MINIREVIEW

Dengue and dengue haemorrhagic fever: implications of host genetics

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Abstract

Little is known of the role of human leucocyte antigen (HLA) alleles or non-HLA alleles in determining resistance, susceptibility or the severity of acute viral infections. Dengue fever (DF) and dengue haemorrhagic fever (DHF) are suitable models for immunogenetic studies, yet only superficial efforts have been made to study dengue disease to date. DF and DHF can be caused by both primary and secondary infection by any of the four serotypes of the dengue virus. Differences in host susceptibility to infectious disease and disease severity cannot be attributed solely to the virus virulence. Variations in immune response, often associated with polymorphism in the human genome, can now be detected. Data on the influence of human genes in DF and DHF are discussed here in relation to (1) associations between HLA polymorphism and dengue disease susceptibility or resistance, (2) protective alleles influencing progression to severe disease, (3) alleles restricting CD4+ and CD8+ T lymphocytes, and (4) non-HLA genetic factors that may contribute to DHF evolution. Recent discoveries regarding genetic associations in other viral infections may provide clues to understanding the development of end-stage complications in dengue disease. The scanty positive data presented here indicate a need for detailed genetic studies in different ethnic groups in different countries during the acute phase of DF and DHF on a larger number of patients.

Introduction

Dengue produces a mild acute febrile illness, dengue fever (DF), and a life-threatening severe illness, dengue haemorrhagic fever (DHF; Agarwal et al., 1999a). DHF has emerged as the most important arbovirus disease in humans in the last three decades. It has been estimated that about 50–100 million cases of DF occur every year, and about 250 000–500 000 cases of DHF (Rigau-Perez et al., 1998), affecting a very large proportion of children (Halstead, 2002; Chaturvedi & Shrivastava, 2004). The years 2001–2003 witnessed unprecedented global dengue epidemics, with a large number of DHF cases (Chaturvedi & Shrivastava, 2004). DHF has been classified into four grades: the mildest is grade I and the most severe is grade IV. The classical features of DHF are increased capillary permeability without morphological damage to the capillary endothelium, altered number and functions of leucocytes, and increased haematocrit and thrombocytopenia (Halstead, 1993; Chaturvedi et al., 1997). Extensive plasma leakage in various serous cavities of the body in DHF grades III and IV may result in profound shock, the dengue shock syndrome (DSS). The risk factors for DHF are infestation with Aedes mosquito, a hot and humid climate promoting mosquito breeding, mosquito density, the presence of all four serotypes of the dengue virus (DV) with secondary infection in the host, poor-quality water storage facilities in people’s homes, a high population density and large movement of people towards urban areas. The sequence of infection with different DV serotypes may be important, e.g. DV-1 then DV-2 or DV-1 then DV-3 are capable of inducing DHF/DSS (reviewed by Chaturvedi & Shrivastava, 2004; Chaturvedi et al., 2005). Antibody-dependent enhancement (ADE), a phenomenon first described by Halstead and colleagues (Halstead, 1970; Halstead et al., 1970) in which DV replication is increased by immune sera (IgG), has been observed in vitro for a large number of viruses. ADE via IgM and complement C3 receptors has also been reported (Cardosa et al., 1983).
Using a dynamic system model of co-circulating dengue serotypes, Cummings et al. (2005) have shown that ADE may provide a competitive advantage to those serotypes that undergo enhancement compared with those that do not, and that this advantage increases with increasing numbers of co-circulating serotypes. Paradoxically, there are limits to the selective advantage provided by increasing levels of ADE, because greater levels of enhancement induce high-amplitude oscillations in incidence of all DV infections, threatening the persistence of both the enhanced and the nonenhanced serotypes. These results suggest that enhancement is most advantageous in settings where multiple serotypes circulate and where a large host population is available to support pathogen persistence during the deep troughs of ADE-induced high-amplitude oscillations of virus replication. Ferguson et al. (1999) have suggested that enhancement may frequently generate complex and persistent cyclical or chaotic epidemic behaviour. Furthermore, enhancement acts to permit the coexistence of all strains where in its absence only one or a subset would persist.

At present, there is no vaccine or specific therapy available for DHF. Pre-existing heterotypic dengue antibody is a risk factor for DHF, and therefore an effective vaccine will have to be tetravalent and needs to prevent infection with all four DV serotypes. Natural DV infection induces long-lasting protective immunity only to the same serotype. A tetravalent formulation that retains the immunogenicity of all four serotypes has proven difficult to produce, requiring the use of more complicated, multiple-dose immunization regimens. A more significant obstacle is the current inability to predict whether candidate DV vaccines will be at all effective in preventing DHF. Studies of candidate vaccines have analysed efficacy only in experimental animal models, none of which faithfully reproduce the DHF syndrome seen in humans. Therefore, selection of the most promising DV vaccine candidates relies on comparing vaccine-induced immune responses with a profile of protective immunity developed from natural DV infections (reviewed by Chaturvedi et al., 2005). Appropriate symptomatic treatment has been successful in reducing the mortality associated with DHF (Chaturvedi & Shrivastava, 2004).

