

HLA and atopic dermatitis with high serum IgE levels

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*Patients with atopic dermatitis usually exhibit allergen-specific IgE antibodies against several environmental antigens. HLA restriction is presumed to be involved in the recognition of such antigens, but several previous reports have so far failed to find a significant association between atopic dermatitis and HLA antigens. In this study we examined 38 unrelated Japanese patients with severe atopic dermatitis and high serum IgE levels (greater than 8000 U/ml). We investigated the serological HLA types and HLA class II alleles in this group of patients with atopic dermatitis. Frequencies of HLA-A24, A33, Cwblank, B44, DR13 and HLA-DRB1*1302, DQB1*0604, DPB1*0301 alleles were increased in the patients. In contrast, frequencies of HLA-Cw1, Bw6, DR4, DR53, and HLA-DQB1*0302 allele were decreased. However, none of these remained significant after p values were corrected. Further study on HLA association with atopic dermatitis through characterization of specific antigens or antigen epitopes is needed. (J ALLERGY CLIN IMMUNOL 1994;94:575-83.)*

Key words: HLA, polymerase chain reaction, atopic dermatitis, IgE

Atopic dermatitis (AD) is one of the common allergic diseases prevalent throughout the world. Patients with this disorder produce allergen-specific IgE antibodies against several environmental antigens such as mites, house dust, pollens, and fungi. Genetic susceptibility has been investigated to disclose the immunologic alterations of AD. Association studies between HLA and AD have been reported by several investigators. Ozawa et al.¹ reported that patients with AD and bronchial asthma and allergic rhinitis showed a significant increase in frequency in both HLA-B12 and B40, although there was no overall difference in the HLA antigen frequencies in patients with AD only. Svejgaard et al.² reported that the frequency of HLA-DR7 antigen was decreased in patients

Abbreviations used

AD:	Atopic dermatitis
bp:	Base pairs
PCR:	Polymerase chain reaction
RFLP:	Restriction fragment length polymorphism
SSO:	Sequence-specific oligonucleotide

with AD, although the significance of this deviation disappeared when *p* values were corrected. Larsen and Grunnet³ were also unable to demonstrate any significant associations between AD and the frequencies of HLA-A, B, or C.

The diagnosis of AD is based on clinical criteria,⁴ and thus this disease is presumed to be composed of several subgroups according to the age of onset, clinical severity, serum IgE levels, family history, and coexistence of bronchial asthma and/or allergic rhinitis. The previous reports on HLA association might have examined heterogeneous groups of patients. Furthermore, patients with AD exhibited several specific IgE antibodies against environmental antigens. These may explain the failure to demonstrate a significant association between AD and HLA. In addition, there have been no reports so far on HLA association with

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TABLE I. A panel of oligonucleotides

Nucleotide sequences	
Primers	
DR13 group	
DRBAMPA3	5'-TACTTCCATAACCAGGAGGAGA-3'
DRBAMPA4	5'-GTTTCTTGGAGTACTCTACGTC-3'
DR13AMPB1	5'-CCCGCTCGTCTTCCAGGAT-3'
DR13AMPB2	5'-TGTTCAGTACTCGGCGCT-3'
DR14AMPB2	5'-TCCACCGCGGCCCCGCC-3'
DRBAMPB4	5'-CTGCACTGTGAAGCTCTCAC-3'
DRBAMPB5	5'-CTGCACTGTGAAGCTCTCCA-3'
DR2 group	
DR2B1AMPA	5'-TTCCTGTGGCAGCCTAAGAGG-3'
DRBAMPB	5'-AGAGCTTCACAGTGCAGCGGC-3'
DQB1	
GH28	5'-CTCGGATCCGCATGTGCTACTTCACCAACG-3'
GH29	5'-GTGTGCAGACACAACCTACCTGCAGCTC-3'
DPB1	
DPBAMPA	5'-GAGAGTGGCGCCTCCGCTCAT-3'
DPBAMPB	5'-GAGTGAGGGCTTTGGGCCGGC-3'
Probes	
DR2B1	
DRB28-13	5'-GTTCTTGGACAGATACTT-3'
DRB70-2	5'-GACTTCCTGGAAGACAGG-3'
DRB70-3	5'-GACCTCCTGGAAGACAGG-3'
DRB70-11	5'-GACATCCTGGAGCAGGCG-3'
DRB86-1	5'-AACTACGGGGTTGGTGAG-3'
DRB86-3	5'-AACTACGGGGTTGTGGAG-3'

