

Genetic Influences on Dengue Virus Infections

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Abstract

Dengue virus infections are an important cause of morbidity and mortality in the tropics, with 100 million people infected annually and an estimated 2.5 billion people at risk. Human infections can be asymptomatic or can manifest as the self-limited febrile dengue fever, or the more severe and life-threatening dengue haemorrhagic fever (DHF). There are several possible reasons why some infected individuals might develop a more severe form of the disease than others. Antibody enhancement and viral virulence have been implicated in the pathogenesis of DHF but host genetic factors may also be relevant and predispose some individuals to DHF. This review discusses the possible involvement of a variety of genetic polymorphisms on the course of dengue virus infections. It has been shown that several common genetic polymorphisms can influence the susceptibility to dengue haemorrhagic fever. Gene polymorphisms concerning human leucocyte antigens, antibody receptors, inflammatory mediators and other factors with immunoregulatory effects are described. The study of genetic polymorphisms might provide important insights into the pathogenesis of a more severe disease and could have an impact on the design of future vaccines.

Keywords: Dengue haemorrhagic fever, genetic polymorphisms.

Introduction

Dengue has become one of the most important arthropod-borne diseases in tropical and subtropical regions of the world. Approximately 100 million cases of dengue infections occur annually, and an estimated 2.5 to 5 billion people are at risk of dengue virus infection^[1]. The four serotypes of dengue virus (DEN-1, 2, 3 and 4) are transmitted to humans through the bite of an infective female *Aedes* mosquito and may result in dengue fever (DF), an acute viral infection characterized by fever, rash,

headache and muscle and joint pain. Occasionally, dengue virus infections progress to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). These are potentially life-threatening illnesses characterized by haemorrhagic manifestations and the loss of plasma from the vascular compartment, which may give rise to shock in severe cases.

Central in the pathogenesis of DHF and DSS is the loss of endothelial integrity that is believed to be the result of an abnormal immune response and a disturbance in

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immune regulation. Elevated levels of several cytokines and chemical mediators, which cause capillary leakage and may lead to shock, have been found in those suffering from DHF and DSS. Replication of dengue viruses occurs primarily in mononuclear phagocytes, which are a major source of tumor necrosis factor (TNF)- α and other vasoactive inflammatory mediators. Several studies have demonstrated that TNF- α and other cytokines that are produced downstream of TNF- α in the inflammatory cascade, e.g. IL-1 β , IL-6 and IL-8, are related with disease severity^[2-4]. Other inflammatory mediators, like IL-2 and interferon (IFN)- γ , are released from T lymphocytes that are activated during dengue virus infections. The levels of these cytokines are significantly higher in DHF and DSS patients than in DF patients^[5,6].

There are several possible reasons why some infected individuals might produce a greater inflammatory response than others. The most favoured hypothesis concerns the antibody-dependent enhancement theory. Several epidemiological studies demonstrate that prior infection with a different viral serotype constitutes the largest risk factor for DHF^[7-11]. In vitro studies demonstrated that the presence of anti-dengue antibodies at sub-neutralizing concentrations augment dengue virus infection of Fc γ receptor-positive cells, such as monocytes^[12,13]. Based on these epidemiological and laboratory observations, it has been hypothesized that dengue cross-reactive antibodies may increase the number of dengue virus-infected monocytes during secondary infections, and lysis of these dengue virus-infected monocytes may lead to DHF and DSS. Another possibility why some infected individuals might produce a greater

inflammatory response is related to viral virulence. Several studies have found that infection with DEN-2 caused more severe disease than other serotypes, suggesting that the virus phenotype influences the outcome^[7,8,11]. In addition, genetic variations within a specific serotype may also account for differences in disease severity, although reports remain scanty^[14].

Hyperendemic transmission of multiple DEN serotypes in a Haitian population and the apparent absence of DHF and DSS, in addition to the observation that black people were hospitalized less frequently with DHF and DSS than the whites during epidemics in Cuba, led to the hypothesis that human genetic factors, e.g. gene mutations and gene polymorphisms, may contribute to variable susceptibility^[15-17]. Genetic polymorphisms are stable gene variants that usually have minor effects on the regulation or function of proteins. These subtle changes might very well have important consequences for susceptibility to the disease^[18]. Several studies have confirmed that some genetic polymorphisms may protect or predispose an individual to DHF and DSS. Understanding the molecular basis for these differences in susceptibility should provide useful insight in the pathogenesis of DHF and DSS and aid in the development of effective therapies and vaccines. This review attempts to describe the current knowledge of the role of genetic influences on dengue virus infections.

Human leucocyte antigen genes

The function of the human leukocyte antigens (HLAs), encoded by the major histocompatibility complex (MHC) and whose genes are on chromosome 6, are to

display antigen-proteins to antigen receptors of host T-lymphocytes in order to activate cellular host immune responses. HLA genes show great variability and it could well be that specific polymorphisms seen in human

HLA gene regions influence peptide epitope binding^[18]. A number of studies have looked at the variation in HLA genes and found some of them to be associated with the severity of dengue virus infections (Table).

