HLA-DRB1*15021 Is the Predominant Allele in Japanese Patients with Juvenile Dermatomyositis

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ABSTRACT. ***Objective.*** To investigate HLA molecules and genes in Japanese patients with juvenile dermatomyositis (JDM).

Methods. Twelve patients (8 girls and 4 boys) with ages of onset between 3 and 15 years were included. HLA class I antigen phenotypes were serologically typed by the Terasaki-NIH standard method. DNA was extracted from peripheral blood leukocytes using the phenol-chloroform extraction procedure, and stored at –70°C until use. Genomic DNA for HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles in JDM patients and controls was determined by the direct sequence method.

Results. HLA-A24 and B52 were each detected in 7 cases (OR = 0.86, 5.02, p = 0.930, 0.006, respectively). HLA-DRB1*15021 was observed in 7 patients. This was significantly more frequent than occurred in the controls (OR = 5.72, p = 0.002). Seven patients out of 12 (58%) had the combination HLA-B52, DRB1*15021, DQA1*0103, and DQB1*0601.

Conclusion. Our results suggest that the susceptibility gene for JDM either is HLA-DRB1*15021 or is present near the HLA-DRB1 locus. This differs from previous reports that describe the association with HLA-DQA1*0501 in Caucasian patients with JDM. The combination HLA-B52, DRB1*15021, DQA1*0103, and DQB1*0601 may contribute to the pathogenesis of JDM in Japanese patients. (J Rheumatol 2004;31:1847–50)

Key Indexing Terms:
JUVENILE DERMATOMYOSITIS HLA SUSCEPTIBILITY GENE JAPANESE

Polymyositis (PM) is an inflammatory myopathy of unknown cause to which the term dermatomyositis is applied in the presence of a characteristic rash: either Gottron's papules or heliotrope rash. Both diseases are included in a group of idiopathic inflammatory myopathies classified by Bohan and Peter[1]. According to this classification, both juvenile dermatomyositis (JDM) and juvenile PM were classified in one group: childhood dermatomyositis and polymyositis with vasculitis. This disease entity has been characterized by widespread necrotizing vasculitis that may occur with intimal proliferation in small blood vessels, thromboses, and multiple infarctions.

Although the etiologies of JDM and juvenile PM are unknown, the pathogenesis of each of these 2 diseases is mediated by different lymphocyte types; CD4+ T cells and B cells show a perivascular distribution in JDM[2] and CD8+ T cells and B cells in juvenile PM in the same area. The presence of antinuclear antibody (ANA) suggests that this disorder could be a kind of autoimmune disease[3]. HLA class II molecules are expressed on the antigen-presenting cell by which the processed foreign antigen can be recognized by T cell receptors. Alternatively, the restricted T cell repertoire of the specific antigen might influence the immune response afterwards in cooperation with co-stimulating antigens. It was recently revealed that the polymorphism of such molecules is associated with certain autoimmune diseases including myasthenia gravis[4] and insulin-dependent diabetes mellitus[5].

The association of Caucasian JDM patients with a certain HLA type, HLA-DR3, was first reported by Friedman, *et al*[6]. Later, Reed, *et al*[7,8] reported that the susceptibility gene for JDM is HLA-DQA1*0501 in Caucasian, African-American, and Hispanic JDM patients; it is known that this allele is linked with HLA-DR3 in a strong linkage disequilibrium. This was confirmed by Rider, *et al* in Caucasian American patients with idiopathic inflammatory myopathy (IIM), but not in Korean patients with similar distribution of clinical characteristics[9]. However, HLA-DR3 is quite rare in the Japanese population (0.12%)[10], although it is found in about 10% of Caucasians[11]. There has been no report about the contribution of HLA to JDM in Japanese patients. Therefore this study was undertaken to determine whether HLA-DQA1*0501 confers susceptibility to JDM in Japanese populations, or whether another HLA gene could be the primary susceptibility allele.

