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Influence of Human Leukocyte Antigens on Altered Immunopathology of Dendue Virus

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ABSTRACT Dengue fever has become one of the most important human viral diseases transmitted by arthropod vector in tropical and subtropical countries of the world. As many as 100 million people are infected yearly resulting in estimated 21,000 deaths. DENV has four antigenically related but genetically distinct serotypes (DENV 1-4). Infection with any of the four serotypes may present as asymptomatic or self limiting mild febrile illness known as dengue fever (DF). However, in some dengue-infected individual, the disease progresses to its severe, life-threatening form, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which may prove fatal without proper early intervention. The pathogenesis of DHF/DSS, however, is not yet completely understood. There are several possible reasons why the primary mild disease progresses to severe form of hemorrhagic manifestations in some individuals. The immune response, Antibody dependent enhancement, virus virulence, and host genetic background are considered risk factors contributing to disease severity. Genetic polymorphisms concerning human leukocyte antigens (HLA) with immunomodulatory effect in dengue infection have been determined in various studies. Human leukocyte antigens (HLA) are highly polymorphic group of genes located on chromosome 6 of human major histocompatibility complex (MHC). HLAs expressed on the cell surface function as antigen presenting molecules and that polymorphism can change individual's immune response. The development of dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) has been associated with HLA alleles. Several studies demonstrate the association of various identified versions (alleles) of HLA class I and class II with conferring susceptibility or protection to DHF/DSS. This review discusses recently identified associations of various HLA class I and class II alleles on enhanced or diminished immune response in the course of dengue viral infection, in ethnically and geographically distinct populations.

INTRODUCTION

Dengue viral infections have become one of the most important mosquito borne viral infections in the world and is one of the major emerging infectious diseases. Early descriptions of the condition date from 1779, and its viral cause and the transmission were elucidated in the early 20th century. Dengue has become a worldwide problem since the Second World War and is endemic in more than 110 countries. In the past fifty years, its incidence has increased 30-fold with significant outbreaks occurring in five of six WHO regions. According to WHO the early 20th century. Dengue has become a worldwide problem since the Second World War and is endemic in more than 110 countries. In the past fifty years, its incidence has increased 30-fold with significant outbreaks occurring in five of six WHO regions. According to WHO some 2.5 billion people, two fifths of the world's population, are now at risk from dengue. DENV infection is a major cause of disease in tropical and subtropical areas, with an estimated 50 - 100 million infections occurring each year and about 2.1 million cases of dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) occur every year resulting in 21,000 deaths (Callaway 2007). According to WHO, despite the over increasing burden of dengue, dengue infection is one of the most neglected disease and even after immense research in the field, we don't have any specific anti viral drug treatments for dengue infection. Treatment depends on the symptoms, varying from oral rehydration therapy at home with close follow-up, to hospital admission with administration of intravenous fluids and/or blood transfusion. Moreover, there are no approved vaccines for the dengue virus. Prevention thus depends on control of and protection from the bites of the mosquito that transmits it.

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The Virus

Based on neutralization assay data, four serotypes DENV-1, DENV-2, DENV-3, and DENV-4 are distinguished which are closely related antigenically and share 60-80% homology between each other (Fu et al. 1992). They belong to the genus *Flavivirus,* family *Flaviviridae,* which contains about 69 viruses. Dengue virus is a small virus (40–50 mm), spherical with a lipid envelope that carries a single strand of RNA as its genome. The genome encodes only ten proteins. Three of these are structural proteins that form the coat of the virus and deliver the RNA to target cells, and seven of them are nonstructural proteins that orchestrate the production of new viruses once the virus gets inside the cell.

Viral Transmission

Dengue viruses are transmitted to humans through the bite of infected female mosquitoes of the genus *Aedes (A).* The primary mosquito vector is *A aegypti* and more rarely *A albopictus*. Only the females seek blood meals, and they feed primarily during the day (Solomon and Mallewa 2001). After virus incubation for 8-10 days, an infected mosquito is capable of transmitting the virus to susceptible individuals for the rest of her life. *A aegypti* breed in collections of clean water (storage jars, containers, etc.) hence prevention becomes difficult.

