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Original Article

HLA class II association with Type I allergy to house dust mite and Japanese cedar pollen in Japanese subjects

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Abstract

We evaluated the incidence of the association of HLA class II phenotype and specific IgE responsiveness against house dust mite (HDM) and/or Japanese cedar pollen (Jc) in 176 patients with allergic rhinitis, with or without bronchial asthma, and 107 nonallergic subjects. Specific IgE antibody titration against the purified allergens *Der f1* and *Der f2* from HDM, and against *Cry J1* and *Cry J2* from Jc, was performed by using enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) in sera from all subjects. HLA class II oligotyping was performed by the polymerase chain reaction sequence specific oligonucleotide (PCR-SSO) method on the DRB1*, DQA1*, DQB1* and DPB1* alleles using peripheral blood cells. The high IgE responders ≥ class 4 to the purified allergens were identified by using the IgE antibody reference concentration obtained by ELISA, RIA and routine IgE CAP RAST. Compared to the controls, the patients with both rhinitis and asthma showed significantly higher frequencies of DRB1* 0901, DQB1* 0303, and DPB1* 0401 alleles. High IgE responsiveness to HDM was associated with DRB1* 1101, 0901, DQB1* 0303, and DPB1*0401 alleles. The patients with anti-*Der f1* IgE antibody concentration exceeding 72.2 ng/mL showed significantly elevated frequencies for DQB1*0401 and DPB1*0401 alleles, and those with anti *Der f2* IgE antibody concentration exceeding 46.2 ng/mL showed significantly elevated frequencies for DPB1*0401 and 0901 alleles. High IgE responsiveness to Jc with *Cry j1* and *Cry j2* was associated with the DRB1* 1201 alleles.

Key words: allergic rhinitis, HLA class II, HLA oligotyping, PCR-SSO, Type I allergy.

Introduction

Atopic diseases such as hay fever and asthma are considered to result from an immune reaction to some allergen.

They have a hereditary basis, as first pointed out by Coca and Cooke in 1923.[1] In support of this, Levine reported that the onset of ragweed hay fever was genetically controlled by HLA haplotype-linked genes.[2] There exist marked genetic polymorphisms in HLA class II alleles, which show individual expression as well as many differences in their phenotype frequencies in various ethnic groups. Investigations of particular HLA phenotype and haplotype associations to an allergic disease may be useful in clarifying the genetic mechanism underlying allergic disease.

Numerous reports have identified an association between a specific HLA allele and an allergic disease. Marsh *et al.* reported that HLA DR2 was associated with responsiveness to short ragweed pollen allergen (Amba 5) in ragweed-allergic patients, and HLA DR5 with the immune responsiveness to short ragweed pollen allergen (Ra 6) in allergic human subjects.[3,4] Sasazuki *et al.* reported that

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the production of IgE antibodies specific to Japanese cedar (Jc) pollen antigen was controlled by the HLA-linked immunosuppression gene (Is gene) in linkage disequilibrium with HLA DQw3.[5,6] More recently, Marsh *et al*. reported positive association between Fel d1 and Alt a1 IgE responses, and DRB1*04 defined by DNA analysis in allergic Japanese subjects.[7] We have also reported the positive association of DQB1*03 and/or *0303 (subtype) molecules with allergic rhinitis in Japanese subjects with allergy to house dust mite (HDM), Jc pollen and orchard grass.[8–10]

All these studies have focused on the marked association between particular HLA molecules and a specific IgE response to major allergens. This illustrates the utility of the allergic model for basic immunogenetic studies of human immune responsiveness. In particular, HLA class II molecules are important for antigen presentation to activated T cells in allergic reactions.

The purpose of this study was to analyze the association between certain specific HLA class II molecules and IgE responsiveness to HDM and Jc, HDM and Jc being the major allergens in allergic Japanese subjects as identified by comparison with control groups at clinical allergy laboratories.

Previous studies used poorly defined nonallergic control groups, in part because the onset of allergic rhinitis and atopic asthma ranges from 20 to 30 years of age. Thus, we carefully defined the criteria for inclusion in the control group for comparison with the patients at clinical allergy laboratories.

METHOD

Subjects

We selected 176 patients with allergic rhinitis with (n = 41) or without (n = 135) asthma for the study. Allergic subjects were diagnosed based on the symptoms of sneezing episodes, watery nasal discharge and nasal obstruction, and then on whether they had a class of two or higher IgE RAST to at least one of HDM and Jc. The control group comprised 107 individuals over 20 years of age without allergic symptoms, and who had a negative IgE RAST for both HDM and Jc (Table 1).