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MATERIALS AND METHODS

Study subjects. Twelve patients seen in the Immunology/Rheumatology division of the Pediatric Department of Yokohama City University School of Medicine (8 girls and 4 boys) were enrolled in this study. They fulfilled the criteria of Bohan and Peter[1]. The average onset age was 8.7 ± 1.0 years, range 3–15 years. Six patients (50%) had elevated ANA. Enzyme-linked immunosorbent assay (ELISA) showed that all cases were negative for antibodies to Jo-1, Scl-70, Sm, RNP, SSA, and SSB, and that all had normal levels of anti-DNA antibodies. The maximum levels of serum creatinine kinase (CK) ranged from 593 to 31,940 IU/l (mean 5,766 IU/l, normal 39-163 IU/l). Blood samples were taken between February and May 1999. Control data on the Japanese population were obtained from more than 1000 healthy bone marrow donors[10].

Laboratory assays. The HLA class I antigen phenotypes were typed by the Terasaki-NIH method[12]. Briefly, genomic DNA was extracted from peripheral whole blood leukocytes using a standard phenol-chloroform extraction procedure, and was stored at –70°C before DNA typing. DNA from patients with JDM and controls was genotyped for alleles at the following major histocompatibility complex (MHC) loci: HLA-DRB1 (HLA-DRB1), HLA-DQa (HLA-DQA1), and HLA-DQb (HLA-DQB1). All loci were analyzed by the direct sequence method using an ABI Prism 377 DNA Sequencer™ (Perkin-Elmer Japan). HLA-DRB1 alleles were matched by the software Match Maker™ version 1.1 (Perkin-Elmer, Foster City, CA, USA).

Statistical analysis. Frequencies of HLA molecules and alleles were compared between patients and controls using the chi-squared test with Yates' continuity correction or, when appropriate, Fisher's exact test (2-tailed); p values were corrected (pc) by Svejgaard's methods[13], in which pc values were obtained by multiplying observed p values by the number of alleles examined at each HLA locus (9 for A, 21 for B, 6 for Cw, 23 for DRB1, 8 for DQA1, and 12 for DQB1). The odds ratios (OR) with a 95% confidence interval (95% CI) were calculated by Haldane's modification of Woolf's method[14] when one square in a 2 × 2 table contained a value of 0.

RESULTS

Serological data of HLA class I antigens are listed in Tables 1 and 2. Although HLA-A24 was observed in 6 cases, this frequency was not significantly greater than that of the controls (OR = 0.86, p = 0.930). HLA-B52 was seen more frequently in the patients than in the controls (OR = 5.02, p = 0.006, pc = 0.121). There was no patient with HLA-B7.

Genotypes of HLA-DRB1, HLA-DQA1, and DQB1 of the patients are shown in Tables 3 and 4. Some specific

Table 1. HLA class I genotypes of patients with JDM.

Patient	HLA Serological Type A Locus		B Locus		Cw Locus	
1	2	26	52	61	3	—
2	2	—*	13	62	3	—
3	11	24	51	54	1	—
4	24	26	52	61	3	—
5	24	31	35	52	4	—
6	2	24	46	52	1	—
7	11	26	54	61	1	3
8	2	33	44	46	1	—
9	26	31	51	61	3	—
10	11	24	52	67	7	—
11	24	—	52	—	—	—
12	24	—	52	61	—	—

* Not detected.

Table 2. Frequencies and odds ratios (OR) of the HLA class I genotypes of patients with JDM.

HLA type	Cases	Controls	OR (95% CI)	p	pc
A2	0.33	0.42	0.74 (0.10–5.58)	0.769	1.000
A24	0.58	0.61	0.86 (0.03–24.08)	0.930	1.000
A26	0.33	0.21	2.03 (0.29–13.99)	0.473	0.997
B52	0.58	0.21	5.02 (1.58–15.90)	0.006	0.121
B61	0.33	0.24	1.69 (0.15–18.86)	0.672	1.000
Cw1	0.60	0.40	1.63 (0.11–24.29)	0.724	1.000
Cw3	0.40	0.30	2.19 (0.46–10.49)	0.327	0.907

HLA-DRB1 molecules were observed among the patients more than once: HLA-DRB1*15021 in 7 patients, DRB1*15011 in 2, DRB1*04051 in 2, DRB1*08032 in 2, and DRB1*0901 in 5. HLA-DRB1*15021 was seen significantly more often in the patients than in the controls (OR = 5.72, p = 0.002). However, after correction of the p value by Svejgaard's methods, pc was 0.055, which was not significant with 95% confidence. The OR of the other HLA-DRB1 alleles were not significantly high compared to those of the controls. There was no patient with HLA-DR3.

