Dengue HLA Associations in Jamaicans

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ABSTRACT

Background: *Polymorphisms in the human leukocyte antigen (HLA) genes might predispose certain individuals to dengue fever (DF) and the severe forms of the disease: dengue haemorrhagic fever/ dengue shock syndrome (DHF/DSS).*

Subjects and Method: *A DNA-based HLA typing method was used to determine the HLA class I and II alleles in 50 patients with dengue, including 45 cases of DF, 5 cases of DHF and 177 healthy individuals in Jamaica.*

Results: *HLA-A*24 and – DRβ5*01/02 were significantly associated with dengue infection while possession of HLA-A*23, – CW*04, -DQβ1*02, -DQβ1*03 and DQβ1*06 were protective. No other significant associations were found after correction for the number of alleles tested at each HLA-locus.*

Conclusion: *This is the first study to report a significant association with HLA-A*24 and DF although this allele is associated with DHF and DSS in Vietnamese patients. The other HLA associations observed in the Jamaican cohort also are different from those reported in other ethnic groups. Further studies which involve larger numbers of patients with DHF and explore functional aspects of HLA allelic associations with dengue in Jamaicans are necessary.*

Keywords: Alleles, cohort, dengue infection, ethnicity, human leukocyte antigen, immune response, polymorphisms

Asociaciones del HLA con el Dengue en los Jamaicanos

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RESUMEN

Antecedentes: *Los polimorfismos de los genes del antígeno leucocitario humano (HLA) podría predisponer a ciertos individuos a la fiebre de dengue (FD) y a las formas severas de esta enfermedad: la fiebre hemorrágica de dengue y el síndrome de choque por dengue (FHD/SCD).*

Sujetos y Método: *Se usó un método de tipificación HLA basado en el ADN con el propósito de determinar los alelos HLA clase I y II en 50 pacientes con dengue, incluyendo 45 casos de FD, 5 casos de FHD y 177 individuos saludables en Jamaica.*

Resultados: *HLA-A*24 y – DRβ5*01/02 estuvieron significativamente asociados con la infección de dengue en tanto que la posesión de HLA-A*23, -CW*04, -DQβ1*02, -DQβ1*03 y DQβ1*06 tenía carácter protector. No se halló ninguna otra asociación significativa tras la corrección en relación con el número de alelos probados en cada locus de HLA.*

Conclusión: *Este es el primer estudio que reporta una asociación significativa de HLA-A*24 y FD, aunque este alelo se halla asociado con FHD y SCD en pacientes vietnamitas. Las otras asociaciones observadas en la cohorte jamaicana son también diferentes de las que se reportan para otros grupos étnicos. Se requieren estudios ulteriores que comprendan grandes números de pacientes con FHD y exploren los aspectos funcionales de las asociaciones alélicas de HLA con el dengue en los jamaicanos.*

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Palabras claves: Alelos, cohorte, infección por dengue, etnicidad, antígeno leucocitario humano, respuesta inmunológica, polimorfismos.

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INTRODUCTION

Dengue occurs globally in the Tropics and Subtropics and is considered the most important arthropod-borne infection as it continues to affect several million persons with increasing morbidity and mortality (1, 2). Most persons infected with any one of the 4 serotypes of dengue virus (DENV-1-4) remain asymptomatic or present with a mild nonspecific febrile illness or classic dengue fever, an acute self-limited febrile illness. A smaller proportion of cases develop dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), the more severe life-threatening forms of disease, and about 5% of dengue cases result in death (1−4). Infection with any one dengue serotype provides complete immunity against that serotype and a partial immunity against other serotypes which might exacerbate secondary infections (3−5). Both cell mediated and humoral immunity contribute substantially to the pathogenesis of dengue although this is not well understood (3−5).

Susceptibility to dengue virus infection and the severity of the disease are believed to be multifactorial involving certain viral genetic, environmental, host genetic and vector related factors (4, 6, 7). Ethnicity appears to play a role in susceptibility and resistance to dengue highlighting the involvement of host genetic factors. While all age groups might be affected, some ethnic populations may be at lower risk of DHF (4, 8, 9).

The human leukocyte antigens (HLA) are cell surface molecules which are involved in antigen processing and presentation of antigenic peptides to T lymphocytes (4, 10). Therefore, polymorphisms in HLA and other immune response genes might be predisposing factors to DF and DHF (4, 11). The results of the limited number of dengue –HLA association studies implicate these genes in susceptibility to DF and DHF. The HLA-allelic associations reported for DF tend to be different from those reported for DHF suggesting that these may be separate clinical entities (4, 10−14). These studies have been limited by small cohort sizes and so far no single HLA allele has been consistently associated with dengue. There is a lack in functional studies to validate the involvement of the specific HLA polymorphisms in susceptibility and severity of dengue (4). This study was undertaken to investigate the HLA dengue associations in a Jamaican cohort of patients with dengue.