Measurement of serum IgE and IgE antibodies against house dust mite and Japanese cedar

Total serum IgE and specific IgE antibody levels were measured by radioimmunosorbent tests, namely, the CAP-RAST[11] and the MAST[12] system, respectively.

Assay of anti-*Der f1* and anti-*Der f2* IgE antibodies

IgE antibodies against the purified HDM allergens *Der f1* and *Der f2* were measured by ELISA.[13] Isolation of *Der f1* and *Der f2* was carried out by a method described previously.[14,15] For anti-*Der f1* and *2* IgE antibody measurements, microtiter plates (Immulon 2; Dynatech Laboratories, Chantilly, VA, USA) were incubated with 100 μL of 10 μg/mL *Der f1* or 50 μg/mL of *Der f2* in 0.1 mol carbonate buffer (pH 9.6) overnight in the cold in a humidified box. After three washes with phosphate-buffered saline (PBS)/Tween, protein-binding sites of the wells were blocked with 100 μL of Black Ace (Yukijirushi, Tokyo, Japan). Diluted 100 μL serum samples (1/2 dilution for IgE) were applied to the wells. After incubation for 1 h at room temperature, the serum samples were removed and washed three times. Then 100 μL of 1/3000-diluted biotin-labeled affinity-purified goat anti-human IgE F(ab) `2 fragment (Tago, Burlingame, CA, USA) was added to each well and incubated at room temperature for 1 h.

After three washes, 100 μL of 1/4000-diluted peroxidase-avidin D (Vector Laboratories, Burlingame, CA, USA) was added to each well and incubated further for 1 h. Then 100 μL of o-phenylenediamine dihydrochlorine (Sigma Chemicals, St Louis, MO, USA) substrate solution was added to the wells after three washes with PBS/Tween, followed by the addition of 100 μL of 4N sulfuric acid to terminate the reaction. Absorbance at 490 nm was read with an automatic ELISA reader (model MTP-32; Corona Electric, Ibaragi, Japan). For the standardization of the assay, a preparation of atopic serum which contained 526 ng/mL of anti-*Der f1* and 139 ng/mL of anti-*Der f2* IgE antibodies was employed as a standard.

Table 1. Background of allergic subjects (n = 176) and controls (n = 107)

	No.	Age	Sex (m:f)	Positive IgE RAST to HDM:Jc
AR alone	125	31.0 ± 13.1	1:1.1	73:95
AR + BA	41	35.2 ± 12.2	1:1.3	41:21
AR + AD	10	15.4 ± 10.1	1:0.9	10:6
Controls	107	29.0 ± 13.7	1:0.76	

AR, allergic rhinitis; BA, bronchial asthma; AD, atopic dermatitis; HDM, house dust mite; Jc, Japanese cedar.

Assay for anti-*Cry j1* and anti-*Cry j2* IgE antibodies

Radio-immunoassay for IgE antibodies to the purified Jc allergens *Cry j1* and *Cry j2* was performed. *Cry j1* was purified from Japanese cedar pollen as previously described.[16] *Cry j2* was provided by Dr Y Taniguchi, Hayashibara Biochemical Laboratories.[17] IgE antibodies to *Cry j1* and *Cry j2* were measured by RAST. CNBr-activated paper disks were coupled with 2 μg/mL of the purified *Cry j1* or *Cry j2* preparation. Allergen disks were incubated for 3 h at room temperature with 50 μL of serum. When necessary because of high IgE antibody concentration, the serum sample was diluted with horse serum. The disks were then washed and 50 μL of ^{125}I-anti IgE (Phadebas RAST; Pharmacia, Uppsala, Sweden) were added. After an overnight incubation, the disks were washed and counted in a gamma counter. The results were expressed in Phadebas RAST Units per milliliter (PRU/mL), using the Phadebas RAST Reference System.