Dengue Pathogenesis

Substantial gaps remain in the basic understanding of the pathogenesis of dengue disease. Infection with one dengue serotype provides lifelong immunity to that serotype, but there is no cross protective immunity to the other serotypes (Gubler 1997). Infection with any of the four serotypes causes a similar clinical presentation that may vary in severity, depending on a number of risk factors. DENV infection may be asymptomatic in the majority of cases or may result in a spectrum of illness ranging from unapparent or mild febrile illness called Dengue Fever (DF) to severe and fatal hemorrhagic manifestation of the disease known as Dengue Hemorrhagic Fever (DHF) (Gubler 2007). In 20-30% of DHF cases, the patient develops shock, known as the dengue shock syndrome (DSS). In Asia, the risk of developing severe disease is greater in DENV-infected children (below 15 years) than in adults whereas in the western countries mainly the adult population progresses toward DHF/DSS (Pinheiro 1997; Halstead 2006; Kittigul et al. 2007).

The clinical features of dengue fever (DF) vary according to the age of the patient. Infants and young children may have a fever with rash. Older children and adults may have either a mild fever or the classical incapacitating disease with abrupt onset and high fever, severe headache, pain behind the eyes, muscle and joint pains, and measles like rash (Gubler 2007; Byron et al. 2009; Whitehorn 2010). DHF is a critical clinical condition which is characterized by early symptoms similar to those of dengue fever in combination with hemorrhagic manifestations evidenced by positive tourniquet test or spontaneous bleeding, thrombocytopenia (platelet count 100+109/L or less). During this phase, there may be significant fluid accumulation in the chest and abdominal cavity due to increased capillary permeability and leakage. This leads to depletion of fluid from the circulation and decreased blood supply to vital organs. During this phase, organ dysfunction and severe bleeding, typically from the gastrointestinal tract, blueness around the mouth (circumoral cyanosis) may occur. There is bleeding with easy bruising, blood spots in the skin (petechiae), spitting up blood (hematemesis), blood in the stool (melena), bleeding gums and nosebleeds (epitasis). Pneumonia and heart inflammation (myocarditis) is observed in some cases. Shock, dengue shock syndrome (DSS) may occur after 2 to 6 days in some severe cases, characterized by symptoms of DHF with the additional presence of circulatory failure manifested by rapid and weak pulse, and narrowing pulse pressure (<20 mmHg) or manifested by hypotension for age and cold, clammy skin and restlessness. If patients do not receive prompt and appropriate treatment, a stage of profound shock may set in, in which pulse and blood pressure become undetectable, resulting in death within 12 to 36 h after onset of shock (Gubler 2007; Byron et al. 2009; WHO 2009). The World Health Organization (WHO) classifies DHF in four grades (I to IV). DHF grades I and II represent relatively mild cases without shock, whereas grade III and IV cases are more severe and accompanied by shock. The four criteria in

the WHO case definition for DHF (fever, hemorrhage, thrombocytopenia and plasma leakage) were claimed not so practical because serious cases sometimes lack one or more of them, particularly hemorrhage and thrombocytopenia. Instead, the Integrated Management of Childhood Illness (IMCI) has recently proposed the use of the terms 'dengue' and 'severe dengue' for the symptomatic patients with no emphasis on those two signs (WHO 2005; Lan et al. 2007).

Currently, the pathophysiology of dengue viral infections and factors that result in severe clinical disease is poorly understood. For years, DHF pathogenesis has been a controversial matter and current hypothesis that it occurs as a consequence of a very complex mechanism were virus, host, and host immune response interact to give this severe disease (Guzman and Kouri 2008, Chaturvedi et al, 2006). There are several possible reasons why some infected individuals might produce a greater inflammatory response than others. The most favored hypothesis concerns the antibody-dependent enhancement (ADE) theory. Dengue virus is the most widely known example of antibody-dependent enhancement. ADE is a phenomenon, which tends to occur in individuals who have been previously infected by one of the serotype of dengue virus and encountered a secondary infection by a different serotype (King et al. 2002). ADE results in increased viremia and severe clinical presentations of the disease. The primary dengue infection induces both innate and adaptive immune responses. During adaptive immune response, neutralizing homotypic immunoglobulin (IgG) antibodies are produced which provide lifelong immunity against the infecting serotype. Infection with DENV also produces some degree of cross protective (neutralizing heterotypic) antibodies against the other serotypes. However, in addition to inducing heterotypic neutralizing antibodies, infection with dengue can also induce heterotypic antibodies which neutralize the virus only partially or not at all (Goncalvez et al. 2007). It is believed that by binding to but not neutralizing the virus, these antibodies bring the virus in close proximity to the WBC where it gets an easy access in the cells. The interaction is mediated between the exposed Fc region of the antibody and the Fc receptor on the Fc^{+} cells that include wide variety of cells of the immune system, like macrophages, B cells, neutrophils, monocytes and granulocytes. This enhancement is facilitated by the fact that the dengue virus-specific CD4+ T lymphocytes produce IFN-g, which in turn up-regulates the expression of FC-γ receptors (Kurane 2007). Once inside the WBC, the virus replicates undetected, eventually generating very high virus titers. It is believed that these cells produce and secrete vasoactive mediators in response to dengue infection, which causes increased vascular permeability leading to severe hemorrhagic conditions (Kurane and Ennis 1997).