Emergence and re-emergence of DF and DHF continue to be a global challenge. Evolution of the genetics of hosts, pathogens and vectors has been phenomenal and the extensive growth of genetic studies has greatly increased our understanding of the transmission and pathogenicity of infectious diseases. The profound influence of the host’s genetic makeup on resistance to infections has been established in numerous studies. It has been shown that susceptibility to many infectious and parasitic diseases has a genetic basis, and the molecular epidemiology and virulence of pathogenic agents, as well as their resistance to drugs, vaccines and antibiotics, are well known in certain conditions (Abel & Dessein, 1998; McNicoll, 1998). The present paper reviews the existing knowledge dealing with the host genetics and its implication in the pathogenesis of severe dengue disease. The discussion includes genetic association with the clinical disease and the genetic restriction of cells such as CD4+ and CD8+ lymphocytes and the dendritic cells (DCs) that appear to play an important role in the pathogenesis of DHF. Because of space constraints only selected work has been cited.

**Immune response to DV**

Dengue virus has 10 viral proteins including the core (C) and membrane (M) proteins, the envelope (E) glycoprotein and seven non-structural (NS) proteins. Anti-E antibodies inhibit DV binding to cells, neutralize viral infectivity in vitro, show a variable degree of cross-reactivity among the DV serotypes and protect mice from DV challenge on passive transfer (Kaufman et al., 1987; Roehrig et al., 1998). Envelope protein domain III (E-D3) is conserved between different flaviviruses. E-D3 is immunogenic, is highly stable and is the cell receptor binding domain recognized by polyclonal and monoclonal antibodies. Thullier et al. (2001) have suggested that the E (306–314) segment is critical for the infectivity of all DV serotypes and that murine monoclonal antibody 4E11 neutralizes DV of all serotypes by binding to the 296–400 segment of the major DV E glycoprotein. The uptake of DV into mononuclear cell lines and primary human monocytes in vitro is enhanced through binding of antibody to virus at non-neutralizing epitopes with cell-surface Ig receptors or at concentrations below the neutralization endpoint. This is known as antibody-dependent enhancement of infection (Morens & Halstead, 1990). NS1 is expressed on the surface of the virus-infected cells, is secreted into the circulation as a soluble multimer and is an important target of antibodies against DV (Young et al., 2000). Antibodies against NS1 can trigger complement-mediated lysis of DV-infected cells in vitro and protect mice from DV challenge (Schlesinger et al., 1987). At the same time, these antibodies may cross-react with endothelial cells, leading to their activation and expression of cytokines, chemokines and adhesion molecules and resulting in cell damage (Lin et al., 2005). NS3 protein is the main antigen that stimulates DV-reactive CD4+ and CD8+ T cell cells that produce high levels of interferon (IFN)-γ as well as tumour necrosis factor (TNF)-α, TNF-β, and chemokines including macrophage inhibitory protein-1β upon interaction with DV-infected antigen presenting cells (APCs), and are efficient at lysis of DV-infected cells in vitro (Kurane et al., 1991a; Gagnon et al., 1999; Loke et al., 2001). Shresta et al. (2004a) reported that IFN-α/β is critical for early immune responses to DV infection while IFN-γ-mediated
immune responses are crucial for both early and late clearance of DV infection in mice; thus, the IFN system plays a more important role than T- and B-cell-dependent immunity in resistance to primary DV infection in mice. In another study, Shresta et al. (2004b) concluded that the early activities of natural killer (NK) cells, B cells and IgM, and later actions of IFN-γ and IgG probably have a role in the defence against DV infection. Contribution of the immune responses to the long-term protective immunity by natural primary DV infection is not fully known.

**Pathogenesis of DHF**

Despite extensive studies, the pathogenesis of DHF is still not fully understood. Views have been expressed on the effect of viral and host factors on disease severity. In Asia, risk of severe disease is greater in children than in adults, and outcome is worse in younger children. By contrast, American strains of DV largely affect the adult population and produce milder disease. This correlates with the structural difference in the two strains of DV (Leitmeyer et al., 1999; Watts et al., 1999; Rico-Hesse, 2003; Cologna et al., 2005).

Once a virus reaches its target cell, a cascade of events generally starting with the interaction of viral envelope glycoproteins with specific entry receptors and co-receptors is necessary in order to trigger the virus–cell membrane glycoproteins with specific entry receptors and co-receptors generally starting with the interaction of viral envelope glycoproteins with specific entry receptors and co-receptors. DV enters the macrophage (Mφ) through a virus receptor or the Fc-receptor as an immune complex. Schlesinger & Chapman (1999) have reported that the FcγRI extracellular domain is sufficient for internalization of infectious DV immune complexes through a mechanism that does not involve classical immunoreceptor tyrosine-based activation motif-dependent signalling. Moreno-Altimirano et al. (2002) have described putative receptors for DV in primary cultures of human Mφ, and Reyes-Del Valle et al. (2005) have shown that heat shock protein 90 (HSP90) and HSP70 act as a receptor complex in human cell lines and in Mφ. Furthermore, both HSPs are associated with membrane microdomains in response to DV infection, and cholesterol-rich membrane fractions are important in DV entry.