Procedure of HLA oligotyping

The DNA typing of HLA class II was performed using the polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) method in DRB*1, DQA*1, DQB*1 and DPB*1 alleles.[18] The sequence specific oligonucleotides (SSO) used for this study were selected by Dr A Kimura, Genetics Medical Institute of Bioregulation, Kyusyu University Fukuoka. Genomic DNA was isolated from peripheral blood cells obtained from both allergic patients and controls. One microgram of sample was amplified by a PCR procedure with 0.2 mmol of dNTP, 25 pmol of each primer pair, and 1 unit of Taq-polymerase (Taq DNA Polymerase; Pharmacia, Uppsala, Sweden). The mixed samples were then subjected to 30 cycles of amplification by denaturing, primer annealing and polymerase extension at suitable temperature and time conditions. Two μL of the amplified DNA samples were denatured and loaded onto a nylon membrane (Hybond-N, Amersham, UK) using a dot blotter (BIO DOT; Bio-Rad, Tokyo Japan). The filters were prehybridized at 54°C for at least 30 min in solution containing 6x SSPE, 5x Denhardt's solution, 0.5% SDS and 100 μg/mL heat-denatured salmon sperm DNA, and then hybridized for 2 h with ^{32}P labeled SSO probes using T_4 polynucleotide kinase. The filters were then washed at room temperature for 15 min in 2x SSPE plus 0.1% SDS. This was repeated before they were washed another two times at 56–58°C in TMAC solution. They were then exposed to an X-ray film (Medical X-ray film; Fuji, Tokyo Japan) with an intensifying screen at room temperature. We made two autoradiograms with short (30 min to 2 h) and long (16–24 h) exposure.

We first ascertained that the amount of amplified DNA for oligotyping obtained was sufficient for the whole procedure of the study by monitoring the framework SSO:control SSO-1 and DQA-LSO. The oligotyping was carried out for the 24 DRB, 8 DQA, 14 DQB, and 22 DPB alleles.

Statistical analysis

For statistical analysis we used Fisher's exact probability test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Titration of serum IgE antibody and HDM CAP RAST class to HDM (DF)

The frequency distribution of the CAP RAST class to HDM (DF) in the patients with allergic rhinitis is shown in Table 2. The mean class value to HDM (DF) of these subject was 3.1 (n = 176). The numbers of allergic subjects that were categorized as over class 2 and 4 were 124 and 52, respectively. We determined that patients with a CAP RAST class of 4 or more were chosen as IgE high responder.

Titration of *Der f1* and *Der f2* by ELISA and DF RAST class

The relationship of each of anti-*Der f1* and *Der f2* to the DF RAST class is shown in Fig. 1(a,b). The CAP RAST class of 4 corresponded to concentration of 72.2 ng/mL for anti *Der f1* IgE and 46.2 ng/mL for anti *Der f2* IgE.

Titration of serum IgE antibody against Jc CAP RAST class to Jc

The CAP RAST class to Jc for the subjects with allergic rhinitis is shown in Table 2. The mean class value for these

Table 2. Serum IgE CAP RAST class in allergic subjects

CAP RAST class	Number of patients with allergy to HDM (DF)		Jc	
0	12		34	
1	40		20	
2	16		59	
3	56		46	
4	21		14	
5	20	n = 52	2	n = 17
6	11		1	
Total	176		176	

HDM, house dust mite; DF, *Dermatophagoides farinae*; Jc, Japanese cedar. The CAP RAST class was 0 or 1 in all of the control subjects.

subjects was 2.6. The class was 2 or higher in 122 of the subjects and 4 or higher in 17 of the subjects. We determined that patients with a CAP RAST class of 4 or more were chosen as IgE high responder.

Titration of *Cry j1* and *Cry j2* by RIA and CAP RAST class

The relationship between each of anti-*Cry j1* and *Cry j2* to the Jc RAST class is shown in Fig. 2(a,b). The CAP RAST class of 4 corresponded to a concentration of 14.7 PRU/mL for anti-*Cry j1* IgE and 13.6 PRU/mL for anti-*Cry j2* IgE.

HLA class II association in allergic subjects

Compared to the controls, the patients with allergic rhinitis without asthma showed a significantly higher frequency of DQB1* 0303 and DPB1* 0401 alleles, and patients with both rhinitis and asthma showed a significantly greater frequency of DRB1* 0901, DQB1* 0303, and DPB1* 0401 alleles (Table 3).

