

Analysis of disease-associated amino acid epitopes on HLA class II molecules in atopic dermatitis

Hidehisa Saeki, MD,^a Shoji Kuwata, MD,^b Hidemi Nakagawa, MD,^a
Takafumi Etoh, MD,^a Masami Yanagisawa, BS,^b Mitsuko Miyamoto, BS,^b
Katsushi Tokunaga, PhD,^c Takeo Juji, MD,^c and Yoichi Shibata, MD^b
Tokyo, Japan

*We investigated the association between HLA class II alleles and severe atopic dermatitis with high serum IgE levels (greater than 8000 U/ml). The frequencies of HLA-DRB1*1302 and DQB1*0604 were increased, whereas the frequency of HLA-DQB1*0302 was decreased. A strong haplotype, HLA-DRB1*1302-DQB1*0604, has been reported in the Japanese population, and this haplotype is conserved in patients with AD. Further analysis of the amino acid epitopes on the HLA-DRB1 and DQB1 domains revealed that DRB1 ⁷¹Glu (RR = 5.71, $p < 0.05$) and DQB1 ³⁰His (RR = 3.25 $p < 0.01$), and ⁵⁷Val (RR = 3.13, $p < 0.05$) were increased in frequency. DRB1 ⁷¹Glu was exclusively unique to DRB1*1302. DQB1 ³⁰His was shared by the following alleles: DQB1*0501, *0502, *0503, and *0604, which were all increased in patients with AD. DQB1 ⁵⁷Val was shared by DQB1*0501 and *0604. A well-known haplotype, HLA-DRB1*1302-DQB1*0604, contains DRB1 ⁷¹Glu and DQB1 ³⁰His and ⁵⁷Val. Therefore HLA-DRB1 ⁷¹Glu and/or DQB1 ³⁰His/⁵⁷Val are considered to play the most important role in the development of atopic dermatitis. (J ALLERGY CLIN IMMUNOL 1995;96:1061-8.)*

Key words: HLA, epitope, amino acid, atopic dermatitis, IgE

Patients with atopic dermatitis (AD) usually exhibit allergen-specific IgE antibodies against several environmental antigens such as mites, house dust, pollens, and fungi. Although HLA restriction is presumed to be involved in the recognition of such antigens, several previous reports have so far failed to find a significant association between AD and HLA antigens. Svejgaard et al.¹ reported that the frequency of HLA-DR7 antigen was decreased in patients with AD, although the significance of this deviation disappeared after correction of p values. Larsen and Grunnet² were unable to demonstrate any significant association between AD

Abbreviations used

AD:	Atopic dermatitis
PCR:	Polymerase chain reaction
RFLP:	Restriction fragment length polymorphism
RR:	Relative risk

and HLA class I antigens. Recently, we examined the association between HLA and AD in patients with high serum IgE levels (greater than 8000 U/ml) and reported that the HLA-B44-DR13-DQ1 haplotype was increased and the HLA-B62-DR4-DQ3 haplotype was decreased in these patients.³ DNA typing of HLA alleles revealed that HLA-DRB1*1302, DQB1*0604 alleles were increased, whereas frequencies of HLA-DQB1*0302 allele were decreased. However, none of these findings remained significant after correction of p values.

The "World Health Organization Nomenclature Committee for Factors of the HLA system, 1994" has identified some new alleles⁴ since our previous

From ^aDepartment of Dermatology, Faculty of Medicine, University of Tokyo; ^bDepartment of Transfusion Medicine and Immunohematology, Faculty of Medicine, University of Tokyo; and ^cJapanese Red Cross Central Blood Center, Tokyo. Supported in part by a research grant from New Trends in Immunopharmacology and a research grant for Intractable Disease from Ministry of Health and Welfare, Japan. Reprint requests: Hidehisa Saeki, MD, Department of Dermatology, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, Japan 113.

