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 thrombocytopoenia (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 monocytic 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 cytokine, chemokine 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 fusion. 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-Altamirano *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-DRA, and HLA-DRB1 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

Table 1. Human leucocyte antigen (HLA) alleles associated with protection against dengue haemorrhagic fever

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

between $CD8^+$ T cells and target cells. To escape recognition and destruction by $CD8^+$ T lymphocytes, viruses have developed strategies to inhibit the expression and/or function 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, regardless 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 populations. 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. Furthermore, dengue mainly affects adults in Brazil but predominant children in Asia (Phuong *et al*., 2004; Siqueira *et al*., 2005).

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 homozygous individuals are 11.6 times less likely to develop DHF in comparison with DR4-negative persons These data suggest 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 significant (Polizel *et al*. 2004). Associations with HLA class II alleles have been shown to play a role in other infections (Abel & Dessein, 1998; McNicoll, 1998; Thursz, 2001).

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 relationship 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

Class	Allele	Population	References
Class I	A1	Cubans	Paradoa Perez *et al*. (1987)
	A2	Thai	Stephens *et al*. (2002)
	A*0207	Thai	Stephens *et al*. (2002)
	A24	Vietnamese	Loke *et al*. (2001)
	B blank	Thai/Cubans	Chiewsilp *et al*. (1981); Paradoa Perez *et al*. (1987)
	B46	Thai	Stephens *et al*. (2002)
	B51	Thai	Stephens *et al*. (2002)
Class II	DQ1	Brazil	Polizel *et al*. (2004)
	DR1?	Brazil	Polizel *et al*. (2004)
Class III	TNF-α-308A	Venezuela	Fernandez-Mestre *et al*. (2004)

(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*., 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

Source	HLA-allele	Epitope	Specificity	Reference
DV-immune patient PBMCs	DPw2	NS3	DV-1-2-3-4 cross-reactive	Kurane *et al*. 1991a
DV-immune patient PBMCs	DR-15	NS3	Flavivirus cross-reactive	Kurane *et al*. 1995
DV-immune patient PBMCs	DR-15	NS3	Flavivirus cross-reactive	Zeng *et al*. 1996
DV vaccine-immunized PBMCs	DR1	NS1/NS2a	DV-1-specific	Green *et al*. 1997
DV vaccine-immunized PBMCs	DPw3	NS1/NS2a	DV-1-3 cross-reactive	Green *et al*. 1997
DV-immune patient PBMCs	DR-15	NS3	DV-2-3-4 cross-reactive	Kurane *et al*. 1995
DV-immune patient PBMCs	DR7	NS1+NS2a/NS3	DV-1-2-3-4 cross-reactive	Mathew *et al*. 1998

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 be explained by their higher efficacy in presenting DV-1 epitopes to CD4 cells.

$CD8^+$ 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,

Table 4. Human leucocyte antigen (HLA)-allele restriction of $CD8^+$ lymphocytes from dengue immune donors

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

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

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-α 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 IFNR-dependent control of primary DV infection involves both STAT1-dependent and STAT1-independent mechanisms. The STAT1 pathway is necessary

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 subclasses 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 susceptiblity 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).

Table 5. Effects of non-human leucocyte antigen alleles on the severity of dengue virus infections

Allele	Effects	Population	Reference
Fc gamma-receptor	Resistance	Vietnamese	Loke *et al.* 2002
Vitamin D receptor	Resistance	Vietnamese	Loke *et al.* 2002
Mannose-binding lectin	No effect	Vietnamese	Loke *et al.* 2002
DCSIGN1-336	Resistance	France	Sakuntabhai *et al.* 2005

Mannose-binding lectin

Several mutations in the MBL gene have been associated with several viral infections (Ji *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). The 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 (Pacsa *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 promotor 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 design of new strategies to inhibit the escape mechanisms used by viruses. These studies may lead 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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References

Abel L & Dessein AJ (1998) Genetic epidemiology of infectious diseases in humans: design of population-based studies. *Emerg Infect Dis* **4**: 593–603.