TABLE II. HLA-DR13-related alleles defined by sequence-specific amplification

DR13 group*	DRB1 alleles	Forward primers		Reverse primers				
		D	D	D	D	D	D	D
		R	R	R	R	R	R	R
		B	B	1	1	1	B	B
		A	A	3	3	4	A	A
		M	M	A	A	A	M	M
		P	P	M	M	M	P	P
		A	A					
		3	4	P	P	P	B	B
				B	B	B	4	5
				1	2	2		
DR13-1	DRB1*1301	+	+	+	-	-	-	+
	DRB1*1302	+	+	+	-	-	+	-
DR13-2	DRB1*1303	-	+	-	+	-	+	-
	DRB1*1304	-	+	+	+	-	-	+
DR13-3	DRB1*1305	+	+	-	-	+	+	-
	DRB1*1306	+	+	-	-	+	-	+

*HLA-DR13 specificities are tentatively subdivided into three groups according to exon 2 nucleotide sequences of the DRB1 gene.
+, Amplified; -, not amplified.

AD with respect to serum IgE levels. In this study we selected patients with severe AD and high serum IgE levels (greater than 8000 U/ml) and examined HLA antigens and HLA class II alleles in this group of patients.

METHODS

Patients

This study included 38 unrelated Japanese patients with AD and high serum IgE levels. The study group consisted of 27 males and 11 females, aged 11 to 61

TABLE III. Digestion patterns of DQB1 and DQB2

	Restriction endonucleases			
	HinI1	HhaI	Cfr13I	MspI
DQB1 alleles				
0201	I1	H1	C1	M1
0301	I4	H2	C2	M2
0302	I2	H2	C2	M3
03031	I4	H2	C3	M3
03032	I4	H2	C2	M3
0304	I2	H2	C2	M2
0401	I5	H3	C4	M3
0402	I5	H4	C4	M3
0501	I3	H1	C5	M2
0502	I3	H5	C5	M2
05031	I5	H1	C5	M3
05032	I3	H1	C5	M3
0504	I3	H5	C6	M2
0601	I4	H1	C7	M4
0602	I2	H6	C7	M3
0603	I2	H6	C7	M3
0604	I2	H4	C7	M2
0605	I2	H4	C7	M2
DQB2* alleles				
0101	I6	H7	C8	M5
0201	I6	H8	C8	M5
0202	I6	H8	C8	M5

Each digestion pattern produces the following DNA fragment sizes. I1:230 bp. I2:123,107 bp. I3:123,66,41 bp. I4:123,88,19 bp. I5:123,47,41,19 bp. I6:227 bp. H1:142,48,40 bp. H2:190,40 bp. H3:142,86,2 bp. H4:142,46,40,2 bp. H5:86,56,48,40 bp. H6:113,46,40,29,2 bp. H7:139,42,40,6 bp. H8:139,48,40 bp. C1:230 bp. C2:132,49 bp. C3:101,49,31 bp. C4:151,48,31 bp. C5:173,48,8,1 bp. C6:174,48,8 bp. C7:181,49 bp. C8:184,43 bp. M1:117,113 bp. M2:113,72,45 bp. M3:185,45 bp. M4:230 bp. M5:227 bp.

*DQB2 pseudogene contains three alleles, which we tentatively named as follows: DQB2*0101, *0201, and *0202. These alleles correspond to the sequences reported by Okada et al.,²³ Berdoz et al.,²⁴ and Kimura (unpublished data, GenBank Database accession no. M83891), respectively.

years (mean age, 25.2 ± 8.5 years). Their serum IgE levels varied from 8440 to 75,600 U/ml (mean level, $18,830 \pm 12,715$ U/ml). All patients showed a severe clinical form of AD on the basis of extent of inflammatory lesions, intensity of exanthema (e.g., erythema, edema, papules, excoriation, lichenification), and subjective symptoms such as pruritus.^{5,6} All patients had high levels of anti-mite and anti-*Pityrosporum ovale* IgE and also had high levels of IgE for at least one of the following allergens: house dust, cedar pollen, and *Can-dida*. Thirty-one patients had a personal history of atopic respiratory diseases (asthma and/or rhinitis), and in 24 of these, a family history of atopic diseases was also noted. In four of the other seven patients with AD, there was a family history of atopic diseases. Fifty-one healthy Japanese persons with no atopic diathesis served as control subjects. All patients and control subjects were HLA-typed by the standard lymphocyte microcytotoxicity test. After informed consent was obtained, venous blood was drawn from each individual.

