need to be resolved. Many institutions have taken up this challenge, and currently there are several dengue vaccine candidates undergoing phase 1a to phase 3 clinical trials [40]. Until such time a vaccine becomes available, prevention will continue to depend on vector control.

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