Agarwal R, Elbishbishi EA, Chaturvedi UC, Nagar R & Mustafa AS (1999a) Profile of transforming growth factor-beta1 in patients with dengue haemorrhagic fever. *Int J Exp Pathol* **80**: 143–149.

Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, Misra AK, Srivastava KL & Chaturvedi UC (1999b) A clinical study of the patients with dengue haemorrhgic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J Trop Med Publ Health* **30**: 735–740.

Cardosa MJ, Porterfield JS & Gordon S (1983) Complement receptor mediates enhanced flavivirus replication in macrophages. *J Exper Med* **158**: 258–263.

Catherinot E, Fieschi C, Feinberg J, Casanova JL & Couderc LJ (2005) Genetic susceptibility to mycobacterial disease: Mendelian disorders of the interleukin-12 – interferon-gamma axis. *Rev Male Respir* **22**: 767–776.

Chaturvedi UC, Agarwal R, Elbishbishi EA & Mustafa AS (2000) Cytokine cascade in dengue haemorrhagic fever: implications for pathogenesis. *FEMS Immunol Med Microbiol* **28**: 183–188.

Chaturvedi UC, Dhawan R & Mukerjee R (1997) Immunosuppression and cytotoxicity of dengue infection in the mouse model. *Dengue and Dengue Haemorrhagic Fever* (Gubler DJ & Kuno G, eds), pp. 289–309. CAB International Press, Wallingford, Oxon, UK.

Chaturvedi UC, Raghupathy R, Pacsa AS, *et al*. (1999) Shift from a Th1-type response to Th2-type in dengue haemorrhagic fever. *Curr Sci* **76**: 63–69.

Chaturvedi UC & Shrivastava R (2004) Dengue haemorrhagic fever: a global challenge. *Indian J Med Microbiol* **22**: 5–6.

Chaturvedi UC, Shrivastava R & Nagar R (2005) Dengue vaccines: prospects and problems. *Indian J Med Res* **121**: 639–652.

Chiewsilp P, Scott RM & Bhamarapravati N (1981) Histocompatibility antigens and dengue hemorrhagic fever. *Am J Trop Med Hyg* **30**: 1100–1105.

Chinen J & Shearer WT (2005) Basic and clinical immunology. *J Allergy Clin Immunol* **116**: 411–418.

Cologna R & Rico-Hesse R (2003) American genotype structures decrease dengue virus output from human monocytes and dendritic cells. *J Virol* **77**: 3929–3938.

Cologna R, Armstrong PM & Rico-Hesse R (2005) Selection for virulent dengue viruses occurs in humans and mosquitoes. *J Virol* **79**: 853–859.

Cooke GS & Hill AV (2001) Genetics of susceptibility to human infectious disease. *Nat Rev Genet* **2**: 967–977.

Cummings DA, Schwartz IB, Billings L, Shaw LB & Burke DS (2005) Dynamic effects of antibody-dependent enhancement on the fitness of viruses. *Proc Natl Acad Sci USA* **102**: 15259–15264.

Ferguson N, Anderson R & Gupta S (1999) The effect of antibody-dependent enhancement on the transmission dynamics and persistence of multiple-strain pathogens. *Proc Natl Acad Sci USA* **96**: 790–794.

Fernandez-Mestre MT, Gendzekhadze K, Rivas-Vetencourt P & Layrisse Z (2004) TNF-alpha-308A allele, a possible severity risk factor of hemorrhagic manifestation in dengue fever patients. *Tissue Antigens* **64**: 469–472.

Fijen CA, Bredius RG & Kuijper EJ (1993) Polymorphism of IgG Fc receptors in meningococcal disease. *Ann Intern Med* **119**: 636–645.

Gagnon SJ, Leporati A, Green S, Kalayanarooj S, Vaughn DW, Stephens HA, Suntayakorn S, Kurane I, Ennis FA & Rothman AL (2001) T cell receptor Vbeta gene usage in Thai children with dengue virus infection. *Am J Trop Med Hyg* **64**: 41–48.

Green S, Kurane I, Pincus S, Paoletti E & Ennis FA (1997) Recognition of dengue virus NS1-NS2a proteins by human $CD4^+$ cytotoxic T lymphocyte clones. *Virology* **234**: 383–386.

