

HLA-DPB1 alleles in house dust mite allergic patients

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Summary

Associations were sought between the presence of allergic sensitivity to Der p 1, a major allergen from the house dust mite, and the HLA-DPB1 genotype. Whilst allergic patients did not differ from controls in DPB1 allelic distribution, there was a correlation of DPB1*11011 with strongly reactive T-cell proliferative responses to Der p 1 and high titre specific IgE to *Dermatophagoides pteronyssinus*.

Introduction

The association of immune responses with HLA class II alleles has been characterized for a number of antigens, including common environmental allergens. The majority of published correlations have been with polymorphisms of the DRB1 gene, and grass, ragweed and tree pollen associations have been demonstrated repeatedly (Marsh, 1990). A comprehensive investigation was undertaken at the 11th International Histocompatibility Workshop, and associations were also confirmed with a number of allergens including the mould spore allergen Alt a 1 (Marsh *et al.*, 1992).

The house dust mite (HDM) is one of the most important perennial allergens, and up to 85% of allergic asthmatics are sensitive to allergens from *Dermatophagoides pteronyssinus* (Dpt) (Smith *et al.*, 1969; Krilis *et al.*, 1985). A number of studies have attempted to identify HLA class II alleles associated with reactivity to major HDM allergens, but no clear picture has emerged. At the 11th Workshop, weak associations with DRB1*04 and *03 were seen in some subjects but not in others. Furthermore, in a large British study, no associations were found with either DR or DP alleles and Der p 1 or Der p 2 reactivity (Young *et al.*, 1994).

Alleles from the HLA-DPB1 locus have also been associated with allergic disease. A negative association was found between DPB1*0401 and allergic asthma in

a Mulatto population in Colombia (Caraballo *et al.*, 1991). More recently, a study of aspirin-induced asthma revealed an increased frequency of the DPB1*0301 allele (Dekker *et al.*, 1997). The potential importance of these associations is underscored by the fact that *in vitro* studies have revealed that at least some of the T-cell reactivity to the major allergen from the HDM, Der p 1, is restricted by DPB1 alleles (Higgins *et al.*, 1992, 1994). In this report we have looked at a further group of 31 HDM-allergic patients and correlated their HLA-DPB1 genotype with their *in vitro* T-cell reactivity to Der p 1, along with the strength of their specific IgE reactivity.

Patients and methods

After obtaining informed consent, 31 symptomatic HDM positive Caucoid patients attending the Allergy Clinic at the Royal Melbourne Hospital were enrolled for this study. Patients were referred for management of one or more of the following conditions: rhinitis, conjunctivitis, asthma or eczema. History and clinical findings were recorded for each patient and these are detailed in Table 1. Immediate-type hypersensitivity to Dpt was determined by skin prick testing (SPT) according to the method of Pepys (1975) with extracts purchased from Dome/Hollister-Stier Laboratories (Sydney, Australia). A positive control with histamine acid phosphate (1 mg mL⁻¹) and a negative control with a buffer solution of the preservative alone were also performed for each of the subjects.

For the proliferation assays, 50 mL of blood was taken into ACD tubes (Becton Dickinson) including 20 mL for HLA typing. Mononuclear cells were isolated from the remainder by density centrifugation and 7-day proliferation assays were performed as previously described (O'Brien *et al.*, 1995). Briefly, the cultures were set up in triplicate in 96-well round-bottom trays in 200-μL volumes, generally at 10⁶ cells mL⁻¹. The Der p 1 was added at the commencement of the culture and proliferation was determined by thymidine incorporation during the final 16 h of culture. As background stimulation varied, results are presented as a stimulation index (SI), calculated as the ratio of the mean (of triplicate) counts min⁻¹ of culture wells containing allergen with that of wells containing medium alone. Positive proliferative responses to Der p 1 were defined as occurring when the SI ≥ 3. In previous research studies, we showed that the allergen-specific proliferation of PBMC

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Table 1 Clinical, immunologic and immunogenetic characteristics of the HDM-sensitive patients

