

Association of HLA-B Alleles With Human Immunodeficiency Virus Type 1 Infection in the Yi Ethnic Group in Sichuan Province¹

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Objective To determine the distribution of HLA-B alleles in the Chinese Yi ethnic group and its association with HIV infection. **Methods** One hundred and six unrelated healthy HIV negative and 73 HIV positive Chinese Yi ethnic individuals were typed by PCR-SSP. **Results** The frequency of alleles B*07, B*35, and B*46 were increased in HIV-1-positive subjects, whereas the alleles B*55, B*44 and B*78 were absent in the HIV-infected persons studied. The B*46 allele was present in a significantly higher gene frequency among HIV-1-positive individuals ($P=0.02$, $OR=3.32$, 95% $CI=1.13-9.78$) compared with control subjects. **Conclusion** HLA-B*46 may be associated with its susceptibility to HIV-1 infections.

Key words: HIV infections; HLA-B alleles; Association

INTRODUCTION

AIDS, a severe immunodeficiency syndrome, is caused by human immunodeficiency virus type 1 (HIV-1). It has caused more deaths than any other disease epidemic in the recorded history^[1]. As is the case for all infectious diseases, the level of susceptibility or resistance to HIV among exposed individuals is a function of the variability of the virus, environment, and host genetics. It is increasingly clear that host genetic factors such as HLA alleles are important in HIV-1 infection and in AIDS progression^[2].

HLA alleles determine the molecular targets of the cellular immune response. Genetic polymorphisms of the HLA gene result in concentrated amino-acid substitutions in the peptide-binding groove that produce variability in peptide epitope binding and presentation to T cells. At a population level, HLA diversity provides protection against epidemic infection, with the differing precise molecular targets of individuals' immune responses ensuring that immune-escape-adaptive mechanisms of an infectious organism are countered.

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This is particularly demonstrable for human infections, such as malaria^[3], that affect mortality and is likely true for all infectious organisms that have an impact on reproductive fitness.

With respect to HIV-1 infection, many HLA alleles have been linked to rapid and slow progression or non-progression to AIDS^[2]. However, there are certain different HLA alleles involved in the susceptibility and/or resistance to HIV infection in individuals of various ethnic backgrounds^[4]. The Yi ethnic group, with a population of 6 578 500, is mainly distributed over the provinces of Sichuan, Yunnan and Guizhou, and the Guangxi Zhuang Autonomous Region. There are more than one million people of the Yi ethnic in Sichuan Province, and most of them live in an area south of the Dadu River and along the Anning River. The influence of HLA alleles on HIV infection /AIDS in the Yi ethnic group has not been reported. The objective of this study was to identify the distribution of HLA-B alleles in HIV-1-positive individuals and healthy control subjects of the Yi ethnic group in Sichuan Province, where the prevalence of HIV infection is high, and consequently to determine whether the presence of certain HLA-B alleles could be a factor affecting the susceptibility to or protection against HIV-1 infection.

MATERIALS AND METHODS

Subjects

One hundred and six healthy and 73 HIV-1-positive blood samples of the Yi ethnic group were randomly collected from Lianshan Yi Autonomous Prefecture in Sichuan Province, which holds the single largest Yi community in China. The ethnicity of the subjects' parents was identified using a questionnaire. All the subjects in this study did not report admixture outside their ethnic groups over at least one generation, and did not have any sib relationships over at least three generations. All blood samples were collected after obtaining written informed consent. HIV serostatus was tested using Vironostika HIV Uni-Form II plus O kit (BioMerix, France) and confirmed by GENELABS HIV BLOT 2.2 kit (Genelabs, USA).

DNA Extraction

Genomic DNA was isolated from leukocytes obtained from anticoagulated peripheral blood of healthy and HIV positive individuals using the QIAamp blood DNA mini kit (Qiagen, USA).

HLA-B Genotyping

Low-resolution HLA-B genotyping was carried out using PCR-SSP. Forty-three separate PCR reactions (including 42 allele PCR reactions and 1 negative PCR reaction) were performed for each sample. The HLA-B loci sequence specific primers and internal positive control primers were designed on the basis of the published sequence^[5] (Sunbiotech Company, Beijing, China). The internal control primers produced an amplicon of 256 bp from exon15 of the adenomatous polyposis (APC) gene. The final reaction volume (20 µL) contained 50-100 ng of genomic DNA, 10×PCR buffer 2 µL, 2.5 mmol/L dNTPs 1.6 µL, 25 mmol/L MgCl₂ 1.6 µL, 1 µmol/L of each locus specific primers, 0.8 µmol/L of each internal control primer, 1 IU of Taq DNA polymerase (TW-biotech, Beijing, China). The DNA amplifications were performed on a Gene Amp PCR system 9700 (Perkin-Elmer