Oligonucleotides

Oligonucleotides were synthesized by the methoxy-phoramidite method on an Applied Biosystems 381A

DNA Synthesizer (Applied Biosystems, Inc., Foster City, Calif.). Because serological typing revealed an increase of HLA-DR13 and -DR15(2) in the patients, we prepared seven primers to identify DR13-related alleles⁷ and also designed primers for DR2 group-specific amplification⁸ and six sequence-specific oligonucleotide (SSO) probes for the DR2-associated DRB1 gene. Primers for DQB1 (GH28, GS29)⁹ and DPB1 (DPBAMPA, DPBAMPB) were synthesized for generic amplification. Table I shows nucleotide sequences of these primers and SSO probes. All the primers correspond to the second exon of each gene.

Polymerase chain reactions

Genomic DNA was extracted from peripheral blood leukocytes according to the method described by Maniatis et al.¹⁰ Polymerase chain reaction (PCR) was carried out as described by Saiki et al.¹¹ with minor modifications. The DRB1 gene for DR2 and DR13, the DQB1 gene, and the DPB1 gene were amplified by PCR. One microgram of genomic DNA was suspended in 100 μ l of 50 mmol/L Tris/HCl (pH 8.3), 1.5 mmol/L MgCl₂, 200 nmol/L of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 50 pmol of

TABLE IV. Serological HLA types in atopic dermatitis

Antigen HLA	Patients (n = 38)		Control subjects (n = 51)		Relative risk	Chi square	p Value
	No.	%	No.	%			
A2	13	34	25	49	0.54	1.9518	0.1623
A3	1	3	3	6	0.43	0.0462*	0.8297
A11	6	16	14	27	0.49	1.6997	0.1923
A24	26	68	21	41	3.09	6.4857	0.0108†
A26	11	29	12	24	1.32	0.3335	0.5635
A31	7	18	7	14	1.41	0.3621	0.5472
A33	10	26	5	10	3.28	4.2365	0.0395†
Cw1	5	13	16	31	0.33	4.0073	0.0453†
Cw9	11	29	15	29	0.97	0.0022	0.9619
Cw10	1	3	7	14	0.16	2.0602*	0.1511
Cw4	1	3	9	18	0.12	3.5321*	0.0601
Cw5	0	0	1	2	0.00	0.0000*	1.0000
Cw6	1	3	1	2	1.35	0.0000*	1.0000
Cw7	12	32	14	27	1.21	0.1794	0.6718
Cw8	2	5	1	2	2.77	0.0676*	0.7947
blank	10	26	4	8	4.19	4.2985*	0.0381†
B7	3	8	7	14	0.53	0.2727*	0.6014
B13	2	5	3	6	0.88	0.0000*	1.0000
B35	4	11	9	18	0.54	0.4063*	0.5238
B39	5	13	3	6	2.42	0.6599*	0.4165
B44	10	26	5	10	3.28	4.2365	0.0395†
B46	1	3	8	16	0.14	2.7727*	0.0958
B48	3	8	4	8	1.00	0.0000*	1.0000
B51	8	21	12	24	0.86	0.0766	0.7818
B52	15	39	14	27	1.72	1.4328	0.2313
B54	1	3	4	8	0.31	0.3490*	0.5546
B55	1	3	3	6	0.43	0.0462*	0.8297
B58	1	3	0	0	4.12	0.0220*	0.8819
B59	1	3	0	0	4.12	0.0220*	0.8819
B60	3	8	5	10	0.78	0.0000*	1.0000
B61	8	21	8	16	1.43	0.4252	0.5143
B62	5	13	12	24	0.49	1.5158	0.2182
B67	1	3	0	0	4.12	1.3573	0.2439
B70	1	3	2	4	0.66	0.0000*	1.0000
B75	0	0	1	2	0.00	0.0000*	1.0000
Bw4	28	74	30	59	1.96	2.1185	0.1455
Bw6	28	74	46	90	0.30	4.2365	0.0395†

*Chi-square tests with Yates' correction.