Patient number	Clinical features*	Cutaneous sensitivities†	S.I. for Der p 1‡	HLA class II alleles		
				DRB1	DQB1	DPB1
1	R	Dpt 2+ (9)	4.6	3, 7	2	0201, 0401
2	R, A	Dpt 3+ (11), GP 3+, Cat 2+, Dog 2+	3.2	7, 13	1, 2	0401
3	R/C	Dpt 4+ (15), Cat 2+	7.5	1, 4	1, 7	0401
4	R, A, E	Dpt 3+ (12)	8.8	4, 13	2, 8	0402, 1101
5	R/C	Dpt 2+ (7)	2.7	7, 15	1, 2	0401, 0402
6	R/C, A	Dpt 2+ (8), Cat 2+, Dog 2+, Mould 1+	4.7	1, 7	2, 5	0401, 1501
7	R	Dpt 2+ (6)	1.3	7, 11	2, 7	0401
8	R/C, A	Dpt 2+ (9)	3.8	7	2, 9	0401
9	R/C, A	Dpt 3+ (10), GP 2+	10.3	7	2	0401, 1101
10	R/C	Dpt 2+ (8)	5.1	7	2	0401, 0901
11	R/C, A	Dpt 3+ (14), Cat 2+, Dog 2+	14.2	4, 11	7	0401
12	R	Dpt 2+ (8), Dog 1+	1.1	2, 7	1, 2	0401
13	A, R	Dpt 2+ (7), Cat 1+, GP 2+	3.8			0401, 0402
14	R/C, A	Dpt 2+ (8)	4.9	7, 13	1, 9	0401
15	R, A	Dpt 1+ (5), GP 1+, Mould 2+	2.3	11, 13	1, 7	0402
16	R, A, E	Dpt 3+ (13)	7.7	3, 15	1, 2	0401, 0301
17	R/C	Dpt 2+ (8), GP 2+, Mould 2+	5.3	1, 13	1, (5)	0201, 0402
18	A	Dpt 1+ (4)	3.5	3, 11	2, 7	0101, 0402
19	A, E	Dpt 2+ (9), GP 2+, Cat 1+	4.2	4, 15	1, 8	0401
20	R	Dpt 4+ (16), Cat 3+	8.9	7, 15	1, 9	0201, 1101
21	R	Dpt 2+ (6), GP 2+	5.5	4, 7	2, 7	0401
22	R/C, E	Dpt 2+ (9), GP 2+, Cat 2+, Dog 2+	0.8	4, 13	6, 7	0401, 0402
23	A	Dpt 1+ (5)	4.0	3, 13	1, 2	0401, 0402
24	R, A	Dpt 3+ (11)	6.4	7, 11	2, 7	0201, 1101
25	R, E (mild)	Dpt 3+ (11)	5.6	1, 10	1	0201, 0401
26	A	Dpt 1+ (4), GP 1+, Clad 1+	1.9	1, 8	4, 5	0401, 1301
27	R/C	Dpt 2+ (7)	2.6			0201, 0401
28	R, A	Dpt 3+ (10), GP 2+, Mould 2+	3.2	4, 13	1, 8	0201, 0401
29	R, A	Dpt 3+ (13), GP 3+, Dog 2+	5.8	3, 4	2, 7	0101, 0401
30	A	Dpt 3+ (12)	8.4	15, 13	1, 7	0201, 1301
31	R/C	Dpt 2+ (6), Cat 2+	3.0	7, 11	2, 7	0301, 1701

*A, asthma; E, eczema; C, conjunctivitis; R, rhinitis.

†Skin prick test (mean of maximal diameter and its orthogonal) 1+, 3–5 mm > histamine (1 mg mL⁻¹) control; 2+, 6–9 mm; 3+ 10–14 mm; 4+, > 15 mm. The mean diameter for Dpt is presented in parentheses.

‡Results are means (of 2) of stimulation indices of PBMC from HDM-sensitive individuals.

is due almost entirely to CD4+ T cells, and when those cells are depleted there is minimal residual reactivity (O'Brien *et al.*, 1992).

For HLA-DRB1 and DQB1 alleles, B cells were isolated from anti-coagulated blood samples by use of magnetic beads and typing was performed by conventional microlymphocytotoxicity using 120 antisera. DPB1 typing was performed by a PCR–SSO technique (Kimura & Sasazuki, 1992). Briefly, locus-specific primers were used in the PCR followed by dot-blotting and hybridization with a battery of digoxigenin-labelled probes defining the known hypervariable regions. The end point readout was chemiluminescence detected on X-ray film. Normal population DPB1 allele frequencies were obtained from a group of 122 healthy individuals.

Results

Results of the HLA-DPB1 genotyping for the 31 allergic patients and the 122 control patients are presented in

Tables 1 and 2. Overall, there was no significant difference between the two groups, although there was a trend for a reduction in the frequency of DPB1*0301 in the HDM-allergic patients. As we had previously shown that the presence of specific HLA-DQ alleles in allergic patients could affect the degree of their T-cell responsiveness to HDM allergens *in vitro* (O'Brien *et al.*, 1995), we now sought correlations between the presence of particular DPB1 alleles and the strength of reactivity to the major HDM allergen Der p 1.