Halstead SB (1970) Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *Yale J Biol Med* **42**: 350–362.

Halstead SB (1993) Pathophysiology and pathogenesis of dengue haemorrhagic fever. *Monograph on Dengue/Dengue Haemorrhagic Fever* (Thongcharoen P, ed.), pp. 80–103. WHO-SEARO, New Delhi.

Halstead SB (2002) Dengue. *Curr Opin Infect Dis* **15**: 471–476.

Halstead SB & O'Rourke EJ (1977) Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. *J Exp Med* **146**: 218–229.

Halstead SB, Nimmannitya S & Cohen SN (1970) Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J Biol Med* **42**: 311–328.

Ho LJ, Wang JJ, Shaio MF, Kao CL, Chang DM, Han SW & Lai JH (2001) Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. *J Immunol* **166**: 1499–506.

Ho LJ, Shaio MF, Chang DM, Liao CL & Lai JH (2004) Infection of human dendritic cells by dengue virus activates and primes T cells towards Th0-like phenotype producing both Th1 and Th2 cytokines. *Immunol Invest* **33**: 423–437.

Ho LJ, Hung LF, Weng CY, Wu WL, Chou P, Lin YL, Chang DM, Tai TY & Lai JH (2005) Dengue virus type 2 antagonizes IFN-alpha but not IFN-gamma antiviral effect via down-regulating Tyk2-STAT signaling in the human dendritic cell. *J Immunol* **174**: 8163–8172.

Hoebee B, Bont L, Rietveld E, van Oosten M, Hodemaekers HM, Nagelkerke NJ, Neijens HJ, Kimpen JL & Kimman TG (2004) Influence of promoter variants of interleukin-10, interleukin-9, and tumor necrosis factor-alpha genes on respiratory syncytial virus bronchiolitis. *J Infect Dis* **189**: 239–247.

Huber S & Schramm C (2006) TGF-beta and $CD4^+CD25^+$ regulatory T cells. *Front Biosci* **11**: 1014–1023.

Hughes AL (2001) Evolutionary change of predicted cytotoxic T cell epitopes of dengue virus. *Infect Genet Evol* **1**: 123–130.

Ji X, Olinger GG, Aris S, Chen Y, Gewurz H & Spear GT (2005) Mannose-binding lectin binds to Ebola and Marburg envelope glycoproteins, resulting in blocking of virus interaction with DC-SIGN and complement-mediated virus neutralization. *J Gen Virol* **86**: 2535–2542.

Kaufman BM, Summers PL, Dubois DR & Eckels KH (1987) Monoclonal antibodies against dengue 2 virus E-glycoprotein protect mice against lethal dengue infection. *Am J Trop Med Hyg* **36**: 427–434.

King NJC & Kesson AM (2003) Interaction of flavivirus with cells of the vertebrate host and decoy of the immune response. *Immunol Cell Biol* **81**: 207–216.

Kurane I, Brinton MA, Samson AL & Ennis FA (1991a) Dengue virus-specific, human $CD4^+$ CD8- cytotoxic T-cell clones: multiple patterns of virus cross-reactivity recognized by NS3-specific T-cell clones. *J Virol* **65**: 1823–1828.

Kurane I, Innis BL, Nimmannitya S, *et al.* (1991b) Activation of T lymphocytes in dengue virus infections. High levels of soluble interleukin 2 receptor, soluble CD4, soluble CD8, interleukin 2, and interferon-γ in sera of children with dengue. *J Clin Invest* **88**: 1473–1480.

Kurane I, Dai LC, Livingston PG, Reed E & Ennis FA (1993) Definition of an HLA-DPw2- restricted epitope on NS3, recognized by a dengue virus serotype-cross-reactive human $CD4^+$ CD8 − cytotoxic T-cell clone. *J Virol* **67**: 6285–6288.

Kurane I, Okamoto Y, Dai LC, Zeng LL, Brinton MA & Ennis FA (1995) Flavivirus-cross-reactive, HLA-DR15-restricted epitope on NS3 recognized by human $CD4^+$ CD8 − cytotoxic T lymphocyte clones. *J Gen Virol* **76**: 2243–2249.

