



In Silico Analysis of HLA Polymorphism of Dengue Virus Serotypes

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ARTICLE DETAILS

Article history:

Received 31 December 2015

Accepted 12 January 2016

Available online 03 February 2016

Keywords:

Alleles

Dengue Serotypes

Dengue Virus

E-Proteins

Genetic Polymorphism

ABSTRACT

Differences in host susceptibility to dengue infection and disease severity cannot be attributed solely to the virus virulence. On the other hand variations in immune response are often associated with polymorphism in the human genome alleles. Identified peptide vaccine candidates of E proteins of Dengue virus (DENV) serotypes were used to predict their binding abilities with 798 Human leukocyte antigen (HLA) alleles in the population and their index score was calculated. Out of 20 HLA-A alleles and 29 HLA-B alleles used for the present study, 14 alleles of HLA-A were positive indicating the resistance and only 6 of them were negative indicating susceptible nature of the alleles, and all the 29 HLA-B alleles were positive for DENV-1. The vaccine candidates of DENV-2, DENV-3 and DENV-4 also showed more positive and the resistant against dengue virus infection.

1. Introduction

Genetic polymorphisms of human leukocyte antigens (HLA) in dengue infection have been reported by few studies. Human leukocyte antigens (HLA) are highly polymorphic group of genes located on chromosome 6 of human MHC [1]. The development of dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) has been associated with HLA alleles [2]. Several studies have reported the association of various identified alleles of HLA class I and class II with the susceptibility or protection to DHF/DSS. Enhanced or diminished immune response of HLA class I and II in the course of dengue viral infection have been reported in ethnically and geographically distinct populations [3]. 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. 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 [4]. In the present study various HLA alleles in the population have been analyzed for their ability to induce dengue or protect from dengue infection.

2. Experimental Methods

Amino acid sequences of E proteins of dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4 were retrieved from NCBI [5]. The sequences were converted into FASTA format. Each full length protein sequence was subjected to BCPreds analysis for the prediction of B-cell epitopes, and the predicted B-cell epitopes (20 mers) having a BCPreds cutoff score >0.8 were selected [6]. The selected B-cell epitopes were subjected to predict T-cell epitopes using Propred-I (for MHC class I) [7] and Propred [8] (for MHC class II) servers were used to identify common epitopes that bind to the MHC class I and II molecules. Inhibitory concentration (IC₅₀) of selected T-cell epitope was calculated using MHCpred v.2 server [9]. Resistance and susceptibility of HLA alleles was

analyzed through T-epitope Designer [10]. The server screen peptides for 798 HLA alleles. The detailed method has been represented in Fig. 1.

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1      11      21      31      41      51      60
MRCVIGISGRDFEVLGSGATWVDVLEHSGCVTTMAKDKPTLDIELLKTEVTNPAVLRKLC 60
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
IEAKISNTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWNGCGLFGKGSLLTCAKPK 120
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
CVTKLEGKIVQYENLKYSVIVTVHTGDQHQVGNSETEHGTATITPQAPTEIQLTDYGA 180
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
LTLDCSRPTGLDFNEMVLLTMKEKSWLVHKQWFLDPLPWTSGASTSQRTWNRQDLLVTF 240
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
KTAHAKKQEVVVLGSGQEGAMHTALTGATEIQTSGTTTIFAGHLKCRCLKMDKLTLMGMSYV 300
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
MCTGSPFLEKEVAETQHGTVLVQIKYEGCTDAPCKIPFSTQDEKGVTONGRILITANPIVTD 360
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
KEKPNVIEAEPPFGESYITVIGAGEKALKLSWFKGSSIGKMEATARGARRMAILGDTAW 420
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
DFGSIQGVFTSVGKLVHQIFGTAYGVLP SGVSWTMKIGIGVLLTWGLNRSRSTLSMTCI 480
..... EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
AVGLVTLYLGMVQA 495

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Fig. 1 Location of B-cell epitope on whole antigen of DENV-1

3. Results and Discussion

The role of HLA alleles in determining resistance, susceptibility or the severity of acute viral infections is known. 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.

