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中国新疆维族人群HLA-B等位基因与HIV-1感染易感性或抗性*

许铭炎[1,2#]，马 军[3#]，洪坤学[2]，邓小玲[1]，刘永超[2]，阮玉华[2]，
邢 辉[2]，张远志[3]，徐小虎[1]，邵一鸣[2**]

(1.汕头大学医学院病原生物学教研室，广东汕头 515041；2.中国疾病预防控制中心性病艾滋病预防控制中心，北京 100050；3.新疆维吾尔族自治区疾病预防控制中心，乌鲁木齐 830011.)

摘要：通过对中国维吾尔族人群HLA-B等位基因的分布频率的研究，探讨HLA-B等位基因与HIV感染的易感/或抵抗性的相关性。本研究用PCR-SSP的方法对新疆维吾尔族110例无相关的健康对照者(HIV阴性)和128例HIV阳性感染者进行HLA-B等位基因分型。用POPGEN软件对健康对照者人群进行Hardy-Weinberg平衡检测，用卡方检验分析HLA-B等位基因在健康对照者和HIV阳性感染者频率分布的差异。在HIV-1阳性感染者中，B*4901等位基因频率显著性增加(B*4901：$P=0.02$，OR=3.06，95%CI=1.16～8.10)。而在健康对照者中，B*40等位基因频率增加具有统计意义(B*40：$P=0.02$，OR=0.39，95%CI=0.07～0.92)。由此可见，B*4901等位基因可能与HIV-1感染的易感性有关，而B*40等位基因可能与与HIV-1感染的抵抗性有关。

关键词：HIV感染，HLA-B抗原，维吾尔族，多态性
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HLA-B Alleles Associated with Susceptibility or Resistance to Human Immunodeficiency Virus Type 1 in a Xinjiang Uygur Population, China*

XU Ming-yan[1,2#], MA Jun[3#], HONG Kun-xue[2], DENG Xiao-ling[1], LIU Yong-chao[2],
RUAN Yu-hua[2], XING Hui[2], ZHANG Yuan-zhi[3], XU Xiao-hu[1], SHAO Yi-ming[2**]

(1. *Department of Pathogenic Biology, Shantou University Medical College, Shantou* 515041, *China*; 2. *Division of Research on Virology and Immunology, National Center for STD/AIDS Control and Prevention, Beijing* 10050, *China*; 3. *Xinjiang Center for Disease Control and Prevention, Urumqi* 830011, *China*)

Abstract: Host genetic factors, such as human leukocyte antigen (HLA) alleles, are important in Human immunodeficiency virus (HIV) infection and its progression to AIDS. HLA class I genes, especially highly polymorphic HLA-B genes, are involved in the activation of HLA-restricted cytotoxic T lymphocytes (CTLs) against HIV, and thus control susceptibility to or protect against this virus. The present study was aimed to determine the distribution of HLA-B alleles in the Chinese Uygur ethnic group and its association with HIV infection. One hundred ten healthy control (HIV negative) and 128 HIV positive Chinese Xinjiang Uygur ethnic individuals were used in this study. HLA typing for B allele was performed by polymerase chain reaction (PCR) with sequence-specific primers (SSP). Hardy-Weinberg equilibrium was calculated using POPGENE software for the healthy control group. The HLA-B frequency of each allele was compared between the patients and the controls using the chi-square test. In HIV-1-positive group, gene frequency of allele B*4901 was significantly higher compared to the healthy control subjects ($P=0.02$, OR=3.06, 95%CI=1.16～8.10 for B*4901). In contrast, the gene frequency of B*40 in healthy controls was significantly higher than in the HIV-positive patients ($P=0.02$, OR=0.39, 95%CI=0.07～0.92 for B*40).

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作者简介：许铭炎(1972－)，男，汉，福建人，讲师，博士，研究方向：病毒免疫。
同为第一作者。
** 通迅作者. Corresponding author. Tel: 010-63166184. E-mail: yshao@public3.bta.net.cn
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In this study, HLA allele B * 4901 may be associated with increased susceptibility to HIV-1 infection, whereas the B * 40 allele may be associated with resistance to H HIV-1 infection.

Key words: IV infection, HLA-B antigens, Uygur ethnic group, polymorphism

The scourge of AIDS, discovered three decades ago, is the most devastating epidemic of our time. The cumulative death toll is approaching 20 million, and the World Health Organization estimates that 37.8 million people (range: 34.6-42.3 million) worldwide today are living with HIV[1]. And still, AIDS expands relentlessly, destroying people's lives and in many cases seriously damaging the fabric of societies. These statistics stimulated intensive research conducted over the past two decades to understand HIV pathogenesis in order to control infection and disease progression. It has been found that some individuals remain seronegative or uninfected despite high-risk behavior and /or multiple exposures to HIV-1. This considerable heterogeneity in the epidemic seems to imply a differing genetic background. It has been demonstrated extensively that host genetic factors such as HLA alleles affect the risk of HIV-1 infection and AIDS progression[2].

