

HLA-DM gene polymorphisms in atopic dermatitis

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*HLA-DM molecules are involved in the antigen-processing pathway of HLA class II-restricted antigen presentation. We investigated polymorphisms of HLA-DM genes in atopic dermatitis by using the polymerase chain reaction–restriction-fragment length polymorphism method to examine a possible contribution of these genes to the pathogenesis of atopic dermatitis. Genomic DNA was extracted from 37 Japanese patients with atopic dermatitis and 52 control subjects. After polymerase chain reaction amplification of the polymorphic third exon of DMA and DMB genes, amplified products were digested with restriction endonucleases to determine HLA-DM alleles. FokI, HinfI, AclI and SfaNI were used for DMA; HhaI, BstI, ApaLI, and Bsp1286I for the DMB gene. We identified three DMA alleles and also three DMB alleles. One of 37 patients possessed the DMA*0103 allele, which has been reported as a rare allele in Caucasian populations. Any DMA and DMB alleles were not increased in the patients. The DMA*0102 allele was estimated to constitute a haplotype with DRB1*1201/DQB1*0301 and DRB1*0901/DQB1*0301 in a Japanese population. HLA-DM genes are not considered to contribute primarily to the susceptibility of atopic dermatitis. Further investigation of the functional roles of HLA-DM gene polymorphisms will be useful for a better understanding of susceptibility loci in HLA class II-associated disease. (J Allergy Clin Immunol 1996;98:S192-200.)*

Key words: HLA, HLA-DM, PCR, PCR-RFLP, atopic dermatitis, haplotype, linkage disequilibrium, Japanese population

Atopic dermatitis is provoked by several environmental allergens, and patients with this disorder generate allergen-specific immunoglobulin E antibodies.^{1,2} CD4⁺ helper T cells contribute to B cell-mediated immunity in combination with the HLA class II-restricted antigen recognition pathway. Antigenic peptides presented by the HLA class II molecules are recognized by self-restricted helper T cells. Before antigen presentation, these

Abbreviations used

CLIP:	Class II associated invariant chain peptides
HF:	Haplotype frequency
Ii chain:	Invariant chain
PCR:	Polymerase chain reaction
PCR-RFLP:	Polymerase chain reaction–restriction-fragment length polymorphism

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peptides are degraded from cytosolic proteins and assembled with HLA class II molecules in the compartment for peptide loading and then are transported to the cell surface.³

The endocytotic pathway generates and delivers antigenic peptides to HLA class II molecules provided by the biosynthetic pathway. HLA class II molecules are α/β dimers that assemble in the endoplasmic reticulum with invariant (Ii) chains. The Ii chains block the binding of antigenic peptides to HLA class II molecules and stabilize the unoccupied α/β dimers while escorting them through the biosynthetic pathway to the endoso-