In the present study, the whole antigens of E-proteins of four dengue virus serotypes were subjected to 20-mer B-cell epitope prediction (Fig. 2). From the predicted B-cell epitopes, 9-mer T-cell epitopes were predicted through MHC class I and II binding (Figs. 3 and 4). Inhibitory ability of the T-cell epitopes was identified using MHCpreds (Fig. 5). Identified peptide vaccine candidates (T-cell epitope) of E proteins of DENV serotypes were used to predict their binding abilities with 798 HLA alleles using T-Epitope Designer (Figs. 6 and 7). From the predicted HLA alleles A and B alleles of the population related to dengue disease were identified for their susceptible or resistant nature. Based on the prediction, some of the HLA alleles were resistant to dengue while others were susceptible. Thus the virulence of the disease is based on the virulence of the virus and the dengue related HLA alleles in the population.

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HLA-A1	DKPTLDIELLKTEVTNP
HLA-A2	DKPTLDIELLKTEVTNP
HLA-A*0201	DKPTLDIELLKTEVTNP
HLA-A*0205	DKPTLDIELLKTEVTNP
HLA-A*1101	DKPTLDIELLKTEVTNP
HLA-A24	DKPTLDIELLKTEVTNP
HLA-A3	DKPTLDIELLKTEVTNP
HLA-A*3101	DKPTLDIELLKTEVTNP
HLA-A*3302	DKPTLDIELLKTEVTNP
HLA-A68.1	DKPTLDIELLKTEVTNP
HLA-A20 Cattle	DKPTLDIELLKTEVTNP
HLA-A2.1	DKPTLDIELLKTEVTNP
HLA-B14	DKPTLDIELLKTEVTNP
HLA-B*2702	DKPTLDIELLKTEVTNP
HLA-B*2705	DKPTLDIELLKTEVTNP
HLA-B*3501	DKPTLDIELLKTEVTNP
HLA-B*3701	DKPTLDIELLKTEVTNP
HLA-B*3801	DKPTLDIELLKTEVTNP
HLA-B*3901	DKPTLDIELLKTEVTNP
HLA-B*3902	DKPTLDIELLKTEVTNP
HLA-B40	DKPTLDIELLKTEVTNP
HLA-B*4403	DKPTLDIELLKTEVTNP
HLA-B*5101	DKPTLDIELLKTEVTNP
HLA-B*5102	DKPTLDIELLKTEVTNP
HLA-B*5103	DKPTLDIELLKTEVTNP
HLA-B*5201	DKPTLDIELLKTEVTNP
HLA-B*5301	DKPTLDIELLKTEVTNP
HLA-B*5401	DKPTLDIELLKTEVTNP
HLA-B*51	DKPTLDIELLKTEVTNP
HLA-B*5801	DKPTLDIELLKTEVTNP
HLA-B60	DKPTLDIELLKTEVTNP
HLA-B61	DKPTLDIELLKTEVTNP
HLA-B62	DKPTLDIELLKTEVTNP
HLA-B7	DKPTLDIELLKTEVTNP

Fig. 2 T-cell epitope prediction from B-cell epitope of DENV-1 after binding with MHC-I alleles