The mechanisms that have been considered in the pathogenesis of DHF include immune complex disease, antibodies cross-reacting with vascular endothelium, enhancing antibodies, complement and its products, various soluble mediators including cytokines, selection of virulent strains and virus virulence (Halstead, 1993; Chaturvedi et al., 1997, 2000, 2005; Cologna et al., 2005; Lin et al., 2005). DV actively replicates in B cells and induces them to produce interleukin (IL)-6 and TNF-α. In addition, a heterologous antibody is able to enhance both virus and cytokine production in B cells. These results suggest that B cells may play an important role in DV pathogenesis (Lin et al., 2002). The most significant role in enhancing the severity of the disease is played by the cascade of cytokines, including a shift from the predominant helper T-cell type 1 (Th1) response observed in cases of DF to the Th2-type in severe cases of DHF grade IV (Chaturvedi et al., 1999, 2000, 2005). Patients with defects in the IL-12 receptor (IL-12R) or IFN-γ receptor (IFN-γR) have abnormal responses to IL-12 or IFN-γ and fail to produce normal levels of IFN-γ (Catherinot et al., 2005; Wood et al., 2005). Current paradigms of T-helper subset balance predict a high prevalence of DHF in this group.

**Human leucocyte antigen**

The human leucocyte antigen (HLA) system is the group of genes located on chromosome 6 in the human major histocompatibility complex (MHC) that encodes the cell-surface antigen-presenting proteins. HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRα, and HLA-DRβ1 are the most thoroughly studied of these genes. HLAs can be further classified into MHC class I, II and III. Additionally, HLA-DM and HLA-DO are important in loading the antigenic peptides generated from pathogens onto the HLA molecules of APCs. The proteins encoded by HLA are present on the cell surface of all the nucleated cells and platelets. HLA is used by the cells of the immune system to differentiate self-and non-self antigens. Proteins produced inside most cells are displayed on HLA antigens (specifically class I MHC) on the cell surface. Infected cells can be recognized and destroyed by components of the immune system (Cooke & Hill, 2001). A number of studies have looked at the variation in HLA genes and found some of them to be associated with severity of DV infections. Little is known of the role of classical HLA-A and -B class I alleles in determining resistance, susceptibility or severity of acute viral infections.

**HLA and dengue fever**

Increased expression of HLA class I and II molecules on infected cells has been reported for Flaviviruses, including DV infection. It is possible that the level of the immune response generated against virus peptides presented by HLA molecules may be responsible for the immunopathology of DV infection (King & Kesson, 2003).

**HLA class I**

CD8+ cytotoxic T lymphocytes (CTLs) play an important in controlling virus-infected cells. The HLA class I antigens loaded with viral antigen-derived peptides along with co-stimulatory receptor/ligand stimuli mediate interactions...
HLA Class II DRB1/C3

2005).

nant children in Asia (Phuong

more, dengue mainly affects adults in Brazil but predomi-

nations. In this context, it would also be interesting to know

different DV serotypes present in the two patient popula-

be due to differences in ethnicity of the populations or the

lack of a negative association with HLA-I in this study could

positively protective against developing

infection showed that HLA-DR4 homo-

gzous individuals are 11.6 times less likely to develop DHF

in comparison with DR4-negative persons These data sug-

that in Mexicans, HLA-DR4 may be a genetic factor that is

protective against DHF (LaFleur et al., 2002). Loke et al.

(2001) have studied polymorphisms in the HLA-DRB1 gene

in Vietnamese patients with DHF and did not find any

association. A study of HLA class II specificities for 14 HLA-

DR and four HLA-DQ and DF patients in a Brazilian

population showed a positive association of HLA-DQ1 with

DF and although HLA-DR1 also showed an increased

frequency in the DF group, this was not statistically sig-

significant (Polizel et al. 2004). Associations with HLA class II

alleles have been shown to play a role in other infections


HLA class II

HLA class II molecules are involved in the presentation of
‘exogenous’ antigens to T helper cells. Class II HLA products

consist of HLA-D, -DR, -DP and -DQ. They are distributed

on B cells, macrophages, dendritic cells, Langerhan’s cells

and activated T cells. Studies carried out in Mexico on

patients with DV infection showed that HLA-DR4 homo-

zous individuals are 11.6 times less likely to develop DHF

in comparison with DR4-negative persons These data sug-

Hence, dengue is predominantly affecting adults in Brazil

and remains mild in children. However, in South America,

be due to ethnic susceptibility differences (Correa et al., 2002).

ence of HLA class I antigens. Thus, HLA class I molecules

restrict CD8+ CTL function and mediate immune responses

against ‘endogenous’ antigens and virally infected targets.

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.

The data summarized in Table 1 show the HLA alleles

that have been found to be associated with patients with

DF but not in patients with DHF. In some of the studies, the

number of patients was small. In a case-control study of

263 ethnic Thai patients infected with either DV-1, -2, -3 or

-4, an HLA class I association with secondary infections

was detected, but not in patients with primary infections.