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TABLE I. A panel of oligonucleotides for PCR amplification

Primers		Nucleotide sequences
DRB1 low resolution primers*		
5' primers		
DR1 group	DR1B1AMPA	5'-TTCTTGTTGGCAGCTTAAGTT-3'
DR2 group	DR2B1AMPA	5'-TTCCTGTGGCAGCCTAAGAGG-3'
DR4 group	DR4B1AMPA	5'-GTTCTTGGAGCAGGTTAAAC-3'
DR3, 5, 6, 8 group	DRBAMPA2	5'-CACGTTTCTTGAGTACTCTAC-3'
3' Primer		DRBAMPB
		5'-GCCGCTGCACTGTGAAGCTCT-3'
DRB1 higher resolution primers		
DR3 group		
5' primer	DRBAMPA3	5'-TACTTCCATAACCAGGAGGAGA-3'
3' primer	DR3B1AMPB	5'-TGCAGTAGTTGTCCACCCG-3'
DR11 group		
5' primer	DRBAMPA2	5'-CACGTTTCTTGAGTACTCTAC-3'
3' primer	DR11AMPB	5'-CCAGTACTCCTCATCAGGCCG-3'
DR8-DR12 group		
5' primer	DR8B1AMPA	5'-GAGTACTCTACGGGTGAGTGT-3'
3' primer	DRBAMPB	5'-GCCGCTGCACTGTGAAGCTCT-3'
DQB1 primers		
5' Primer	DQBAMPA	5'-AGGGATCCCCGCAGAGGATTCGTG-3'
3' Primers		
DQ1 group	DQBAMPB1	5'-CACCTGCAGATCCCGCGGTACGCCACCTC-3'
DQ2, 3, 4 group	DQBAMPB2	5'-CACCTGCAGTGCAGGCTCCAACCTGGTA-3'

*DRB1 alleles corresponding to DR7, DR9, and DR10 specificities were excluded in this study.

report; therefore, we examined, again, the HLA-DRB1 and DQB1 alleles with the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method with modified primers. Recent studies showed that specific HLA-D residue positions or sequences were implicated in susceptibility to certain autoimmune disorders, including insulin-dependent diabetes mellitus,⁵ pemphigus vulgaris, and rheumatoid arthritis.⁶ Ansari et al.⁷ demonstrated a strong association between a specific HLA sequence and immune responsiveness to the grass pollen allergen Lol p III. Previously, we estimated the frequencies of the epitopes related to increased HLA class II alleles in the patient group.³ In this report we evaluated amino acid epitopes of HLA-DRβ1 and DQβ1 domains associated with AD.

METHODS

Patients

Thirty-eight unrelated Japanese patients with AD who had high serum IgE levels were examined. The group consisted of 27 male and 11 female patients, aged 11 to 61 years (mean age, 25.2 ± 8.5 years). Their serum IgE levels varied from 8440 to 75,600 U/ml (mean level, $18,830 \pm 12,720$ U/ml). All patients had severe AD on the basis of extent of skin lesions, intensity of exanthema (e.g., erythema, edema, papules, excoriation, lichenification), and subjective symptoms such as pruritis.^{8,9} Fifty-

one healthy Japanese individuals, who had no atopic diathesis, served as control subjects. After informed consent was obtained, venous blood was drawn from each subject.

Genotyping of HLA-DRB1 alleles

We previously examined HLA-DRB1 alleles related to DR13 and DR2, which were increased in frequency in the patient group. We identified DR13-related alleles by using PCR amplification with sequence-specific primers and DR2-related alleles by using hybridization of PCR-amplified product with sequence-specific oligonucleotide probes. In this study we evaluated all of the HLA-DRB1 alleles using the PCR-RFLP method. The synthesis of oligonucleotides, extraction of genomic DNA, and PCR-RFLP were carried out as described previously.³ We performed group-specific amplification by using four pairs of DRB1 low-resolution primers shown in Table I.¹⁰ DR3, 5, 6, 8 group DRB1 alleles, except for DRB1*1410, were amplified by a set of primers, DRBAMPA2 and DRBAMPB. DRB1*1410 was amplified by a set of primers, DR4B1AMPA and DRBAMPB. Because DR3, 5, 6, 8 group contains so many alleles, it is relatively troublesome to determine all alleles together with PCR-RFLP. Thus further group-specific amplification was performed with higher resolution primers shown in Table I.¹⁰ By using these primer pairs, DR3, 5, 6, 8 group alleles were classified into DR3, DR11, DR8-DR12, and DR6 groups. DR3 group alleles were amplified by a set of primers, DRBAMPA3 and