DRB1_0101	DKPTLDIELLKTEVTNP
DRB1_0102	DKPTLDIELLKTEVTNP
DRB1_0301	DKPTLDIELLKTEVTNP
DRB1_0305	DKPTLDIELLKTEVTNP
DRB1_0306	DKPTLDIELLKTEVTNP
DRB1_0307	DKPTLDIELLKTEVTNP
DRB1_0308	DKPTLDIELLKTEVTNP
DRB1_0309	DKPTLDIELLKTEVTNP
DRB1_0311	DKPTLDIELLKTEVTNP
DRB1_0401	DKPTLDIELLKTEVTNP
DRB1_0402	DKPTLDIELLKTEVTNP
DRB1_0404	DKPTLDIELLKTEVTNP
DRB1_0405	DKPTLDIELLKTEVTNP
DRB1_0408	DKPTLDIELLKTEVTNP
DRB1_0410	DKPTLDIELLKTEVTNP
DRB1_0421	DKPTLDIELLKTEVTNP
DRB1_0423	DKPTLDIELLKTEVTNP
DRB1_0426	DKPTLDIELLKTEVTNP
DRB1_0701	DKPTLDIELLKTEVTNP
DRB1_0703	DKPTLDIELLKTEVTNP
DRB1_0801	DKPTLDIELLKTEVTNP
DRB1_0802	DKPTLDIELLKTEVTNP
DRB1_0804	DKPTLDIELLKTEVTNP
DRB1_0806	DKPTLDIELLKTEVTNP
DRB1_0813	DKPTLDIELLKTEVTNP
DRB1_0817	DKPTLDIELLKTEVTNP
DRB1_1101	DKPTLDIELLKTEVTNP
DRB1_1102	DKPTLDIELLKTEVTNP
DRB1_1104	DKPTLDIELLKTEVTNP
DRB1_1106	DKPTLDIELLKTEVTNP

Fig. 3 T-cell epitope prediction from B-cell epitope of DENV-1 after binding with MHC-II alleles

The HLA allele used in the test is: DRB101				
The query sequence				
LKTEVTNPA				
Amino acid groups	Predicted -log(Cp (d))	Predicted IC ₅₀ Value (nM)	Confidence of prediction (Max = 1)	
LKTEVTNPA	7.25	56.23	0.89	

Fig. 4 Inhibitory ability of T-cell epitope (LKTEVTNPA) of DENV-1 using MHCpred

T-epitope Designer

Limited by number of k-top score results: 10

Summary	
HLA molecule selected	B*1568
Total number of 9-mer subsequences	1
Number of results (based on limit options)	1
Number of (+) scores	1
Number of (-) scores	0
Average score	1484.41
Maximum score	1484.41
Minimum score	1484.41

Scoring Results				
Rank	Start position	Allele	9-mer peptide	Score
1	1	B*1568	LKTEVTNPA	1484.41

Fig. 5 Finding of resistance (+)score of HLA allele (HLA-B*1568) score using T-epitope designer

Limited by number of k-top score results: 10

Summary	
HLA molecule selected	A*0101
Total number of 9-mer subsequences	1
Number of results (based on limit options)	1
Number of (+) scores	0
Number of (-) scores	1
Average score	-457.61
Maximum score	-457.61
Minimum score	-457.61

Scoring Results				
Rank	Start position	Allele	9-mer peptide	Score
1	1	A*0101	LKTEVTNPA	-457.61

Fig. 6 Finding of susceptibility (-) score of HLA allele (HLA-B*1568) using T-epitope designer

For the present study 49 dengue related alleles in the population of different ethnics were selected. Among them 20 HLA-A alleles and 29 HLA-B alleles were used for the binding ability of the peptide vaccine candidate predicted in the present study. DENV1 peptide vaccine candidate was made to bind with the HLA-A and B alleles. Among the 20 HLA-A alleles 14 alleles were positive indicating the resistance by the population having those alleles and only 6 of them were negative indicating susceptible nature of the alleles. All the 29 HLA-B alleles were positive and resistant against dengue virus infection. The vaccine candidate of DENV-2 showed positive response to population with 18 out of the 20 HLA -A and 27 out of 29 HLA-B alleles in the population were positive and resistant to dengue virus infection. Only two alleles from HLA-A and B were alone negative and susceptible to the disease. The peptide vaccine of DENV-3 was also more resistant to dengue virus infection. In this study, only 2 HLA-A alleles were negative and susceptible to dengue disease remaining 18 HLA-A alleles and all the 29 HLA-B alleles were positive and resistant to dengue virus infection. The vaccine candidate for DENV-4 was more positive and the resistant to dengue virus infection. Among the 20 HLA-A alleles only 3 of them were negative and susceptible. Remaining 17 of the HLA-A alleles and all the HLA-B alleles were fully positive and resistant to the infection of dengue virus (Table 1). From the above study, the HLA-A and B alleles, related to susceptible and resistant nature, were mostly resistant to dengue virus indicating the successful nature of the peptide vaccine candidate of the E-proteins for dengue infection.

