Association between HLA-DRB1*01 and HLA-Cw*08 and Outcome Following HTLV-I Infection

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ABSTRACT

Background: Human T cell lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is an inflammatory disease which occurs in less than 2% of HTLV-I -infected individuals. High proviral load, high HTLV-I-specific $CD8^+$ cytotoxic T lymphocyte frequency (CTL) and host genetic factors such as HLA all appear to be associated with HTLV-I infection. Previous studies have shown that HLA-DRB1*01 increases the risk of HAM/TSP in Japanese HTLV-I infected individuals. **Objective:** To investigate the association between HLA class II DRB1 alleles and HLA class I alleles (HLA-Cw*08, B54, A*02 and A-30) in HTLV-I infected individuals in Mashhad. **Methods:** Here we determined the frequency of HLA class II DRB1, using INNO-LIPA reverse hybridization line probe assay, and HLA class I alleles (HLA-Cw*08,B54, A*02 and A-30) by PCR-SSCP method in healthy controls, HAM/TSP patients and HTLV-I infected individuals born and resident in Mashhad. **Results:** The frequency of HLA-DRB1*01 alleles in this population was different from other areas of Iran. The frequency of HLA-DRB1*01 was significantly increased in HAM/TSP patients compared with carriers (p 0.028; OR=9.4). The frequency of HLA-Cw*08 was also significantly increased in HAM/TSP patients compared with controls (p=0.03; OR=13.5). **Conclusion:** Our results may suggest that possession of HLA-DRB1*01 increases the risk of HAM/TSP in HTLV-I-infected individuals and HLA-Cw*08 correlates with low CTL immune response in HAM/TSP patients.

Keywords: HLA, HTLV-I, HAM/TSP

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INTRODUCTION

HTLV-I induces two main types of diseases in humans; adult T cell leukaemia (ATL) and HAM/TSP (1, 2). HAM/TSP is a chronic degenerative disease which occurs in HTLV-I endemic areas. HTLV-I is endemic in northeast Iran (Mashhad) and the prevalence of HTLV-I infection is estimated to be 2-3% in the whole population and 0.7% in blood donors (3, 4).

Host genetic factors are believed to be important in determining whether an individual will be susceptible or resistant to particular infectious diseases (5). Genes within the HLA complex have been widely studied in this context as they are important factors involved in both the specificity and regulation of immune responses. The interaction between MHC-peptide complex and TCR determines the specific pattern of the immune response against the peptide. Viral peptides, which do not bind tightly to HLA molecules, are not recognised by CTL (6).

MHC class I and class II polymorphisms which reside across the peptide binding site are responsible for affecting antigen binding, the specificity of recognition by T cells and the response to particular foreign antigens (7, 8). Both MHC class I and class II molecules are likely to be important in determining the recognition of HTLV-I peptides and for the generation of an effective immune response. The predominant CTL responses against Tax protein, which is essential for viral transcription, can be restricted by a wide variety of MHC class I molecules in HAM/TSP patients and in HTLV-I carriers. Studies have suggested that no single MHC class I allele is associated with HAM/TSP (9, 10). Previous HLA studies in HTLV-I-infected individuals in Japan indicated that expression of HLA-A*02 and HLA Cw*08 alleles reduced the proviral load of HTLV-1 and consequently the risk of HAM/TSP. In contrast the expression of HLA-B*5401 increased the proviral load and the risk of HAM/TSP in HTLV-I carriers, and expression of HLA-DRB1*0101 predisposed asymptomatic carriers to develop HAM/TSP in the absence of HLA-A*02. (11- 13). A similar study in Iranian HTLV-I infected individuals in the cities of Mashhad and Neishabour revealed that HLA-DRB*0101 is associated with disease susceptibility in the absence of HLA-A*02 (14).