SUBJECTS AND METHODS

The study population comprised 50 consecutive unrelated patients (mean age 22 years, range 6 months − 64 years) who presented at the University Hospital of the West Indies during 2007 and 177 healthy blood donors. The serum was separated from 5−10 ml clotted blood samples, which had been collected from each patient and submitted to the microbiology laboratory for viral investigations, and stored at -70°C until tested. The patients' dengue status was confirmed by dengue IgG and IgM testing using commercially prepared enzyme-linked immunosorbent assay (ELISA) kits (Focus Diagnostics, Cypress CA), viral cultures in mosquito cells and RNA detection by reverse transcriptase-polymerase reaction (RT-PCR) assay as previously described (15, 16). A 3 ml EDTA blood specimen was collected from each control subject after informed consent was obtained. The peripheral blood mononuclear cells (PBMC) were separated and stored at -20°C until tested. Ethical approval was obtained for the study and clinical data were obtained from the patients' hospital records.

For HLA typing, the DNA was extracted from serum or PBMC using commercially prepared reagents (QIAamp DNA Blood Mini Kit; Qiagen, Germany) according to the manufacturer's instructions and stored at −20°C until tested. The HLA-A, -B, -C, -DRβ1, -DRβ3, -DR β4, -DR β5 and –DQβ1 alleles were determined using RELI™ SSO HLA-Typing Kits (Dynal/Invitrogen, Bromborough, Wirral, UK). The HLA-test procedure was based on three major processes including PCR amplification of exons 2 and 3 of the gene encoding the alpha chain of HLA class I and exon 2 of the gene encoding the alpha chain of the HLA class II molecule using biotin labelled primers, hybridization of the amplified products to an array of immobilized sequence specific oligonucleotide probes (SSOP) and detection of the probe-bound amplified product by colour formation. The manufacturer's instructions for the manual procedure were followed and the results of the assay analysed using the interpretation software and tables provided.

The HLA allele frequencies in the patient and control groups were compared by Chi-square or Fisher exact tests where appropriate. *P*-values were corrected for the number of comparisons made at each locus (p_C) by multiplying the number of alleles tested according to the Bonferonni inequality method (17, 18). Relative risks were calculated using Woolf's method (18, 19).

RESULTS

The prevalence of haemorrhagic, gastrointestinal and central nervous system (CNS) manifestations in the 50 patients with dengue is shown in Table 1. The suspected diagnosis was dengue haemorrhagic fever (DHF) in 5(10%) patients; 10 patients with DF (20%) had thrombocytopenia; 2(4%) had gastroenteritis and 1(2%) had viral encephalitis.

Table 1: Suspected clinical diagnosis, haemorrhagic and other manifestations in 50 dengue patients for HLA-typing*

Manifestation	Frequency (%)
Dengue fever	50 (100)
DHF	5 (10)
Thrombocytopenia	10 (20)
Gastroenteritis	2 (4)
Viral Encephalitis	1 (2)

* DHF = dengue haemorrhagic fever

Dengue virus infection was confirmed by serology in all but three cases which were confirmed by RNA testing. The infecting dengue serotypes isolated were DENV-1 (2 cases), DENV-2 (5 cases), DENV-4 (4 cases) and 40 patients were negative in the dengue RNA assay.

The comparative frequencies of HLA class I and class II alleles in dengue cases and controls are shown in Tables 2−6. As shown in Table 2, HLA-A* 24 was statistically

Table 2: Comparative HLA-A allele frequencies in dengue cases and healthy controls*

HLA-A allele	Frequency (%) Dengue cases n = 43	Controls n = 127	RR	*P*	P_C
A*01	3 (7)	17 (13)	0.5		
A*02	17 (40)	31 (24)	2.1		
A*03	11 (26)	17 (13)	2.4		
A*11	2 (5)	2 (2)	2.6		
A*23	1 (20)	40 (31)	0.05	0.0001	0.002
A*24	8 (19)	2 (2)	11.5	0.0001	0.002
A*25	0 (0)	1 (1)	0		
A*26	1 (2)	1 (1)	2		
A*29	5 (12)	4 (3)	4.4	0.05	
A*30	12 (28)	30 (23)	1.3		
A*31	0 (0)	1 (1)	0		
A*32	1 (2)	2 (2)	1		
A*33	10 (23)	8 (6)	4.7	0.01	
A*34	0 (0)	7 (6)	0		
A*36	0 (0)	8 (6)	0		
A*38	0 (0)	0 (0)	0		
A*43	0 (0)	0 (0)	0		
A*66	1 (2)	4 (3)	0.8		
A*68	2 (5)	23 (18)	0.2		
A*69	0 (0)	0 (0)	0		
A*74	1 (2)	16 (13)	0.1	0.025	
A*80	0 (0)	1 (1)	0		

