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Association of HLA class Ⅰ and class Ⅱ genes with severe acute respiratory syndrome in the northern Chinese population

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ABSTRACT

Severe acute respiratory syndrome (SARS) was a major epidemic at the beginning of the 21st century. This highly infectious disease is caused by a novel coronavirus (SARS-CoV), whose immune reaction is still not completely understood. This study described the genetic patterns of HLA-A, -B, and -DRB1 loci in patients from Beijing who survived SARS, and examined whether an association between HLA genes and susceptibility/resistance to SARS exists. A total of 148 Chinese Han SARS survivors were recruited to donate convalescent plasma in 2003. HLA low-resolution genotyping was carried out using PCR-SSP. Allele frequencies were compared with published frequencies of HLA alleles from 11 755 unrelated northern Chinese Han bone marrow donors by Fisher´s exact test. In this cohort, 13, 25 and 13 alleles were observed at HLA-A, -B, and -DRB1 loci respectively. Fisher´s exact tests revealed four alleles (A*26, DRB1*04, DRB1*09, and DRB1*16) that showed a nominal association significance with the SARS virus (P<0.05), yet none of these associations remained significant after correction. Our study suggests that HLA polymorphisms were unlikely to have contributed significantly to either the susceptibility or resistance to the SARS-Cov infection in patients who survived SARS in the Northern Chinese population, thus leaving an open question for future studies into a possible association HLA class Ⅰ and class Ⅱ genes with SARS in patients who were unable to survive the infection.

Keywords: dHLA, polymorphism, severe acute respiratory syndrome

INTRODUCTION

Severe acute respiratory syndrome (SARS) first emerged as an epidemic in southern China in late 2002 and swiftly spread across five continents, with a mortality rate of almost 10%[1]. Between November 2002 and July 2003, this outbreak caused at least 8 096 infections and 774 deaths worldwide[2], mainly affecting East Asia. With China the major epidemic area, Beijing reported over 30% (2 521 cases) of global cumulative affected cases with a mortality rate of 7.3%. This confined geographical and racial spread of SARS suggested a potential underlying genetic predisposition[1]. After a remarkable global collaborative effort, the SARS coronavirus (SARS-CoV) was identified as the causative agent, with scientists able to sequence its entire genome soon afterwards[2–4].

The human leucocyte antigen (HLA) system plays

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an essential role in the host´s response to virual infections. HLA class I or Ⅱ gene present antigenic peptides to T cells, initiate immune responses and remove foreign materials by neutralizing antibodies, cytokines, and activating cytotoxic T cells. Therefore the virus and host´s immune condition interactively determine the clinical outcome. Polymorphisms in HLA system may be important immunogenetic determinants that attribute to variations in individual susceptibility or resistance to immune-mediated/controlled diseases. To date, hundreds of autoimmune and infectious diseases have been associated with HLA polymorphisms[5]. For example, altered resistance to viral infections have been reported in the hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human tuberculosis[5–7]. These genetic polymorphisms have also been studied in relation to the susceptibility or resistance to SARS-CoV[8–16], however no consensus has yet been reached. A number of studies reporting possible SARS-associated HLA types have been conducted on populations in the southern China area (Hong Kong, Taiwan, Guangzhou)[8–14, 16], however few studies have been conducted on larger samples from northern China. Therefore, in this study we evaluated the possible association between HLA polymorphisms and susceptibility or resistance to SARS in a cohort of patients from Beijing, northern China, to gain further insight and find a way to limit any potential SARS virus spread in the future.

MATERIALS AND METHODS

Subjects

This study was conducted on 148 Chinese Han SARS patients; 67 females and 79 males (with 2 missing basic information); age range, 19 to 74 with an average of 35.5 years, who had been hospitalized in multiple hospitals in Beijing, and later recovered. All included individuals were unrelated, and were diagnosed according to the World Health Organization (WHO) case definition for SARS[17]. Participants were recruited by the Beijing Red Cross Blood Center in 2003, for the purpose of exploring the potential treatment of SARS with a neutralizing antibody[18]. All donors met the physical examination criteria, and all screening results for HIV-Ab, HCV-Ab, HBsAg, syphilis, and alanine transaminase (ATL) were negative. This study was performed with approval of the Institutional Review Board of Beijing Red Cross Blood Center. Written informed consent was obtained from each participant using the pre-donation questionnaire. All procedures were in accordance with the ethical standards of the appropriate committee on human experimentation and Helsinki Declaration.