HLA-A*0203 was associated with the less severe DF, regard-

less of the secondary infecting virus serotype, and HLA-

B*52 was associated with DF in patients with secondary

DV-1 and DV-2 infections. Moreover, HLA-B44, -B62, -B76

and -B77 also appear to be protective against developing

clinical disease after secondary DV infection (Stephens

et al., 2002). By contrast, Polizel et al. (2004) reported no

association with any of the 55 HLA class I antigens tested in

patients with DF in a Caucasian Brazilian population. The

lack of a negative association with HLA-I in this study could

be due to differences in ethnicity of the populations or the

different DV serotypes present in the two patient popula-

tions. In this context, it would also be interesting to know

whether susceptibility to either DF or DHF in Brazilians

reveals any differential association with ethnicity, and thus

perhaps with some HLA haplotypes. Note that World

Health Organization definitions do not fit exactly with

patients with DHF in South American countries. Further-

more, dengue mainly affects adults in Brazil but predomi-

nant children in Asia (Phuong et al., 2004; Siqueira et al.,

2005).

Table 1. Human leucocyte antigen (HLA) alleles associated with protec-

tion against dengue haemorrhagic fever

<table>
<thead>
<tr>
<th>Class</th>
<th>Allele</th>
<th>Population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA Class I</td>
<td>A*0203</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
</tr>
<tr>
<td>A29</td>
<td>Cubans</td>
<td>Paradoa Perez et al. (1987)</td>
<td></td>
</tr>
<tr>
<td>A33</td>
<td>Vietnamese</td>
<td>Loke et al. (2001)</td>
<td></td>
</tr>
<tr>
<td>B13</td>
<td>Thai</td>
<td>Chiewsilp et al. (1981)</td>
<td></td>
</tr>
<tr>
<td>B14</td>
<td>Cubans</td>
<td>Paradoa Perez et al. (1987)</td>
<td></td>
</tr>
<tr>
<td>B44</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>B52</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>B62</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>B76</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>B77</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>HLA Class II</td>
<td>DRB1*04</td>
<td>Mexicans</td>
<td>LaFleur et al. (2002)</td>
</tr>
</tbody>
</table>

**HLA and DHF**

**HLA class I**

Polymorphisms in the HLA class I region gene are associated

with DHF disease susceptibility (Table 2). Chiewsilp et al.

(1981) were the first to report an association between HLA

class I and the severity of DV infection. HLA-A and -B typing

on lymphocytes from 87 unrelated Thai children with DSS

and/or DHF was compared with that in 138 controls who

had no clinical dengue infection. A positive association was

seen for HLA-A2 and HLA-B blank and a negative relation-

ship for HLA-B13 (Chiewsilp et al., 1981). Paradoa Perez

et al. (1987) determined the frequency of HLA antigens in 82

Cuban patients with DHF/DSS. The HLA-A1, HLA-B blank,

HLA-Cw1 and HLA-A29 antigens showed a significant

difference when their values were compared with the normal

control group. A study of Thai patients with secondary DV

infections showed that HLA-A*0207 is associated with

susceptibility to the more severe DHF in patients with

secondary DV-1 and DV-2 infections only. Conversely,

HLA-B*51 is associated with the development of DHF in

patients with secondary infections. These results confirm that

classical HLA class I alleles are associated with the clinical

outcome of exposure to DV, in previously exposed and

immunologically primed individuals (Stephens et al., 2002).

**HLA class II**

T lymphocyte activation during dengue is thought to

contribute to the pathogenesis of DHF. Gagnon et al.
Table 2. Human leucocyte antigen alleles associated with susceptibility to dengue haemorrhagic fever

<table>
<thead>
<tr>
<th>Class</th>
<th>Allele</th>
<th>Population</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>A1</td>
<td>Cubans</td>
<td>Paradoa Perez et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>A*0207</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>A24</td>
<td>Vietnamese</td>
<td>Loke et al. (2001)</td>
</tr>
<tr>
<td>B blank</td>
<td>Thai/Cubans</td>
<td>Chiewsilp et al. (1981); Paradoa Perez et al. (1987)</td>
<td></td>
</tr>
<tr>
<td>B46</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>B51</td>
<td>Thai</td>
<td>Stephens et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>Class II</td>
<td>DQ1</td>
<td>Brazil</td>
<td>Polizel et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>DR17</td>
<td>Brazil</td>
<td>Polizel et al. (2004)</td>
</tr>
<tr>
<td>Class III</td>
<td>TNF-α-308A</td>
<td>Venezuela</td>
<td>Fernandez-Mestre et al. (2004)</td>
</tr>
</tbody>
</table>

(2001) examined T-cell receptor Vβ gene use by a reverse transcriptase-PCR assay during infection and after recovery in 13 children with DHF and 13 children with DF. There was no deletion of specific Vβ gene families. A significant expansion was detected in use of single Vβ families in six subjects with DHF and three subjects with DF over the course of infection, but these did not show an association with clinical diagnosis, viral serotype or HLA alleles. Differences in Vβ gene use between subjects with DHF and subjects with DF were of borderline significance (Gagnon et al., 2001). These data suggest that the differences in T-cell activation in DHF and DF are quantitative rather than qualitative and that T cells are activated by conventional antigen(s) and not a viral superantigen.

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 and heat shock proteins (Cooke & Hill, 2001). Loke et al. (2001) studied promotor polymorphisms in the TNF-α gene but did not find an association with DHF, and Fernandez-Mestre et al. (2004) studied a single-nucleotide polymorphism and reported a significant increase of the TNF-308A allele in patients with DHF.

