

Association between HLA Antigens and Birch Pollen Allergy in Japanese Subjects

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ABSTRACT

Birch pollen is a very common cause of nasal allergy (pollinosis) not only in Scandinavia, Europe, Canada, and the northern part of the United States but also in Hokkaido, Japan. Although there are many papers describing the association of HLA antigens and pollinosis caused by many other allergens, there is no report about the association of HLA antigens and birch pollen allergy. In this paper, we performed an HLA population study of birch pollen-allergic patients in Japan and their pattern analysis of HLA-DRB and HLA-DQB gene by the Southern blot hybridization technique. Our population study data indicated that the HLA-DR9 and HLA-DQw3 phenotypes strongly associated with the development of birch pollen allergy in Japanese subjects. By restriction fragment length polymorphism pattern analysis, some restriction fragments of the HLA-DQB gene were detected only in the patient group. These data suggested that there was a significant association between HLA class II antigens and the development of birch pollen allergy in Japanese subjects.

Birch pollen is a very common cause of nasal allergy in Hokkaido, Japan. Birch pollen nasal allergy (pollinosis), which is diagnosed by eosinophils in the nasal discharge, a skin test, and a radioallergosorbent test (RAST), is found in about 15% of all patients with nasal allergies seen at Asahikawa Medical College Hospital.

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Birch pollen is also a very common cause of pollinosis, hay fever, asthma, and allergic conjunctivitis in Scandinavia,^{1,2} Europe,^{3,4} the northern parts of the United States⁵ and Canada.

There have been many reports describing about the association between a certain HLA antigen and nasal allergy caused by *Dermatophagoides farinae*,^{6,7} Japanese cedar,⁸ or grass pollen.⁹⁻¹² Several reports suggest that HLA class II antigens play an important role in influencing the genetic regulation of IgE reactivity against a certain antigen such as Japanese cedar and grass pollen.¹³⁻¹⁶ However, up to now, there has been no report for the association of HLA and allergic reaction to birch pollen.

In this paper, which is the first report describing the association between HLA and nasal allergy to birch pollen, we investigated whether birch pollen allergy in Japanese was associated with a certain HLA antigen by HLA population study and restriction fragment length polymorphism (RFLP) pattern analysis of HLA-DRB and HLA-DQB genes. Our data suggest that patients' immune responsiveness to the birch pollen was strongly associated with the expression of HLA-DR9 and HLA-DQw3 antigens in Japanese patients.

MATERIALS AND METHODS

Study Subjects

For the HLA population study, 12 subjects (3 men, 9 women; mean age 34.2 ± 8.1 years; range 27-56 years) were sensitized by birch pollen and 192 healthy adult were control subjects. Ten of the 12 subjects were sensitized by multiple antigens, such as house dust (mite), timothy grass, and/or orchard grass. None of

the allergens were cross-reacted to each other. Diagnosis was made in all subjects by clinical symptoms, eosinophilic cells in the nasal discharge, intradermal skin tests or the scratch test for birch allergen (Hollister-Stier, Spokane, WA), nasal provocation tests using a disc soaked with allergen, serum IgE and specific IgE antibody to birch pollen using RAST.

HLA Typing

HLA typing was carried out by a standard method at the Red Cross Blood Center in Sapporo.

HLA class I (A, B, and C) determination was performed by means of a standard microdroplet lymphocytotoxicity test. Briefly, lymphocytes were separated from the peripheral blood of the patients and the control subjects using the column technique. Lymphocyte suspension (concentrated to $2 \times 10^6/\text{ml}$) was added to each well of a round-bottomed microplate, previously set with anti-HLA serum. After incubation at room temperature, rabbit serum was added to each well. Next, 5% eosin-Y was put into each well, and the reaction was stopped by adding neutral formaldehyde. Cytotoxicity was observed under a phase-contrast microscope.