Blood monocytes are the primary targets of dengue viral infection which are the major source of tumor necrosis factors (TNF-α), IFN-α, IL-1, IL-1B, IL-6, platelet activating factors (PAF) and other vasoactive inflammatory mediators. Moreover, CD4+ T lymphocytes produce a number of cytokines, including gamma interferon (IFN-g), IL-2, IL-4, IL-5, IL-6, IL-10, lymphotoxin, and histamine. Finally, cytokine, complement activation products C3a and C5a, and chemical mediator production is induced by other cytokines. Thus, once cytokines are produced, a complex network of induction may further increase the levels of cytokines and chemical mediators. The levels of these cytokines are significantly higher in DHF and DSS patients than in DF patients (Kurane et al. 1991; Green et al. 1999) hence it has been postulated that excessive production of these proinflammatory cytokine and chemical mediators by the virus specific T lymphocytes, can provoke endothelial cell damage and vascular leakage, distinctive feature of DHF (Suharti et al. 2003; Hober et al. 1993). This pathology is typically observed during secondary dengue infection and believed to be the consequence of cross reactive memory T cells elucidated by the primary infection.

Another possibility why some infected individuals might produce a greater inflammatory response is related to viral virulence (Leitmeyer et al. 1999). Several studies have found that infection with DEN-2 caused more severe disease than other serotypes, suggesting that the virus phenotype influences the seriousness of the disease **(**Burke et al. 1988; Vaughn et al. 2000).

The postulated theories and explanations may sound convincing and cross reactive T cells and cross reactive antibodies may contribute to disease pathogenesis, but these cannot be the only explanation for the immunopathological mechanisms leading to severe disease, as severe clinical disease is known to occur even during

primary dengue viral infections, especially infants (Chau 2008) and in pregnant women (Waduge 2006). Furthermore, the majority of individuals who are infected with the dengue virus develop mild or asymptomatic disease, only a relatively small proportion that is, approximately 1% of primary infections and 2–9% of secondary infections of affected persons develop DHF, which suggests that there are host risk factors for the development of the complications among the infected individuals (Kuno et al. 1998).

Despite of hyper endemic transmission of multiple DENV serotypes in a Haitian population very few cases of DHF and DSS are identified, in addition black people were hospitalized less frequently with DHF and DSS than the whites during epidemics in Cuba, this observation led to the hypothesis that human genetic factors, for example, gene mutations and gene polymorphisms, may contribute to variable susceptibility (Halstead et al. 2001). Genetic polymorphisms are stable gene variants that usually have minor effects on the regulation or function of proteins. These subtle changes might very well have important consequences for susceptibility to the disease (Cooke and Hill 2001). Several studies have confirmed that some genetic polymorphisms may protect or predispose an individual to DHF and DSS. Certain HLA- class I and class II alleles (Stephens 2002; Appanna 2010; Malavige 2011) polymorphisms in the tumor necrosis factor alpha (TNF-a), Vitamin D receptor (Loke et al. 2002), CTLA-4, and transforming growth factor β (TGF-b) (Chen et al. 2009) have been shown to be associated with development of DHF/DSS.

Variations in immune response, often associated with polymorphism in the HLA alleles, can now be detected. HLA-controlled immune response may be responsible for the immunopathology of DV infection (King et al. 2003). This review attempts to describe the current knowledge of the association of HLA polymorphisms in the clinical spectrum of dengue infection with relation to associations between HLA polymorphism and dengue disease susceptibility or resistance.

HLA Genes and Significance in Dengue Pathogenesis

The Human Leukocyte Antigen (HLA) system is a group of genes located on the chromosome 6 in humans that encodes the cell surface antigen presenting protein. The HLA complex helps the immune system distinguish the body's own proteins from proteins made by foreign invaders such as viruses and bacteria. They achieve this by displaying fragmented pieces or antigens on the host cell's surface which are recognized by the cells of immune system. These antigens may be self or nonself.