HLA and high IgE responsiveness to house dust mite

The patients with CAP RAST class of 4 or more for HDM showed significantly elevated frequencies of DRB1* 1101,

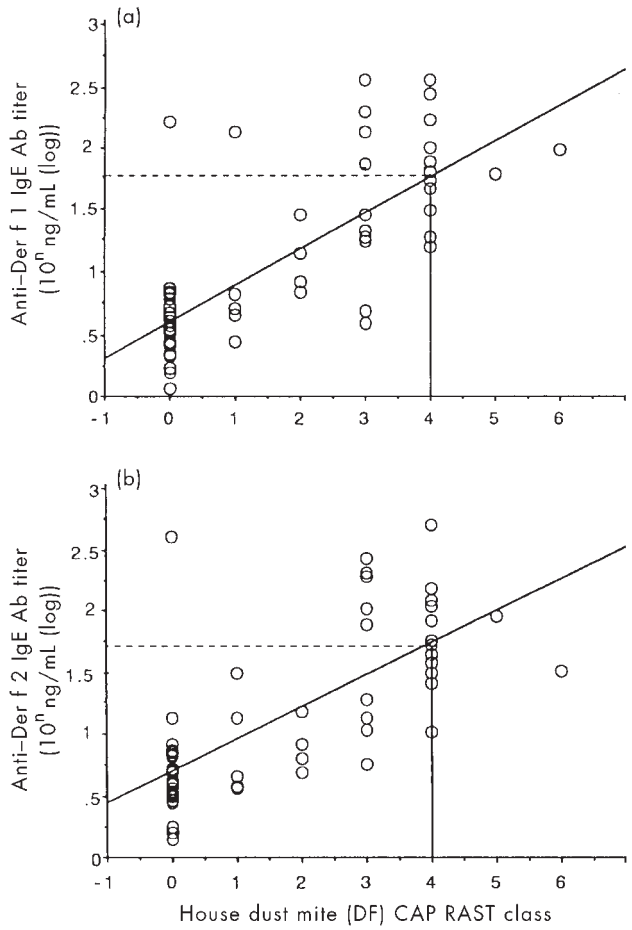

Fig. 1 Relationship between each of (a) anti-*Der f1* and (b) anti-*Der f2* IgE antibody and HDM (DF) CAP radioallergosorbent (RAST) test. The CAP RAST class of 4 corresponded to titer of 72.2 ng/mL for *Der f1* and 46.2 ng/mL for *Der f2* on each dot linear. (a) y = 0.292x + 0.605; x, DF CAP RAST class; y(ng/mL), anti-*Der f1* IgE antibody titer by the ELISA; r^2 = 0.556. (b) y = 0.26x + 0.698; x, DF CAP RAST class; y(ng/mL), anti-*Der f2* IgE antibody titer by ELISA; r^2 = 0.49.

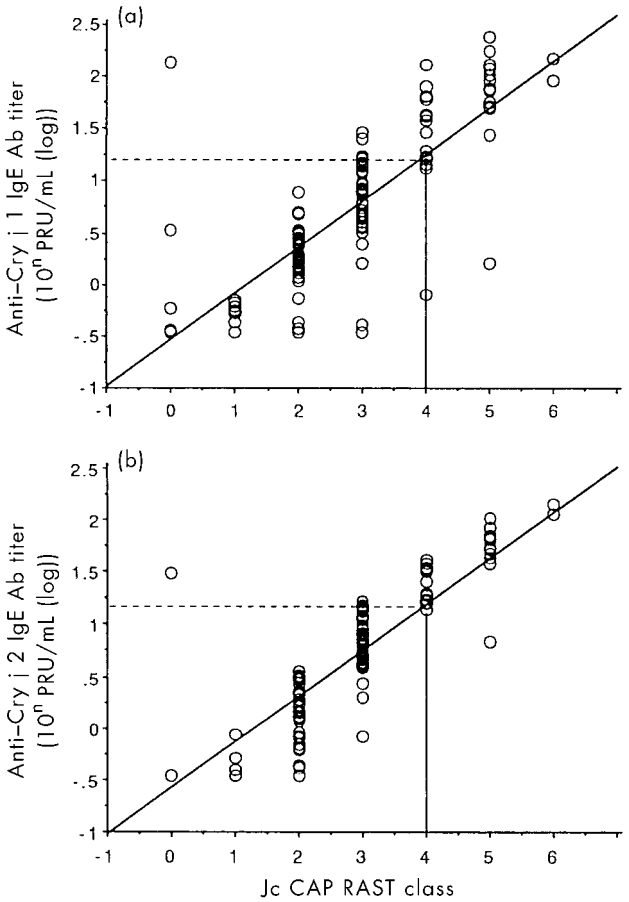

Fig. 2 Relationship between each of (a) anti-*Cry j1* and (b) anti-*Cry j2* IgE antibody and Jc CAP radioallergosorbent test (RAST) class. The CAP RAST class of 4 corresponded to titer of 14.7 PRU/mL for *Cry j1* and 13.6 PRU/mL for *Cry j2* on each dot linear. (a) y = 0.459x –0.575; x, Jc CAP RAST class; y(PRU/mL), anti-*Cry j1* IgE antibody titer by the RIA; r^2 = 0.782. (b) y = 0.451x –0.611; x, JC CAP RAST class; y(PRU/mL), anti-*Cry j2* IgE antibody titer by the RIA; r^2 = 0.868. PRU, Phadebas RAST Units.