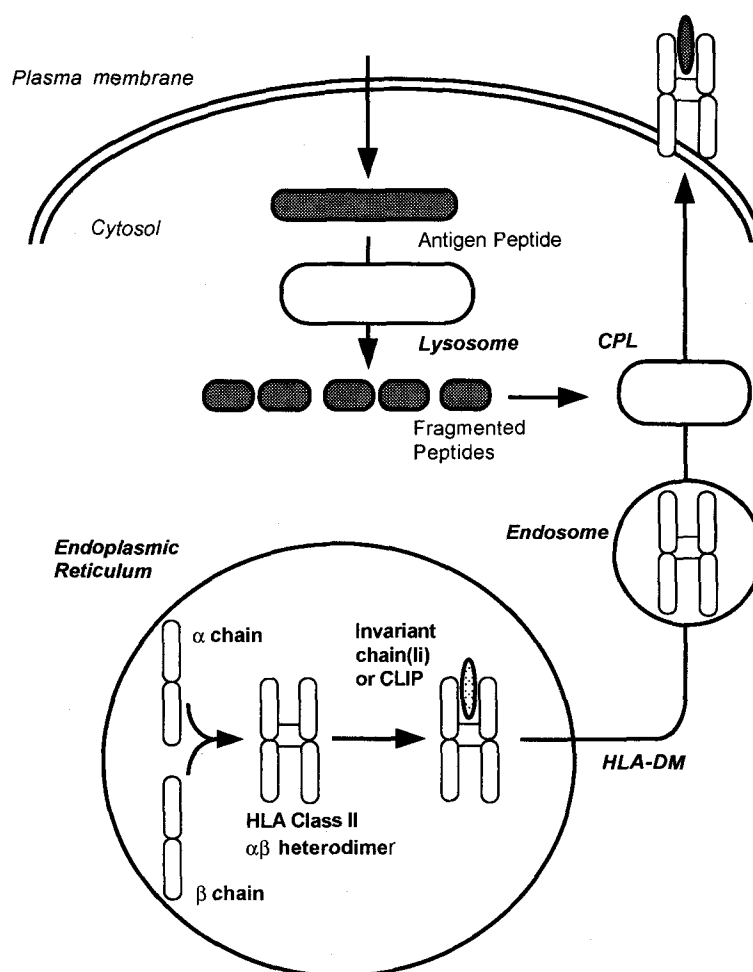


FIG. 1. HLA-DM molecules in HLA class II-restricted antigen processing pathway. HLA-DM molecules are involved in HLA class II-restricted antigen processing pathway. Ii chain blocks binding of antigenic peptides to HLA class II molecules and stabilizes unoccupied HLA class II α/β dimers while escorting them through biosynthetic pathway to endosomal compartment and compartment for peptide loading (CPL). HLA class II molecules can bind antigenic peptides after removal of Ii chain. Peptide-loaded HLA class II complexes are then transported to cell surface. HLA-DM molecules are involved in processing pathway of removal of Ii chain and loading of antigenic peptides.

mal compartment. After removal of Ii in the acidic and proteolytic environment of the endosomal compartment, HLA class II molecules can bind antigenic peptides.⁴ Peptide-loaded HLA class II complexes are then transported to the cell surface for inspection by helper T cells. HLA-DM molecules are involved in the processing pathway of detachment of the Ii chain and loading of the antigenic peptides⁵ (Fig. 1).

Two genes, DMA and DMB, are included in HLA-DM genes. Both genes exhibit genetic polymorphisms in the third exon. Three polymorphic sites have been found in the DMA gene and two polymorphic sites in the DMB gene.^{6,7} Different combinations of polymorphism at these sites con-

stitute four possible alleles at DMA and four possible alleles at DMB^{6,7} (Fig. 2). HLA-DM gene polymorphisms are thought to cause alterations in the peptide-processing pathway. Therefore HLA-DM genes are regarded as a candidate for disease-susceptibility genes or one of the additional genes conferring susceptibility. However, no investigators have yet reported on the association between HLA-DM alleles and diseases.

We investigated the DMA and DMB allele frequencies in the patients with atopic dermatitis in this study. Documentation of HLA-DM allele frequencies have been so far confined in Caucasian populations.^{6,7} We explored whether any differences exist in the frequencies of HLA-DM alleles

exon3	codon 140	179
DMA*0101	Val	Ile
0102	Ile	Ile
0103	Val	Thr
0104	Ile	Thr

exon3	codon 144	179
DMB*0101	Ala	Ile
0102	Glu	Ile
0103	Ala	Thr
0104	Val	Thr

FIG. 2. HLA-DM alleles. DMA and DMB genes contain four alleles each. Polymorphic sites are located in third exon of both genes. DMA gene possesses three polymorphic regions: codons 140, 155, and 184. DMB gene possesses two polymorphic regions: codons 144 and 179.

between the Caucasian and Japanese populations. We previously reported that Japanese patients with atopic dermatitis showed an increase in DRB1*1302/DQB1*0604 alleles^{8,9} and that genes for the transporter associated with antigen processing (TAP), which are genes involved in the HLA class I-restricted antigen processing pathway, did not contribute primarily to the development of atopic dermatitis.^{10,11} Therefore analysis of the HLA-DM genes will provide more precise insights on the susceptibility of this disorder. In this study we used the polymerase chain reaction–restriction-fragment length polymorphism (PCR-RFLP) method by using restriction endonucleases to discriminate HLA-DM alleles. Because HLA-DM genes are located in the HLA class II region between DPB1 and DQB1 loci, we also estimated extended HLA haplotypes, including HLA-DM genes, in a Japanese population.