HLA allele genotyping

Genomic DNA was extracted from the peripheral whole blood of subjects. Low-resolution HLA-A, -B, and -DRB1 genotyping was carried out by polymerase chain reaction based on the sequence-specific primers (PCR-SSP) method with Olerup SSP HLA-A, -B, and -DRB1 kits (Olerup SSP AB, Hasselstigen 1, Sweden). Briefly, genomic DNA (2 μL) was added to each 96-well microplate, and DNA amplification was performed in 10 μL system using an ABI 9700 thermal cycler (Applied Biosystems). The thermocycler program started with an initial step of denaturation at 94 °C for 35 s, followed by 36 cycles of denaturation (94 °C, 30 s), annealing (60 °C, 45 s), extension (72 °C, 35 s) and a final extension step (72 °C, 5 min) which terminated the reaction, and holding at 4 °C. The PCR products (15 μL) were separated by electrophoresis using standard 1.5% agarose gel containing 0.5 μg per mL ethidium bromide. The genotyping results were examined under UV light trans-illumination and analyzed according to DNA band profile. Inadequate results were obtained from 9 samples resulting in their HLA-DRB1 genotyping failure.

Controls

The frequencies of HLA alleles of the SARS patients were then compared with published frequencies of matching alleles in 11 755 unrelated northern Chinese Han bone marrow donors[19]. These donors were collected between 2002 and 2005 through the China Marrow Donor Program, which comprised of individuals with an age range of 18-45 years. HLA genotyping was carried out using PCR-SSP and sequence-specific oligonucleotide primed PCR (PCR-SSO). Allele frequencies were caculated by direct counting. No significant deviation from the Hardy-Weinberg equilibrium was observed in the sample population. As Beijing is located in northern China and the majority ethnicity is Han, this control population was considered to be reliable and suitable for comparison.

Statistical analysis

HLA-A, -B, and -DRB1 allele frequencies were estimated by directly counting observed alleles. Odds ratios (OR) and the corresponding 95% confidence intervals were obtained from the 2-way contingency table analysis. Statistical significance was performed by a two-tailed Fisher´s exact test to compare the allele frequencies observed in patients, versus the allele frequencies in the control group. Bonferroni correction for multiple tests was used to account for inflated type

I error rate, according to which the significance level was set as 0.05 divided by the number of independent tests at each locus[20]. Statistical analyses were done in the open source environment R (Version 3.1.0).

RESULTS

The allelic frequencies of HLA-A, -B, and -DRB1 in the 148 SARS patients and 11755 control subjects are listed in ***Table 1***. There were 13, 25 ,13 low-resolution alleles observed in the patient group at HLA-A, -B, and -DRB1 loci respectively. At the HLA-A locus, three alleles; A*02, A*24, and A*11 accounted for over 70% of the SARS group. The most frequent alleles with a frequency of over 10% at the HLA-B locus were B*15, B*40, and B*13, while at the HLA-DRB1 locus the most frequent alleles were DRB1*15, DRB1*9, and DRB1*12. Fisher´s exact tests showed that in SARS patients, the frequencies of A*26 and DRB1*04 were lower, and the frequencies of DRB1*09 and DRB1*16 were higher than those in the control population at the nominal significance level (P<0.05). The highest significant difference was found at HLA-DRB1*16 with P=0.007 (OR=2.30, 95% CI: 1.23-3.97). However, after Bonferroni correction, there were no significant differences in HLA gene frequencies between the two groups.

DISCUSSION

This study investigated the possible influence of HLA alleles on susceptibility or resistance to the SARS infection in a cohort of recovered SARS patients from northern China. One HLA-Aallele and three HLA-DRB1 alleles showed nominal significance, yet none of them withstood Bonferroni correction for multiple tests. To the best of our knowledge, this study included the largest number of SARS cases from northern China and although the patients were recruited over a decade ago (and genotyping was conducted at that time) the well-preserved original materials and data ensured the quality of current analyses.

The association between HLA genes and SARS has been previously studied in patients from Taiwan, Hong Kong, Guangzhou (southern China), Tianjin (northern China), and Vietnam, showing largely inconsistent results. Similarly to our findings, a previous report of a SARS cohort comprised of 95 recovered SARS patients from Guangzhou demonstrated no significant association[8]. However, the nominal significant HLA alleles found in our study and the Guangzhou study had no overlap at all. The study of 90 patients from Hong Kong revealed a significant association of B*0703 and DRB1*0301 with SARS infection[9]. The same research group subsequently published a replication study using an additional independent SARS cohort, but failed to confirm their previous findings[10]. Another study in Hong Kong demonstrated no significant association, despite incorporating a larger cohort of patients than previous reports[11]. DRB1*0301 also attained significance (P=0.04) in a study of 130 cases in Taiwan[12]. In our study, B*07 and DRB1*03 did not seem to be key factors, and we were unable to look deeper without high resolution genotyping data. The Tianjin studies identified several significant HLA-A and HLA-B alleles (P<0.05)[13,14], which also failed to show significance in our study despite the genetic homogeneity of these two populations. Another study in the Southeast Asian population was performed on 44 Vietnamese SARS patients, which reported DRB1*1202 as a new possible allele involved in SARS development[15]. However, although populations from mainland China, Hong Kong, and Taiwan largely share common origins and the ethnicity of the Vietnamese population has some Chinese influence dating back to the Bronze Age[21], reported SARS-associated HLA alleles from any single population could not be verified in any of the other populations. These inconsistencies among studies may have resulted from the limited amount of samples available, different genotyping methods, various choices of control, multiple test correction, or diverse SARS-Cov strains.