Corporation, USA). The cycling parameters were as follows: 1 minute denaturation at 96°C, followed by 5 cycles of 25 seconds at 96°C, 45 seconds at 70°C, 30 seconds at 72°C, 21 cycles of 25 seconds at 96°C, 45 seconds at 65°C, 30 seconds at 72°C, 4 cycles of 25 seconds at 96°C, 60 seconds at 55°C, 2 minutes at 72°C, and 10 minutes at 72°C. The amplification products (10 µL) were visualized on 1.5% agarose gels containing 0.5 µg/mL ethidium bromide after the addition of 2 µL 6×loading buffer. The gels were run at 150 V for approximately 20 minutes in 1×TAE buffer and visualized using UV illumination.

For samples with previously unknown alleles or ambiguous PCR-SSP typing results, sequencing analysis for exon2 and exon3 was performed^[6].

Statistical Analysis

Hardy-Weinberg equilibrium was calculated using POPGENE software in healthy control subjects. To examine the association between allele prevalence and HIV-1 positive status, the proportion of HIV-1-positive patients and control subjects with each HLA-B allele was compared. The degree of association in an HLA-B allele was expressed as the odds ratio (OR), which was calculated according to Woolf's formula^[7] and Haldane's modification of the formula was used in sets containing 0. An OR<1 and 95% confidence interval (CI) of OR < 1 indicate protection, whereas an OR of >1 and CI of OR>1 indicate increased risk. Statistical analysis was performed by the Chi-square test. The *P*-value was calculated by using Fisher's test for which theoretical value was below 5. *P*<0.05 was considered statistically significant.

$$\text{Woolf's formula: OR} = \frac{\text{No. of patients with alleles} \times \text{No. of controls without alleles}}{\text{No. of patients without alleles} \times \text{No. of controls with alleles}}$$

Haldane's modification formula:

$$\text{OR} = \frac{(\text{No. of patients with alleles} + 0.5) \times (\text{No. of controls without alleles} + 0.5)}{(\text{No. of patients without alleles} + 0.5) \times (\text{No. of controls with alleles} + 0.5)}$$

RESULTS

DNA samples from 106 healthy control subjects and 73 HIV-1-positive individuals of the Yi ethnic group from Sichuan Province were typed by PCR-SSP. Table 1 shows the distribution of HLA-B as well as the odds ratio, 95% confidence interval, and *P* values for alleles presented significant differences compared with the healthy population. The population of 106 healthy control subjects was in Hardy-Weinberg equilibrium ($\chi^2=153.46$, df= 190, *P*=0.9759).

In HIV-1-positive groups, increased gene frequencies of B*07 and B*46 were observed compared to healthy control subjects (*P*=0.04, OR=2.58 for B*07; *P*=0.02, OR=3.32 for B*46). In addition, the gene frequency of B*35 trended towards an increase in HIV positive subjects (*P*=0.08, OR=2.25). Of these three alleles, only B*46 had a significantly higher gene frequency among HIV-1-positive subjects (*P*=0.02, OR=3.32, 95% CI=1.13-9.78).

On the other hand, B*55, B*44, and B*78 alleles were absent in the HIV-positive individuals studied. While B*55 was statistically significant (*P*=0.04, one-tailed Fisher's exact test), but the 95% CI of B*55 was higher than 1(95% CI 0.01-1.12). None of HLA-B

alleles in this study was significantly higher among healthy control subjects.

TABLE 1

Distribution of HLA-B Alleles in 106 Healthy Control Subjects and 73 HIV-positive Individuals

	Healthy Control Subjects		HIV-positive Individuals		P Value	OR	95% CI
	n	GF	n	GF			
B*40	42	0.1981	28	0.1892	0.83		
B*15	29	0.1368	19	0.1284	0.81		
B*51	27	0.1274	20	0.1351	0.72		
B*5201	15	0.0708	5	0.0342	0.13		
B*38	9	0.0425	4	0.0274	0.44		
B*56	9	0.0425	8	0.0548	0.61		
B*35	8	0.0377	12	0.0822	0.08	2.25	0.90-5.65
B*39	8	0.0377	4	0.0274	0.58		
B*5401	8	0.0377	6	0.0411	0.89		
B*07	7	0.0330	12	0.0822	0.04	2.58	0.99-6.72
B*13	7	0.0330	5	0.0342	0.80		
B*5801	7	0.0330	1	0.0068	0.19		
B*48	6	0.0283	1	0.0068	0.28		
B*55	6	0.0283	0	0	0.04	0.11	0.01-1.12
B*46	5	0.0236	11	0.0753	0.02	3.32	1.13-9.78
B*47	4	0.0189	3	0.0205	0.77		
B*27	8	0.0425	3	0.0205	0.25		
B*44	3	0.0142	0	0	0.20		
B*18	2	0.0094	3	0.0205	0.68		
B*78	1	0.0047	0	0	0.59		
B*57	1	0.0047	1	0.0068	0.64		