HLA class II (DR, DQ, and DP) determination was performed by a B cell cytotoxicity assay. Briefly, the B lymphocytes were purified by the column technique and T cell elimination was performed with monoclonal anti-T lymphocyte antibody and complement. Antisera against HLA-DR or HLA-DQ or HLA-DP were incubated with the B lymphocyte, followed by incubation with complement. Phenotypes were determined by the eosin exclusion test.

Southern Blotting

We performed Southern blot analysis by a standard method.¹⁷ Briefly, genomic DNA was extracted from peripheral blood leukocytes and lymphocytes of patients and nonallergic control subjects. Each 10- μg sample of DNA was digested overnight (10–14 hours) by the restriction enzymes, *EcoRI*, *BamHI*, *KpnI*, *SstI*, and *TaqI*, under a condition recommended by the manufactures. These DNA samples were separated in 0.8% agarose gel in TAE by electrophoresis and transferred on to a nylon membrane filter. After baking, the filters were incubated for 20–24 hours at 65°C in a prehybridized solution [20 $\times$ SSPE, 10% sodium dodecyl sulfate (SDS), 10% skim milk, and single-stranded DNA, 10 ng/ml]. The probe of hybridization was the cDNA clone (pDC β -101) which contained most parts of the sequences for an HLA-DQw1B (kindly provided by Dr. Inoko) and full-length cDNA clone of the DR9 β chain, DRB9.¹⁸ The purified cDNA fragment of β -101 (1.2 kb) and the DR9B cDNA fragment (1.1 kb)

were labeled with α -[³²P]dCTP by the multiple primer method (Boehringer-Mannheim, Mannheim, West Germany). The filters hybridized with ³²P-labeled cDNA probe were followed by washing under a stringent condition (0.1% SDS, 1 $\times$ SSC at room temperature for 5 minutes twice, 0.1% SDS, 0.1 $\times$ SSC at 42°C for 15 minutes twice, and 0.1% SDS, 0.1 $\times$ SSC at 65°C for 3 minutes). The labeled fragments were exposed to Kodak X-AR films with an intensifying screen for 1 or 2 days at -70°C.

RESULTS

HLA Population Study

We carried out an HLA population study of 12 patients and 192 healthy control subjects. The 12 patients included 2 birch pollen-allergic patients and 10 multi-antigen-allergic patients (sensitized by birch pollen and another allergens). As seen in Table I, the incidences of HLA-DR9 and HLA-DQw3 were apparently higher in patients than in healthy control subjects

TABLE 1
HLA Typing of 12 Patients with Nasal Allergy to Birch Pollen and 192 Healthy Control Subjects

	Patients		Control Subjects	
	No.	%	No.	%
DR1	1	8.3	25	13.0
DR2	4	33.3	75	39.1
DR3	0	0	0	0
DR4				
DR4.1	3	25.0	41	21.4
DR4.2	1	8.3	24	12.5
DR5			34	17.7
DRw11	1	8.3		
DRw12	2	16.6		
DRw6				
DRw13	1	8.3	25	13.0
DRw14	0	0	18	9.4
DR7	0	0	2	1.0
DRw8				
DR8.1	0	0	21	10.9
DR8.2	0	0	17	8.9
DR9	10	83.3*	73	38.0
DRw10	0	0	0	0
DQw1	5	41.6	135	70.3
DRw2	0	0	2	1.0
DQw3	10	83.3†	84	43.8
DQw4	3	25.0	46	24.0
DQw7	0	0	35	18.2

(Table I, *,†). The statistical analysis revealed that only HLA-DR9 and HLA-DQw3 were significantly correlated with the patient group. The χ^2 values were 9.61 and 7.12, and the relative risks were 3.06 and 3.89, respectively (Table II). These values indicated the possibility that the susceptibility for nasal allergy to birch pollen might associate with HLA-DR9 and/or HLA-DQw3 antigen expression. There was no negative correlation in this study.