TABLE II. A panel of endonucleases for PCR-RFLP analysis of HLA-DRB1 alleles

DRB1	Endonucleases
DR1 group	<i>HphI</i> , <i>MnII</i> , <i>PstI</i>
DR2 group	<i>Cfr13I</i> , <i>FokI</i> , <i>HphI</i> , <i>XmnI</i>
DR4 group	<i>AvaII</i> , <i>HaeII</i> , <i>HinfI</i> , <i>HphI</i> , <i>MnII</i> , <i>SacII</i>
DR3, 5, 6, 8 group	
DR11 group	<i>BsrBI</i> , <i>DdeI</i> , <i>HphI</i> , <i>XmnI</i>
DR8-DR12 group	<i>FokI</i> , <i>HphI</i> , <i>SacII</i> , <i>SfaNI</i>
DR6 group	<i>AccII</i> , <i>ApaI</i> , <i>AvaII</i> , <i>DdeI</i> , <i>HaeII</i> , <i>HhaI</i> , <i>HphI</i> , <i>RsaI</i> , <i>XmnI</i>

DR3B1AMPB. Likewise, DR11 group alleles were amplified by DRBAMPA2 and DR11AMPB, DR8-DR12 were amplified by DR8B1AMPA and DRBAMPB. Because DR6 group alleles could not be amplified specifically by higher resolution primers, the other alleles, which were not amplified by those three higher resolution primer pairs, were classified into the DR6 group. This subgroup information facilitates the determination of alleles. Products amplified with DRB1 low-resolution primers were digested by several restriction endonucleases, shown in Table II, according to the reports by Ota et al.¹¹ and Senger et al.,¹² with some modifications.

Genotyping of HLA-DQB1 alleles

For HLA-DQB1 genotyping, we previously digested amplified DNA with *HinfI*, *HhaI*, *Cfr13I*, and *MspI* to discriminate 18 DQB1 alleles.³ However, DQB1*0602 and *0603, as well as *0604 and 0605, cannot be discriminated by this PCR-RFLP method. Sengar and Goldstein¹³ and Mitsunaga et al.¹⁴ reported that by performing PCR-RFLP with DQ1 group (DQ5 and DQ6)-specific primer pairs, DQB1*0602 and DQB1*0604 could be distinguished from *0603 and *0605 by the digestion patterns of *RsaI* and *HaeIII*, respectively. Furthermore, because the report of the World Health Organization Nomenclature Committee for Factors of the HLA System, 1994 contains some additional alleles, we reexamined DQB1 alleles by PCR-RFLP by using different group-specific primer pairs, shown in Table I. The digestion patterns of DQ1 group DQB1 alleles by four endonucleases are shown in Table III. DQB1*0602 produces a polymorphic band at 110, 81, 39, 30, 19 bp on *RsaI* digestion; in contrast, DQB1*0603 produces a polymorphic band at 84, 81, 39, 30, 26, 19 bp on *RsaI*. DQB1*0604 produces a polymorphic band at 166, 113 bp on *HaeIII* digestion; in contrast, DQB1*0605 produces a polymorphic band at 127, 113, and 39 bp on *HaeIII* digestion. We need three additional endonucleases to distinguish among several alleles. DQB1*0605 and *0606 are discriminated by the digestion pattern of *BsoFI*. DQB1*0603, *0607, and *0608 are discerned by endonucleases *BsmFI* and *SfaNI*. Although DQB1*0604 and *0609 could not be distinguished by endonucleases,

TABLE III. Digestion patterns of DQ1 group DQB1 alleles

DQB1 alleles	Restriction endonucleases			
	<i>RsaI</i>	<i>HaeIII</i>	<i>BsaHI</i>	<i>HhaI</i>
0501	R4	Ha3	B3	Hh1
0502	R4	Ha3	B3	Hh3
05031	R3	Ha3	B4	Hh1
05032	R3	Ha3	B3	Hh1
0504	R4	Ha2	B3	Hh3
0601	R1	Ha2	B2	Hh1
0602	R2	Ha1	B1	Hh2
0603	R4	Ha1	B1	Hh2
0604	R4	Ha1	B1	Hh1
0605	R4	Ha2	B1	Hh1
0606	R4	Ha2	B1	Hh1
0607	R4	Ha1	B1	Hh2
0608	R4	Ha1	B1	Hh2
0609	R4	Ha1	B1	Hh1