Table 3. HLA Class II association in subjects with allergic rhinitis

HLA allele	Allergic rhinitis alone	Allergic rhinitis with bronchial asthma
DRB1*		0901 (P = 0.04)
DQB1*	0303 (P = 0.04)	0303 (P = 0.03)
DPB1*	0401 (P = 0.008)	0401 (P = 0.009)
Total	n = 125	n = 41

In the subjects with allergic rhinitis, significantly more frequent positiive associations were found for allele frequencies of DRB1* 0901, DQB1* 0303 and DPB1* 0401, when compared to the non-allergic controls (n = 107).

Table 4. HLA Class II association to high IgE responsiveness against HDM and Jc

	CAP RAST Class	
HLA allele	HDM (DF) ≥ 4 (n = 52)	Jc ≥ 4 (n = 17)
DRB1*	1101 (P = 0.04)	
	0901 (P = 0.02)	1201 (P = 0.04)
DQB1*	0303 (P = 0.003)	
DPB1*	0401 (P = 0.003)	

HDM, house dust mite; DF, *Dermatophagoides farinae*; Jc, Japanese cedar. In high IgE responsiveness against house dust mite, positive associations were statistically found in allele frequencies of DRB1* 1101, 0901, DQB1* 0303 and DPB1* 0401. In high IgE responsiveness against Japanese cedar, positive associations were also statistically found in allele frequency of DRB1* 1201.

0901, DQB1* 0303, and DPB1* 0401 alleles (Table 4). Significantly elevated frequency was also observed in patients whose anti-*Der f1* IgE antibody concentration exceeded 72.2 ng/mL for DQB1* 0401 and DPB1* 0401 alleles, and in patients whose anti-*Der f2* IgE antibody concentration exceeded 46.2 ng/mL for DPB1* 0401 and 0901 alleles.

HLA and high IgE responsiveness to Jc

The patients with CAP RAST class of 4 or more for Jc showed a significantly elevated frequency of DRB1* 1201 allele (Table 4). Significant elevation was also observed in the patients whose anti-*Cry j1* IgE antibody concentration exceeded 14.7 PRU/mL for the frequency of DRB1* 1201 allele, and in the patients whose anti-*Cry j2* IgE concentration exceeded 13.6 PRU/mL for frequency of the DRB1* 1201 allele.

DISCUSSION

There have been many reports worldwide on the association between HLA and allergy. After Marsh *et al*. documented a strong association of HLA-DR2/Dw2 to short ragweed allergy, positive associations between the frequencies of HLA alleles and the IgE immune responsiveness to common airborne-allergens have been documented in many studies. These associations include HLA-DR2/Dw2 with IgE responses to ragweed allergen Amba5,[19] Ambt5 and Ambp5,[3,4,20] HLA-DR3/Dw3 with Lolp1,2,3,[21,22] DRB1*01 with Fel d1-, DRB1* 04,14 and DQB1* 0401 with Alt a1-, DRB1* 15 with Fel d1 and Amba5-, DRB1* 08 with Bet v1,[7] and HLA-DR1-DQ5 and Chi t1.[23] However, to date there have been few studies conducted in Japanese subjects with allergies to the major aero-allergens HDM and Jc in comparison with nonallergic control groups instead of ethnic groups. Our laboratory analyzed samples obtained from allergic Japanese patients for the associations between HLA and IgE-mediated Type I allergy in collaboration with 7 allergic laboratories and clinics in Japan.

Patients with CAP RAST class of 4 or more were chosen as high responders for two reasons. Firstly, the histograms of the patients with HDM and Jc stratified by CAP RAST class showed an almost normal distribution (data not shown), with the average HDM class in allergy group being 3.1 and that of Jc being 2.6. Therefore, it was considered necessary for IgE high responders to at least exceed both of the average values. Secondly, the number of HDM class of 4 or more was 52, which accounted for 30% of the total of 176 patients, and that of Jc was 17, which accounted for 10%. These numbers were considered adequate for comparison with normal individuals, although the numbers differed between the two groups.