HLA and dengue-primed cells

It is highly likely that DV-reactive T cells may mediate DHF pathogenesis by cell apoptosis and by inducing secretion of cytokines that increase vascular permeability (Chaturvedi et al., 2000, 2005). Analyses of cross-reactivity patterns of CD4+ and CD8+ T-cell responses to dengue virus, which is consistent with the higher level of soluble CD8 in DHF, points to an important role of T cells in the pathogenesis of severe dengue disease (Kurane et al., 1991b; Mathew et al., 1998; Spaulding et al., 1999; Loke et al., 2001).

CD4+ T Lymphocyte

CD4+ T lymphocytes play a central role in regulating the cell-mediated immune response to infection. These cells are also known as ‘helper’ T cells, as they act on other cells of the immune system to promote various aspects of the immune response, including immunoglobulin isotype switching and affinity maturation of the antibody response, macrophage activation, and enhanced activity of NK cells and CTLs. Some CD4+ T cells can develop into CTLs, but they can attack only those cell types (e.g. B cells, macrophages, dendritic cells) that express class II MHC molecules (Chinen & Shearer, 2005; Wing et al., 2005).

CD4+ T cells recognize antigen that has been processed and is presented in association with a self-class II MHC molecule to APCs – B cells, macrophages and dendritic cells – which take up, process and present the relevant antigen. The activated CD4+ T cell is capable of recognizing the antigen presented by any cell that expresses the appropriate class II MHC molecule. CD4+ T cells act by releasing cytokines in response to antigenic stimulation. The interaction between antigen-specific CD4+ T cells and macrophages forms the basis of the delayed-type hypersensitivity response, which is one of the main effector mechanisms in eliminating infections with intracellular organisms (Wing et al., 2005; Huber & Schramm, 2006).

The role of DV-specific serotype cross-reactive T lymphocytes in recovery from and pathogenesis of DV infections is not fully known. The human CD4+ CD8-CTL clone JK34 cross-reacts for DV types 1, 2, 3 and 4 and recognizes NS3 (Kurane et al., 1991a). Genotypic typing further revealed that HLA-DPw2 is the restriction allele for recognition of an epitope on NS3 by JK34 (Kurane et al., 1991a). Genotypic typing further revealed that HLA-DPw2 is the restriction allele for recognition of an epitope on NS3 by JK34 (Kurane et al., 1993) (Table 3). The majority of T-cell clones derived from a donor who received a live experimental DV-3 vaccine cross-reacts with all four serotypes of DV, but some are serotype specific or only partially cross-reactive. NS3 is immunodominant in the CD4+ T-cell response of this donor. JK15 and JK13 recognize only DV-3 NS3, JK44 recognizes DV-1, DV-2 and DV-3 NS3, and JK5 recognizes DV-1, DV-3 and West Nile virus NS3 (Zeng et al., 1996). A study of amino acids critical for T-cell recognition revealed that clones JK13 and JK5 recognize the same core epitope that is critical for recognition by both clones. Sequence analysis of the T-cell receptors of these two clones showed that they utilize different VP chains (Zeng et al., 1996). A single epitope can induce T cells with different virus specificities despite the restriction of these T cells by the same HLA-DR15 allele. This suggests complex interactions between human T-cell receptors and viral epitopes with very similar sequences on infected cells (Zeng et al., 1996). The clones JK36 and JK46 are cross-reactive for DV-2, 3 and 4, but not for type 1, and recognize NS3. The smallest synthetic peptide recognized by
Table 3. Human leucocyte antigen (HLA)-allele restriction of CD4+ lymphocytes from dengue immune donors

<table>
<thead>
<tr>
<th>Source</th>
<th>HLA-allele</th>
<th>Epitope</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DV-immune patient PBMCs</td>
<td>DPw2</td>
<td>NS3</td>
<td>DV-1-2-3-4 cross-reactive</td>
<td>Kurane et al. 1991a</td>
</tr>
<tr>
<td>DV-immune patient PBMCs</td>
<td>DR-15</td>
<td>NS3</td>
<td>Flavivirus cross-reactive</td>
<td>Kurane et al. 1995</td>
</tr>
<tr>
<td>DV-immune patient PBMCs</td>
<td>DR-15</td>
<td>NS3</td>
<td>Flavivirus cross-reactive</td>
<td>Zeng et al. 1996</td>
</tr>
<tr>
<td>DV vaccine-immunized PBMCs</td>
<td>DR1</td>
<td>NS1/NS2a</td>
<td>DV-1-specific</td>
<td>Green et al. 1997</td>
</tr>
<tr>
<td>DV vaccine-immunized PBMCs</td>
<td>DPw2</td>
<td>NS1/NS2a</td>
<td>DV-1-3 cross-reactive</td>
<td>Green et al. 1997</td>
</tr>
<tr>
<td>DV-immune patient PBMCs</td>
<td>DR-15</td>
<td>NS3</td>
<td>DV-2-3-4 cross-reactive</td>
<td>Kurane et al. 1995</td>
</tr>
<tr>
<td>DV-immune patient PBMCs</td>
<td>DR7</td>
<td>NS1 + NS2a/NS3</td>
<td>DV-1-2-3-4 cross-reactive</td>
<td>Mathew et al. 1998</td>
</tr>
</tbody>
</table>

DV, dengue virus; PBMCs, peripheral blood mononuclear cells.