The aim of this study was to examine the frequency of HLA class I alleles (HLA-A*02, HLA-A*30, HLA-Cw*08 and HLA-B*54) and HLA-DRB1 alleles in HTLV-I-infected individuals and healthy controls resident in Mashhad.

MATERIALS AND METHODS

Study Population. All subjects in this study were living in Mashhad. For analysis of HLA-DRB1 alleles, 72 healthy controls, 36 patients with HAM/TSP and 34 randomly selected HTLV-I carriers participated in this study. Due to rareness of the disease, the sample size of HTLV-I infected individuals did not attain the same size as the healthy controls. For HLA class I the study population included 91 healthy controls, 71 HTLV-I carriers and 61 patients with HAM/TSP. All patients fulfilled established criteria for HAM/TSP (15). The epidemiological and clinical features of HAM/TSP patients have been reported elsewhere (16). All of the HAM/TSP patients, HTLV-I carriers and healthy controls had the same ethnic background. The study protocol was approved by the ethics committee of Ghaem Hospital, Mashhad University of Medical Sciences and

Mashhad blood transfusion center. After receiving informed consent, blood was obtained from all cases and controls.

HLA Genotyping. DNA was extracted from 5 ml EDTA anti coagulated whole blood samples using a standard phenol-chloroform method. HLA-DRB1 alleles were determined by a commercial method (INNOLiPA DRB, Innogenetics, Belgium). PCR using sequence specific primers (PCR-SSP) was performed to determine HLA-A*02, A*30, Cw*08 and B*54 status. Previous studies in Japanese HTLV-I infected individuals had shown that these alleles are associated with HTLV-I infection, thus we selected these alleles to examine their relationship with HTLV-I infection in HTLV-I infected individuals born and resident in Mashhad. The method and the design of amplification primers were based on the procedures of Bunce et al (17).

Statistical Analysis. Differences in HLA allele frequencies among HAM/TSP patients, HTLV-I carriers and healthy controls were performed using STATA version 8 (STATA Corp.). The level of statistical differences in HLA allele frequencies between groups was established using the Fisher Exact test. Strength of association of alleles was examined by calculation of odds ratios (OR) with a confidence interval (CI) of 95%. A p value of less than 0.05 was considered to be significant.

RESULTS

Phenotype Distribution of HLA-DRB1 in HTLV-I Infection. HLA-DRB1 phenotype frequencies were determined in healthy controls, HAM/TSP patients and HTLV-I carriers (Table 1). The frequency of the HLA-DRB1*01 allele was significantly increased in HAM/TSP patients (22.2%) compared with carriers (2.9%) (p=0.028; OR=9.4 [95% CI: 1.11-430.68]). HLA-DRB1*01 was also more frequent in HAM/TSP patients than in controls (11.2%), however, the difference was not significant. The frequency of HLA-DRB1*16 was non-significantly higher in HAM/TSP patients compared with carriers (11.1% vs. 2.9%).

Table 1. HLA-DRB1 phenotype frequencies in healthy controls, HTLV-I carriers and HAM/TSP patients

HLA-DRB1	Controls n=72		HAM/TSP n=36		HTLV-I Carriers n=34	
	n	%	n	%	n	%
*01	8	11.1	8	22.2[a]	1	2.9[a]
*15	13	18.1	6	13.9	6	17.6
*16	6	8.3	4	11.1	1	2.9
*03	12	16.7	2	5.6	6	17.6
*04	19	26.4	10	27.8	7	20.6
*11	23	31.9	12	33.3	11	32.3
*12	3	4.2	0	0	0	0
*13	25	34.7	12	33.31	12	35.3
*14	5	6.9	1	2.8[b]	6	17.6[b]
*07	11	15.3	8	22.2	5	14.7
*08	1	1.4	2	5.6	2	5.9
*09	4	5.6	0	0	2	5.9
*10	3	4.2	3	8.3	2	5.9

a: HAM/TSP vs carriers (p=0.028, OR=9.4, 95% CI: 1.11-430.68).
b: HAM/TSP vs carriers (p=0.05, OR=7.5, 95% CI: 0.8-354.5).