Differences in susceptibility to disease can be seen at the level of individuals and populations. Major susceptibility genes account for a significant proportion of the genetic contribution to disease susceptibility. HLA loci evolve very rapidly, as a result of selective pressure from pathogens, and polymorphisms in these loci have been associated with susceptibility to infectious diseases. The role of HLA alleles in determining resistance, susceptibility or the severity of acute viral infections is well established. Differences in host susceptibility to infectious disease and disease severity cannot be attributed solely to the virus virulence. Variations in immune response are associated with polymorphism in the human genome. At the population level, considerable differences in allele frequencies are found within different ethnic groups. The extreme polymorphism of HLA classes I and II genes has an influence on the biological function of their products. Most polymorphism results in significant amino acids changes in the protein molecule of HLA. Thus HLA class I and II molecules clearly play an important role in the host immune response to DENV.

Table 1 Identification of resistance and susceptible nature of E-proteins (T-cell epitopes) with selected dengue related HLA alleles in the population

Dengue HLA alleles	HLA (+ or -) Binding score				Total number of positive response with Dengue
	DENV-1 Response	DENV-2 Response	DENV-3 Response	DENV-4 Response	
A*0102	122.29 (+)	-67.26 (-)	309.29 (+)	473.25 (+)	3
A*0201	-322.81 (-)	724.35 (+)	659.65 (+)	23.73 (+)	3
A*0203	-333.60 (-)	935.90 (+)	697.35 (+)	-137.44 (-)	2
A*0207	-388.59 (-)	710.93 (+)	806.65 (+)	92.46 (+)	3
A*0301	54.58 (+)	1182.83 (+)	542.16 (+)	815.67 (+)	4
A*1101	291.92 (+)	533.97 (+)	255.21 (+)	620.65 (+)	4
A*2304	192.79 (+)	567.84 (+)	74.44 (+)	152.78 (+)	4
A*2402	106.71 (+)	-15.78 (-)	-203.76 (-)	-319.68 (-)	1
A*2501	54.99 (+)	578.04 (+)	639.00 (+)	388.38 (+)	4
A*2601	112.78 (+)	897.40 (+)	845.76 (+)	465.23 (+)	4
A*2901	512.82 (+)	1062.71 (+)	1109.08 (+)	979.60 (+)	4
A*3001	203.14 (+)	770.96 (+)	268.45 (+)	177.84 (+)	4
A*3108	535.21 (+)	807.99 (+)	174.85 (+)	207.56 (+)	4
A*3207	302.52 (+)	556.59 (+)	643.20 (+)	555.39 (+)	4
A*3301	-326.18 (-)	709.38 (+)	-203.90 (-)	-61.29 (-)	1
A*3401	575.69 (+)	1357.57 (+)	1078.57 (+)	299.10 (+)	4
A*3601	-204.44 (-)	209.40 (+)	84.55 (+)	759.88 (+)	3
A*6601	339.01 (+)	1176.66 (+)	957.75 (+)	481.29 (+)	4
A*6801	86.95 (+)	807.47 (+)	268.84 (+)	415.49 (+)	4
A*7401	-195.14 (-)	1089.65 (+)	150.58 (+)	758.9 (+)	3
B*0702	867.18 (+)	1047.36 (+)	1258.74 (+)	1358.2 (+)	4
B*0801	1488.58 (+)	428.53 (+)	1801.20 (+)	1774.2 (+)	4
B*1304	1089.94 (+)	649.19 (+)	1304.12 (+)	1718.6 (+)	4
B*1401	261.92 (+)	963.14 (+)	692.55 (+)	927.83 (+)	4
B*1501	877.38 (+)	1222.58 (+)	1254.90 (+)	1331.3 (+)	4
B*1801	686.84 (+)	901.31 (+)	1398.42 (+)	810.56 (+)	4
B*2702	1100.65 (+)	1493.00 (+)	1199.23 (+)	1041.8 (+)	4
B*3501	576.69 (+)	778.39 (+)	1246.47 (+)	1663.5 (+)	4
B*3702	903.91 (+)	1172.26 (+)	1310.93 (+)	1033.0 (+)	4
B*3802	451.08 (+)	329.26 (+)	823.18 (+)	644.80 (+)	4
B*3901	656.44 (+)	901.29 (+)	1199.10 (+)	1258.1 (+)	4
B*4001	820.51 (+)	763.68 (+)	805.99 (+)	540.72 (+)	4
B*4101	885.79 (+)	120.53 (+)	830.65 (+)	139.62 (+)	4
B*4201	943.54 (+)	480.24 (+)	1140.71 (+)	1662.8 (+)	4
B*4402	1251.67 (+)	395.44 (+)	1143.78 (+)	447.06 (+)	4
B*4506	1062.11 (+)	926.64 (+)	542.63 (+)	459.39 (+)	4
B*4601	1121.04 (+)	1401.90 (+)	1330.53 (+)	569.29 (+)	4
B*4701	652.87 (+)	1342 (+)	912.68 (+)	938.32 (+)	4
B*4902	1293.45 (+)	11397 (+)	1291.36 (+)	203.42 (+)	4
B*5001	1186.69 (+)	86.46 (+)	1150.87 (+)	279.82 (+)	4
B*5101	430.23 (+)	1 6.94 (+)	818.11 (+)	868.85 (+)	4
B*5201	754.26 (+)	279.93 (+)	988.70 (+)	480.74 (+)	4
B*5301	529.96 (+)	405.62 (+)	1062.56 (+)	1154.7 (+)	4
B*5402	583.24 (+)	1068.25 (+)	1136.58 (+)	1465.1 (+)	4
B*5501	669.10 (+)	1248.45 (+)	886.71 (+)	1584.5 (+)	4
B*5601	915.62 (+)	1100.92 (+)	861.25 (+)	1666.9 (+)	4
B*5701	320.40 (+)	-511.41 (-)	917.02 (+)	750.90 (+)	3
B*5801	408.18 (+)	-317.42 (-)	1331.00 (+)	300.91 (+)	3
B*7801	789.08 (+)	1163.42 (+)	1518.44 (+)	1914.7 (+)	4