In the present study, 4 alleles showed statistical significance in our sample. A*26 and DRB1*04 were less frequently observed, while DRB1*09 and DRB1*16 were more frequently observed in the affected population compared to the control. As far as we know, these potential signals have not been reported in any other populations so far. The largest difference was seen in DRB1*16, despite the relatively low allele frequency in the control population (2.3%), with an absolute difference of 2.7% between the SARS patients and controls (P=0.007, OR=2.30, 95% CI:1.23-3.97). DRB1*16 has been previously reported to implicate a heightened risk to infectious diseases such as leprosy[22], tuberculosis[23], and hepatitis B virus infection[24]. However, the hepatitis B virus and SARS coronavirus belong to different virus families and may induce different host immune responses. The nominal significance found in our study needs to be further examined using independent samples.

Several studies have investigated HLA polymorphisms in severe SARS patients, usually defined as enduring ICU-admission or death. While studies in Hong Kong, Guangzhou, and Tianjin showed no correlation between HLA genotypes and disease severity [8,9,11,14], the study of Taiwanese SARS patients

Table 1 **Frequencies of HLA-A, -B, and -DRB1 alleles in SARS patients and in northern Chinese Han population**

HLA alleles		SARS patients		ontrol(2N=23510)[b]	OR(95%CI)	*P* value[c]
		Alleles count	Frequency %	Frequency %		
HLA-A	01	9	3.0	5.4	0.55 (0.25, 1.07)	0.089
(2N=296)	02	97	32.8	29.3	1.18 (0.91, 1.51)	0.199
Significance level=0.0038[a]	03	17	5.7	5.2	1.12 (0.64, 1.84)	0.597
	11	55	18.6	17.9	1.04 (0.76, 1.41)	0.761
	23	1	0.3	0.5	0.73 (0.02, 4.21)	1.000
	24	56	18.9	16.1	1.21 (0.89, 1.63)	0.203
	26	3	1.0	3.4	0.29 (0.06, 0.86)	0.022
	29	3	1.0	1.1	0.89 (0.18, 2.65)	1.000
	30	17	5.7	6.7	0.85 (0.49, 1.39)	0.638
	31	13	4.4	3.8	1.17 (0.61, 2.04)	0.540
	32	2	0.7	2.0	0.33 (0.04, 1.20)	0.139
	33	20	6.8	7.2	0.94 (0.56, 1.48)	0.910
	68	2	0.7	1.2	0.56 (0.07, 2.05)	0.591
HLA-B	07	9	3.0	4.5	0.66 (0.30, 1.28)	0.260
(2N=296)	08	8	2.7	1.3	2.14 (0.91, 4.33)	0.061
Significance level=0.0020[a]	13	39	13.2	11.1	1.21 (0.84, 1.71)	0.264
	14	2	0.7	0.6	1.09 (0.13, 4.04)	0.707
	15	47	15.9	13.8	1.17 (0.84, 1.61)	0.310
	27	3	1.0	2.1	0.48 (0.10, 1.43)	0.298
	35	14	4.7	6.0	0.78 (0.42, 1.34)	0.458
	37	7	2.4	1.9	1.27 (0.50, 2.67)	0.513
	38	11	3.7	2.9	1.29 (0.63, 2.35)	0.383
	39	5	1.7	1.8	0.95 (0.30, 2.26)	1.000
	40	41	13.9	13.0	1.08 (0.75, 1.51)	0.663
	41	1	0.3	0.1	2.57 (0.06, 15.52)	0.330
	44	9	3.0	5.1	0.58 (0.26, 1.13)	0.140
	46	26	8.8	7.2	1.25 (0.80, 1.88)	0.258
	48	9	3.0	3.2	0.94 (0.42, 1.81)	1.000
	49	1	0.3	0.3	1.20 (0.03, 7.00)	0.568
	50	2	0.7	0.9	0.76 (0.09, 2.80)	1.000
	51	17	5.7	7.4	0.77 (0.44, 1.25)	0.368
	52	9	3.0	3.6	0.83 (0.38, 1.61)	0.753
	54	16	5.4	3.3	1.67 (0.94, 2.78)	0.070
	55	2	0.7	2.0	0.33 (0.04, 1.20)	0.139
	57	2	0.7	1.4	0.47 (0.06, 1.72)	0.452
	58	12	4.1	4.2	0.96 (0.49, 1.71)	1.000
	67	2	0.7	0.9	0.77 (0.09, 2.85)	1.000
	81	2	0.7	0.1	5.7 (0.66, 22.80)	0.053
HLA-DRB1	01	8	2.9	4.1	0.70 (0.30, 1.41)	0.441
(2N=278)	03	9	3.2	4.2	0.77 (0.35, 1.49)	0.545
Significance level=0.0038a	04	20	7.2	11.5	0.60 (0.36, 0.94)	0.023
	07	27	9.7	11.4	0.84 (0.54, 1.25)	0.446
	08	16	5.8	6.1	0.94 (0.53, 1.56)	0.900
	09	50	18.0	12.9	1.49 (1.07, 2.03)	0.015
	10	8	2.9	1.8	1.65 (0.70, 3.33)	0.164
	11	12	4.3	7.0	0.60 (0.31, 1.07)	0.095
	12	31	11.2	10.7	1.05 (0.70, 1.53)	0.770
	13	12	4.3	5.3	0.81 (0.41, 1.45)	0.588
	14	16	5.8	6.3	0.91 (0.51, 1.52)	0.901
	15	55	19.8	16.8	1.22 (0.89, 1.65)	0.196
	16	14	5.0	2.3	2.30 (1.23, 3.97)	0.007