*P_C = p-value corrected by multiplying the number of alleles tested for at each locus (22 alleles for HLA-A). Only significant P and P_C values are shown.

significantly increased in dengue cases (8/43, 19% *vs* 2/127, 2%, $\chi^2 = 27.8$, $p = 0.0001$, $p_C = 0.002$, RR, 11.5). Also HLA-A*29 (5/43, 12% *vs* 4/127, 3%, $\chi^2 = 4.1$, $p = 0.05$, $p_C = 0.22$, RR, 4.4) and HLA-A*33 (10/43, 22% *vs* 8/127, 6%, $\chi^2 = 6.3$, $p = 0.025$, $p_C = 0.55$, RR, 4.7) were statistically significantly increased in patients compared to controls, while HLA-A*74 (1/43, 2%, *vs* 16/127, 13%, $\chi^2 = 5.5$, $p = 0.025$, $p_C = 0.73$, RR, 0.14) was statistically significantly decreased but these differences became insignificant following correction for the number of alleles tested. The frequency of HLA- A*23 was statistically significantly decreased in dengue cases compared with control subjects (1/43, 2% *vs* 40/127, 31%, $\chi^2 = 12.3$, $p = 0.001$, $p_C = 0.02$, RR, 0.05). As shown in Table 3.

Table 3: Comparative HLA-B allele frequencies in dengue cases and healthy controls*

HLA-B allele	Frequency (%) Dengue cases (n = 50)	Controls (n = 127)	RR	*P*
B*07	15 (30)	23 (18)	1.9	
B*08	5 (10)	7 (6)	1.7	
B*13	2 (4)	3 (2)	2.0	
B*14	0 (0)	7 (7)	0	
B*15	11 (22)	26 (20)	1.1	
B*18	2 (4)	9 (7)	0.6	
B*27	3 (6)	1 (1)	6.3	
B*35	14 (28)	13 (10)	3.5	0.025
B*37	1 (2)	2 (2)	1	
B*38	1 (2)	1 (7)	0.27	
B*39	1 (2)	2 (2)	1	
B*40	4 (8)	4 (3)	2.8	
B*41	0 (0)	1 (1)	0	
B*42	0 (0)	0 (0)	0	
B*44	7 (14)	12 (9)	1.6	
B*45	2 (4)	5 (4)	1	
B*46	1 (2)	1 (1)	2.0	
B*48	0 (0)	0 (0)	0	
B*49	4 (8)	5 (4)	2.1	
B*50	0 (0)	0 (0)	0	
B*51	3 (6)	3 (2)	3.1	
B*52	3 (6)	3 (2)	3.1	
B*53	2 (4)	8 (6)	0.7	
B*55	0 (0)	0 (0)	0	
B*56	1 (2)	1 (1)	2	
B*57	5 (10)	6 (5)	2.1	
B*58	1 (2)	10 (8)	0.2	
B*78	0 (0)	1 (1)	0 (0)	
B*81	0 (0)	3 (2)	0 (0)	

*HLA-B35 was significantly increased in patients compared to controls but the p-value became insignificant after correction for the number of alleles tested (29 alleles for the HLA-B locus).

there was a statistically significant increase in HLA-B*35 in dengue cases compared to controls (14/50, 28% *vs* 13/127, 10%, $\chi^2 = 6.3$, $p = 0.025$, $p_C = 0.73$, RR, 3.5) however, the increase did not remain significant after correction for the number of alleles tested at this locus. The frequency of HLA-CW*04 was statistically significantly decreased in patients compared to controls (7/33, 21% *vs* 20/30, 65%, $\chi^2 = 11.1$, $p = 0.001$, $p_C = 0.015$, RR, 0.14) as shown in Table 4.