METHODS

Patients

Thirty-seven Japanese patients with atopic dermatitis who were outpatients in the Department of Dermatol-

ogy at the University of Tokyo Hospital were enrolled in this study. Their clinical characteristics are described elsewhere.^{10,11} This patient group consisted of 27 males and 10 females, aged 11 to 42 years (mean age 27.0 ± 6.2 years). All of the patients manifested the severe clinical form of atopic dermatitis and showed high serum IgE levels, in a range of 8000 to 75,600 U/ml (mean level $25,400 \pm 28,200$ U/ml). Fifty-two Japanese persons with no atopic constitutions served as control subjects. Venous blood was drawn from each participant after informed consent was obtained.

HLA typing

Serologic HLA typing was performed in all subjects by using the standard lymphocyte microcytotoxicity test. We further extracted genomic DNA from peripheral blood leukocytes by using the standard phenol–chloroform–proteinase K method. HLA-DRB1,^{12,13} HLA-DQA1,¹⁴ HLA-DQB1,¹⁵ and HLA-DPB1¹⁶ alleles also were determined by the PCR-RFLP method.

PCR-RFLP analysis of DMA and DMB genes

We amplified the polymorphic third exon of DMA and DMB genes by using the polymerase chain reaction (PCR) technique. The DMA and DMB genes contain four alleles each. The DMA gene contains polymorphic sites at codons 140, 155, and 184 and the DMB gene at codon 144 and 179. Four restriction endonucleases were used for the determination of DMA and DMB alleles: *FokI*, *HinfI*, *AciI*, and *SfaNI* were used for DMA alleles, and *HhaI*, *BsrI*, *ApaLI*, and *Bsp1286I* were used for DMB alleles. Alignments and nucleotide sequences of PCR primers and the amplified fragment of the PCR product in each DM gene are shown in Fig. 3.

PCR was carried out as described by Saiki et al.¹⁷ with minor modifications. We suspended 0.2 μ g of genomic DNA in 20 μ l of 50 mmol/L Tris/HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 200 nmol/L each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 10 pmol each primer, and 0.4 U Taq DNA polymerase (Takara Shuzo, Tokyo, Japan). PCR cycles consists of the initial 2-minute denaturation at 95°C and 30 cycles of 1-minute denaturation at 95°C, 1-minute annealing at 56°C, and 1-minute extension at 72°C, followed by 5-minute extension at 72°C. This cycling was performed in a programmable DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, Conn.). Amplified PCR products were subjected to digestion with restriction endonucleases. Five microliters of PCR amplified products was digested with 5 U of each enzyme in a total volume of 10 μ l with the buffer of the manufacturer's recommendation. After the digestion reaction was performed for 2 hours at 37°C for *FokI*, *HinfI*, *AciI*, *SfaNI*, *HhaI*, *ApaLI*, and *Bsp1286I* and at 65°C for *BsrI*, digested samples were subjected to electrophoresis in 10% polyacrylamide gel in 1× TBE buffer at 200 V. Digested fragments were visualized by staining with ethidium bromide.

We determined the exact fragment size of the PCR

products and the polymorphic sites by using the direct-sequencing method and confirmed the sequences with those reported by Kelly et al.⁵ and Carrington M et al.^{6,7} Fractionated PCR fragments were cut in 1.5% Nusieve GTG agarose gel (FMC BioProducts, Rockland, Me.) and purified. Direct sequencing was performed with Ampli-Taq facilitated cycle-sequencing reaction by using the dye deoxy terminator method (Applied Biosystems, Foster City, Calif.) according to the manufacturer's recommendations. Nucleotide sequences were obtained by using an Applied Biosystems 373A automated DNA sequencer (Applied Biosystems). Both strands were sequenced by using the same primers as in the PCR amplification.

Genotyping of DMA and DMB alleles

Four possible DMA alleles were determined by a combination of three polymorphic sites at codons 140, 155, and 184. As for DMB alleles, four possible alleles were determined by a combination of two polymorphic sites at codons 144 and 179. Dimorphisms at DMA codon 140 (Val-Ile) and at DMA codon 155 (Gly-Ala) were discerned by digestion with *FokI* and *HinfI*, respectively. Polymorphism at DMA codon 184 (Arg-His/Cys) was discerned by digestion with *AclI* and *SfaNI*. Likewise, polymorphism at DMB codon 144 (Ala-Glu-Val) was discerned by digestion with *HhaI*, *ApaLI*, and *BspI286I*. Dimorphism at DMA codon 179 (Ile-Thr) was discerned by digestion with *BsrI*.