Southern Blot Analysis of HLA-DRB and HLA-DQB

From our population study data, there are allergic patients and nonallergic healthy individuals who have same HLA-DR9 or HLA-DQw3 haplotypes. Many previous reports revealed that these HLA class II genes have marked genetic polymorphisms, especially in their β chain gene. We considered the possibility that this discordance to the reactivity to birch pollen within the same HLA haplotype is restricted by the genetic variation of these HLA genes. To investigate this possibility, we performed Southern blot analysis of these HLA genes. First, we focused on the HLA-DRB gene using an HLA-DR9B cDNA probe. Ten patients and 2 healthy control subjects who had the HLA-DR9 haplotype were tested. We could not find any differences in RFLP patterns with five different restriction enzyme digestions among the patients and the healthy control subjects (Fig. 1). Next, we focused on the HLA-DQB genes of the 8 HLA-DQw3-positive patients and 2 healthy control subjects using the pDC β -101 cDNA probe. In this gene, we found some differences in RFLP patterns among the patients and the control subjects. With *Bam*HI digestion, 6.6- and 11.5-kb fragments were detected only in the patient group; on the other hand, 4.7- and 10.5-kb fragments were detected only in the control group (Fig. 2). With *Kpn*I digestion, a 5.5-kb fragment was seen only in the control group (data not shown). However, there was no difference in digestion by *Eco*RI, *Sst*I and *Taq*I (data not shown).

TABLE 2

Significant Association between HLA-DR9,-DQw3 Phenotype and Nasal Allergy to Birch Pollen

	No. Tested	HLA-DR9		HLA-DQw3	
		No.	%	No.	%
Nasal allergy	12	10	83.3	10	83.3
Control	192	73	38.0	84	43.8
χ^2		9.61		7.12	
		$(p < 0.01)$		$(p < 0.01)$	
Relative risk		3.06		3.89	

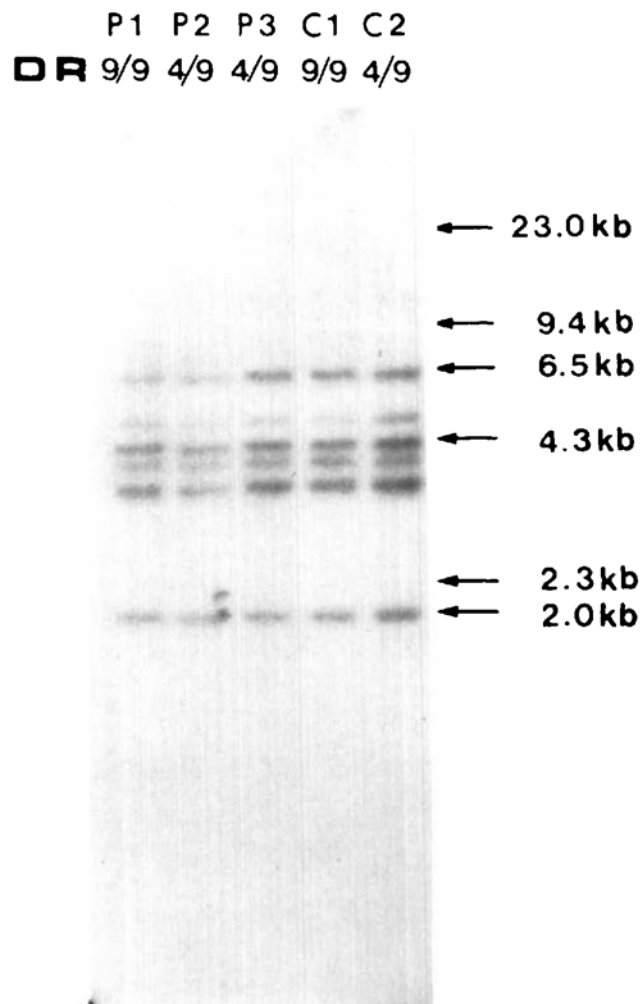


Figure 1. Southern blot patterns of DNA in patients (P1-P3) and control subjects (C1-C2) digested with *Bam*HI using an HLA-DRB probe [DR9B cDNA fragment (1.1 kb)]. All of them have the DR9 phenotype, but there is no difference in the RFLP pattern among the patients and the control subjects.