Kurane I, Zeng L, Brinton MA & Ennis FA (1998) Definition of an epitope on NS3 recognized by human $CD4^+$ cytotoxic T lymphocyte clones cross-reactive for dengue virus types 2, 3, and 4. *Virology* **240**: 169–174.

LaFleur C, Granados J, Vargas-Alarcon G, *et al.* (2002) HLA-DR antigen frequencies in Mexican patients with dengue virus infection: HLA-DR4 as a possible genetic resistance factor for dengue hemorrhagic fever. *Hum Immunol* **63**: 1039–1044.

Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon, Ramos C & Rico-Hesse R (1999) Dengue virus structural differences that correlate with pathogenesis. *J Virol* **73**: 4738–4747.

Lin YW, Wang KJ, Lei HY, Lin YS, Yeh TM, Liu HS, Liu CC & Chen SH (2002) Virus replication and cytokine production in dengue virus-infected human B lymphocytes. *J Virol* **76**: 12242–12249.

Lin CF, Chiu SC, Hsiao YL, *et al.* (2005) Expression of cytokine, chemokine, and adhesion molecules during endothelial cell activation induced by antibodies against dengue virus nonstructural protein 1. *J Immunol* **174**: 395–403.

Livingston PG, Kurane I, Dai LC, Okamoto Y, Lai CJ, Men R, Karaki S, Takiguchi M & Ennis FA (1995) Dengue virus-specific, HLA-B35-restricted, human $CD8^+$ cytotoxic T lymphocyte (CTL) clones. Recognition of NS3 amino acids 500 to 508 by CTL clones of two different serotype specificities. *J Immunol* **154**: 1287–1295.

Loke H, Bethell DB, Phuong CX, Dung M, Schneider J, White NJ, Day NP, Farrar J & Hill AV (2001) Strong HLA class I-restricted T cell responses in dengue hemorrhagic fever: a double-edged sword? *J Infect Dis* **184**: 1369–1373.

Loke H, Bethell D, Phuong CX, Day N, White N, Farrar J & Hill A (2002) Susceptibility to dengue hemorrhagic fever in Vietnam: evidence of an association with variation in the vitamin d receptor and Fc gamma receptor IIa genes. *Am J Trop Med Hyg* **67**: 102–106.

MacDonald PN, Dowd DR & Haussler MR (1994) New insight into the structure and functions of the vitamin D receptor. *Semin Nephrol* **14**: 101–118.

Mangada MM & Rothman AL (2005) Altered cytokine responses of dengue-specific $CD4^+$ T cells to heterologous serotypes. *J Immunol* **175**: 2676–2683.

Mathew A, Kurane I, Green S, Stephens HA, Vaughn DW, Kalayanarooj S, Suntayakorn S, Chandanayingyong D, Ennis FA & Rothman AL (1998) Predominance of HLA-restricted cytotoxic T-lymphocyte responses to serotype-cross-reactive epitopes on nonstructural proteins following natural secondary dengue virus infection. *J Virol* **72**: 3999–4004.

McNicoll J (1998) Host genes and infectious diseases. *Emerg Infect Dis* **4**: 423–426.

Mehra NK, Kaur G & Jaini R (2002) Genetic diversity in the human major histocompatibility complex: lessons for vaccination approaches to HIV infection. *Community Genet* **5**: 162–166.

Moreno-Altamirano MM, Sanchez-Garcia FJ & Munoz ML (2002) Non Fc receptor-mediated infection of human

macrophages by dengue virus serotype 2. *J Gen Virol* **83**: 1123–1130.

Morens DM & Halstead SB (1990) Measurement of antibody-dependent infection enhancement of four dengue virus serotypes by monoclonal and polyclonal antibodies. *J Gen Virol* **71**: 2909–2914.

Nakayama EE, Hoshino Y, Xin X, *et al.* (2000) Polymorphism in the interleukin-4 promoter affects acquisition of human immunodeficiency virus type 1 syncytium-inducing phenotype. *J Virol* **74**: 5452–5259.

Nieto G, Barber Y, Rubio MC, Rubio M & Fibla J (2004) Association between AIDS disease progression rates and the Fok-I polymorphism of the VDR gene in a cohort of HIV-1 seropositive patients. *J Steroid Biochem Mol Biol* **89–90**: 199–207.

