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Association of human leukocyte antigen class II alleles with severe acute respiratory syndrome in the Vietnamese population

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ABSTRACT

Excessive immune response is believed to play a role in the development of severe acute respiratory syndrome (SARS). Inhomogeneous spread of SARS led one to think of an Asian genetic predisposition and contribution of human leukocyte antigen (HLA) to the disease susceptibility. However, past case-control studies showed inconsistent results. In Viet Nam, of 62 patients with SARS, 44 participated in the present study together with 103 individuals who had contact with SARS patients and 50 without contact history. HLA-DRB1*12 was more frequently shown in SARS patients than in controls (corrected $p = 0.042$). HLA-DRB1*1202, the predominant allele in the Vietnamese population showed the strongest association with SARS in a dominant model (corrected $p = 0.0065$ and 0.0052, depending on the controls to be compared). Our results and accumulated data on HLA in the Asian populations would help in the understanding of associations with emerging infectious diseases.

1. Introduction

Severe acute respiratory syndrome (SARS) originated in southern China in November 2002, reaching Hong Kong in February 2003 and thereafter spreading rapidly to other countries in Asia, Europe, and North America; it ended by July 2003 [1]. In total, 8098 individuals had SARS worldwide, with the majority of the patients confined to regions around southeastern or eastern Asia (Mainland Chinese, Hong Kong residents, Vietnamese, Singaporeans, and Taiwanese), which raised the question as to the possibility of an Asian-specific genetic predisposition to SARS [2–5].

This emerging disease was caused by a novel coronavirus (SARS-CoV) and was characterized by extensive inflammatory damage of alveolar epithelium in the lung, resulting in death in 10% of the patients. Because the lung lesions develop approximately 1 week after the peak of viral replication in the lung, hyperimmune response has been believed to play a role in the progress of the disease, although details of the immunologic mechanism and effective therapeutic measures for acute lung injury caused by emerging viruses such as SARS-CoV and H5N1 remain unknown [6–8].

Human leukocyte antigen (HLA) variations are often associated with susceptibility or resistance to a wide range of infections, including malaria, tuberculosis, leprosy, HIV and virus-induced hepatitis [9,10]. In this context, HLA was the first human gene to be investigated immediately after the SARS outbreak. However, such reports from Taiwan [2,4], a study in Hong Kong [3], and a study in mainland China [5] showed disease association of different alleles and no consensus has been reached yet for interpretation of the overall data.

In the present study, we presented genotyping data of HLA class I and class II genes in Vietnamese SARS patients and controls, after which we gained insight into the overall association studies relating to HLA allele and haplotype distribution in Asian populations.

2. Subjects and methods

2.1. Subjects

This study was reviewed and approved by ethics committees in the Ministry of Health in Viet Nam as well as the International

Naoto Keicho and Satoru Itoyama contributed equally to this work.

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Medical Center in Japan. Written informed consent had been obtained from all subjects, and detailed demographics of the subjects had been described beforehand [11]. In brief terms, the study population comprised 44 SARS patients, 103 staff members of the same hospital as control subjects who had come into contact with SARS patients but had not developed SARS, and 50 healthy individuals having had no contact history with SARS patients. All participants were unrelated Vietnamese. No samples from deceased patients were available for this study.

2.2. HLA typing

Genomic DNA was extracted from the whole blood by using the QIAampTM DNA Blood Midi Kit (Qiagen Sciences, Germantown, MD). Plasma samples six months on average after the outbreak were tested for anti–SARS-CoV antibodies by SARS ELISA (Genelabs Diagnostics Pty Ltd, Singapore) from all participants [12]. DNA-based HLA typing was performed by Luminex Multi-Analyte Profiling system (xMAP) with WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan) as described elsewhere [13]. Briefly, highly polymorphic exons 2 and 3 of HLA-A, -B, and -C genes and exon 2 of HLA-DRB1 and -DQB1 genes were amplified using primer pairs attached to the kit. Each PCR product was hybridized with sequence-specific oligonucleotide probes, complementary to the allele-specific sequences. Reproducibility was checked between two independent measurements of randomly chosen samples and the level of agreement was more than 99% (183/184 alleles). Samples showing ambiguous patterns were subjected to sequence-based typing by using AlleleSEQR HLA typing kit (Abbott JAPAN, Tokyo, Japan) and analyzed on an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.3. Statistical analysis

Disease association was assessed by the χ^2 test. When any expected number in the 2×2 contingency table was less than 5, the p value was directly calculated by Fisher's exact test. Values of $p < 0.05$ are shown. Corrected p values (p_c), p values multiplied by the number of comparisons in each locus, are also shown. Values of $p_c < 0.05$ were considered to be statistically significant.