The quantitative results of the T-cell assays for reactivity to Der p 1 are presented in Table 1 as stimulation indices, a value of 3 being chosen as a cut-off for a positive response. In Table 3, T-cell reactivity is listed in rank order of strength, along with the patient's HLA-DPB1 genotype. The degree of reactivity amongst the positive T-cell responders was arbitrarily divided into three equal groups of eight and there were a further seven non-responders. The distribution of DPB1 alleles within these groups was evaluated, and it is evident that

Table 2 DPB1 allele frequencies in house dust mite positive allergic patients and controls

DPB1 allele	Allergic patients (no. alleles = 62)		Controls (no. alleles = 244)	
	<i>n</i>	%	<i>n</i>	%
0101	2	3.2	10	4.1
0201	7	11.3	24	9.8
0202	0	0	2	0.8
0301	2	3.2	19	7.8
0401	33	53.2	116	47.5
0402	9	14.5	32	13.1
0501	0	0	7	2.9
0601	0	0	4	1.6
0901	1	1.6	0	0
1001	0	0	6	2.5
11011	4	6.5	8	3.3
1301	2	3.2	2	0.8
1401	0	0	3	1.2
1501	1	1.6	2	0.8
1601	0	0	1	0.4
1701	1	1.6	2	0.8
2001	0	0	2	0.8
2101	0	0	1	0.4
2301	0	0	2	0.8
6901	0	0	1	0.4

No significant differences were found in allele frequencies between patients and controls (χ^2 test).

HLA-DPB1*11011 is strongly associated with a high response, being present in 50% of the high responders and in none of the medium or low responders or in the patients with negative responses ($P < 0.005$; χ^2 with Yates' correction). No trend was evident with any other DPB1 alleles.

The strength of the IgE reactivity to Dpt was determined by quantitative skin prick test analysis, and the mean diameters of the responses are presented in Table 1.

Overall there was a strong correlation between the SPT result and the T-cell reactivity to Der p 1 ($R = 0.701$). As with T-cell reactivity, the four patients with DPB1*11011 had high responses with a mean SPT size of 12.25 ± 2.63 (SD) vs. 8.59 ± 2.99 for the remainder ($P = 0.014$; t -test).

Discussion

The major finding from this study was that, although there was no association between specific HLA-DPB1 alleles and allergic sensitivity to the house dust mite Dpt, there was a strong association between the presence of the DPB1*1101 allele and high T-cell reactivity to the major mite allergen Der p 1. In fact, 50% of high T-cell responders to Der p 1 were DPB1*1101 positive, whereas none of the medium, low or non-responders were DPB1*1101 positive. The lack of association between HLA-DPB1 and HDM reactivity is in accord with findings of other researchers (Kimura & Sasazuki, 1992; Young *et al.*, 1994) and the association between

T-cell reactivity to Der p 1 and the DPB1*1101 allele is in keeping with our earlier research showing an association between HLA-DQ alleles and reactivity to the Der p 2 molecule (O'Brien *et al.*, 1995). Interestingly, in this study there was no association evident between HLA-DR/DQ and T-cell reactivity or DPB1 alleles.

There are two identically expressed alleles, DPB1*11011 and DPB1*11012, which differ by a single synonymous substitution. The four samples identified in this group were all DPB1*11011. The fact that this allele was associated with a strong T-cell response to Der p 1 suggests that the relevant peptide is presented to the responding CD4+ T cell more effectively in the context of DPB1*11011. A search of the MHCPEP (Brusic *et al.*, 1994) database, however, has failed to reveal any data on peptide binding to this molecule. In the absence of any peptide binding rules it is difficult therefore to correlate strength of binding with the Der p 1 sequence in order to identify any potential epitopes. The small

Table 3 T-cell reactivity to Der p 1 and DPB1 alleles in house dust mite positive allergic patients

	Stimulation index (S.I.)	DPB1 allele
High	14.2	0401
	10.3	0401 11011
	8.9	0201 11011
	8.8	0402 11011
	8.4	0201 1301
	7.7	0401 0301
	7.5	0401
	6.4	0402 11011
Medium	5.8	0101 0401
	5.6	0201 0401
	5.5	0401
	5.3	0201 0402
	5.1	0401 0901
	4.9	0401
	4.7	0401 1501
	4.6	0201 0401
Low	4.2	0401
	4.0	0401 0402
	3.8	0401
	3.8	0401 0402
	3.5	0101 0402
	3.2	0401
	3.2	0201 0401
	3.0	0301 1701
Negative	2.7	0401 0402
	1.3	0401
	1.1	0401
	2.3	0402
	0.8	0401 0402
	1.9	0401 1301
	2.6	0401 0201

DPB1*11011 was associated with a high response. Allele frequency was 25% vs. 3.3% in controls ($P < 0.001$; χ^2 with Yates' correction). DPB1*11011 frequency was significantly higher in high responders vs. medium, low and non-responders ($P < 0.0051$; χ^2 with Yates' correction).

number of patients in this study also demands caution in the interpretation of the findings. Additional high responder patients are required to confirm the observed association.

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