Okamoto Y, Kurane I, Leporati AM & Ennis FA (1998) Definition of the region on NS3 which contains multiple epitopes recognized by dengue virus serotype-cross-reactive and flavivirus-cross-reactive, HLA-DPw2-restricted $CD4^+$ T cell clones. *J Gen Virol* **79**: 697–704.

Pacsa AS, Agarwal R, Elbishbishi EA, Chaturvedi UC, Nagar R & Mustafa AS (2000) Interleukin-12 in patients with dengue haemorrhagic fever. *FEMS Immunol Med Microbiol* **28**: 151–155.

Paradoa Perez ML, Trujillo Y & Basanta P (1987) Association of dengue hemorrhagic fever with the HLA system. *Haematologia (Budap)* **20**: 83–87.

Phuong CX, Nhan NT, Kneen R, *et al.* (2004) Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the World Health Organization classification system helpful? *Am J Trop Med Hyg* **70**: 172–179.

Polizel JR, Bueno D, Visentainer JEL, Sell AM, Sueli Borelli SD, Tsuneto LT, Dalalio MM, Coimbra MTM & Ricardo Alberto Moliterno (2004) Association of human leukocyte antigen DQ1 and dengue fever in a white Southern Brazilian population. *Mem Inst Oswaldo Cruz* **99**: 559–562.

Pryor MJ, Carr JM, Hocking H, Davidson AD, Li P & Wright PJ (2001) Replication of dengue virus type 2 in human monocyte-derived macrophages: comparisons of isolates and recombinant viruses with substitutions at amino acid 390 in the envelope glycoprotein. *Am J Trop Med Hyg* **65**: 427–434.

Raghupathy R, Chaturvedi UC, Al-Sayer H, Elbishbishi EA, Agarwal R, Nagar R, Nusrat H, Mustafa AS, Azizieh F & Khan MAY (1998) Elevated levels of IL-8 in dengue haemorrhagic fever. *J Med Virol* **56**: 280–285.

Reyes-Del Valle J, Chavez-Salinas S, Medina F & Del Angel RM (2005) Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. *J Virol* **79**: 4557–4567.

Rico-Hesse R (2003) Microevolution and virulence of dengue viruses. *Adv Virus Res* **59**: 315–341.

Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ & Vorndam AV (1998) Dengue and dengue haemorrhagic fever. *Lancet* **352**: 971–977.

Roehrig JT, Bolin RA & Kelly RG (1998) Monoclonal antibody mapping of the envelope glycoprotein of the dengue 2 virus, Jamaica Virology. **246**: 317–328.

Saito M, Eiraku N, Usuku K, Nobuhara Y, Matsumoto W, Kodama D, Sabouri AH, Izumo S, Arimura K & Osame M (2005) ApaI polymorphism of vitamin D receptor gene is associated with susceptibility to HTLV-1-associated myelopathy/tropical spastic paraparesis in HTLV-1 infected individuals. *J Neurol Sci* **232**: 29–35.

Sakuntabhai A, Turbpaiboon C, Casademont I, *et al.* (2005) A variant in the CD209 promoter is associated with severity of dengue disease. *Nat Genet* **37**: 507–513.

Sanders LA, van de Winkel JG, Rijkers GT, Voorhorst-Ogink MM, de Haas M, Capel PJ & Zegers BJ (1994) Fc gamma receptor IIa (CD32) heterogeneity in patients with recurrent bacterial respiratory tract infections. *J Infect Dis* **170**: 854–861.

Schlesinger JJ & Chapman SE (1999) Influence of the human high-affinity IgG receptor FcgammaRI (CD64) on residual infectivity of neutralized dengue virus. *Virology* **260**: 84–88.

Schlesinger JJ, Brandriss MW & Walsh EE (1987) Protection of mice against dengue 2 virus encephalitis by immunization with the dengue 2 virus non-structural glycoprotein NS1. *J Gen Virol* **68**: 853–857.