Four types of HLA-DQA1 alleles were observed among the patients: HLA-DQA1*0101, DQA1*0102, DQA1*0103, and DQA1*0301/0302. HLA-DQA1*0102 were seen in 3 cases, DQA1*0103 in 8, and DQA1*0301/0302 in 7. None of the OR of these HLA-DQA1 alleles was significantly higher than those of the controls, because of the limited number of cases. There was only one patient with HLA-DQA1*0501. It was not found more often in the patients (OR = 2.24, p = 0.840). The following 7 HLA-DQB1 alleles were observed in the patient group: HLA-DQB1*0302 was seen in one patient, DQB1*0303 in 5, DQB1*0304 in one, DQB1*0401/0402 in 2, DQB1*0501 in one, DQB1*0601 in 8, and DQB1*0602 in 3. None of the OR of these HLA-DQB1 alleles was significantly higher than those of the controls, again because of the limited number of cases.

Of interest, the combination B52-DRB1*15021-DQA1*0103-DQB1*0601 was seen in 7 patients. The OR of this combination was 6.46, significantly higher than in the controls (p = 0.001). The mean of the maximum CK was 6,912 IU/l in patients with HLA-DRB1*15021 and 798 IU/l in those without.

DISCUSSION

In our study, HLA-DRB1*15021, but not HLA-DQA1*0501, was observed significantly more frequently in the Japanese patients with JDM than in controls (OR = 5.72, p = 0.002, pc = 0.055). Only one patient in this study had the HLA-DQA1*0501 allele. On the other hand, the combination of HLA-B52, DRB1*15021, DQA1*0103, and DQB1*0601 was observed in 7 patients out of 12 (58%).

Table 3. HLA class II genotypes of the patients with JDM.

Patient	HLA Class II Genotype					
	DR B1*		DQ A1*		DQ B1*	
1	15021	04051	0103	0103	0601	0401/0402
2	15011	0901	0102	0301/0302	0602	0303
3	0101	0901	0101	0301/0302	0501	0303
4	15021	0410	0103	0301/0302	0601	0401/0402
5	15021	1201	0103	0501	0601	0304
6	15021	08032	0103	0103	0601	0601
7	04051	08021	0301/0302	0301/0302	0401	0302
8	1302	08032	0102	0103	0601	0602
9	15011	0901	0102	0301/0302	0602	03032
10	15021	0901	0103	0301/0302	0601	03032
11	15021	15021	0103	0103	0601	0601
12	15021	0901	0103	0301/0302	0601	03032

0301/0302: HLA-DQA1*0301 or 0302; 0401/0402: HLA-DQA1*0401 or 0402.

Table 4. Frequencies and odds ratios (OR) of the HLA class II genotypes of the patients with JDM.

HLA Allele	Cases	Controls	OR (95% CI)	p	pc
DRB1*15011	0.18	0.14	1.49 (0.00–783.13)	0.900	1.000
DRB1*15021	0.64	0.19	5.72 (1.85–17.66)	0.002	0.055
DRB1*0803	0.18	0.16	1.26 (0.31–5.03)	0.746	1.000
DQA1*0103	0.67	0.47	2.14 (0.54–8.55)	0.280	0.928
DQA1*0301/0302	0.58	0.65	0.75 (0.02–34.87)	0.882	1.000
DQA1*0501	0.08	0.07	2.24 (0.00–5525.24)	0.840	1.000
DQB1*0601	0.67	0.45	2.34 (0.61–9.00)	0.217	0.947
DQB1*0602	0.25	0.13	2.42 (0.26–22.61)	0.437	0.999

0301/0302: HLA-DQA1*0301 or 0302.

This is the first study to find this combination among JDM patients.