Each digestion pattern produces the following DNA fragment sizes. R1: 179, 81, 19 bp. R2: 110, 81, 39, 30, 19 bp. R3: 84, 81, 69, 26, 19 bp. R4: 84, 81, 39, 30, 26, 19 bp. Ha1: 166, 113 bp. Ha2: 128, 113, 38/127, 113, 39 bp. Ha3: 127, 67, 46, 39 bp. B1: 152, 127 bp. B2: 152, 108, 19 bp. B3: 152, 66, 61 bp. B4: 152, 61, 47, 19 bp. Hh1: 162, 69, 48/162, 69, 46, 2 bp. Hh2: 133, 69, 46, 29, 2 bp. Hh3: 106, 69, 56, 48 bp.

we regarded them as DQB1*0604 because there are no reports of DQB1*0609 in Japanese subjects.

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.¹⁵

Nomenclature

Nomenclature for HLA specificities and HLA class II alleles is described according to the system of the World Health Organization Nomenclature Committee HLA, 1994.⁴

RESULTS

We evaluated the HLA-DRB1 alleles with the PCR-RFLP method. Fig. 1 shows an example of PCR-RFLP pattern digested with nine endonucleases in DRB1*1302. Because this allele was amplified by DR3, 5, 6, 8 group-specific low-resolution primer pairs but not by any of three higher resolution primer pairs shown in Table I, it was classified into the DR6 group. This allele produced a polymorphic band at 207, 60 bp on *AccII* digestion; at 267 bp on *ApaI* digestion; at 267 bp on *AvaII* digestion; at 242 bp on *DdeI* digestion; at 267 bp on *HaeII* digestion; at 164, 103 bp on *HhaI* digestion; at 146, 109 bp on *HphI* digestion; at 150, 102 bp on

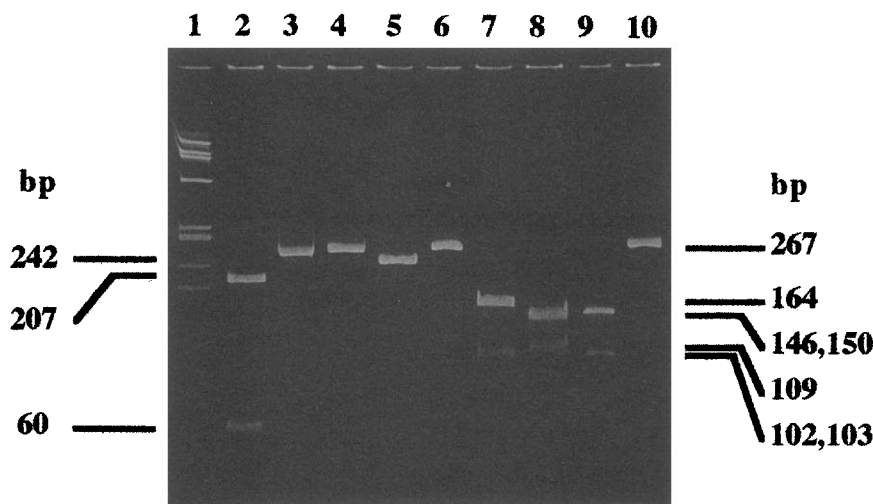


FIG. 1. PCR-RFLP of HLA-DRB1 alleles. An example of PCR-RFLP in DRB1*1302. This allele produced a polymorphic band at 207, 60 bp on *AcclI* digestion; at 267 bp on *Apal* digestion; at 267 bp on *AvalI* digestion; at 242, 25 bp on *Ddel* digestion; at 267 bp on *HaeII* digestion; at 164, 103 bp on *HhaI* digestion; at 146, 109, 12 bp on *HphI* digestion; at 150, 102, 15 bp on *RsaI* digestion; at 267 bp on *XmnI* digestion. Lane 1, Molecular weight size marker, *HaeII*-digested ϕ x 174; lane 2, digestion by *AcclI*; lane 3, digestion by *Apal*; lane 4, digestion by *AvalI*; lane 5, digestion by *Ddel*; lane 6, digestion by *HaeII*; lane 7, digestion by *HhaI*; lane 8, digestion by *HphI*; lane 9, digestion by *RsaI*; lane 10, digestion by *XmnI*.