Human leukocyte antigen (HLA) is the human version of the major histocompatibility complex (MHC), a gene family that occurs in many species. In humans, the MHC complex consists of more than 200 genes located close together. Genes in this complex are categorized into three basic groups: class I, class II, and class III.

Human have three main MHC class I genes, known as *HLA-A*, *HLA-B*, and *HLA-C*. The proteins produced from these genes are present on the surface of all nucleated cells and on platelets. On the cell surface, these proteins are bound to protein fragments (peptides) that have been exported from within the cell 'endogenous'. Antigens associated with MHC class I products interact with CD8+ cells. If the immune system recognizes the peptides as foreign (such as viral or bacterial peptides), it responds by triggering the infected cell to self-destruct. There are six main MHC class II genes in humans: *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRA*, and *HLA-DRB1*. MHC class II genes provide instructions for making proteins that are present on the surface of certain immune system cells like B cells, macrophage, dendritic cells langerhans cells and activated T cells. HLA class II present antigenic molecules derived from extracellular proteins to CD4+ T cells. HLA class III includes genes that code for several secretary proteins with immune functions. The proteins produced from HLA class III genes have somewhat different functions; they are involved in inflammation and other immune system activities. The functions of some HLA genes are unknown (Shankarkumar 2004).

The HLA loci evolve very rapidly, probably as a result of selective pressure from pathogens. HLA genes have many possible variations, allowing each person's immune system to react to wide range of foreign invaders. Some HLA genes have hundreds of identified versions (alleles), and these polymorphisms have been associated with altered susceptibility to infectious diseases (Shankarkumar 2004). More than 100

diseases have been associated with different alleles of HLA genes. Most polymorphisms result in significant changes in the organization of HLA molecules, which affect the binding and subsequent presentation of microbial peptide to antigen specific T cells (Hertz et al. 2011).

HLA Association in Dengue Disease Severity

Dengue virus, like other members of *Flaviviridae* family increase the expression of HLA class I and HLA class II expression on infected cells (King et al. 2003). Thus HLA class I and HLA class II molecules clearly play an important role in host immune response to DENV infection, which is also supported by a number of studies that have looked at variations in HLA genes and found that host HLA allele profile influenced the reactivity of DENV specific T cells. Favoring the verdict are studies suggesting associations of polymorphisms in HLA class I and class II with an altered risk of developing severe disease. Certain HLAs are found to be associated with decreased risk of DHF while others are with increased risk (Stephens 2002; Appanna 2010; Malavige 2011). Two biological explanations are proposed for the association of HLA with DHF, that protective HLA allele's exhibit reduced ability to present cross reactive peptides while HLA alleles associated with susceptibility show an increased ability. Therefore, individuals carrying protective alleles would actually elicit a weaker T cell response because fewer memory T cells would be engaged and vice versa. Also protective HLA alleles may select for T cell epitopes that are associated with greater protection. In this case, the memory response would provide more effective control of the virus. Likewise, individuals with susceptible alleles display a diminished capacity to present protective epitopes (Simmons et al. 2005). However, the majority of studies have been based on limited numbers of patients and selective groups of population. Understanding the molecular basis for these differences in susceptibility should provide useful insight in the pathogenesis of DHF and DSS and aid in the development of effective therapies and vaccines (see Table 1).

HLA Class I

Class I HLA products consists of HLA-A, HLA-B and HLA-C that have wide distribution on all the nucleated cells. Cytotoxic T lymphocytes (CTL) recognizes the antigenic peptides presented by HLA class I molecules which normally triggers protective immune response, but can result in immune enhancement of disease. The degree of effectiveness of the CTL response in protection against infection is strongly determined by the viral peptide selection and its presentation by the HLA class I molecules (Simmons et al. 2005). Infection with a single dengue serotype induces both serotype-specific and serotype cross-reactive CD8+ memory T cells and it is assumed that cross-reactive T cells of low avidity for heterologous virus are not protective (Martina et al. 2009). Certain Class I HLAs were found to be associated with decreased risk of DHF while others were associated with increased risk. Protective HLA allele's exhibit reduced ability to present cross-reactive peptides while HLA alleles associated with susceptibility show an increased ability. Several studies demonstrate the association of polymorphisms in the HLA class I genes with conferring protection or susceptibility towards DF and progression of severe dengue disease DHF. Once activated, the CTLs undergoes clonal expansion with the help of a cytokine called Interleukin-2 (IL-2) that is a growth and differentiation factor for T cells and also production of effector cytokines such as TNF-a or IFN-γ which may contribute to disease severity.