Nomenclatures for HLA class II DMA and DMB alleles were described according to the World Health Organization 1995 HLA nomenclature committee.¹⁸

Statistics

Frequencies of each allele were compared by the chi-square test between patients and control subjects. Yates' correction was used on necessary. The odds ratio of the disease was calculated according to the method of Svejgaard et al.¹⁹ and expressed as relative risk.

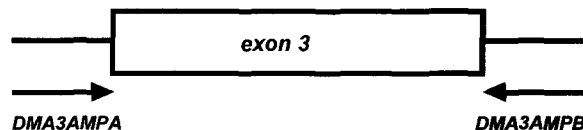
$$\text{Relative risk} = \frac{a/b}{c/d} = \frac{ad}{bc}$$

where *a* is the number of patients with the allele; *b* is the number of patients without the allele; *c* is the number of control subjects with the allele; and *d* is the number of control subjects without the allele. When *abcd* = 0,

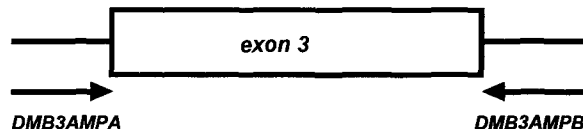
$$\text{Relative risk} = \frac{(2a + 1)(2d + 1)}{(2b + 1)(2c + 1)}$$

Because DMA and DMB genes are located in the HLA class II region, possible linkage disequilibrium between HLA-DM genes and the classic HLA class II genes were also investigated. We estimated haplotype including DRB1, DQB1, DPB1, and HLA-DM genes in

DMA gene



DMB gene



DMA3AMPA: AGTCTCTTTTCCCTACAC
DMA3AMPB: ATCTATCCCTTTTGCCCCCA

DMB3AMPA: GTCACCTCCTTCTAAACAT
DMB3AMPB: CCATCCATCTGCCATACCTT

FIG. 3. Panel of PCR primers for HLA-DM gene amplifications. Polymorphic third exon of both DMA and DMB genes were amplified by PCR. PCR primers for each gene were located in second and third introns. Predicted fragment sizes of amplified products are 370 bp for DMA gene and 348 bp for DMB gene.

the patients and the control subjects. Δ Values and haplotype frequencies (HF) were calculated as follows²⁰:

$$\Delta = \frac{\sqrt{nd} - \sqrt{(b+d)(c+d)}}{\sqrt{n}}$$

$$HF = \frac{\sqrt{n} - \sqrt{b+d} - \sqrt{c+d} - \sqrt{d}}{\sqrt{n}}$$

In cases of two alleles A and B located on adjacent but separate genes, where *a* is observed numbers of A+B+; *b* is observed numbers of A-B+; *c* is observed numbers of A+B-; and *d* is observed numbers of A-B-, *n* = *a* + *b* + *c* + *d*.

Linkage disequilibrium was determined by using the chi-square test with 2 × 2 tables. Statistical analysis was regarded as significant at *p* values < 0.05.

RESULTS

Genotyping of DMA and DMB alleles

We determined the expected size of digested fragments by using PCR-RFLP based on the obtained nucleotide sequences of DMA and DMB genes. PCR-RFLP patterns of HLA-DM genes are shown in Table I. *FokI* digestion of DMA gene gave an undigested fragment of 370 bp for codon 140 Val

TABLE I. PCR-RFLP patterns of HLA-DM genes

DMA gene	DMA	<i>FokI</i>	<i>HinfI</i>	<i>AccI</i>	<i>SfaMI</i>
PCR products: 370 bp Restriction fragments	0101	0	2	1	1
	0102	1	2	1	1
	0103	0	1	0	2
	0104	1	2	0	2
	F0: 370	HF1: 277, 93	AC0: 370	S1: 210, 160	
	F1: 224, 146	HF2: 207, 93, 70	AC1: 292, 78	S2: 177, 160, 33	
DMB gene	DMB	<i>HhaI</i>	<i>BsrI</i>	<i>ApaLI</i>	<i>Bsp1286I</i>
PCR products: 348 bp Restriction fragments	0101	1	1	0	1
	0102	0	1	0	2
	0103	1	2	0	1
	0104	0	2	1	2
	HH0: 348	BR1: 220, 105, 23	AP0: 348	BP1: 290, 40, 10, 8	
	HH1: 189, 159	BR2: 220, 77, 28, 23	AP1: 187, 161	BP2: 191, 99, 40, 10, 8	