DISCUSSION

AIDS epidemic is characterized by extreme heterogeneity in the clinical course as well as in the incidence of HIV-1 infection among exposed individuals^[8]. These differences are in part a result of HLA-restricted CTL activity against HIV infection. HLA-B, as the classical class I loci, encodes molecules that bind to antigenic epitopes usually derived from intracellular pathogens (such as HIV virus) and present them to cytotoxic T-cell lymphocytes, thereby initiating a cytotoxic T cell response. Controlling CD4⁺ depletion by virus-specific CTLs is an important immunogenetic response toward protecting individuals both from infection of HIV and progression to AIDS. Allelic variants of the HLA molecule can bind to and display various antigenic peptides with differing affinities, thereby influencing the efficiency of immune protection by both the specificity and affinity of peptide binding and recognition by T cells. Evolution and population studies have led to the concept that the distribution of allelic frequencies observed in HLA genes are maintained through selective forces (i.e. morbidity from infectious diseases). Therefore, a change in the frequency of HLA-B alleles in HIV-1-positive versus control subjects would suggest a role in susceptibility to or resistance against this viral infection.

In this study, we found that the allele HLA-B*46 occurred more frequently in HIV-1-positive individuals than in control subjects, suggesting a role in increasing its

susceptibility to HIV-1 infection.

To obviate the effect of complex ethnic backgrounds, the subjects in this study were the entire Yi ethnic group. To increase the resolution and accuracy of HLA-B typing, we used DNA-based methods of HLA typing involving the polymerase chain reaction with sequence-specific primers (PCR-SSP), which has been shown to be more specific and reliable than serological typing methods^[9]. Moreover, the result of sequence-based typing confirmed the results of the PCR-SSP typing.

Several authors have reported that HLA-B alleles such as B*08, B*18, B*22, B*29, B*35, B*39 and B*51 are associated with a rapid progression to AIDS, whereas alleles B*14, B*27, B*44, B*55 and B*57 are associated with protection against infection and with slow progression to AIDS^[10]. A strong association of B*35 with rapid progression to AIDS has been observed in many studies on a wide variety of risk groups. The B*35 subtypes were divided into two groups according to peptide-binding specificity: the HLA- B*35-PY group and HLA- B*35-PX group. It was found that the B*35-PY group was associated with resistance to AIDS, while the B*35-Px group was associated with susceptibility to AIDS^[11]. In our study, the frequencies of B*35 in HIV-positive groups were higher than those in control groups, although the difference appeared no significant. However, we were unable to divide the B*35 genotype into B*35-Px group or B*35-PY group by our “low resolution” typing method.

Most HLA-B alleles identified as having protective effects (e.g. HLA-B*27, B*57) carry the Bw4 specificity, whereas alleles associated with susceptibility (e.g. HLA-B*35, B*08) tend to have the Bw6 specificity^[12]. In this study, the B*46 allele associated with susceptibility to HIV-1 infection was of the Bw6 specificity. It is possible that viral peptides that bind to HLA-B alleles sharing the Bw6 specificity are unable to elicit an effective HIV-1-specific CTL response. Another mechanism to explain the susceptibility to HIV infection with certain HLA genotypes may involve the regulation of natural killer (NK) cell activity^[13]. Like CTLs, NK cells are involved in surveillance and killing of foreign or infected cells through a mechanism involving HLA molecules. It may be possible that the B*46 allele can reduce NK cell number and activity, thus being associated with susceptibility to HIV infection.

It has been suggested that other host factors, such as co-receptor CCR5, CCR2 and CX₃CR₁, also play an important role in viral infection and in the progression to AIDS. However, we were unable to find any significant SNP of co-receptor (CCR5, CCR2 and CX₃CR₁) associated with susceptibility or resistance to HIV-1 infection in this study (data not shown). Taken together, our findings seem to indicate that HLA molecules can exercise a crucial function in resistance to HIV infection in the Yi ethnic population.

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