JK36 has eight amino acids, whereas that by JK46 has 11 (Kurane et al., 1998). Two CD4+ CD8-CTL clones, JK4 and JK43, established from the peripheral blood T lymphocytes of a DV-4-immune donor cross-reacted with DV-1, 2, 3 and 4, yellow fever virus and West Nile virus, and recognized NS3. The smallest synthetic peptide recognized by these T cell clones is an identical nine amino acid peptide of DV-4 NS3. HLA-DR15 is the restriction allele for recognition of this epitope by JK4 and JK43 clones (Kurane et al., 1995). Green et al. (1997) examined nine DV-specific human CD4+ CD8-CTL clones for protein recognition, using recombinant vaccinia viruses that contain genes coding for DV proteins. These clones were established from peripheral blood mononuclear cells (PBMCs) of a donor previously immunized with a live-attenuated experimental DV-1 vaccine. Of nine CD4+ T-cell clones, seven were DV-1-specific and two were DV-1,-3 cross-reactive. Four DV-1-specific clones and one DV-1,-3 cross-reactive clone recognized epitopes within the NS1 or NS2a proteins. Analysis of HLA restriction revealed that three DV-1-specific clones are HLA-DR1-restricted and one DV-1,-3 cross-reactive clone is HLA-DPw3-restricted (Green et al., 1997). These results indicate that NS1 and NS2a proteins as well as C, E and NS3 proteins contain one or more epitopes recognized by DV-specific human CD4+ T lymphocytes.

Okamoto et al. (1998) reported that a region on NS3 contains multiple epitopes recognized by DV serotype-cross-reactive and flavivirus-cross-reactive CD4+ CTLs in an HLA-DPw2-restricted fashion. Numerous CD4+ CTL lines against DV NS proteins were generated from the bulk cultures of PBMCs of two patients, KPP94-037 and KPP94-024, which were specific for NS1 and NS2a collectively and NS3, respectively. All CTL lines derived from both patients were cross-reactive with other DV serotypes. The CD4+ CTL lines from patient KPP94-037 were HLA DR7 restricted (Mathew et al., 1998). Mangada & Rothman (2005) measured the frequencies and characterized the cytokine responses of DV-specific memory CD4+ T cells in PBMCs of six volunteers who received experimental, live attenuated monovalent DV vaccines. They demonstrated epitope sequence-specific differences in T-cell effector function that may play a role in the immunopathogenesis of DHF. Thus, DV-specific CD4 lymphocytes may contribute to immunopathology by lysing virus-infected monocytes, the primary site of virus replication. Although HLA class I restricted CD8+ CTLs are the major cells responsible for clearing virus-infected cells, CD4+ DV-specific, HLA class II restricted T-cell clones with cytolytic activity have been raised from individuals infected or vaccinated with DV as cited above. The HLA-DQ1 and/or DR1 associations may play a role in the immunopathogenesis of DHF. Thus, DV-specific CD4 lymphocytes may contribute to immunopathology by lysing virus-infected monocytes, the primary site of virus replication. Although HLA class I restricted CD8+ CTLs are the major cells responsible for clearing virus-infected cells, CD4+ DV-specific, HLA class II restricted T-cell clones with cytolytic activity have been raised from individuals infected or vaccinated with DV as cited above. The HLA-DQ1 and/or DR1 associations may play a role in the immunopathogenesis of DHF.

**CD8+ T lymphocytes**

The CD8+ T lymphocytes represent the antigen-specific CTLs, which respond to and kill cells that are infected with intracellular pathogens such as viruses. Virtually every cell in the body expresses class I MHC molecules and can be a target of CD8+ CTLs. In general, the role of the CD8+ T cells is to monitor all the cells of the body, ready to destroy any that express foreign antigen fragments in their class I molecules.

Livingston et al. (1995) reported that all four DV-specific CD8+ CD4-CTL clones established from lymphocytes of a DV-4-immune adult were HLA-B35 restricted and recognized NS3 epitopes. Memory CTL responses of PBMCs obtained from patients in Thailand 12 months after natural symptomatic secondary DV infection were studied in four patients (Mathew et al., 1998). The CD4+ and CD8+ CTL lines generated from the bulk cultures of two patients, KPP94-037 and KPP94-024, which were specific for NS1 and NS2a collectively and NS3 proteins respectively, cross reacted with other DV serotypes. All CTL lines derived from both patients were cross-reactive with other DV serotypes. The CD8+ NS1.2a-specific lines from patient KPP94-037 were HLA B57 restricted, and the CD8+ NS3-specific lines from patient KPP94-024 were HLA B7 restricted. A majority of the CD8+ CTLs isolated from patient KPP94-024 were found to recognize amino acids on NS3 (Mathew et al., 1998) (Table 4). These results demonstrate that in Thai patients after symptomatic secondary natural DV infections,
CTLs are mainly directed against NS proteins and are broadly cross-reactive.