In a case control study on distinct ethnic Thai patients undergoing secondary infection, but not in immunologically naive patients with primary infection, HLA-A* 0203, HLA-B*44, HLA-B*62, HLA-B*76, HLA-B*77 were perceived to confer resistance to DF, regardless of the secondary infecting virus serotype. Conversely HLA-B*52 was associated positively with DF in patients with secondary DEN 1 and DEN 2 infections. HLA-A*0207 and HLA-B*51 were associated with susceptibility to more severe DHF in patients with secondary infection (Stephens et al. 2002). Similarly, a hospital based case control study was carried out on children under age group of 15 years belonging to distinct ethnicity, Kinh race in Vietnam. The result demonstrated an increase in the frequency of allele HLA-A*24 in both DHF and DSS patients i.e. HLA –A*24 illustrated a strong positive association with DHF and DSS (Lan et al. 2008). Also, HLA-A*24 was strongly associated with the development of DHF during primary

Table 1: HLA alleles associated with susceptibility/protection against development of DF and/or DHF/DSS

HLA alleles	*Effect*	*Country (Population)*	*Reference*
Class I			
HLA-A*0203	Protection	Thai	Stephens et al. 2002
HLA-A*0207	Susceptibility	Thai	Stephens et al. 2002
HLA-A*03	Protection	Malaysia	Appanna et al. 2010
	Protection	Venezuela	Mestre et al. 2009
HLA-A*24	Susceptibility	Sri Lanka	Malavige et al. 2011
	Susceptibility	Vietnam	Lan et al. 2008
HLA-A*31	Susceptibility	Sri Lanka	Malavige et al. 2011
	Susceptibility	Cuba	Sierra et al. 2007
HLA-B*13	Susceptibility	Malaysia	Appanna et al. 2010
HLA-B*15	Protection	Venezuela	Mestre et al. 2009
	Susceptibility	Cuba	Sierra et al. 2007
HLA-B*18	Protection	Malaysia	Appanna et al. 2010
HLA-B*40	Susceptibility	Venezuela	Mestre et al. 2009
HLA-B*44	Protection	Thai	Stephens et al. 2002
HLA-B*49	Protection	Venezuela	Mestre et al. 2009
HLA-B*51	Susceptibility	Thai	Stephens et al. 2002
HLA-B*52	Susceptibility	Thai	Stephens et al. 2002
HLA-B*53	Susceptibility	Malaysia	Appanna et al. 2010
HLA-B*57	Susceptibility	Venezuela	Mestre et al. 2009
HLA-B*62	Protection	Thai	Stephens et al. 2002
HLA-B*76	Protection	Thai	Stephens et al. 2002
HLA-B*77	Protection	Thai	Stephens et al. 2002
Class II			
HLA-DRB1*0901	Protection	Vietnam	Lan et al. 2008
HLA-DRB1*02	Protection	Venezuela	Mestre et al. 2009
HLA-DRB1*03	Protection	Venezuela	Mestre et al. 2009
HLA-DRB1*04	Protection	Cuba	Sierra et al. 2007
HLA-DRB1*07	Protection	Cuba	Sierra et al. 2007
HLA-DRB1*08	Susceptibility	Sri Lanka	Malavige et al. 2011
HLA-DRB1*12	Susceptibility	Sri Lanka	Malavige et al. 2011
HLA-DRB1*15	Susceptibility	Venezuela	Mestre et al. 2009

dengue infection in a study carried out lately in cohort of patients with DHF in Sri Lankan population. The investigators also found that HLA-A*31 allele frequency increased significantly in DHF patients who developed shock when compared to those who did not develop shock (Malavige et al. 2011). In Cuban individuals the allele frequency of HLA-A*31 in DHF cases was appreciably higher than in healthy controls, that is, HLA-A*31 might be associated with susceptibility to DSS during secondary dengue infection in Sri Lankans while show positive association with development of DHF in Cubans (Sierra et al. 2007; Malavige et al. 2011). Also, HLA-B*15 was found to be associated with Venezuelan population as HLA-B*15 and HLA-B*49 were significantly reduced in frequency in patients with dengue infection compared with healthy individuals (Mestre et al. 2009). This signifies that HLA-B*15 might confer protection against development of dengue fever in Venezuelans but on the other hand shows susceptibility towards progression of DHF in Cuban population (Sierra et al. 2007; Mestre et al. 2009). Also, HLA-B*57 was significantly increased in patients with dengue when compared with the healthy controls in Venezuelans. HLA-B*40 showed a positive association with progression of DHF whereas HLA-B*03 was absent in patients with DHF when compared with healthy control group. Although the associations revealed in this study came from a very small case control population and all the HLA class I frequency differences lost significance after Bonferroni correction, the data suggests that HLA class I alleles can possibly play role in resistance or susceptibility to dengue virus infection and development of DHF (Mestre et al. 2009). Subsequently a study was carried out to investigate genotype variants of HLA class I (A and B) of DENV infected patients with different ethnic groups (Malay, Chinese and Indian) in Malaysia. HLA-A*03 and HLA-B*18 were found to be associated with protection from progression to severe disease, which holds up with the observation of similar previous study on