Concerning class II alleles (Tables 5−6), no significant differences were found in the frequencies of HLA-DRβ1 alleles in patients and controls. However HLA-DRβ5*01/02 (13/43, 38% *vs* 27/177, 15%, $\chi^2 = 3.9$, $p = 0.05$, $p_C = 0.05$, RR, 3.5) was statistically significantly increased (Table 5)

Table 4: Comparative HLA-CW allele frequencies in dengue cases and healthy controls*

HLA-CW allele	Patients (n = 33)	Controls (n = 30)	RR	*P*	P_C
CW*01	0 (0)	7 (22)	0		
CW*02	3 (9)	5 (17)	0.48		
CW*03	4 (12)	1 (4)	3.3		
CW*04	7 (21)	20 (65)	0.14	0.001	0.015
CW*05	0 (0)	0 (0)	0		
CW*06	1 (3)	3 (10)	0.27		
CW*07	17 (52)	7 (22)	3.8		
CW*08	1 (3)	0 (0)			
CW*12	0 (0)	0 (0)	0		
CW*13	0 (0)	0 (0)	0		
CW*14	5 (15)	1 (4)	4.2		
CW*15	2 (6)	0 (0)			
CW*16	5 (15)	3 (10)	1.6		
CW*17	0 (0)	0 (0)	0		
CW*18	0 (0)	1(4)	0		

*P_C = *p*-value corrected by multiplying the number of alleles tested for at each locus (15 alleles for HLA-CW). Only significant *P* and P_C values are shown.

Table 5: Comparative HLA-DRβ1, DRβ3, DRβ4 and DRβ5 allele frequencies in dengue cases and healthy controls

	Frequency (%)				
HLA	Patients (n = 43)	Controls (n = 177)	RR	*P*	P_C
DRβ1*01	6 (18)	16 (9)	2.2		
DRβ1*03	10 (29)	44 (25)	1.2		
DRβ1*04	3 (9)	15 (9)	1		
DRβ1*07	3 (9)	13 (7)	1.3		
DRβ1*08	2 (6)	9 (5)	1.2		
DRβ1*09	2 (7)	23 (13)	0.5		
DRβ1*10	1 (3)	10 (6)	0.5		
DRβ1*11/12	11 (27)	30 (17)	1.8		
DRβ1*13/14	11 (27)	65 (37)	0.6		
DRβ1*15/16	7 (23)	53 (30)	0.7		
DRβ3*01/02/03	21 (62)	72 (40)	2.5		
DRβ4*01/02/03	5 (15)	35 (20)	0.7		
DRβ5*01/02	13 (38)	27 (15)	3.5	0.05	0.05

*P_C = *p*-value corrected by multiplying the number of alleles tested for at each locus (10 alleles for DRβ1, and 1 each for DRβ3, DRβ4 and DRβ5). Only significant *P* and P_C values are shown.

while HLA-DQβ1*02 (4/50, 8% *vs* 35/90, 39%, $\chi^2 = 13.6$, $p = 0.0001$, $p_C = 0.0006$, RR, 0.14), HLA-DQβ1*03 (13/50, 26% *vs* 35/90, 39%, $\chi^2 = 9.1$, $p = 0.001$, $p_C = 0.006$, RR, 0.52) and HLA-DQβ1*06 (20/50, 40% *vs* 65/90, 73%, $\chi^2 = 14.0$, $p = 0.0001$, $p_C = 0.0006$, RR, 0.23) were statistically significantly decreased in patients compared to controls (Table 6).

Several other class I and II alleles conferred increased or decreased relative risk for dengue without achieving statistical significance (Tables 2−6). No HLA associations were found with any clinical manifestation of dengue. The significant enhancing and protective dengue HLA associations are summarized in Table 7.

Table 6: Comparative HLA-DQβ1 allele frequencies in dengue cases and healthy controls*

	Frequency %				
HLA-DQβ1 allele	Dengue case (n = 50)	Controls (n = 90)	RR	*P*	*Pc*
DQβ1*01	0 (0)	0 (0)	0		
DQβ1*02	4 (8)	35 (39)	0.4	0.0001	0.0006
DQβ1*03	13 (25)	35 (39)	0.52	0.001	0.006
DQβ1*04	1 (2)	10 (11)	0.17		
DQβ1*05	21 (40)	32 (36)	1.2		
DQβ1*06	20 (38)	65 (73)	0.23	0.0001	0.0006

*P_C = *p*-value corrected by multiplying the number of alleles tested for at each locus (6 alleles for DQβ1). Only significant *P* and P_C values are shown.

Table 7: Dengue HLA associations in Jamaicans*

HLA –Allele	Relative Risk conferred	Comment
Class I		
A*23	0.05	decreased/protective
A*24	11.5	increased/enhancing
CW*04	0.14	decreased/protective
Class II		
DRβ5*01/02	3.5	increased/enhancing
DQβ1*02	0.4	decreased/protective
DQβ1*03	0.52	decreased/protective
DQβ1*06	0.23	decreased/protective

*Includes HLA-class 1 and class II allele frequencies which diferred significantly in patients with dengue compared to healthy controls after *p*-values were corrected for the number of alleles tested at each HLA locus.