RsaI digestion; at 267 bp on *XmnI* digestion. The pattern of HLA-DR2 and DR13 alleles determined with the PCR-RFLP method completely coincided with that obtained when the PCR-sequence-specific oligonucleotide probe and PCR-sequence-specific primer methods were used, previously. One allele of the DR11 group, found in a patient (TUHAD28), showed an unknown digestion pattern, which we tentatively termed *DRB1*11New*. Nucleotide sequence of this *DRB1*11New* allele is now under investigation.

Because HLA-DQB1*0602 and *0603, as well as *0604 and *0605, could not be distinguished by previous PCR-RFLP,³ we examined, again, the DQB1 alleles by PCR-RFLP by using different primers. Fig. 2 shows an example of the PCR-RFLP pattern digested with *RsaI*, *HaeIII*, *BsaHI*, and *HhaI* in DQB1*0601, *0602, and *0604. DQB1*0602 produced a polymorphic band at 110, 81 bp on *RsaI* digestion; at 116, 113 bp on *HaeIII* digestion; at 152, 127 bp on *BsaHI* digestion; and at 162, 69 bp on *HhaI* digestion. DQB1*0604 produced a polymorphic band at 84, 81 bp on *RsaI* digestion; at 166, 113 bp on *HaeIII* digestion; at 152, 127 bp on *BsaHI* digestion; and at 162, 69, 46 bp on *HhaI* digestion.

Table IV shows the frequencies of HLA-DRB1 and DQB1 alleles in the patients with AD. The frequencies of HLA-DRB1*1302 (relative risk =

5.71, $p < 0.05$), and DQB1*0604 (RR = 5.71, $p < 0.05$) were increased in the patient group. A strong haplotype, HLA-DRB1*1302-DQB1*0604, has been reported in the Japanese population, and this haplotype is conserved in patients with AD. In contrast, the frequencies of HLA-DQB1*0302 (RR = 0.22, $p < 0.05$) were decreased. However, none of these findings remained significant after correction of p values.

To examine the epitopes of the HLA class II molecules associated with AD, we evaluated the phenotypic frequencies of the epitopes of HLA-DR β 1 and DQ β 1 domains. Table V presents the significantly associated epitopes. The frequencies of ⁹EYSTS, ⁶⁷ILEDE of HLA-DR β 1 domain, and ²⁶LVTRH, ⁵³QGRP of HLA-DQ β 1 domain were increased in the patient group. In contrast, the frequencies of ⁹EQVKH of HLA-DR β 1 domain and ²⁶LVTRY, ⁵³LGPPA of HLA-DQ β 1 domain were decreased in the patient group.

DISCUSSION

There have been several reports on the significant association between allergic diseases and HLA class II antigens or molecules. Kumai et al.¹⁶ reported that HLA-DR9 and HLA-DQ3 phenotypes were strongly associated with the development of birch pollen allergy in Japanese subjects. Kitano et al.¹⁷ clarified that allergic rhinitis in