Table. Effect of HLA and non-HLA alleles on the severity of dengue virus infections

Alleles	Class	Effect	Population	Reference
HLA alleles	Class I			
	A1	Susceptibility	Cubans	20
	A2	Susceptibility	Thai	19, 22
	A*0203	Protective	Thai	22
	A*0207	Susceptibility	Thai	22
	A24	Susceptibility	Vietnamese	21
	A29	Protective	Cubans	20
	A33	Protective	Vietnamese	21
	B blank	Susceptibility	Cubans/Thai	19, 20
	B13	Protective	Thai	19
	B14	Protective	Cubans	20
	B44	Protective	Thai	22
	B46	Susceptibility	Thai	22
	B51	Susceptibility	Thai	22
	B52	Protective	Thai	22
	B62	Protective	Thai	22
	B76	Protective	Thai	22
B77	Protective	Thai	22	
	Class II			
	DRB1*04	Resistance	Mexicans	29
Non-HLA alleles	Fc gamma-receptor	Resistance	Vietnamese	30
	Vitamin D receptor	Resistance	Vietnamese	30

HLA class I

HLA class I alleles consist of HLA-A, -B, and -C; its products have a wide distribution and are present on the surface of all nucleated cells and on platelets. Antigens associated with HLA class I products will interact with CD8 cells during an immune response.

Polymorphisms in this class I region gene were found to be associated with DHF disease susceptibility. Chiewslip and Paradoa were the first to report an association between HLA class I and the severity of dengue virus infection^[19,20]. In two ethnically and geographically distinct populations evidence was presented suggesting that HLA-

A1, HLA-A2 and HLA-B blank increased in frequency in DHF patients. A negative relationship was found for HLA-B13, HLA-B14 and HLA-A29. However, these studies had a small sample size and additional studies with a larger number of patients were needed. Subsequently, a large case control study in 560 study subjects was performed, which mainly confirmed the observations made by the two previous studies that HLA class I was important^[21]. The data demonstrated that polymorphisms in the HLA class I region, particularly of the HLA-A gene, were significantly associated with susceptibility to DHF. Of the 15 alleles studied, two particular alleles were relevant: patients with HLA-A33 were less likely to develop DHF (odds ratio 0.56; 95% confidence interval 0.34-0.39), whereas those with HLA-A24 were at an increased risk to develop DHF (odds ratio 1.54; 95% confidence interval 1.05-2.25). The HLA-B alleles were not associated with DHF disease susceptibility.

Another case control study, in a Thai population, also demonstrated that the HLA-A2 locus serotype was associated with disease susceptibility^[22]. HLA-A*0203 was associated with the less severe DF, whereas HLA-A*0207 was associated with susceptibility to the more severe DHF. Interestingly, these associations were only found in immunologically primed persons, but not in immunologically naïve patients with primary infection. Dengue virus-specific associations were also observed within the HLA-B5 group of related alleles, whereby molecularly-determined HLA-B51 alleles were associated with the development of DHF after secondary infections. HLA-B51-restricted CTL responses to a variety of viruses have been described, including Hantaan virus which also causes a

haemorrhagic fever^[23]. HLA-B52 showed a strong association with less severe DF. The reduced frequency of the HLA-B15 group of serotypes, including HLA-B62, B76 and B77, in patients with secondary infections, suggests that they may be protective against developing clinical disease in immunologically primed individuals. By contrast, HLA-B46 that also belongs to the HLA-B15 group of serotypes, was increased in DHF patients with secondary infections. Since HLA-B46 is in strong equilibrium with HLA-A*0207, it is believed that the effect of B46 was likely to be an adjunct to that of A*0207. Finally, HLA-B44 appeared also to be protective against the development of severe disease in patients with secondary dengue virus infections.

HLA class II

Class II HLA products consist of HLA-D, -DR, -DP, and -DQ; they have a more limited distribution on B-cells, macrophages, dendritic cells, Langerhans cells and activated T cells. HLA class II alleles have shown to play a role in mycobacterial diseases, and their association with hepatitis clearance is also established^[24-26]. HLA-DRB1, which is one of the most polymorphic loci of the HLA complex in the Mexican population^[27,28], was studied in Mexican patients suffering from a dengue virus infection^[29]. Although the sample size was relatively small, the investigators found that the frequency of HLA-DRB1*04 was lower in DHF patients. Persons homozygous for DRB1*04 were less likely to develop DHF than persons who were DRB1*04 negative (odds ratio 0.28; 95% confidence interval 0.12-0.66), suggesting a protective effect. The envelope protein (E) of the virus is responsible for viral entry into target cells. The immunological determinants of protein E are probably

processed and presented by HLA class II antigens. The HLA-DRB1*04 molecule may present these viral antigens to CD4+ lymphocytes leading to an effective immune response and therefore protection from DHF. These findings are in contrast to the findings of Loke et al., who studied polymorphisms in the HLA-DRB1 gene but did not find an association^[21].