Flaviviruses have evolved a set of strategies for interaction with the immune response. Interaction of virus with the innate and adaptive immune system inhibit the generation of the antiviral immune response resulting the generation of low affinity, self-reactive T-cells which clear very low virus load as part of the survival strategy of the dengue virus serotypes. This enables viral growth and immune escape leading to immunopathology caused by autoimmune cross reactive damage of uninfected high MHC complex [11].

In the preset investigation, identified peptide vaccine candidates of E proteins of DENV serotypes were used to predict their binding abilities to 798 HLA alleles in the population. From the predicted HLA alleles A and B, alleles of the population related to dengue disease were identified for their susceptible or resistant nature. Based on the prediction some of the HLA alleles were resistant to dengue while others were susceptible. For the present study 49 dengue related alleles in the population of different ethnics were selected. Among them 20 HLA-A alleles and 29 HLA-B alleles were used for the binding ability of the peptide vaccine candidate predicted in the present study. The following dengue related HLA alleles were selected to study the susceptible and resistant nature.

A*0102, A*0201, A*0203, A*0207, A*0301, A*1101, A*2304, A*2402, A*2501, A*2601, A*2901, A*3001, A*3108, A*3207, A*3301, A*3401, A*3601, A*6601, A*6801, A*7401, B*0702, B*0801, B*1304, B*1401, B*1501, B*1801, B*2702, B*3501, B*3702, B*3802, B*3901, B*4001, B*4101, B*4201, B*4402, B*4506, B*4601, B*4701, B*4902, B*5001, B*5101, B*5201, B*5301, B*5402, B*5501, B*5601, B*5701, B*5801 and B*7801

From the study, the HLA-A and B alleles, related to susceptible and resistant nature, were resistant to dengue virus indicating the successful nature of the peptide vaccine candidates from the E-proteins of dengue virus serotypes.