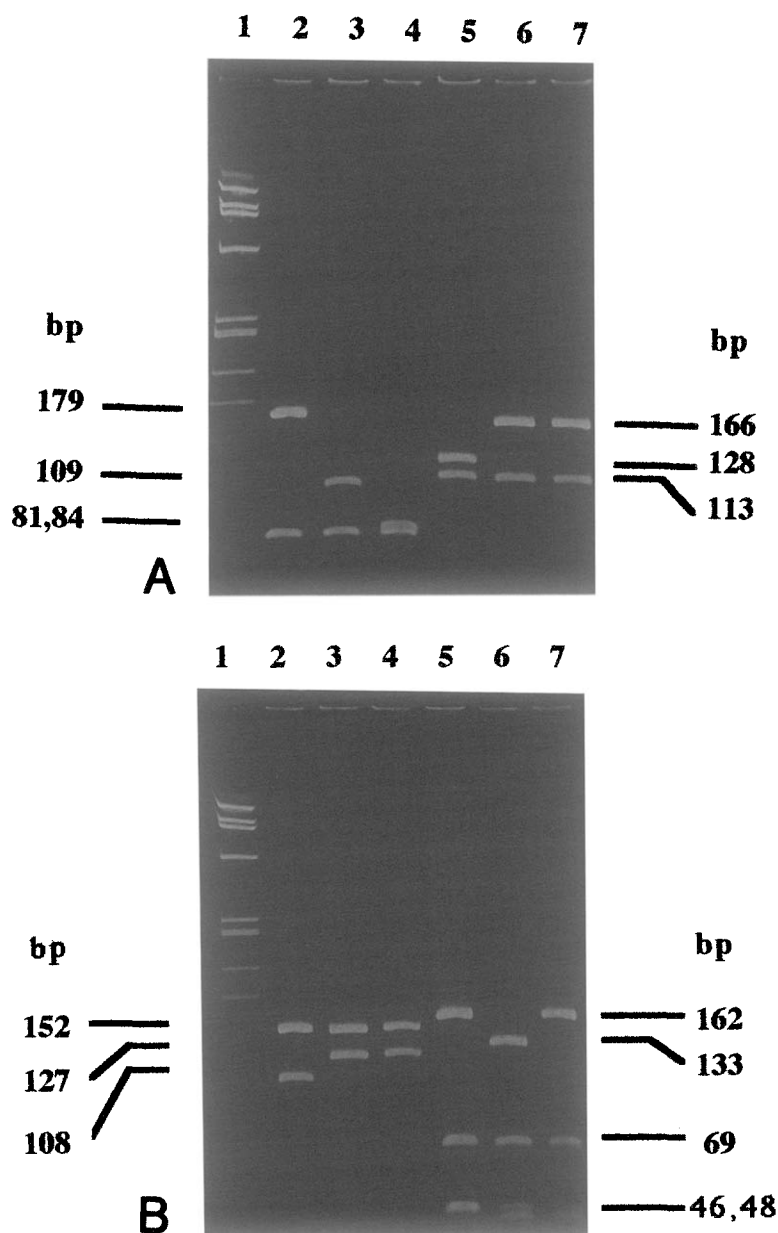


FIG. 2. A, PCR-RFLP of HLA-DQB1 alleles. *RsaI* and *HaeIII* digests of DQB1*0601, *0602, and *0604. DQB1*0601 produced a polymorphic band at 179, 81, 19 bp on *RsaI* digestion and at 128, 113, 38 bp on *HaeIII* digestion. DQB1*0602 produced a polymorphic band at 110, 81, 39, 30, 19 bp on *RsaI* digestion and at 166, 113 bp on *HaeIII* digestion. DQB1*0604 produced a polymorphic band at 84, 81, 39, 30, 26, 19 bp on *RsaI* digestion and at 166, 113 bp on *HaeIII* digestion. *Lane 1*, Molecular weight size marker, *HaeIII*-digested ϕ x 174; *lane 2*, DQB1*0601 digested by *RsaI*; *lane 3*, DQB1*0602 digested by *RsaI*; *lane 4*, DQB1*0604 digested by *RsaI*; *lane 5*, DQB1*0601 digested by *HaeIII*; *lane 6*, DQB1*0602 digested by *HaeIII*; *lane 7*, DQB1*0604 digested by *HaeIII*. **B,** PCR-RFLP of HLA-DQB1 alleles. *BsaHI* and *HhaI* digests of DQB1*0601, *0602, and *0604. DQB1*0601 produced a polymorphic band at 152, 108, 19 bp on *BsaHI* digestion and at 162, 69, 48 bp on *HhaI* digestion. DQB1*0602 produced a polymorphic band at 152, 127 bp on *BsaHI* digestion and at 133, 69, 46, 29, 2 bp on *HhaI* digestion. DQB1*0604 produced a polymorphic band at 152, 127 bp on *BsaHI* digestion and at 162, 69, 46, 2 bp on *HhaI* digestion. *Lane 1*, Molecular weight size marker, *HaeIII*-digested ϕ x 174; *lane 2*, DQB1*0601 digested by *BsaHI*; *lane 3*, DQB1*0602 digested by *BsaHI*; *lane 4*, DQB1*0604 digested by *BsaHI*; *lane 5*, DQB1*0601 digested by *HhaI*; *lane 6*, DQB1*0602 digested by *HhaI*; *lane 7*, DQB1*0604 digested by *HhaI*.