The HLA-DRB1*14 allele was more frequent in HTLV-I carriers (17.6%) compared with both controls (6.9%) and HAM/TSP patients (2.8%), however no significant difference was observed between HLA-DRB1*14 in HAM/TSP patients and controls (p>0.05), but a trend towards a difference was observed between HTLV-I carriers and HAM/TSP patients (p 0.05). Although, HLA-DRB1*03 was at a lower frequency in HAM/TSP patients (5.6%) compared with carriers (17.6%) and controls (16.7%), there was no significant difference among groups (p<0.05).

HLA-A*02, A*30, Cw*08 and B*54 Distribution in HTLV-I Infection. Table 2 shows the frequency of HLA-A*02, A*30, Cw*8 and B*54 in healthy controls, HAM/TSP patients and HTLV-I carriers. The frequency of HLA-A*02 was similar between all of the three groups; controls (42.2%), HTLV-I carriers (31.2%) and HAM/TSP patients (39%). There was no statistically significant differences in HLA-A*02 frequency among groups. A reduction in HLA-A*30 frequency was observed in HAM/TSP (9.4%) compared with carriers (18.2%) and controls (20.5%) (Table 2), but this did not reach a level of significance.

Table 2. Frequency of HLA-A*02, A*30, Cw*08 and B*54 in healthy controls, HAM/TSP patients and HTLV-I carriers

Type of HLA	Controls n=90		HAM/TSP n=64		Carriers n=77	
	n	%	n	%	n	%
HLA-A*02	38	42.2	25	39	24	31.2
HLA-*A30	18	20	6	9.4	14	18.2
HLA-Cw*08	5	5.6	11	17.2*	8	10.4
HLA-*B54	0	0	0	0	0	0

a: HAM/TSP vs. controls (p=0.03, OR=3.5, 95% CI: 1.05-13.5).

The frequency of HLA-Cw*08 was significantly increased in HAM/TSP patients (17.2%) compared with controls (5.6%) (p 0.03; OR; 3.5, [95% CI: 1.05-13.5]). No individuals with positive HLA-B*54 were identified in either the normal population or in HAM/TSP patients and HTLV-I carriers (Table 2).

DISCUSSION

The concept of MHC restriction can provide an explanation for how self/non self recognition is regulated by the immune system (18). The level of response to particular foreign antigen is largely determined by the affinity of MHC binding and thus is clearly influenced by MHC polymorphism. Both HLA class I and class II antigens are involved in the recognition of HTLV-I peptides. The predominant CTL response against Tax can be restricted by a wide variety of MHC class I molecules in HAM/TSP and in HTLV-I carriers (19, 20).

In this study we demonstrated that possession of HLA-DRB1*01 increased the risk of HAM/TSP in HTLV-I-infected individuals. Consistent with our results, it has been reported that the frequencies of HLA-DRB1*01 and DRB1*0803 are significantly higher in HAM/TSP patients than in HTLV-I carriers in Japan (21). HLA-DRB1*01 is also associated with a lower proviral load in HAM/TSP patients compared with HTLV-I carriers. However, this susceptibility has not been observed in HLA-A*02 positive HAM/TSP patients (12). This association was not confirmed in a different Japanese population (22). In a study with a group of HTLV-I-infected individuals from Mashhad

and Neishabour, it was previously shown that HLA-DRB1*01 allele increases the risk of HAM/TSP in HTLV-I carriers (14). We have also confirmed this result in our study of HTLV-I-infected individuals who were born and lived in Mashhad. This may suggest that there is no difference in the ethnic background of people in these two HTLV-I endemic cities. However, the frequencies of HLA-DRB1 alleles in north east Iran was different from those of south west Iran (14, 23).