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. Increased expression of HLA class I and II molecules on infected cells has been reported for Flaviviruses, including DV infection. Polymorphisms in the HLA class I region gene are associated with DHF disease susceptibility.

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. Little is known of the role of HLA alleles or non-HLA alleles in determining resistance, susceptibility or the severity of acute viral infections. Recent discoveries regarding genetic associations in other viral infections may provide clues to understanding the development of end stage complications in dengue disease. There is 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 [12].

Polymorphism at the human leukocyte antigen (HLA) class I loci was significantly associated with DHF disease susceptibility, but polymorphism in the HLA-DRB1 was associated with protection. Amino acid peptides present in the poly-protein of the dengue 2 Jamaica strain, which are able to bind to the HLA class I and class II allotypes associated with susceptibility to or protection against the dengue clinical disease, respectively [13,14]. In a case-control study of 263 ethnic Thai patients infected with either DEN-1, -2, -3 or -4, detected HLA class I associations with secondary infections, but not in immunologically naive patients with primary infections. HLA-A*0203 was associated with the less severe DF, regardless of the secondary infecting virus serotype. By contrast, HLA-A*0207 was associated with susceptibility to the more severe DHF in patients with secondary DEN-1 and DEN-2 infections only. Conversely, HLA-B*51 was associated with the development of DHF in patients with secondary infections, and HLA-B*52 was associated with DF in patients with secondary DEN-1 and DEN-2 infections. Moreover, HLA-B44, B62, B76 and B77 also appeared to be protective against developing clinical disease after secondary dengue virus infection. These results confirm that classical HLA class I alleles are associated with the clinical outcome of exposure to dengue virus [15].

Lan et al [16] investigated the HLA-A, -B (class I), and -DRB1 (class II) polymorphism in Vietnamese children with different severity (DHF/DSS) by a hospital-based case-control study. The study showed persons carrying HLA-A*2402/03/10 are about 2 times more likely to have severe dengue infection than others. On the other hand, HLA-DRB1*0901 persons are less likely to develop DSS with DEN-2 virus infection. These results clearly demonstrated that HLA controlled the susceptibility to severe forms of DV infection.

Biological markers are very useful in clear cut diagnosis of identifying the nature of diseases. In dengue, biological markers are useful in determining the disease nature. On the other hand genetic markers are very useful in determining the susceptibility or protective ability of the infected human population. Many research groups are attempting to assess a variety of potential biomarkers associated with dengue disease. Non- structural proteins-1 (NS-1) levels in the plasma levels have been shown to be correlated with disease severity and therefore NS-1 can be considered as a potential biomarker [17-19]. Numerous studies have investigated associations between altered levels circulating cytokines/chemokines and complement activation markers with dengue severity [20].

Markers of endothelial activation like reduced levels of angiotensin-1, increased level of angiotensin-2 increased levels of circulating endothelial cells and raised circulating heparin sulphate levels during the early phase of dengue. Recently, Yacoub and Willis [21] while discussing about the predicting markers for dengue, they have discussed the potential of viral, immunological and vascular biomarkers.

The human leukocyte antigen alleles have been implicated as probable genetic markers in predicting the susceptibility and/or protection to severe manifestations of dengue virus (DENV) infection. In the total population, HLA-B*53 probably involve in disease susceptibility, while the HLA-A*03 and HLA-B*18 may confer protection from progression to

severe disease; (2) In the Malay population, HLA-B*13 and B*18 are probably associated in disease susceptibility and protection, respectively [22].

Large case control gene association studies have been performed on cohorts of dengue virus (DENV) infected patients identified in mainland Southeast Asia, South Asia and the Caribbean. In ethnic Thais with secondary infections a variety of HLA class I alleles (HLA-A*0203, *0207, *A11, B*15, B*44, B*46, B*48, B*51, B*52), DCSIGN promoter polymorphisms and the AB blood group, independently associate with either susceptibility or resistance to dengue fever (DF) and the more severe dengue hemorrhagic fever (DHF). There is also evidence that some HLA associations with disease severity correlate with the DENV serotype inducing secondary infections. Taken together, there is now evidence that allelic variants of multiple gene loci involved in both acquired and innate immune responses contribute significantly to DENV disease outcome and severity [23].