HLA class III

Genes in the class III region encode a number of proteins, including complement proteins (C4A, C4B, C2 and Bf), TNF- α and TNF- β ^[18]. Loke and colleagues studied promotor polymorphisms in the TNF- α gene but did not find an association^[21]. No other studies are reported to have studied HLA class III polymorphisms.

Non-HLA host genetic factors

The number of studies on polymorphisms within non-HLA genes remains low. Loke and colleagues investigated the association between susceptibility to DHF and polymorphic non-HLA alleles: vitamin D receptor (VDR), Fc γ receptor II (Fc γ RII), Interleukin-4 (IL-4), Interleukin-1 repeat alleles (IL-1RA), and mannose-binding lectin (MBL)^[30]. Two of the five genes assessed showed evidence of association with altered risk of severe dengue.

Fc γ receptor

The Fc γ receptor is a widely distributed receptor for all subclasses of IgG and is able to mediate antibody dependent enhancement in vitro by binding to virus-IgG complexes^[31,32]. An arginine to histidine substitution at position 131 of the Fc γ RIIA

gene has been associated with meningococcal disease and recurrent respiratory tract infections^[33,34]. It changes the IgG binding affinity of the receptor with reduced opsonisation of IgG2 antibodies causally associated with the arginine variant. Loke et al. found that homozygotes for the arginine variant at position 131 of the Fc γ RIIA gene may be less susceptible to DHF^[30].

Vitamin D receptor (VDR)

This gene mediates the immuno-regulatory effects of 1,25-dihydroxyvitamin D3, which include activating monocytes, stimulating cellular immune responses and suppressing immunoglobulin production and lymphocyte proliferation^[35]. Recently the tt genotype of a single nucleotide polymorphism at position 352 of the VDR gene has been associated with tuberculoid leprosy, enhanced clearance of HBV infection and resistance to pulmonary tuberculosis^[36,37]. Expression of VDR may affect susceptibility to DHF since activated B and T lymphocytes express VDR and 1,25D3 affects monocytes, the main sites of dengue virus infection and replication^[12]. The t allele at position 352 of the vitamin D receptor (VDR) gene was associated with resistance to severe dengue, although the exact mechanism needs to be explored.

Interleukin-4 (IL-4)

IL-4, primarily produced by Th2 subset of CD4+ T-cells, regulates B-cell growth, IgG class switching and suppresses Th1-type responses as well^[38,39]. Since this gene affects both antibody responses and inflammatory responses during disease, IL-4 promotor polymorphisms were studied in order to find a relationship in susceptibility to DHF. However, no associations were found in this context^[30].

Interleukin-1 repeat allele (IL-1RA)

IL-1RA was thought to be a good candidate gene as well because IL-1RA is involved in the regulation of IL-1-mediated inflammatory responses by competitive binding to IL-receptors^[40]. But no significant difference could be found in the DHF group in addition to the controls^[30].

Mannose-binding lectin (MBL)

Several mutations in the MBL gene, which encodes for a protein involved in the activation of the classical complement pathway^[41,42], have been associated with a marked reduction in serum MBL levels and MBL-mediated complement activation^[43,44]. Polymorphisms in this gene were not proved to have any effect on the susceptibility to DHF. However, this variant allele was relatively low in the observed population, which limits the statistical power of the analysis^[30].

Discussion and future perspective

The number of candidate susceptibility and protective genes is expanding rapidly, but what is the use of studying these genes in relation to DHF? Studying host genetic factors will clearly contribute to our understanding of the pathophysiology of dengue virus

infections but also of viral infections in general. The finding of a protective association with particular HLA or non-HLA-types may encourage the design of future vaccines, whereas polymorphisms associated with the susceptibility to develop a more severe disease may help to identify certain risk groups in a population. It is therefore of great importance to stimulate the study of the interaction of single and multiple polymorphisms in severe dengue virus infections.

The few studies performed thus far have demonstrated that host genetic factors can be important in susceptibility to DHF. It is most likely that classical HLA class I and class II gene products play a crucial role in determining the severity of dengue virus infections. Two polymorphic non-HLA alleles, the Fc γ RII receptor and VDR, could also play an important role in susceptibility to DHF. Some polymorphic HLA alleles were observed in several studies, e.g. HLA-A2 in a Thai and Vietnamese population, but differences in susceptibility to DHF were observed^[19,21,22]. An explanation for the observed difference may be that a genetic polymorphism is more frequent in a population whereas another is relatively infrequent. Overall, such disease associations warrant further analysis, but also emphasize the need to expand the scope of investigation to other candidate genes within and outside of the HLA region.

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