3. Results

Of 62 patient cases corresponding to the World Health Organization case definition of probable SARS, five patients died and another three were not Vietnamese. As a result, 44 of the remaining 54 SARS patients after recovery (Cases), 103 individuals who had contact with SARS patients (Contacts) and 50 without contact (No contacts) were included within this study. DNA samples from two of 103 Contacts showed inconclusive genotyping results and were excluded from the present analysis.

We primarily compared the Cases with Contacts, No contacts, or both. Subsequently all Cases and 16 of the 101 Contacts were revealed to have anti-SARS-CoV antibodies in their blood and we secondarily analyzed them together with SARS patients as the Infected group ($n = 44 + 16$) and compared with the Uninfected group ($n = 101 - 16$). Allele frequencies of HLA class I and class II genes in each group were first represented by low resolution (two-digit) typing and are listed in Tables 1 and 2, respectively. When the number of comparisons was considered at each locus, HLA class I genes did not show any significant association ($p_c > 0.10$). On the other hand, among HLA class II genes, HLA-DRB1*12 showed positive association ($p = 0.0032$, $p_c = 0.042$), and HLA-DRB1*13 showed marginally negative association ($p = 0.0069$, $pc = 0.090$) when the SARS group was compared with the Contacts. A similar tendency was observed when the SARS group was compared with the No-contacts as well. However, the same alleles did not show any strong association when the Infected group was compared with Uninfected.

HLA-DRB1*1202 allele was the only allele of HLA-DRB1*12 identified in this population by higher resolution (four-digit) typing (Table 3). When the Contacts and No-contacts were collected together as a single control group, HLA-DRB1*1202 showed further significant association ($p = 0.0011$, $p_c = 0.014$, data not shown). In our previous population-based study on Vietnamese HLA alleles, the frequency of HLA-DRB1*1202 allele in 170 healthy Hanoi citizens was 0.353 [13]. The frequency of HLA-DRB1*1202 in our SARS patients (0.466) remained higher than that of the general population in Hanoi. Under the dominant model, HLA-DRB1*1202 was the most strongly associated with SARS as shown in Table 4 ($p_c = 0.0065$ and 0.0052 depending on the controls to be compared).

HLA-DRB1*13 was composed of DRB1*1301, *1302 and *1303 but no single alleles were further associated with the disease (Table 3). HLA association between the Infected and Uninfected groups did not exceed any association of HLA alleles observed between the Cases and Contacts (Tables 1 and 2). Possible association of HLA polymorphism with severity of SARS was not investigated in this study because of the small numbers in each subgroup. No significant deviation from Hardy-Weinberg equilibrium was observed in the control populations ($p > 0.10$).

4. Discussion

No particular alleles of class I genes were associated with SARS in the Vietnamese population, whereas HLA-DRB1*1202 showed a significant association with SARS development. In Viet Nam, the majority of the patients were health care staff in one hospital, and our study was the only HLA report from this country.

As far as we know, at the present time HLA reports regarding association with SARS [2–5] have been published in Taiwan, Hong Kong, and southern China. Interestingly, all reports showed inconsistent results, although HLA patterns in the general population of their countries were quite similar, presumably attributable to rather homogenous ancestral gene pool around southern China. It is known that ethnicity of the Vietnamese is also influenced by the population resident in southern China in the Bronze Age [13]. Thus, comparative consideration of the previous four association studies in these Asian populations is worthwhile.

First of all, our data can be interpreted naturally and consistently with a recent large study from Southern China [5], which showed an increase in frequency of several HLA-A and -B antigens and DRB1*12 allele, although the alleles did not reach significant levels when multiple comparisons were made. In case of HLA-DRB1*12, allele frequency was 32.6% in 95 SARS and 22.8% in 403 controls with a value of $p < 0.046$ in their study [5]. We were able to confirm this disease association of HLA-DRB1*12 clearly in the present study.

In the Vietnamese population, the frequency of HLA-DRB1*1202 mainly consisting of DRB1*12 is notably higher than in the Southern Chinese population [13] and therefore the HLA association with SARS might have been more obvious even in the smaller sample study like ours. In particular, our advantage is that the majority of cases and controls were exposed to SARS-CoV during a rather short period inside one hospital through a single spread from Hong Kong [14]. Such homogeneity of environmental and pathogenic factors might have provided a favorable situation to identify host genetic factors without detailed consideration of unknown confounders.

Another recent Taiwanese genome-epidemiologic study demonstrated potential association of HLA-Cw*0801 with SARS infection between seropositive and seronegative cases [4]. In fact, even in our study, HLA-Cw*0801 was more frequently observed in the Infected group (24.2%) than in the Uninfected group (17.1%), although not reaching a significant level, probably because of insufficient statistical power. More interestingly, a five-locus haplotype including both DRB1*1202 and Cw*0801 alleles, A*1101-Cw*0801-B*1502-DRB1*1202-DQB1*0301, is the most frequent haplotype in