†p < 0.05.

each primer, and 2 units of Ampli-Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). PCR conditions were set as follows: 95° C for 60 seconds for denaturing, then at 55° C for 120 seconds for annealing, and at 72° C for 90 seconds for primer extension. This cycling was repeated 30 times in a programmable DNA Thermal Cycler (Perkin-Elmer Cetus). Further incubation for 5 minutes at 72° C was performed to complete the extension after the last cycle. Five-microliter ali-

quots of the amplified products were electrophoresed in 5% polyacrylamide gel to ascertain the amplification.

Genotyping of HLA-DRB1 alleles

Because frequencies of DR13 and DR15(2) specificities were increased in the patients with AD, we examined DRB1 alleles related to DR13 and DR2. HLA-DR2 specificity is encoded by two functional genes, DRB1 and DRB5. The DR2-related DRB1 gene con-

TABLE IV. Cont'd.

Antigen HLA	Patients (n = 38)		Control subjects (n = 51)		Relative risk	Chi square	p Value
	No.	%	No.	%			
DR4	8	21	22	43	0.35	4.7528	0.0292†
DR7	1	3	3	6	0.43	0.0462*	0.8297
DR8	7	18	15	29	0.54	1.4135	0.2344
DR9	7	18	13	25	0.66	0.6245	0.4293
DR11	5	13	4	8	1.78	0.2182*	0.6403
DR12	3	8	4	8	1.00	0.0000*	1.0000
DR13	10	26	3	6	5.71	5.7428*	0.0165†
DR14	7	18	6	12	1.69	0.7734	0.3791
DR15	19	50	18	35	1.83	1.9387	0.1638
DR16	1	3	0	0	4.12	0.0220*	0.8819
DR52	26	68	29	58	1.64	1.2322	0.2669
DR53	15	39	34	68	0.32	6.5073	0.0107†
DQ1	32	84	35	69	2.43	2.8415	0.0918
DQ2	1	3	2	4	0.66	0.0000*	1.0000
DQ3	21	55	35	69	0.56	1.6669	0.1966
DQ4	8	21	12	24	0.86	0.0766	0.7818

*Chi-square tests with Yates' correction.

† $p < 0.05$.

tains five alleles. Six digoxigenin-11-dUTP labeled SSO probes (Table I) were hybridized with slot-blotted samples amplified by DR2 group-specific amplification. Hybridization signals were detected by chemiluminescent assay with hydrolysis of AMPPD (Boehringer, Mannheim GmbH, Mannheim, Germany) with alkaline phosphatase-conjugated anti-digoxigenin antibody.^{12, 13}

HLA-DR13 specificity contains six alleles. We identified DR13-related alleles by sequence-specific amplification. Six alleles related to DR13 were discriminated with seven sets of primers, as shown in Table II.

Genotyping of HLA-DQB1 and DPB1 alleles by PCR-restriction fragment length polymorphism

For HLA-DQB1 genotyping, amplified DNA was digested with *Hin*1I, *Hha*I, *Cfr*13I, and *Msp*I to discriminate 18 DQB1 alleles.¹⁴ For HLA-DPB1 genotyping, amplified DNA was digested with *Apa*I, *Sac*I, *Acc*II, *Fok*I, *Rsa*I, *Eco*NI, *Dde*I, and *Eco*RII to discriminate 20 DPB1 alleles.¹⁵ Ten microliters of amplified DNA solution was digested with each enzyme in a total volume of 20 μ l and analyzed by means of 10% polyacrylamide gel electrophoresis. Genotypes were determined by comparison of estimated fragment sizes with the predicted sizes^{14, 15} (Table III).

DQB1*0602 and *0603, as well as *0604 and *0605, cannot be discriminated by the PCR-restriction fragment length polymorphism (RFLP) method; therefore, the hybridization was further carried out with three

SSO probes DQB26-3 (5'-CGTCTTGTGACCAGAT-AC-3'), DQ26-4 (5'-CGTCTTGATACCAGACAC-3'), and DBQ26-6 (5'-CGTCTTGTAACCAGATAC-3') to discriminate these alleles.