(GTC) or fragments of 224 and 146 bp for codon 140 Ile (ATC). *HinfI* digestion of DMA gene gave fragments of 277 and 93 bp for codon 155 Ala (GGA) or fragments of 207, 93, and 70 bp for codon 140 Gly (GCA). *AccI* digestion of DMA gene gave an undigested fragment of 370 bp for codon 184 His (CAC) or Cys (TGC) and fragments of 292 and 78 bp for codon 184 Arg (CGC). *SfaNI* digestion of DMA gene gave an undigested fragment of 370 bp for codon 184 His (CAC) or Cys (TGC) and fragments of 177, 160, and 33 bp for codon 184 Arg (CGC).

HhaI digestion of DMB gene gave an undigested fragment of 348 bp for codon 144 Glu (GAG) or Val (GTG) and fragments of 189 and 159 bp for codon 144 Ala (GCG). *Bsp1286I* digestion of DMB gene gave also fragments of 290, 40, 10, and 8 bp for codon 144 Glu (GAG) or Val (GTG) and fragments of 191, 99, 40, 10, and 8 bp for codon 144 Ala (GCG). *ApaLI* digestion of DMB gene gave an undigested fragment of 348 bp for codon 144 Ala (GCG) or Glu (GAG) and fragments of 187 and 161 bp for codon 144 Val (GTG). *BsrI* digestion of DMB gene gave fragments of 220, 105, and 23 bp for codon 179 Ile (ATT) and fragments of 220, 77, 28, and 23 bp for codon 179 Thr (ACT). Representative results of PCR-RFLP analysis of DMA and DMB genes are given in Figs. 4 and 5.

Allele frequencies of HLA-DM genes

We determined HLA-DM allele frequencies in the patients and control subjects. We were able to discriminate all combinations of heterozygous alleles by using this PCR-RFLP method. Japanese control subjects exhibited an increased frequency

of the DMB*0102 allele (27.9% in Japanese and 3.0% in Caucasian; $p < 10^{-10}$) and a decreased frequency of the DMAB*0101 allele (50.0% in Japanese and 77.2% in Caucasian; $p < 4 \times 10^{-8}$), compared with the Caucasian population described by Carrington et al.^{6,7} (Table II). Frequencies of DMA alleles and the other remaining DMB alleles were similar between the two populations. Ethnic differences of allele frequencies of HLA-DM genes were confirmed for the first time in this study. When we compare the DMA and DMB allele frequencies between the patients and the control subjects, we observed no significant differences between the two groups (Table III).

Haplotypes between HLA class II and HLA-DM alleles

We examined whether HLA-DM alleles were in linkage disequilibrium with DRB1/DQB1 or DPB1 alleles. The results are summarized in Table IV. We confirmed two haplotype in the control subjects. They were DRB1*1201/DQB1*0301/DMA*0102 (HF = 5.69%, $p = 0.0035$) and DRB1*0803/DQB1*0601/DMA*0102 (HF = 2.83%, $p = 0.0351$). In the patient group no combinations reached statistical significance, probably because of the small number of subjects studied.

DISCUSSION

HLA-DM genes were discovered as the gene products required for HLA class II antigen presentation and processing pathways. HLA-DM genes consist of DMA and DMB genes, and HLA-DM molecules are composed of α/β heterodimer