HLA gene products are fundamental to acquired immune response. As HLA class I molecules, the products of HLA-B genes are present on the surface of all nucleated cells where they present short viral peptide fragments, called epitopes, which elicit immune responses from cytotoxic T lymphocytes (CTLs). Each HLA class I molecule is able to present only a limited range of peptides. The HLA class I genotype of a patient therefore dictates the repertoire of CTL responses he or she is able to mount, which translates into different abilities to cope with a pathogen infection[3]. At the population level, the extraordinary degree of polymorphism in HLA alleles is the result of natural selection through infectious disease morbidity and mortality[4-5]. Therefore, the increased or decreased frequency of certain alleles in a population may suggest that they are a factor in susceptibility to or protection against the pathogen infection[6].

Many HLA alleles have been linked to rapid, slow, or nonprogression to AIDS[7]. Moreover, there is certain to be different HLA alleles involved in the susceptibility and/or resistance to HIV infection in individuals of various ethnic backgrounds[8]. The Uygur ethnic group, with a population of 8,250,000, is mainly distributed over the Xinjiang Uygur Autonomous Region. The HIV epidemic is more severe in Xinjiang autonomous region, especially among Uygur ethnic populations. In 2002, Xinjiang had over 1,000 reported HIV infections and an estimated number of infections of more than 40,000[8]. However, the influence of HLA alleles on HIV infection /AIDS in the Uygur ethnic group has not been reported. The objective of this study is to determine the distribution of the HLA-B allele in HIV-1-positive patients and healthy control subjects of Uygur ethnic group in Xinjiang Uygur Autonomous Region, where there is a high rate of HIV infection, in an effort to determine whether the presence of certain HLA-B alleles could be a factor in susceptibility to or protection against HIV-1 infection.

1 Materials and Methods

1.1 Subjects

One hundred ten Healthy and 128 HIV-1-positive blood samples of Uygur ethnic group were randomly collected from Xinjiang Uygur Autonomous Region, which holds the single largest Uygur community in China. The ethnicities of the subjects were identified using a questionnaire. All of 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 generation. Moreover, subjects with HLA-related disorders such as ankylosing spondylitis and rheumatoid arthritis were excluded. All blood samples were collected after obtaining written informed consent. HIV serostatus was tested using Vironostika HIV Uni-Form II plus O kit (BioMer-

ix, France) and confirmed by GENELABS HIV BLOT 2.2 kit (Genelabs, USA).

1.2 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).

1.3 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 published sequence[9] (Sunbiotech Company, Beijing, China). The internal control primers produce an amplicon of 256 bp from exon 15 of the adenomatous polyposis (APC) gene. The final reaction volume (20μL) contained 50-100ng of genomic DNA, 10×PCR Buffer 2μL, 2.5mmol/L dNTPs 1.6μL, 25mmol/L MgCl2 1.6μL, 1μmol/L of each locus specific primers, 0.8μmol/L of each internal control primers, 1U 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 min denaturation at 96 ℃, followed by 5 cycles of 25s at 96 ℃, 45s at 70 ℃, 30s at 72 ℃, 21 cycles of 25s at 96 ℃, 45s at 65 ℃, 30s at 72 ℃, 4 cycles of 25s at 96 ℃, 60s at 55 ℃, 2 min at 72 ℃, and 10 min at 72 ℃. The amplification products (10μL) 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 150V 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 [10].

1.4 Statistical analysis

Hardy-Weinberg equilibrium was calculated using POPGENE software in healthy control subjects [11]. 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 between an HLA alleles was expressed as the odds ration (OR), which was calculated according to Woolf's formula or Haldane's modification of the formula, and P was determined by $\chi 2$ analysis or, when appropriate, by Fisher's exact test. A P value less than 0.05 was considered statistically significant. An OR of <1 and 95% confidence interval (CI) of OR <1 indicate protection, whereas an OR of >1 and CI of OR >1 indicate increased risk.

2 Results

DNA samples from 110 healthy control subjects and 128 HIV-1-positive subjects of Xinjiang Uygur ethnic background 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 allele present significant differences with respect to the healthy population. The population of 110 healthy control subjects was in Hardy-Weinberg equilibrium ($\chi 2=309.29$, $df=351$, $P=0.9469$).

In HIV-1-positive patients groups, an increased gene frequency of B*38 and B*4901 were observed compared to healthy control subjects ($P=0.04$, OR=3.12, 95%CI=1.00～9.64 for B*38; $P=0.02$, OR=3.06, 95%CI=1.16～8.10 for B*4901). Since CI of B*38 contained 1, only B*4901 had a significantly higher gene frequency among HIV-1-positive subjects.

In contrast, the gene frequency of B*07 and B*40 in healthy control subjects were higher than in the HIV-positive patients ($P=0.05$, OR=0.35, 95%CI=0.12～1.00 for B*07; $P=0.02$, OR=0.39, 95%CI=0.07～0.92 for B*40). But only B*40 was statistically significant for its CI below 1 (95%CI=0.07～0.92 for B*40).