Shresta S, Kyle JL, Robert Beatty P & Harris E (2004a) Early activation of natural killer and B cells in response to primary dengue virus infection in A/J mice. *Virology* **319**: 262–273.

Shresta S, Kyle JL, Snider HM, Basavapatna M, Beatty PR & Harris E (2004b) Interferon-dependent immunity is essential for resistance to primary dengue virus infection in mice, whereas T- and B-cell-dependent immunity are less critical. *J Virol* **78**: 2701–2710.

Shresta S, Sharar KL, Prigozhin DM, Snider HM, Beatty PR & Harris E (2005) Critical roles for both STAT1-dependent and STAT1-independent pathways in the control of primary dengue virus infection in mice. *J Immunol* **175**: 3946–3954.

Simmons CP, Dong T, Chau NV, Dung NT, Chau TN, Thao le TT, Dung NT, Hien TT, Rowland-Jones S & Farrar J (2005) Early T-cell responses to dengue virus epitopes in Vietnamese adults with secondary dengue virus infections. *J Virol* **79**: 5665–675.

Siqueira JB Jr, Martelli CM, Coelho GE, Simplicio AC & Hatch DL (2005) Dengue and dengue hemorrhagic fever, Brazil, 1981–2002. *Emerg Infect Dis* **11**: 48–53.

Spaulding AC, Kurane I, Ennis FA & Rothman AL (1999) Analysis of murine $CD8^+$ T-cell clones specific for the Dengue virus NS3 protein: flavivirus cross-reactivity and influence of infecting serotype. *J Virol* **73**: 398–403.

Stephens HA, Klaythong R, Sirikong M, *et al.* (2002) HLA-A and -B allele associations with secondary dengue virus infections correlate with disease severity and the infecting viral serotype in ethnic Thais. *Tissue Antigens* **60**: 309–318.

Thio CL, Mosbruger T, Astemborski J, Greer S, Kirk GD, O'Brien SJ & Thomas DL (2005) Mannose binding lectin genotypes

influence recovery from hepatitis B virus infection. *J Virol* **79**: 9192–9196.

Thullier P, Demangel C, Bedouelle H, Megret F, Jouan A, Deubel V, Mazie JC & Lafaye P (2001) Mapping of a dengue virus neutralizing epitope critical for the infectivity of all serotypes: insight into the neutralization mechanism. *J Gen Virol* **82**: 1885–1892.

Thursz M (2001) MHC and the viral hepatitides. *Q J Med* **94**: 287–291.

Tosi MF (2005) Innate immune responses to infection. *J Allergy Clin Immunol* **116**: 241–249.

Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S & White JH (2004) Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* **173**: 2909–2912.

Watts DM, Porter KR, Putvatana P, Vasquez B, Calampa C, Hayes CG & Halstead SB (1999) Failure of secondary infection with American genotype dengue 2 to cause dengue haemorrhagic fever. *Lancet* **354**: 1431–1434.

Wing K, Suri-Payer E & Rudin A (2005) $CD4^+CD25^+$-regulatory T cells from mouse to man. *Scand J Immunol* **62**: 1–15.

van de Winkel JG & Capel PJ (1993) Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunology Today* **14**: 215–221.

Wood PM, Fieschi C, Picard C, Ottenhoff TH, Casanova JL & Kumararatne DS (2005) Inherited defects in the interferon-gamma receptor or interleukin-12 signalling pathways are not sufficient to cause allergic disease in children. *Eur J Pediatr* **164**: 741–747.

Young PR, Hilditch PA, Bletchly C & Halloran W (2000) An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *J Clin Microbiol* **38**: 1053–1057.

Zeng L, Kurane I, Okamoto Y, Ennis FA & Brinton MA (1996) Identification of amino acids involved in recognition by dengue virus NS3-specific, HLA-DR15-restricted cytotoxic $CD4^+$ T-cell clones. *J Virol* **70**: 3108–3117.

Zivna I, Green S, Vaughn DW, Kalayanarooj S, Stephens HA, Chandanayingyong D, Nisalak A, Ennis FA & Rothman AL (2002) T cell responses to an HLA-B*07-restricted epitope on the dengue NS3 protein correlate with disease severity. *J Immunol* **168**: 5959–5965.