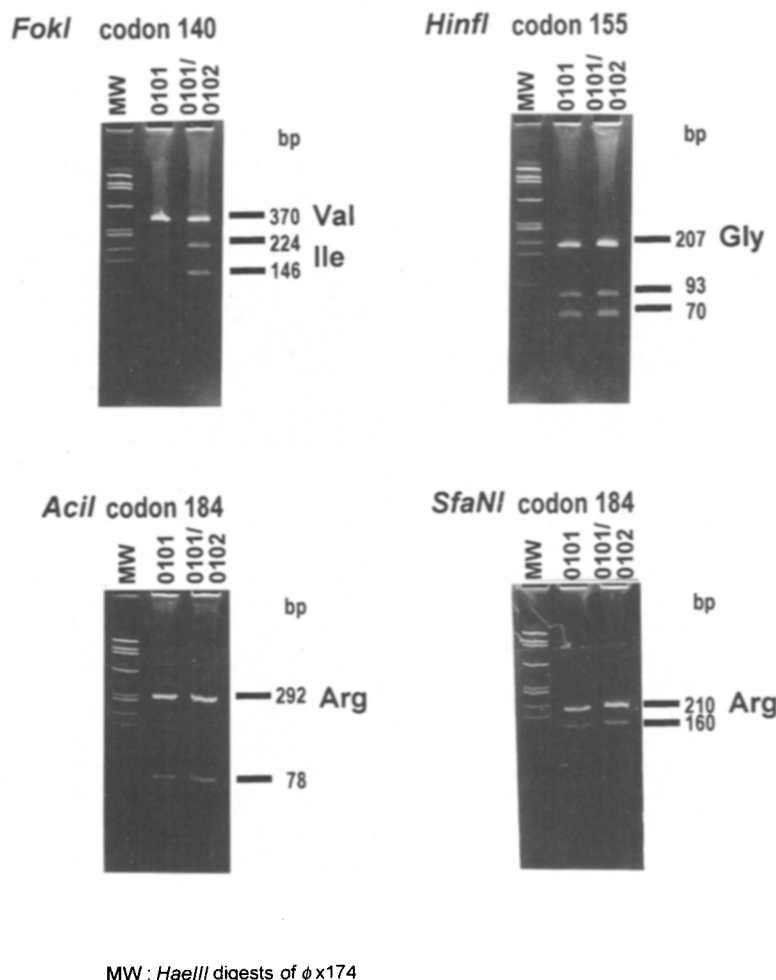


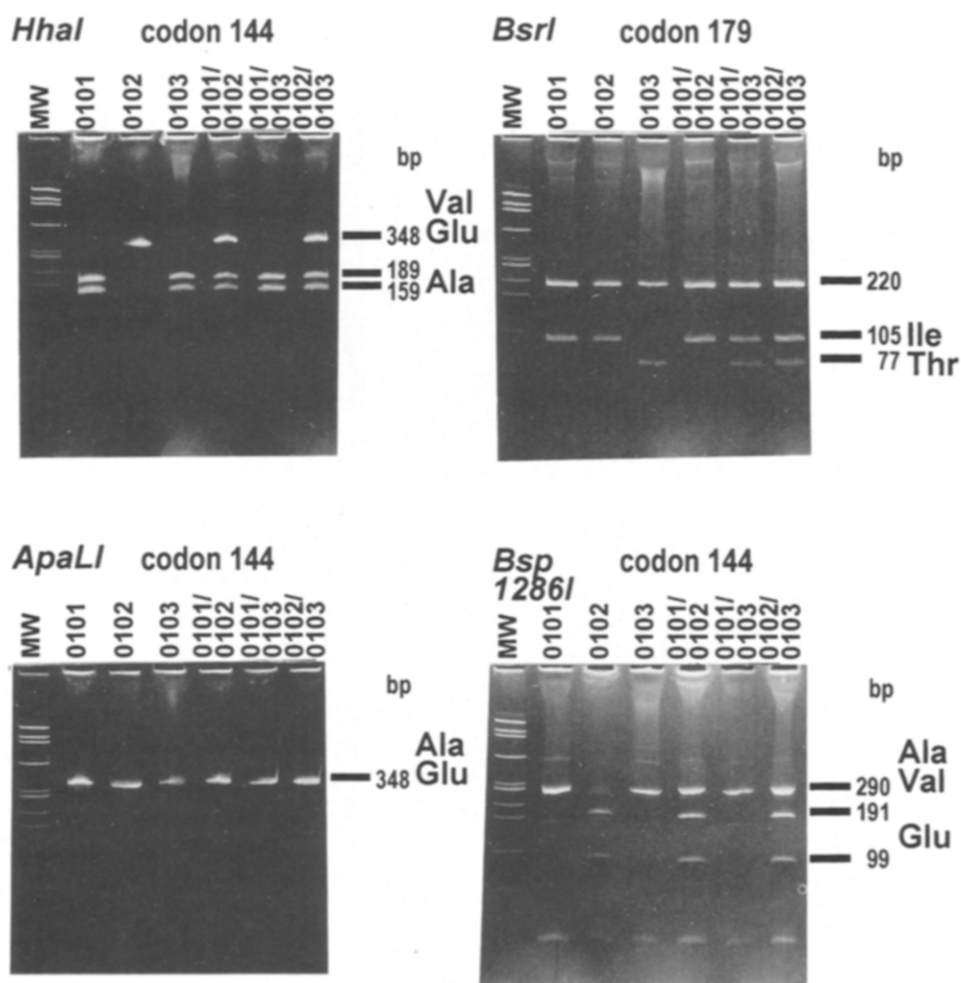
FIG. 4. PCR-RFLP analysis of DMA gene. Third exon of DMA gene was amplified by PCR. Three polymorphic codons were distinguished by using four restriction endonucleases: *FokI*, *HinfI*, *Acil*, and *SfaNI*.