Table1 Distribution of HLA-B alleles among 110 healthy control subjects and 128 HIV-positive individuals in Xinjiang Uygur ethnic group.

Allele	Healthy control subjects		HIV-positive patients		*P* value	OR	95% CI
	N	GF	N	GF			
B*07	14	0.0636	6	0.0234	0.05	0.35	0.12～1.00
B*08	7	0.0318	9	0.0352	0.84		
B*13	21	0.0955	19	0.0742	0.41		
B*14	2	0.0091	9	0.0352	0.11		
B*15	10	0.0455	10	0.0391	0.72		
B*18	4	0.0182	8	0.0312	0.53		
B*27	4	0.0182	5	0.0195	0.82		
B*35	25	0.1136	25	0.0977	0.49		
B*3701	3	0.0136	0	0	0.10		
B*38	4	0.0182	14	0.0547	0.04	3.12	1.00～9.64
B*39	4	0.0132	1	0.0039	0.28		
B*3910	1	0.0045	0	0	0.16		
B*40	17	0.0773	8	0.0312	0.02	0.39	0.07～0.92
B*41	1	0.0045	2	0.0078	0.89		
B*44	13	0.0591	11	0.0430	0.42		
B*4601	7	0.0318	15	0.0508	0.17		
B*47	6	0.0273	4	0.0156	0.38		
B*48	8	0.0364	3	0.0117	0.14		
B*4901	5	0.0227	17	0.0664	0.02	3.06	1.16～8.10
B*50	6	0.0273	9	0.0352	0.62		
B*51	25	0.1136	32	0.1250	0.80		
B*5201	10	0.0455	14	0.0547	0.65		
B*53	10	0.0455	5	0.0195	0.11		
B*55	1	0.0045	8	0.0312	0.07	7.06	0.84～59.43
B*56	1	0.0045	0	0	0.46		
B*57	5	0.0227	5	0.0195	0.94		
B*58	0	0	4	0.0156	0.08		
B*78	2	0.0091	3	0.0117	0.86		
Total	220		256				

HLA: human leukocyte antigen; HIV: human immunodeficiency virus; N: number; GF: gene frequency; OR: odds ratio; CI: confidence interval.

3 Discussion

The AIDS epidemic is characterized by extreme heterogeneity in the clinical course as well as in the incidence of HIV-1 infection among exposed individuals [12]. These differences are in part a result of HLA-restricted CTL activity against HIV infection. Controlling CD4+ depletion by virus-specific CTL is an important immunogenetic response toward protecting individuals both from infection of HIV and progression to AIDS once HIV-infected [13]. 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 [14]. Moreover, HIV-1 has been suggested to down regulate the HLA class I expression on infected cells and thus reduce their lysis by specific CTLs [15]. Recently, studies of comprehensive analysis of the class I restricted CD8 + T-cell response against HIV in southern African demonstrated a dominant role for HLA-B alleles in HIV infection [16]. So we choose HLA-B allele as candidate gene to study.

Association of HLA alleles with susceptibility to HIV infection and progression of disease has been studied in diverse populations. Several authors have reported 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 [17]. However, no single HLA allele so far has been agreed upon by all reports to exert a significant effect during the HIV-1 infection [18]. In this study, our finding that allele B * 4901 occurred more frequently in HIV-1-positive individuals than in healthy control subjects suggests that it play a role in susceptibility to HIV-1 infection. On the other hand, a significant higher gene frequency of B * 40 was observed in healthy control subjects. Therefore, the presence of this allele would suggest protection against HIV-1 infection. We could not correlate their presence with progression to AIDS, because it is necessary to know the evolution of disease for each HIV-1-positive individual. Comparing with what our group [19] and other authors [17] have previously reported , the disparate effects of the HLA-B alleles on the Chinese Uygur ethnic group and other ethnic populations in the world are consistent with previous evidence that HLA genes are subject to varying degrees of gene flow and natural selection in different human populations [20]. This emphasizes that the main HLA-B restrictive elements for HIV-specific T lymphocytes will most likely be different for each population. We address here is the first report on the association of distinct HLA allele subtypes with HIV-1 infection in a Chinese Uygur population.

To obviate the effect of complex ethnic backgrounds, the subjects in this study are the entire Uygur 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 [21]. Moreover, the result of sequence-based typing confirmed the results of the PCR-SSP typing.

Although considerable progress has occurred in the development of therapy for AIDS, there are still many unsolved challenge for drug resistance and vaccine development. The goal of identifying human HLA genes associated with HIV-1 infection and AIDS progression is to further define the regulatory components of an infected individual's cell physiology in HIV infection and disease progression. At the end, we can explore the potential application of predicting AIDS progression for a given HLA genotype combination to inform clinical trials for new drugs and vaccines. Our results may have important implications in understanding the host genetic factors responsible for HIV acquisition and disease progression in the Chinese Uygur ethnic group. Further, the results of this study may be important in designing and testing effective vaccines in this high-risk population.

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