The HLA genotypes of the five patients with DHF are shown in Table 8.

Table 8: HLA-genotype in 5 dengue haemorrhagic fever (DHF) cases

DHF Case	HLA-
Patient 1	A*01 A*33 B* 15 B*03 CW*07 CW*03 DRβ1*03 DRβ1*13 DRβ3*01, DRβ3*02 DQβ*06
Patient 2	A*02 A*30 B*07 B*39 CW*02 CW*07 DRβ1*03 DRβ3*- DRβ4*- DQβ*06
Patient 3	A*02 A*68 B*35, CW* 07 CW*15 DRβ1- DRβ3-DRβ4- DR β5 DQβ *02 DQβ*06
Patient 4	A*29 A*23 B*35 CW-DRβ1*12 DRβ1*13 DRβ3*02 DQβ*06
Patient 5	A- B* 18 B*51 CW- DRβ1*03 DRβ1*11 DRβ3*01 DRβ3*02 DQβ1*03 DQβ1*06

DISCUSSION

This study is the first to report HLA associations with dengue in Jamaicans. Similar studies have been carried out in dengue patients in a few other dengue endemic populations including Thailand, Vietnam, Brazil, Mexico and Cuba (4, 10, 12, 13, 20−23). Some of these studies compared HLA-allele frequencies in DF and DHF/DSS cases, others compared DF with DHF cases and healthy controls. A small number of studies have compared HLA alleles in DF patients and healthy controls. These might be more appropriate for comparing with data from the present study which comprised unselected consecutive dengue cases including patients with DF with or without thrombocytopenia, a small number of DHF cases and healthy controls. This is also the first study to report a significant positive association between HLA-A*24 and DF although HLA-A*24 is associated with DHF and DSS in Vietnamese patients (11, 20). None of the five DHF patients in this Jamaican cohort possessed the HLA*24 allele. However, it might be worth mentioning, that these patients possessed other HLA-A polymorphisms which are associated with DHF/DSS in other populations. These include HLA-A1 and –A2 in Thais (13), HLA-A1 and –A29 in Cubans with DHF/DSS (4, 12, 24). In the present study, HLA-A*29 along with -A*33 were significantly increased in dengue cases before correction for the number of alleles tested. The HLA-A allelic association with DF reported in other populations is that between HLA-A2 and secondary DF (13). The protective association observed with HLA-A*23 in the Jamaican cohort has not been reported in other ethnic groups.

Certain HLA-B allele associations with secondary DF were observed in other populations such as HLA-B*15 in Thai and Cuban patients; -B*52 and -B*77 in Thai patients were either found in insignificant frequencies in the Jamaican cohort or were not observed (12, 13). The statistically significant increase which was observed in HLA-B*35 was of interest as this has not been reported in other populations but this association was not strong enough to withstand correction of the *p*-value in this cohort.

The significant protective association observed with HLA-CW *04 also has not been reported in other ethnic groups although HLA-CW polymorphisms were also evaluated in similar studies conducted in Cuba (12).

The dengue HLA-DRβ5*01/02 association was the only positive HLA-class II association observed. This along with the protective HLA-DQβ1*02, – DQβ1*03 and – DQβ1*06 associations have not been reported in any other ethnic groups. Despite its protective association, HLA-DQβ *06 was well represented in the five Jamaican patients with DHF. In the relatively few studies reported, HLA class II DR alleles have been most consistently associated with dengue severity or protection rather than susceptibility. For example, HLA-DRβ1*04 and -DRβ1*07 were protective for DF in Cubans and -DRβ1*09 in Vietnamese and Mexicans (12, 20, 22, 23). In contrast, positive associations were reported with HLA-DR1 and -DQ1 with DF in Brazilian patients (21).

The major limitation of the present study was the relatively small number of dengue patients which did not allow for stratification by disease severity or primary and secondary infection. A cohort comprising larger numbers of patients might have increased the statistical power of comparisons.

In summary, the results provide preliminary data on the dengue HLA allelic associations at eight HLA-class I and II loci, in Jamaican patients, which differ somewhat from those reported in other ethnic groups. This is in keeping with the complexity of HLA-disease associations and the fact that HLA genes are in linkage disequilibria with other HLA and non-HLA genes. Further studies involving larger groups of patients and exploring the functions of HLA-alleles which are positively associated with dengue in Jamaicans are indicated.

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