DISCUSSION

The three human MHC class II antigens, HLA-DR, HLA-DQ, and HLA-DP, are composed of two subunits, α (~33 kD) and β (~28 kD), and each subunit has two extracellular domains of a highly polymorphic region.¹⁹ This marked polymorphism is mainly defined by the 1st domain of the β chain encoded by the 2nd exon of each gene. These polymorphisms on class II molecules possibly play an important role in the interaction between the immune response cells through antigen recognition and antigen presentation.²⁰

Many groups reported about the relation of the HLA system and an allergic immune response to many allergens.¹⁻¹¹ However, in siblings who have same HLA haplotypes, there exist some allergic patients and non-allergic individuals, and there is a difference in clinical symptoms between individuals who have the same

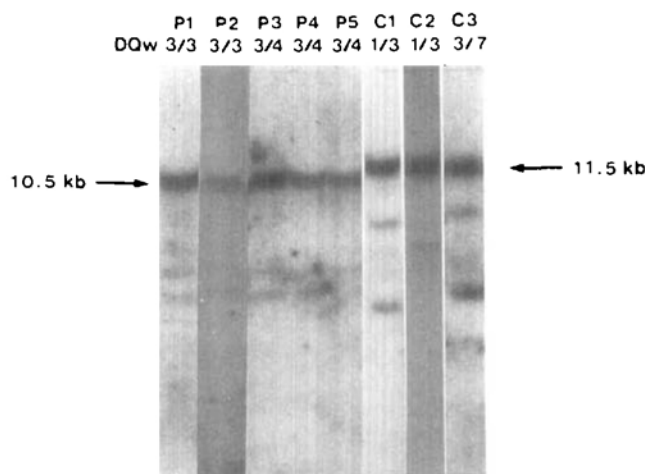


Figure 2. Southern blot patterns of DNA in patients (P1–P5) and control subjects (C1–C3) using an HLA-DQB probe [pDC β -101 (1.2 kb)]. All of them have the DQw3 phenotype. The patients have a 10.5-kb BamHI fragment and the control subjects have a 11.5-kb BamHI fragment.

HLA haplotype. These facts suggest that the genetic control might be present in any step of the development of this allergic reaction.

In this paper, we reported that the susceptibility to development of the allergic reaction to birch pollen also related to the HLA system. Our data indicated that HLA-DR9 and HLA-DQw3 antigens were strongly associated with this allergic reaction in Japanese subjects. From our Southern blot analysis, there were some differences in the RFLP patterns of HLA-DQw3B but no difference in those of HLA-DR9B. Up to now, HLA-DQw3 could be divided into three subtypes, such as HLA-DQw7, HLA-DQw8, and HLA-DQw9, and it is known that the HLA-DQw9 antigen is in linkage disequilibrium with the HLA-DR9 antigen. However, we have no HLA-DQw9 allo-antisera to detect the HLA-DQw9 haplotype, so our HLA-DQw3-positive patients might have HLA-DQw9. Interestingly, all of HLA-DR9-positive patients had HLA-DQw3 and HLA-DR9-positive patients and healthy control subjects had no differences in the RFLP patterns of the DRB gene with any restriction enzyme digestion, but there were some differences on the DQB gene with coupled restriction enzyme digestion (*Bam*HI, *Kpn*I). The difference detected on the HLA-DQB Southern blot might indicate the difference of an HLA-DQ haplotype, such as HLA-DQw3 and HLA-DQw9. This raises the question of which HLA-DQ haplotype, HLA-DQw3 or HLA-DQw9, really could be associated with the development of birch pollen allergy in Japanese subjects. Further study should be performed to resolve this question using purified birch pollen allergen, such as a ragweed purified allergen,^{21–23} and an increased number of patients.

ACKNOWLEDGMENT

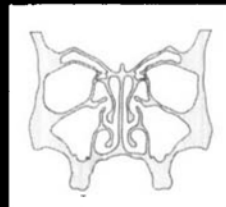
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