TABLE IV. 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							
0101	4	11	5	10	1.08	0.0000	1.0000
1501 (= 1503)†	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
0401	0	0	2	4	0.00	0.2618	0.6088
0403	0	0	5	10	0.00	2.3147	0.1281
0405	5	13	7	14	0.95	0.0060	0.9381
0406	2	5	9	18	0.25	2.0457	0.1526
0410	2	5	1	2	2.77	0.0676	0.7947
1101	4	11	4	8	1.38	0.0039	0.9496
11New	1	3	0	0	4.12	0.0220	0.8819
1201	2	5	4	8	0.65	0.0027	0.9578
1202	1	3	0	0	4.12	0.0220	0.8819
1302	10	26	3	6	5.71	5.7428	0.0165*
1401	3	8	0	0	10.15	2.0954	0.1477
1403	1	3	2	4	0.66	0.0000	1.0000
1405	1	3	4	8	0.31	0.3490	0.5546
1406	2	5	0	0	7.05	0.8726	0.3502
0701	1	3	1	2	1.35	0.0000	1.0000
0802	3	8	4	8	1.00	0.0000	1.0000
0803	4	11	11	22	0.42	1.1886	0.2756
0901	7	8	13	25	0.66	0.6245	0.4293
DQB1							
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 (= 0609)†	10	26	3	6	5.71	5.7428	0.0165*
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	0.9381
0401	5	13	7	14	0.95	0.0060	0.9381
0402	3	8	4	8	1.00	0.0000	1.0000

* $p < 0.05$.[†]DRB1*1501 and *1503, as well as DQB1*0604 and *0609, cannot be distinguished by using available restriction endonucleases at present.

response to *Dermatophagoides farinae* was significantly associated with HLA-DQB1*0303. Ansari et al.⁷ demonstrated that immune responsiveness to the grass pollen allergen Lol pIII was strongly associated with the sequence ⁹EYSTS, present in the first polymorphic regions of HLA-DRβ1 polypeptide chains of DR3, DR11, and DR6. These reports suggest that specific environmental allergens are recognized by unique HLA class II molecules and are presented to T cells, causing

allergic diseases. Therefore recognition of specific environmental allergens by a unique HLA class II molecule is supposed to play a critical role in the development of AD.

Recent studies have shown that specific amino acid residues on a certain HLA molecule determine disease susceptibility. Todd et al.⁵ first reported that HLA-DQB1 alleles that did not have asparaginic acid at position 57 of the DQβ1 domain were significantly enriched in the Caucasian

TABLE V. Epitopes of HLA class II molecules in atopic dermatitis

Amino acid	Patients (n = 38)		Control subjects (n = 51)		Relative risk	Chi square	p Value
	No.	%	No.	%			
DRβ1 domain							
⁹ EYSTS	18	47	12	24	2.92	5.5379	0.0186*
⁹ EQVKH	9	24	24	47	0.34	5.0995	0.0239*
⁶⁷ ILEDE	10	26	3	6	5.71	5.7428	0.0165*
DQβ1 domain							
²⁶ LVTRY	13	34	31	61	0.33	6.1515	0.0131*
²⁶ LVTRH	10	26	3	6	5.71	5.7428	0.0165*
⁵³ QGRPV	14	37	8	16	3.13	5.2372	0.0221*
⁵³ LGPPA	3	8	14	27	0.22	4.1980	0.0404*

* $p < 0.05$.

population with insulin-dependent diabetes mellitus. In our study HLA-DRβ1 ⁹EYSTS, ⁶⁷ILEDE and HLA-DQβ1 ²⁶LVTRH, ⁵³QGRPV were significantly increased in the patient group. In order to distinguish the critical amino acids in the increased epitopes, we also examined the frequencies of each amino acid. The frequencies of ¹³Ser, ⁷¹Glu of HLA-DRβ1 domain and ³⁰His, ⁵⁵Arg, and ⁵⁷Val of HLA-DQβ1 domain were significantly increased in the patient group. Phenotypic frequencies of these five amino acids in the patient group and in the control group are as follows: DRβ1 ¹³Ser, 47% versus 24% (RR = 2.92, $p < 0.05$); DRβ1 ⁷¹Glu, 26% versus 6% (RR = 5.71, $p < 0.05$); DQβ1 ³⁰His, 50% versus 24% (RR = 3.25, $p < 0.01$); DQβ1 ⁵⁵Arg, 95% versus 76% (RR = 5.53, $p < 0.05$); DQβ1 ⁵⁷Val, 37% versus 16% (RR = 3.13, $p < 0.05$).