CI: confidence interval; OR, odds ratio; SARS, severe acute respiratory syndrome.a:Significance level defined by Bonferroni method; b:Data from Wu et al. 2007 (Counts of each allele not available)[19]; c: 2-tailed *P*-value, by Fisher´s exact test.

showed that 6 severe cases had a significantly higher frequency of HLA-B*4601 compared with heathy medical workers[16]. In our study, a trend toward a higher frequency of HLA-B*46 in the SARS patients (8.8%) versus in the control population (7.2%) was observed, although not reaching the pre-defined significance level. As patients included in our study were SARS survivors, they may differ from those who died or who were severely affected in terms of HLA polymorphisms. An alternative explanation was that the frequency of HLA-B*46 allele in our control population was lower (8.8%) compared with the controls of the Taiwan (13.7%, 190 individuals) and Hong Kong (14.7%, 18 774 individuals) studies[11,16]. Moreover, we used a general population as control while the Taiwan study compared severe patients with a group of non-infected health care workers working in a high risk environment, which may have led to a significant difference between cases and controls. Unfortunately, the present study was unable to further investigate HLA-B*46 in groups stratified by disease severity due to inaccessibility of relevant data.

The HLA loci stands out as one of the leading candidates for infectious disease susceptibility due to its important role in the immune response. As cell surface proteins, HLA participate in establishing an antigen specific T-cell repertoire and subsequently activate these T cells during initiation of immune responses. Effective viral antigen presentation to $CD8^+$ and $CD4^+$ T cells by HLA class Ⅰ and Ⅱ complexes play an important role in the regulation of an optimal immune response against viral infections. This has been evidenced by studies showing the implication of HLA polymorphism in the influenza virus infection[25–26]. However, despite the similarity between influenza and SARS, we found no association between HLA and SARS, which is where further research among severely affected SARS patients might give more insight.

Alternatively, it has been suggested that the innate inflammatory immune response, rather than the specific aim of the immune response, takes a more profound effect with more importance during disease course, however we still do not know the molecular process of the immune response to SARS-Cov infection clearly enough[27]. The negative results of the present data are consistent with this view, however, it is not apparent whether naturally acquired immune responses can confer protection from re-infection of SARS-CoV. If this is the case, vaccines are likely to be one effective way to provide protection against a re-emergence of SARS. At this point deeper research into the molecular involvement of HLA in SARS pathogenesis is still required for the future planning of effective vaccination strategy.

In summary, human leucocyte antigen polymorphisms were unlikely to have contributed significantly to the susceptibility or resistance to SARS infection in surviving patients from northern China. As small sample size is a major limitation in this kind of study, it would be worthwhile to combine various studies for a meta-analysis. Our results here were based on a low-resolution platform, however looking further into high resolution genotyping data from this group of patients may be of future interest.

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