chains. DM genes have been confirmed in human and murine genomes, and the genes are located in the MHC class II region in both species.^{10, 21} HLA-DM molecules detach the Ii chains and facilitate binding of antigenic peptides to HLA class II molecules.

Four possible mechanisms are considered to be involved in removing the Ii chain from HLA class II molecules.²² First HLA-DM molecules may be high-affinity receptors for Ii peptides. They may shift the equilibrium of HLA class II molecules from HLA class II-Ii peptide complexes to empty class II molecules by absorbing Ii peptides released from HLA class II molecules. HLA-DM heterodimers are thought to possess an antigen-binding groove for a limited set of peptides. However, such a high-affinity receptor function would necessitate a mechanism by which HLA-DM molecules

could avoid binding Ii in the endoplasmic reticulum. Second, HLA-DM molecules may function as chaperones that induce a conformational change in HLA class II molecules, resulting in the release of Ii peptides to HLA class II molecules. Third, HLA-DM molecules may have peptidase activity, cleaving Ii peptides into the form that is no longer capable of binding HLA class II molecules. Finally, HLA-DM molecules may play an indirect role in the removal of Ii peptides from HLA class II molecules, affecting trafficking or targeting of HLA class II molecules. Interaction of HLA-DM molecules with DR, DQ, and DP molecules may trigger their transport into the compartment where Ii peptides are removed from class II molecules.

HLA-DM genes also exhibited polymorphisms, and both DMA and DMB genes contain four alleles. Analysis of HLA-DM gene polymorphisms



MW : *HaeIII* digests of ϕ x174

FIG. 5. PCR-RFLP analysis of DMB gene. Third exon of DMB gene was amplified by PCR. Two polymorphic codons were distinguished by using four restriction endonucleases: *HhaI*, *BsrI*, *ApaLI*, and *Bsp1286I*.

were first reported by Carrington et al.^{6,7} by the method of sequence-specific oligonucleotide probe hybridization. Analysis by the PCR-RFLP method in this study also was useful for identifying heterozygosity among the participants.

Because HLA-DM genes are located between DQB1 and DPA1 genes, it is thought that there exists possible linkage disequilibrium and haplotype formation between HLA-DM genes and DRB1/DQB1 genes or between HLA-DM genes and the DPB1 gene. Strong linkage disequilibrium has been well known between DRB1 and DQB1 genes.²³ However, some DPB1 alleles exhibited linkage disequilibrium with DRB1/DQB1 alleles and others did not. Therefore it has been hypoth-

esized that the "hot spot" of recombination exists between DQB1 and DPB1 genes. We identified two haplotypes in the Japanese control subjects in this study: DRB1*1202/DQB1*0301/DMA*0102 and DRB1*0803/DQB1*0601/DMA*0102. Further studies, including family studies or a large-scale population studies, will be needed to identify haplotypes.