Statistics

Statistical significance was determined by the chi square test with Yates' correction as necessary. The odds ratio of the disease was calculated according to the method of Svejgaard et al.¹⁶

Nomenclature

Nomenclature for HLA specificities and HLA class II alleles was described according to the system of the World Health Organization Nomenclature Committee HLA 1991.¹⁷

RESULTS

Table IV shows the results of serologically defined HLA types. The frequencies of HLA-A24 A33, Cwblank, B44, and DR13 were increased among the patients with AD. In contrast, the frequencies of HLA-Cw1, Bw6, DR4, and DR53 showed a decrease in the patient group. However, none of these remained significant after p values were corrected.

Because serologic typing revealed an increase of HLA-DR13 in the patients with AD, DR13 alleles were determined by group-specific ampli-

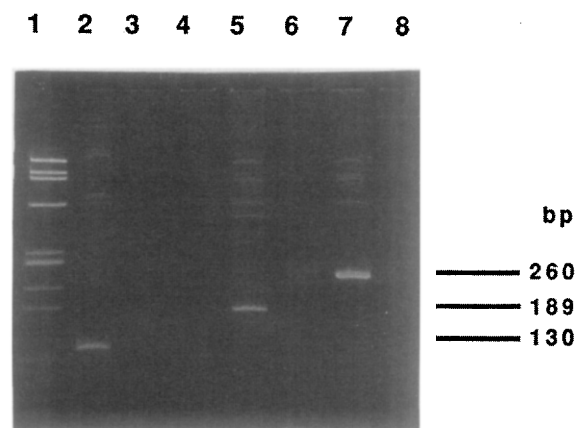


FIG. 1. Sequence specific amplification of HLA-DR13 group. DR13 specificities are tentatively subdivided into three groups. All of the HLA-DR13 found in the patients belong to the DR13-1 group in Table II. DR13-1 group contains two alleles, DRB1*1301 and *1302. Further sequence-specific amplification revealed that all the subjects with DR13-1 had the DRB1*1302 allele. *lane 1*, Molecular weight size marker: HaeIII digested ϕ x174; *lane 2*, 130 bp fragment is amplified with DRBAMPA3 and DR13AMPB1; *lane 3*, DRBAMPA4 and DR13AMPB2; *lane 4*, DRBAMPA3 and DR14AMPB2; *lane 5*, 189 bp fragment is amplified with DRBAMPA3 and DRBAMPB4; *lane 6*, DRBAMPA3 and DRBAMPB5; *lane 7*, 260 bp fragment is amplified with DRBAMPA4 and DRBAMPB4; *lane 8*, DRBAMPA4 and DRBAMPB5.

fication. Fig. 1 shows seven sets of DR13 group-related amplification. DR13 specificities are tentatively subdivided into three groups. All HLA-DR13 found in the patients with AD belong to the DR13-1 group in Table II. The DR13-1 group contains two alleles, DRB1*1301 and *1302. Further sequence-specific amplification revealed that all 10 subjects with DR13-1 had the DRB1*1302 allele. Because DR2 was also increased in the patients, DR2 alleles were discriminated by hybridization. Hybridization study revealed that four subjects with serologically defined DR15 group had the DRB1*1501 allele and 15 subjects had the DRB1*1502 allele. One subject with DR16 had the DRB1*1602 allele. HLA-DQB1 alleles were determined by PCR-RFLP. Fig. 2 shows an example of PCR-RFLP in a heterozygote for DQB1*0601/0604. The hybridization with three SSO probes DQB26-3, DQB26-4, and DQB26-6 revealed that all the subjects who were supposed to carry either DQB1*0602 or *0603 had DQB1*0602 and that those with either DQB1*0604 or *0605 had DQB1*0604.