In a study of Vietnamese patients with DHF, variation at the HLA-A locus was significantly associated with susceptibility to DHF, and specific HLA-A susceptibility and resistance alleles were identified. HLA-A-specific epitopes were predicted from binding motifs, and ELISPOT analyses of patients with DHF revealed high frequencies of circulating CD8+ T lymphocytes that recognized both serotype-specific and cross-reactive DV epitopes. Thus, strong CD8+ T-cell responses are induced by natural DV infection, and HLA class I genetic variation is a risk factor for DHF (Loke et al., 2001). Peptides bound by human class I MHC molecules and presented to CTLs were predicted by a computer algorithm. The changes in these predicted CTL epitopes (pCTLE) were restricted by HLA-B*2705 and were particularly pronounced in DV-1 and DV-3, indicating CTL-driven selection on DV, particularly, DV-1 and DV-3 (Hughes, 2001).

Zivna et al. (2002) studied the response to an HLA-B*07-restricted T-cell epitope of the DV NS3, in 10 HLA-B*07(+)-Thai children during and after acute DV infections. The frequency of peptide-specific T cells was higher in subjects who had experienced DHF than in those who had DF. Also detected were peptide-specific T cells in PBMCs obtained at the time of the acute DV infection in two of five subjects. This suggests that the NS3 epitope is an important target of CD8+ T cells in secondary DV infection and that activation and expansion of DV-specific T cells is greater in subjects with DHF than in those with DF (Zivna et al., 2002). These findings support the hypothesis that activation of DV-specific CD8+ T cells plays an important role in the pathogenesis of DHF. A study of 48 Vietnamese adults with secondary DV infections suggests that cross-reactive T cells dominate the acute response during secondary infection. Acute ELISPOT responses weakly correlate with the extent of the disease. NS3 556-564 and Env 414-422 were identified as novel HLA-A*24- and B*07-restricted CD8+ T-cell epitopes, respectively (Simmons et al., 2005). The results highlight the importance of NS3 and cross-reactive T cells during acute secondary infection but suggest that the overall breadth and magnitude of the T-cell response is not significantly related to clinical disease grade.

**Dendritic cells**

DCs are ‘professional’ APCs required for establishing a primary immune response. Pryor et al. (2001) studied the association of disease severity with replication of DV isolates from Asia or America in Mφ and also the constructed recombinant DV with substitutions at residue 390 in the envelope glycoprotein (E390). The American strain does not replicate as well as the two Asian strains. For the recombinant viruses, substitution of Asn (Asian) at E390 with Asp (American) results in decreased ability to replicate in Mφ. This indicates that the lack of association of native American DV-2 strains with severe disease is linked to reduced ability to replicate in Mφ, and that Asp at E390 may contribute to this reduction. Using human Mφ and DCs, it has been demonstrated that the chimeric DV containing the E mutation has a lower virus output compared with the parental infectious clone. A larger reduction in virus output is observed for the triple mutant and the wild-type, American genotype virus from which chimeric inserts are derived. It appears that the three changes function synergistically, although the E mutation alone gives a lower output compared with the 5′- and 3′-terminal mutations (Cologna & Rico-Hesse, 2003).

The DV-infected DCs show maturation markers such as B7-1, B7-2, HLA-DR, CD11b and CD83, and produce TNF-α and IFN-γ, but not IL-6 and IL-12. Although DCs undergo spontaneous apoptosis in the absence of feeding cytokines, this process appears to be delayed after DV infection (Ho et al., 2001). DV-infected DCs induce the interacting T cells to proliferate and produce IL-2, IL-4, IL-10 and IFN-γ (Ho et al., 2004). Furthermore, preinfection treatment with either IFN-α or IFN-γ effectively arms DCs and limits viral production in infected cells. However, after infection, DV develops mechanisms to counteract the protection from recently added IFN-α, but not IFN-γ. Such a selective antagonism on the antiviral effect of IFN-α, but not IFN-γ, correlates with down-regulated tyrosine-phosphorylation and DNA-binding activities of STAT1 and STAT3 transcription factors by DV (Ho et al., 2005). Shresta et al. (2005) have demonstrated that IFN-α-dependent control of primary DV infection involves both STAT1-dependent and STAT1-independent mechanisms. The STAT1 pathway is necessary.

<table>
<thead>
<tr>
<th>Source</th>
<th>HLA-alleles</th>
<th>Epitope</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DV-immune patient PBMCs</td>
<td>B35</td>
<td>NS3</td>
<td>Cross-reactive</td>
<td>Livingston et al. 1995</td>
</tr>
<tr>
<td>DV-immune patient PBMCs</td>
<td>B7; B57</td>
<td>NS1-NS2a/NS3</td>
<td>Cross-reactive</td>
<td>Mathew et al. 1998</td>
</tr>
<tr>
<td>Computer prediction</td>
<td>B*2705</td>
<td>NS3</td>
<td>Cross-reactive</td>
<td>Hughes 2001</td>
</tr>
<tr>
<td>DV-infected or-immune patient PBMCs</td>
<td>B*07</td>
<td>NS3</td>
<td>Cross-reactive</td>
<td>Zivna et al. 2002</td>
</tr>
<tr>
<td>DV-immune patient PBMCs</td>
<td>A<em>24 and B</em>07</td>
<td>NS3</td>
<td>Cross-reactive</td>
<td>Simmons et al. 2005</td>
</tr>
</tbody>
</table>

DV, dengue virus; PBMCs, peripheral blood mononuclear cells.
for clearing the initial viral load, whereas the STAT1-independent pathway controls later viral burden and prevents dengue disease in mice. The STAT1-independent responses in mice with primary DV infection include the early activation of B and NK cells as well as the up-regulation of MHC class I molecules on DCs.