It is not obvious at whether differences in HLA-DM alleles confer differences in the ability of the Ii chain to detach from HLA class II molecules or differences in selection of the amino acid sequences or length of Ii chains, or further, whether HLA-DM molecules are involved in selection of antigenic peptides. Ii chains are bound to HLA

TABLE II. HLA-DM allele frequencies in a Japanese population

HLA-DM alleles	Japanese subjects	Caucasian subjects*	Chi-square	p Value
DMA	<i>n</i> = 104	<i>n</i> = 180		
0101	93 (89.4%)	152 (83.9%)	1.3791	0.2402
0102	11 (10.6%)	18 (10.0%)	0.02392	0.8770
0103	0 (0.0%)	5 (3.0%)	1.5538	0.2125
0104	0 (0.0%)	5 (3.0%)	1.5538	0.2125
DMB	<i>n</i> = 104	<i>n</i> = 400		
0101	52 (50.0%)	309 (77.2%)	30.1588	4×10^{-8}
0102	29 (27.9%)	12 (3.0%)	68.3944	$<10^{-10}$
0103	23 (22.1%)	73 (18.3%)	3.6375	0.05649
0104	0 (0.0%)	6 (1.5%)	0.5611	0.4538

*Allele frequencies in Caucasian population are based on report of Carrington et al.^{6,7}

TABLE III. HLA-DM allele frequencies in atopic dermatitis

HLA-DM alleles	Atopic dermatitis (<i>n</i> = 74)	Control (<i>n</i> = 104)	Relative risk	Chi-square	p Value
DMA					
0101	66 (89%)	93 (89%)	0.98	0.002480	0.9602
0102	7 (9%)	11 (11%)	0.88	0.05939	0.8074
0103	1 (1%)	0 (0%)	4.27	0.02940	0.8638
DMB					
0101	41 (55%)	52 (50%)	1.24	0.5063	0.4767
0102	21 (28%)	29 (28%)	1.02	0.005218	0.9424
0103	12 (16%)	23 (22%)	0.68	0.9524	0.3290

TABLE IV. Linkage disequilibrium between HLA-DM and HLA class II genes

Allele 1	Allele 2	Δ Value	Chi-square	p Value	HF
Control					
DRB1/DQB1	DMA				
1201/0301	0102	0.0455	8.5036	0.0035	0.0569
0803/0601	0102	0.0239	4.4414	0.0351	0.0283

class II molecules before the binding of antigenic peptides to HLA class II molecules. They prevent antigenic peptides from binding to HLA class II molecules. Human Ii chains consisted of 218 amino acid residues, and 14-mer to 25-mer peptides are obtained by extracting Ii chains bound to HLA class II molecules. They are designated as class II-associated Ii chain peptides (CLIPs).²⁴ CLIPs correspond to the 81st to 104th amino acid sequences of the Ii chain. This CLIP region is supposed to play a critical role in antigen processing by binding to HLA class II molecules. Human Ii chains are a mixture of 33, 35, 43, and 45 kd molecules by alternative splicing. Among them, 33 kd molecules are most abundant and are designated as the p33 Ii chain. Four possible pathways

are considered to be involved in Ii chain functions.²⁵ First, binding of Ii chains to HLA class II molecules stabilizes α/β heterodimer formation of HLA class II molecules in the endoplasmic reticulum. Second, Ii chains prevent antigenic peptides from binding to HLA class II molecules. Third, binding of Ii chains to HLA class II molecules causes exiting of HLA class II molecules from the endoplasmic reticulum. Finally, Ii chains facilitate the movement of HLA class II molecules from the endoplasmic reticulum to endosomal compartments.

Both HLA-DM molecules and Ii chains regulate the binding of antigenic peptides to HLA class II molecules in the HLA class II-restricted antigen processing pathway. Further analysis will be

needed to investigate the precise processing mechanisms by integrated examination of these molecules and peptides. Ii chains bound to HLA class II molecules are degraded through an endosomal component to the compartment for peptide loading. However, these processing pathways have not yet been fully elucidated.²⁶ In addition, no reports are currently available to clarify whether different DMA or DMB alleles contribute to alterations in binding abilities of Ii chains and antigenic peptides. It also will be necessary to clarify the functional role of the HLA-DM molecules.

In conclusion, we determined HLA-DM alleles in Japanese patients with atopic dermatitis. No differences in DMA and DMB allele frequencies were observed between the patients and the control subjects. DM genes are believed not to contribute primarily to the susceptibility of atopic dermatitis. Further investigation of functional roles of DM gene polymorphisms will be useful for a better understanding of susceptibility loci in HLA class II-associated disease.

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