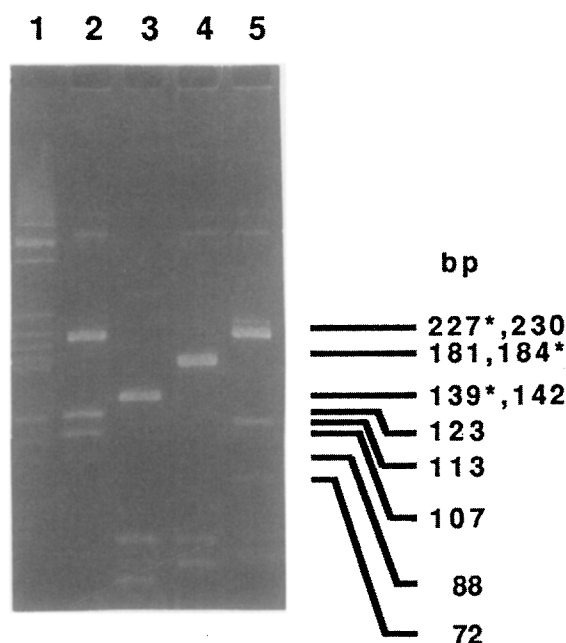


FIG. 2. PCR-RFLP of HLA-DQB1 alleles. An example of PCR-RFLP in a heterozygote for DQB1*0601/0604. This heterozygote produced a polymorphic band at 123, 107, 88 and 19 base pairs (bp) on Hin1I digestion; at 142, 48, 46, 40, and 2 bp on HhaI digestion; at 181 and 49 bp on Cfr 13I digestion; and at 230, 113, 72, and 45 bp on MspI digestion. Fragments derived from pseudogene DQB2 alleles were also visible at 227 bp on Hin1I digestion; at 139, 48, 42, 40, and 6 bp on HhaI digestion; at 184 and 43 bp on Cfr13I digestion; and at 227 bp on MspI digestion. *lane 1*, Molecular weight size marker: HaeIII digested pBR322; *lane 2*, digestion by Hin1I; *lane 3*, digestion by HhaI; *lane 4*, digestion by Cfr13I; *lane 5*, digestion by MspI. Fragments with an asterisk were derived from pseudogene DQB2 alleles.

Table V shows the frequencies of HLA class II alleles in patients with AD. The frequencies of HLA-DRB1*1302, DQB1*0604, and DPB1*0301 were increased in the patient group. In contrast, the frequencies of HLA-DQB1*0302 showed a decrease in the patient group. However, none of these remained significant after *p* values were corrected.

DISCUSSION

HLA molecules are supposed to be involved in presenting environmental antigens to T cells in allergic diseases. There have been several reports on the significant association between allergic rhinitis and HLA class II antigens. For example, Kumai et al.¹⁸ reported that HLA-DR9 and HLA-DQ3 phenotypes were strongly associated with the development of birch pollen allergy in Japa-

TABLE V. HLA class II alleles in atopic dermatitis

Class II alleles	Patients (n = 38)		Control subjects (n = 51)		Relative risk	Chi square	p Value
	No.	%	No.	%			
DRB1							
1302	10	26	3	6	5.71	5.7428*	0.0165†
1501	4	11	8	16	0.63	0.1530*	0.6955
1502	15	39	11	22	2.37	3.3758	0.0661
1602	1	3	0	0	4.12	0.0220*	0.8819
DQB1							
0201	1	3	1	2	1.35	0.0000*	1.0000
0301	10	26	13	25	1.04	0.0077	0.9298
0302	3	8	14	27	0.22	4.1980*	0.0404†
0303	8	21	13	25	0.77	0.2378	0.6257
0401	4	11	7	14	0.73	0.0163*	0.8981
0402	5	13	4	8	1.78	0.2182*	0.6403
0501	4	11	5	10	1.08	0.0000*	1.0000
0502	2	5	0	0	7.05	0.8726*	0.3502
0503	4	11	4	8	1.38	0.0039*	0.9496
0601	18	47	20	39	1.39	0.5915	0.4418
0602	4	11	8	16	0.63	0.1530*	0.6955
0604	10	26	3	6	5.71	5.7428*	0.0165†
DPB1							
0201	7	18	13	25	0.45	2.2259	0.1357
0202	5	13	10	20	0.46	1.6175	0.2034
0301	5	13	0	0	16.91	4.8449*	0.0277†
0401	7	18	2	4	5.53	3.5675*	0.0589
0402	8	21	10	20	1.09	0.0281	0.8667
0501	21	55	32	63	0.73	0.5060	0.4768
0601	0	0	6	12	0.00	3.1051*	0.0780
0901	13	34	10	20	2.13	2.4229	0.1195
1301	0	0	3	6	0.00	0.8597*	0.3538
1401	1	3	2	4	0.66	0.0000*	1.0000
1601	0	0	1	2	0.00	0.0000*	1.0000
1701	1	3	0	0	4.12	0.0220*	0.8819
1901	0	0	1	2	0.00	0.0000*	1.0000

*Chi square tests with Yates' correction.