Analysis of HLA class II antigens in Jamaican HAM/TSP patients, HTLV-I carriers and ATL patients identified that the frequencies of HLA-DRB1*1501 and DRB1*1101 were significantly greater in HTLV-I carriers and ATL patients than in those having HAM/TSP (24). Although, our results do not support their data, but previous observations in Jewish HAM/TSP patients from Mashhad showed that HLA-DRB1*DQB1* (1502-0601 and 1201-0301) is more frequent in these patients (24).

The existence of an HLA class II association with HTLV-I infection may help explain the pathogenesis of HAM/TSP. Immunological evidence has demonstrated that an association between some HLA alleles such as DRB1*01 may contribute to disease by presenting exogenous antigenic peptides to $CD4^+$ T cells. It appears that $CD4^+$ T cells may be involved in the pathogenesis of HAM/TSP through bystander damage to uninfected cells in the CNS (25, 26). The first hypervariable region of the HLA-DRB1*01 allele contains a glutamine at position 10 and lysine at position 12. Although these particular residues are not involved in direct contact with the peptide, they may contribute to the binding of adjacent residues such as tryptophan at position 9 (7, 27).

CTL responses have a critical role to play in HTLV-I infection. Recognition of the Tax11-19 peptide in the context of HLA class I by specific $CD8^+$ T cells is an important event in eliciting the CTL response against HTLV-I. Possession of HLA-A*02 and HLA-Cw*08 alleles among HTLV-I carriers reduces the proviral load and the risk of HAM/TSP in the Japanese population. In contrast HLA-B*54 not only increases the proviral load, but enhances the risk of HAM/TSP in HTLV-I carriers who express this allele (13). We showed that there is no significant association in HLA-A*02 frequencies among any of the groups. Although Jeffery et al. reported that HLA-A*02 is a protective allele in HTLV-I carries, Yashiki et al. showed no significant difference in observed HLA-A*02 frequencies between HAM/TSP patients and HTLV-I carriers in the same population. They concluded that this discrepancy might be attributed to either micro-heterogeneity in southern Japanese or to sampling variation (28).

We found increased frequency of HLA-A*30 in healthy controls and HTLV-I carriers, though not statistically significant. It is likely that low HLA-A*30 frequency in HAM/TSP patients may be associated with poor CTL responses, because this allele presents the HTLV-I pol antigen (29). HAM/TS patients showed a significantly higher frequency of HLA-Cw*08 compared to healthy controls, but not with HTLV-I carriers. Although the expression of HLA-Cw*08 on the surface of the cells is lower than HLA-A and HLA-B, the role of HLA-Cw*08 restricted epitopes have been identified in viral infection and tumour resistance. Such alleles are able to present peptides derived from the gp 120 protein of HIV or melanoma antigens, and may contribute to high immune responses (30, 31). There is no evidence for HLA-Cw*08 HTLV-I specific $CD8^+$ CTL in patients with HTLV-I infection, but it is suggested that this allele is independently associated with low HTLV-I proviral load, thus conferring protection to HTLV-I carriers against developing HAM/TSP (11). In contrast, a high frequency of this allele is reported in an ATL population in Japan, indicating that HLA-Cw*08 is associated with low CTL immune responses in ATL patients (28). Our results may suggest that HLA-Cw*08 is

correlated with low CTL immune response in patients with HAM/TSP.
HLA-B*54 in HTLV-I infection is a known risk factor within HTLV-I carriers for the development of HAM/TSP. The absence of HLA-B*54 in our population in this study again strongly raises the possibility that the association between a complex disease and a single HLA allele or HLA haplotype depends on ethnic background.
The discrepancies between our data and those from Japanese and Jamaican populations may be due to ethnic differences. Other factors such as geographical and environmental differences should be taken into account. Further studies with a large population of patients with HTLV-I infection in Mashhad are needed. Typing of HLA class I (A, B and C) and HLA class II will give informative data which may determine HLA haplotypes in HAM/TSP patients in north east Iran.

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