Modern molecular genetic techniques have made possible the investigation of the association of MHC genes and certain diseases including rheumatoid arthritis and Sjögren's syndrome[15]. JDM has also been the target of such investigations. The strong association with HLA-DR3 was reported in the early days in Caucasian patients[6]. Recently, Reed, *et al* reported that HLA-DQA1*0501, which is linked with HLA-DR3 in linkage disequilibrium in Caucasian populations, is the susceptibility gene for this disease in some races[7,8]. However, these alleles can be observed in few Japanese individuals; HLA-DR3 was reported in about 0.12%[10], and HLA-DQA1*0501 is observed in only 3.3%. It is therefore quite difficult to imagine that this is also a susceptibility gene in Japanese patients. This is the reason we conducted this study.

We found another candidate as the susceptibility gene of JDM: HLA-DRB1*15021. OR were markedly high for the HLA-B allele and HLA-DRB1 allele. Considering the sequence of alleles on chromosome 6, which contains HLA-A, Cw, B, DRB1, DQA1, and DQB1 from the telomere to the centromere, these OR suggested that the susceptibility allele for JDM is located between or around HLA-B and HLA-DRB1 loci, between which there is an HLA class III region with many known and unknown genes modulating immune response.

Consequently, our results lead to 2 possibilities. First, there is an unknown susceptibility allele around the HLA-B and HLA-DRB1 locus, which may be linked in linkage disequilibrium with the HLA-DQA1*0501 allele in Caucasians and with the HLA-DRB1*15021 allele in Japanese. Second, HLA-DRB1*15021 may have similar functions to HLA-DQA1*0501 in terms of pathogenesis of JDM while stimulating certain T cell repertoires.

Considering the first possibility that an unknown allele near HLA-B and HLA-DRB1 can contribute to the pathogenesis of JDM, 7 patients out of 12 (58%) in this study had the combination HLA-B52, DRB1*15021, DQA1*0103, and DQB1*0601. The haplotype HLA-B52-DRB1*15021-DQA1*0103-DQB1*0601 is known to contain the gene C4A3-2-C4BQ0 in the Japanese population[16], and this and nearby genes of the region control the production of protein in the complement system. Robb, *et al*[17] and Reed, *et al*[7] reported the increased prevalence of C4A deletion among JDM patients, although no study of C4B* has been reported. Immune complex deposits with complement on small blood vessels in muscle tissue are thought to be important in the pathogenesis of JDM[18]. Although it has not been confirmed that the patients in this study population had this combination of genes as a haplotype or merely by chance, when considering that this haplotype can be seen in 8.41% of the Japanese population[16], the figure of 58% is much higher than would be suggested by chance.

On the other hand, HLA class II molecules are proteins expressed on the antigen-presenting cell, which has one of the central roles in the control of a subsequent immune response. Tezak, *et al* hypothesized that certain HLA might

induce dynamic interactions between the muscular, vascular, and immune systems, and investigated the gene expression profiles of patients with untreated JDM[19].

It is widely accepted that HLA-DQA1*0501 has a strong association with the pathogenesis of JDM in Caucasians[20]. However, most patients in our study had no such allele. So HLA-DQA1*0501 is not the unique susceptibility gene for JDM, at least in our study group. Indeed, West and Reed reported that HLA-DMA*0103 and HLA-DMB*0102 had increased relative risk ratios[21]. It can therefore be hypothesized that some HLA molecules, including the HLA-DRB1*15021 allele, also cause JDM, perhaps like the shared epitope in rheumatoid arthritis[22]. Furuya, *et al* reported that HLA-DRB1*08 had an association with DM in Japanese adults[23]; this was a different form of HLA accumulation from those in previous reports concerning adult Caucasian populations and JDM patients in both Caucasian[6-8] and Japanese populations (OR = 1.07, p = 0.811, in our study). Therefore, it can be suggested that there are differing varieties of the susceptibility gene for JDM in different races with different genetic backgrounds, and that this gene is different from those of adult patients. This idea is in accord with an ethnogeographic study of patients with IIM[9]. The disease may be classified into subtypes according to genetic background in the future.

Finally, JDM can perhaps be regarded as a multifactorial disease caused by several susceptibility genes and some environmental factors[24]. There are limitations of our study due to the large number of controls and the small number of patients that might lead to false positive showing significance, or to false negative due to small sample size. A study on a wider scale with patients of various races would be helpful for appreciation of the variety of manifestations of the disease.

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