Malavige et al [22] conducted studies to identify possible HLA- class I and class II alleles, which increase the risk of developing DHF/DSS during primary and secondary dengue in the Sri Lankan population. They found that HLA-A*31 and DRB1*08 were significantly associated with susceptibility to DSS when infected with the dengue virus, during secondary dengue infection

Epitopes from all available full length sequences of dengue fever virus (DENV) restricted by Human Leukocyte Antigen class I (HLA-I) alleles covering 12 HLA-I supertypes were predicted. The selected predicted epitopes were synthesized and approximately 75% were found to bind the predicted restricting HLA molecule with an affinity. The immunogenicity of 25 HLA-A*0201, 28 HLA-A*2402 and 28 HLA-B*0702 binding peptides was tested in three HLA-transgenic mice models and led to the identification of 17 HLA-A*0201, 4 HLA-A*2402 and 4 HLA-B*0702 immunogenic peptides. The immunogenic peptides bound HLA significantly stronger than the non-immunogenic peptides. All three peptides that elicited responses had an HLA binding affinity of 2 nM or less. The results indicate the importance of the strength of HLA binding in shaping the immune response [25].

Monteiro et al [26] identified HLA-A alleles in 67 patients with dengue fever and 42 with DHF in Brazil. Statistical analysis revealed an association between the HLA-A*01 allele and DHF, while analysis of the HLA-A*31 allele suggested a potential protective role in DHF. This study provides evidence that HLA class I alleles might be important risk factors for DHF in Brazilian patients.

HLA-B*44 was found to be associated with increased susceptibility to DHF in response to DENV-3 infection. In addition, HLA-B*07 and HLA-DR*13 were found to be associated with resistance to secondary dengue infection by DENV-3. The above results suggest that HLA-B*44 supertype alleles and their respective T-cell responses might be involved in susceptibility to severe dengue infections, whereas the HLA-B*07 supertype alleles and DR*13 might be involved in cross-dengue serotype immunity [27].

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-A0203 was associated with the less severe DF, regardless of the secondary infecting virus serotype, and HLAB 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 [16].

Loke et al [26] 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 HLADR 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 [27].

Polymorphisms in the HLA class I region gene are associated with DHF disease susceptibility. Chiewsilp et al [28] 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 [26]. Paradoa Perez et al [29] 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-A0207 is associated with susceptibility to the more severe DHF in patients with secondary DV-1 and DV-2 infections only. Conversely, HLA-B51 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 [14].

4. Conclusion

Identified peptide vaccine candidates of E proteins of DENV serotypes were used to predict their binding abilities with 798 HLA alleles in the population and their index score was calculated. Out of 20 HLA-A alleles and 29 HLA-B alleles used for the present study, 14 alleles of HLA-A were positive indicating the resistance and only 6 of them were negative indicating susceptible nature of the alleles, and all the 29 HLA-B alleles were positive for DENV-1. The vaccine candidate of DENV-2 showed positive response to population with 18 out of the 20 HLA -A and 27 out of 29 HLA-B alleles in the population were positive and resistant to dengue virus infection. The peptide vaccine of DENV-3 was also more resistant to dengue virus infection. In this study, only 2 HLA-A alleles were negative and susceptible to dengue disease remaining 18 HLA-A alleles and all the 29 HLA-B alleles were positive and resistant to dengue virus infection. The vaccine candidate for DENV-4 was more positive and the resistant to dengue virus infection. Among the 20 HLA-A alleles only 3 of them were negative and susceptible. Remaining 17 of the HLA-A alleles and all the HLA-B alleles were fully positive and resistant to the infection of dengue virus.

Acknowledgement

The authors are grateful to the UGC Major Research project, New Delhi, India (MRP R.No: 41-472/2012(SR)) for providing fund for this project. We specially express our thanks to the management of A.V.V.M. Sri Pushpam College (Autonomous), Poondi, for providing them necessary facilities and support to carry out this work.

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