As for DRβ1 amino acids associated with AD, HLA-DRβ1 ¹³Ser was shared by the following alleles: HLA-DRB1*1101, *1302, *1401, *1403, *1405, and *1406. Among these alleles, HLA-DRB1*1101, *1302, *1401, and *1406 were increased in patients with AD; however, DRB1*1403 and *1405 were decreased. In contrast, HLA-DRβ1 ⁷¹Glu was exclusively unique to HLA-DRB1*1302, which was increased in patients with AD. Therefore DRβ1 ⁷¹Glu is supposed to be one of the candidate positions on the HLA class II molecules involved in the recognition of an environmental allergen in the case of AD. As for DQβ1 amino acids associated with AD, HLA-DQβ1 ³⁰His was shared by the following alleles: HLA-DQB1*0501, *0502, *0503, and *0604. All of these alleles were increased in patients with AD. In contrast, HLA-DQβ1 ⁵⁵Arg was shared by the following alleles: HLA-DQB1*0501,

*0502, *0503, *0601, *0602, *0604, *0401, and *0402. Among these alleles HLA-DQB1*0501, *0502, *0503, *0601, and *0604 were increased in patients with AD, but DQB1*0602 and *0401 were decreased. HLA-DQβ1 ⁵⁷Val was shared by HLA-DQB1*0501 and *0604, which were both increased in patients with AD. Thus HLA-DQβ1 ³⁰His and ⁵⁷Val are supposed to be the candidate positions involved in the development of AD. Because a well-known HLA haplotype, DRB1*1302-DQB1*0604, contains DRβ1 ⁷¹Glu and DQβ1 ³⁰His and ⁵⁷Val, we cannot determine at present which amino acids, DRβ1 ⁷¹Glu or DQβ1 ³⁰His/⁵⁷Val, contribute primarily to the development of AD.

The three-dimensional structure of the HLA class II molecule was clarified by Brown et al.,¹⁸ and several antigen-binding clefts were found in the HLA class II molecule. Some amino acids located in the antigen-binding clefts are involved in the recognition of environmental allergens. HLA-DRβ1 ⁷¹Glu and HLA-DQβ1 ⁵⁷Val are located in the α helix, which is supposed to form the wall of the antigen-binding cleft, and HLA-DQβ1 ³⁰His is located in the β sheet, presumed to form the floor of the antigen-binding cleft. Therefore HLA-DRβ1 ⁷¹Glu and/or HLA-DQβ1 ³⁰His/⁵⁷Val appear to play the most important role in the development of AD.

There are almost no reports on direct analysis of antigen peptides recognized by HLA class II molecules. In contrast, several investigators have identified T-cell epitopes of specific environmental allergens. Yssel et al.¹⁹ reported that when recombinant deletion proteins were used, three T-cell epitopes were identified on the house dust mite Der pI molecule: p45-67, p94-104, and p117-143.

Verhoef et al.²⁰ reported that when clonal analysis was used, four distinct T-cell epitopes were identified on the house dust mite Der pII molecule, located within residues 16-31, 22-40, 82-100, and 111-129. Because patients with AD exhibit allergen-specific IgE antibodies against several environmental allergens, it is not clear that all of these allergens contribute to the pathogenesis of AD or whether some of them are essential. No reports, so far, have documented the pathognomonic peptides in AD. Chicz et al.²¹ extracted naturally processed peptides from immunoaffinity-purified HLA-DR2, DR3, DR4, DR7, and DR8 molecules and obtained a total of 201 peptide sequences. To clarify the pathogenesis of AD, it may be necessary to extract foreign antigenic peptides from HLA class II molecules on the lymphocytes of patients with AD and examine the most pathognomonic antigen or antigen epitope. Association between amino acid epitopes on HLA class II molecules and AD must be reevaluated on the basis of specified and characterized antigen or antigen epitope.

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