Among the antibody responders IgE RAST class of 4 concentration was approximately 72.2 ng/mL, 46.2 ng/mL, 14.7PRU/mL and 13.6PRU/mL, respectively, for anti-*Der f*, *Der f2*, *Cry j1* and *Cry j2* IgE antibody. In the correlation analysis, significantly elevated frequencies were observed in the patients with response to HDM for DRB1* 1101, 0901, DQB1* 0303 and DPB1* 0401 alleles, and in those with response to Jc for DRB1* 1201 allele. There were some discrepancies between the numbers of HDM class of 4 or more and anti-*Der f1* and *Der f2* IgE high responders. In the case between the HDM of 4 or more and the anti-*Der f1* IgE high responder (Fig. 1a), the part indicated with the right side of vertical lines is the patients with HDM class of 4 or more, and a correlation with HLA is noted for DRB1* 1101, 0901, DQB1* 0303, and DPB1* 0401. The part indicated by the upper side of the dotted horizontal lines in Fig. 1(a) reflects anti-*Der f1* IgE high responder than 72.2 ng/mL, and a correlation with HLA was noted for DQB1* 0401 and DPB1* 0401. Therefore, only DPB1* 0401 was common to

the two groups. This may also have partly resulted from the difference in the number of patients between the two groups.

The results in Table 3 did not show that DRB1* 1201 correlated with Jc. They were similar to those obtained from the patients with HDM class of 4 or more. That is, the absence of DPB1* 1201 may have partly resulted from the fact that only 17 high responders existed in the Jc group. Because all the tests of significance in this study were performed in comparison with 107 normal individuals, the 17 cases may be masked (i.e. no longer significant) when compared with all of the allergy patients (125 cases).

Significantly elevated frequency was seen for DQB1* 0303 allele in HDM IgE high responders ($P = 0.003$) and for DPB1* 0401 with anti-*Der f1* and *Der f2* IgE antibody ($P = 0.003$) when compared with the HLA class II phenotype frequencies of nonallergic individuals.

The data for DQB1* 0303 allele frequency and high level of IgE to HDM corroborate our preliminary data indicating positive association between DQB1* 0303 allele and high HDM IgE response.[8,9] From the results obtained by our family study, it could be concluded that allergic rhinitis to HDM was probably inherited in an HLA-linked autosomal-recessive manner.[8] The results of the family study reported by Sasazuki *et al.* suggested that high IgE antibody production to Jc also inherited in an HLA-linked autosomal-recessive manner.[5]

Sasazuki reported that the production of IgE antibody to Jc was controlled by the HLA-linked immunesuppression gene (Is gene) in negative linkage disequilibrium with HLA DQw3.[24]

As regards the DPB allele, we observed strong association between HLA DPB1* 0401 and high IgE response to both *Der f1* and *Der f2* as noted above. Eura *et al.* reported a positive association between DPB1* 0401 and *Der f2* IgE response in Caucasians.[25] Higgins *et al*. demonstrated that for a particular HDM-atopic individual the T cell response to the *Dermatophagoides pteronyssinus* (*Der p1*) is limited to a single region (residues 101–143) of the protein.[26] Analysis of the HLA class II restriction specificity of the T-cell clones revealed that the T-cell epitope, residues107–119 presented by HLA DPB1* 0401.[26] However, racial differences related to DPB1* 0401 have not been thoroughly analyzed. Given that DPB1* 0401 is associated with multiple allergens, as pointed out by Young *et al*.[27] DPB class II HLA restriction for antigen recognition may be insufficient to account for individual differences in reactions to common allergens.[27]

On the other hand, negative associations of HLA class II allele frequencies with particular allergens have also been reported, such as that between HLA-DR4 and the IgE response to mountain cedar pollen,[28] and non-responsiveness to Jc pollen may be associated with HLA-DQw8.[6] There are strong negative associations between the anti-*Der f1* and *Der f2* IgE responses and the DQB1* 0302 allele, which differs from the *0303 allele by one amino acid residue (data not shown). The latter is derived from conversion of aspartic acid to alanine at the 57-amino acid sequence of the domain at the DQB chain. This position is located inside that, as an α-helix on the HLA class II three-dimensional structure. As revealed recently by Brown, this is important in antigen recognition.[29]

The results of correlation studies for HLA DQB1* 0303 and DPB1* 0401 with high IgE response to HDM may help to elucidate the restriction molecules responsible in immune recognition. However, molecular analysis using allergen peptides from HDM is needed.

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