†p < 0.05.

nese subjects. Reid et al.¹⁹ reported that there was a strong negative relationship between HLA-DR4 and subjects with mountain cedar pollinosis. Sasazuki et al.²⁰ showed the association of Japanese cedar pollinosis with HLA-DQ3 and concluded that IgE production against a certain antigen was genetically regulated. Kitano et al.²¹ reported that allergic rhinitis in response to *Dermatophagoides farinae* was significantly associated with HLA-DQ3. Conversely, HLA-A2 was significantly decreased, indicating that this might be a resistant genetic factor. Further molecular genetic studies revealed that HLA-DQB1*0303 was sig-

nificantly increased in the patients with this mite allergy.

In our study of AD, the frequency of HLA-DR13 was increased and that of HLA-DR4 was decreased in the patients with high serum IgE levels, whereas the significance of this deviation disappeared when the *p* value was corrected for the number of comparisons made. Patients with allergic rhinitis are known to show a specific IgE antibody for a certain pollen antigen. On the other hand, patients with AD usually show specific IgE antibodies for several environmental antigens. This may explain, in part, the reason we

failed to find a significant association between AD and HLA antigens.

In this study the frequency of HLA-DR13 showed an increased tendency. Thus in order to determine whether the increased frequency of HLA-DR13 is independent of or dependent on an increase of a certain haplotype, we examined the estimated haplotypes carrying HLA-DR13. The haplotype HLA-A33-B44-Cwblank-DR13-DQ1 in patients was increased (8 of 38 patients, 21%; 3 of 51 control subjects; 6%). However this estimated haplotype that carries DR13 occurs almost exclusively in Japanese persons. Therefore we were unable to determine whether an increased frequency of HLA-DR13 in patients is an independent association or is due to an increase in HLA haplotype.

Recent studies showed that specific amino acid epitopes on a certain HLA molecule determine disease susceptibility. Todd et al.⁹ first reported that insulin-dependent diabetes mellitus is linked to the fifty-seventh amino acid of the HLA-DQB1 domain. In our study, the frequencies of HLA-DRB1*1302 and DQB1*0604 tended to be increased in the patient groups. Therefore we estimated the frequencies of DRB1*1302-related epitopes and DQB1*0604-related epitopes. The DRB1*1302 allele contains two unique amino acid epitopes, ²⁶FLDRYFH and ⁶⁷ILEDE. Phenotypic frequencies of these two epitopes in the patient group and in the control group are as follows; ²⁶FLDRYFH: 34.2% versus 13.7% ($p < 0.01$), ⁶⁷ILEDE: 26.3% versus 5.9% ($p < 0.05$). The DQB1*0604 allele contains four unique amino acid epitopes: ²⁶LVTRH, ⁵³QGRP, ⁷⁰RTRAE, and ⁸⁴EVGYRGI. Phenotypic frequencies of these four epitopes in the patient group and in the control group are as follows: ²⁶LVTRH, 26.3% versus 5.9% ($p < 0.05$); ⁵³QGRP, 36.8% versus 15.6% ($p < 0.05$); ⁷⁰RTRAE, 89.4% versus 86.2% ($p > 0.1$); and ⁸⁴EVGYRGI, 26.3% versus 5.9% ($p < 0.05$). Five amino acid epitopes (DRB1 ²⁶FLDRYFH, DRB1 ⁶⁷ILEDE, DQB1 ²⁶LVTRH, DQB1 ⁵³QGRP, and DQB1 ⁸⁴EVGYRGI) may contribute to susceptibility to AD.

Matsushita et al.²² clarified that the HLA-DR molecule in *Cryptomeria japonica* pollen antigen-driven IgE response is the product of immune response genes, because anti-HLA-DR monoclonal antibody blocked the response, and the interaction between monocyte and monocyte-depleted peripheral blood lymphocytes in response to *C. japonica* pollen antigen was restricted by HLA-DR.

Patients with AD exhibited allergen-specific IgE antibodies against several environmental antigens. Whether all of these antigens contribute to the pathogenesis of AD or some of them are essential remains ambiguous. In the study of AD it is necessary to clarify the most pathognomonic antigen or antigen epitope, and association must be re-evaluated on the basis of specified and characterized antigens. In addition, it may be necessary to examine the in vitro function of the HLA class II molecule to recognize the specific environmental antigen and to present the antigen to T cells, followed by T-cell proliferation and lymphokine production.

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