Malaysian population. Whereas HLA-B*13 and HLA-B*53 were associated with susceptibility towards developing DHF in Malay population (Appanna et al. 2010).

No significant difference was observed in the frequency of any of the HLA-C in any of the studies, which may implement that HLA-C polymorphisms does not play any significant role in determining dengue disease severity.

HLA Class II

Class II HLA genes encode molecule that present antigenic molecules derived from extracellular proteins to T helper cells (CD4T). Polymorphisms in HLA class II result in significant changes in organization of HLA molecules which influence the binding and subsequent presentation of epitopes derived from DENV to T helper cell. As DENV replicates primarily in antigen presenting cells which are the major source of TNFs and other vasoactive inflammatory mediators, HLA class II polymorphisms are believed to influence the altered susceptibility to severe dengue infection.

HLA class II product consists of HLA- DP,-DQ and –DR. Majority of the studies carried out on HLA-class II are focused on HLA-DR as it is established to be mainly associated with determining resistance, susceptibility or the severity of dengue viral infection. Lending credence to the investigation are various studies carried out that prove that HLA class II polymorphisms, especially in HLA-DR genes are associated with varied DHF susceptibility individuals of various ethnicity.

In a case control study on children hospital under age group of 15 at a children's hospital in Southern Vietnam belonging to a distinct Kinh ethnicity, the frequency of HLA-DRB1* 0901 was significantly decreased in secondary infection of DSS that is, DRB-1*0901 shows a negative association with development of severe form of DENV infection (Stephens et al. 2002). Investigation of HLA class II alleles that are positively and negatively associated with development of DSS in a cohort of patients with DHF and also the alleles associated with development of DHF during primary dengue infection in Sri Lankan population highlighted HLA class II alleles HLA-DRB1*08 and DRB1*12. HLA-DRB1*08 was associated with susceptibility to DSS during secondary dengue infection while HLA-DRB1*12 was strongly associated with development of DHF during primary dengue infection (Malavige et al. 2011). The analysis of HLA class II profile in Cubans revealed a significantly increased frequency of HLA-DRB1*07 in healthy control subjects versus those with DF and DHF which means HLA-DRB1*07 might be negatively associated with development of dengue fever as well as in the progression of sever hemorrhagic manifestations. Also, HLA-DRB1*04 was associated with protection against advancement of severe dengue disease as the frequency of HLA-DRB1*04 significantly increased in healthy controls as compared to cases with secondary infection (Sierra et al. 2007).

A related study on ethnically mixed Venezuelans revealed reduced frequencies of HLA class II alleles HLA-DRB1*02/03 and a increased frequency of HLA-DRB1*15 in DF patients when compared with healthy controls. Although the association revealed in this study came from a very small case control population and that after correction for multiple testing only the association with HLA-DRB1*15 is maintained, the data suggests that HLA- class II genes may be associated with genetic diminished and enhanced susceptibility to the development of dengue virus infection and severe form of the disease in the studied population (Mestre et al. 2009).Some alleles observed in ethnically and geographically distinct population showed varied degree of susceptibility to DHF which can be explained by the difference in frequency of genetic polymorphisms in the respected populations. These studies revealed very valuable information and insights on the mysteries of DHF pathogenesis and ascertain the hypothesis that HLA polymorphisms are important associate in altering susceptibility to DHF. These studies are important but have been limited in most cases by relatively small patient groups. Understanding the mechanism underlying the development of DHF is crucial for the development of novel strategies to improve patient management. These results could furnish as a valuable predictive tool to identify ethnically different individuals at risk and/or protection from severe forms of DENV infection and would provide valuable information for the design of future dengue vaccine and development of effective immunotherapies to control viral infections. The relatively sparse data available on different

HLA allele's relation with altered susceptibility exhibits a wide scope and necessity for detailed genetic studies in different ethnic groups in different countries on a larger number of patients.

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