DC-specific ICAM-3 grabbing non-integrin (DC-SIGN1, encoded by CD209), an attachment receptor of DV, is essential for productive infection of DCs. Sakuntabhai et al. (2005) have reported a strong association between a promoter variant of CD209, DCSIGN1-336, and risk of DF compared with DHF or population controls. The G allele of the variant DCSIGN1-336 is associated with strong protection against DF in three independent cohorts from Thailand.

Non-HLA genetic factors

A few studies have investigated the association between susceptibility to DHF and polymorphic non-HLA alleles, for example vitamin D receptor (VDR), Fcg receptor II (FcgRII), IL-4, IL-1 repeat alleles (IL-1RA) and mannose-binding lectin (MBL) (Table 5). All subclasse of IgG have a widely distributed Fcg receptor to mediate antibody-dependent enhancement in vitro (van de Winkel and Capel, 1993). A few infections are associated with an arginine to histidine substitution at position 131 of the FcgRIIA (Fijen et al., 1993; Sanders et al., 1994), whereas less susceptibility to DHF has been reported with the homozygotes for the arginine variant at position 131 of the FcgRIIA gene (Loke et al., 2002).

The immunoregulatory effects of 1,25-dihydroxyvitamin D3 (1,25D3), including activation of monocytes, stimulation of cellular immune responses, and suppression of immunoglobulin production and lymphocyte proliferation, are mediated by the VDR gene (MacDonald et al., 1994). Recently, the tt genotype of a single nucleotide polymorphism at position 352 of the VDR gene has been reported to be associated with progression of several infections. Expression of VDR may affect susceptibility to DHF as activated B and T lymphocytes express VDR and 1,25D3 affects monocytes, the main sites of DV infection and replication (Halstead & O’Rourke, 1977). The t allele at position 352 of the VDR gene was associated with resistance to severe dengue, although the exact mechanism needs to be explored (Loke et al., 2002).

Mannose-binding lectin

Several mutations in the MBL gene have been associated with several viral infections (Li et al., 2005; Thio et al., 2005; Tosi, 2005). Polymorphisms in this gene did not have any effect on susceptibility to DHF. However, this variant allele was relatively low in the observed population, which limits the statistical significance of the data (Loke et al., 2002).

Cytokines

In a number of studies the frequencies of the genotypes associated with polymorphism of the cytokine genes have been determined, and their association with the risk of increasing the severity of various viral infections has been investigated (Nakayama et al., 2000; Hoebee et al., 2004). Among the several hypothesis proposed to explain the pathogenesis of severe dengue disease, the model of cytokine cascade is the most well supported (Chaturvedi et al., 2000). The key cytokines that have been associated with DHF include the shift from Th1-type response in DF to the Th2-type cytokine response in DHF, with increased levels of IL-10 and IL-4 (Chaturvedi et al., 1999). Increased levels of TNF-α (reviewed by Chaturvedi et al., 2000), tumour growth factor (TGF)-β (Agarwal et al., 1999b) and IL-8 (Raghupathy et al., 1998) have been associated with DHF, and that of IL-12 with DF (Pacsas et al., 2000). Polymorphism of the genes of these cytokines have been shown in a number of diseases, including viral infections, but little effort has been made to study cytokine polymorphism in patients with DV infection. Loke et al. (2002) have studied IL-4 promoter and IL-1 repeat allele polymorphisms but found no relationship with susceptibility to DHF. By contrast, Fernandez-Mestre et al. (2004) have studied a single-nucleotide polymorphism of TNF-α, IFN-γ, IL-6, TGF-β1 and IL-10 in patients with DV infections and reported a significant increase of the TNF-308A allele in patients with DHF.

Conclusions

Differences in susceptibility to disease can be seen at the level of individuals and populations. Genetic epidemiology data indicate that there might be major susceptibility genes that account for a significant proportion of the genetic contribution to disease susceptibility. Several single-gene disorders have been implicated in altered susceptibility to many different infectious diseases. HLA loci evolve very rapidly, probably as a result of selective pressure from pathogens, and polymorphisms in these loci have been associated with altered susceptibility to infectious diseases.
Several non-HLA genes have also been linked with increased susceptibility to disease, e.g. TNF-α, VDR, and perhaps other cytokine genes. The findings of several recent studies of the genetic influence on IL-12 and IFN-γ receptor functions in various diseases indicate a need for such studies with regard to DV infection. Our knowledge of the molecular mechanisms used by viruses to inhibit the expression of HLA class I antigen/peptide complexes on the cell surface is not only crucial to understanding the pathogenesis of viral diseases, but may also contribute to the development of effective immunotherapies to control viral infections. The scant positive data presented here indicate a need for detailed genetic studies in different ethnic groups in different countries during the acute phase of DF and DHF on a larger number of patients.

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