Studies of the HLA class II alleles involved in human responses to ragweed allergens *Ambrosia artemisiifolia* V (Ra5S) and *Ambrosia trifida* V (Ra5G)

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Abstract: Previous studies have associated skin test sensitivity and specific IgE response to *Ambrosia artemisiifolia* V (*Amb a* V) with HLA-DR2, and to *Ambrosia trifida* V (*Amb t* V) with HLA-DRw52 haplotypes in atopic individuals. Using HLA class II typing by restriction fragment length polymorphism (RFLP) analysis with DRB, DQB and DQA DNA probes to define the HLA-D alleles, we have demonstrated the association of the DQw6 in 16 out of 16 (100%) *Amb a* V-responsive individuals, compared to 3 out of 18 (17%) ragweed-sensitive but *Amb a* V-nonresponsive individuals ($p = 5.7 \times 10^{-6}$, RR > 75). We suggest that the DQw6 association with *Amb a* V sensitivity may be a reflection of an association with the DQA*0102 allele. This suggests an association of a particular HLA class II allele with an immune response to a well-characterized antigen (*Amb a* V). The HLA-DRw52 haplotypes in the *Amb t* V-sensitive individuals are not of one particular subtype. The HLA-DRw52 association with *Amb t* V sensitivity may reside in homologous DRB1 alleles linked on HLA-DRw52-bearing haplotypes.

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Ragweed allergy has been subdivided into atopy to various protein components of the ragweed pollen. We and others (1–4) have studied specific immune responsiveness to two highly homologous low molecular weight proteins, *Ambrosia artemisiifolia* V (*Amb a* V or Ra5S, MW = 5000) of short ragweed pollen, and *Ambrosia trifida* V (*Amb t* V or Ra5G, MW = 4400) of giant ragweed pollen, and found a strong association of HLA-DR2(DRw15),Dw2 with positive skin test and IgE response to *Amb a* V, and of HLA-DRw52 with positive skin test and IgE response to *Amb t* V (2). A study of HLA class II restriction fragment length polymorphisms (RFLPs) in *Amb a* V-sensitive individuals confirmed the HLA-DR2(DRw15),Dw2 association, but did not account for the non-DR2(DRw15),Dw2 *Amb a* V-responsive patients studied (5). Other allergies have been associated with HLA class II, including allergy to *Lolium perenne* grass pollen allergen *Lol p* III (Rye III) with HLA-DR3 and DR5 (6), and allergic contact nickel dermatitis with a *Taq* I HLA-DQA1 4.5 kb restriction fragment (7). We have used HLA class II typing by DNA RFLP analysis (according to the findings of the Tenth International Histocompatibility Workshop (8, 9)) to define alleles coding for specific HLA class II α and β chains in caucasian patients with ragweed hay fever allergy.

Material and Methods

We studied 34 caucasian patients with ragweed sensitivity who represent a subset of patients from our previous study (2), specifically those patients from the Ottawa area. These patients are mainly Anglo-Saxon in ethnic origin. In that study, patients were tested for cutaneous reactivity to purified *Amb a* V (0.01 μg/ml) and to *Amb t* V (0.1 μg/ml). Tests were defined as positive 20 minutes post-intradermal testing for wheal and flare diameters > 5 and 10 mm respectively. Five patients were reactive to *Amb t* V only, 10 patients were responsive to *Amb a* V, 6 patients were responsive to both *Amb t* V and *Amb a* V, and the remaining 13 patients were responsive to neither ragweed allergen. Serologic HLA class II typing had been performed by standard cytotoxicity methods.

Genomic DNA was extracted from whole blood

and the Southern method was performed (10–13). Probes used were cDNA probes for DQβ (800 bp *Pst I/Eco* RI fragment) obtained from Dr. D. Larhammer (14) and DRβ (700 bp *Pst* I fragment) obtained from Dr. E. Long (15), and a genomic probe for DQα (2.4 kb *Pst* I fragment) obtained from Dr. J. Trowsdale (16). *Taq* I RFLP patterns with the DRβ and DQα probes *Bam* HI, and *Eco* RI patterns with the DQβ probe, were used to assign HLA class II specificities DR1-DRw8, DRw52, DRw52 subtypes Dw24-Dw26, DRw53 and DQw2, DQw4-DQw9 according to the findings of the 10th International Histocompatibility Workshop (8) and as outlined in our previous studies (17). HLA class II haplotypes bearing alleles coding for specific DQα and DQβ chains as previously defined by 2-dimensional protein gel electrophoresis were assigned from DQα and DQβ RFLP patterns (10th International Workshop (9)). The Chi-square test with Yates' correction, or two-tailed Fisher's exact test when appropriate, were used for comparisons between groups. p values less than 0.05 were considered statistically significant. Relative risk (RR) was determined by the odds ratio.

Table 1.
HLA class II specificities by RFLP typing in 34 caucasian patients with ragweed allergy

Subject No.	HLA-DR	HLA-DQw	Allergen reactivity
1	5(w11),4,Dw25,53	7,8	*Amb t* V
2	5(w11),2(w15),Dw2,Dw25	7,6	*Amb t* V
3	5(w11),1,Dw25	7,5	*Amb t* V
4	w8,4,53	4,8	*Amb t* V
5	3(w17),Dw24	2,2	*Amb t* V
6	2(w15),Dw2,w6(w14),Dw9,Dw25	6,5	*Amb t* V+*Amb a* V
7	2(w15),Dw2,w6(w13),Dw19,Dw26	6,6	*Amb t* V+*Amb a* V
8	2(w15),Dw2,3(w17),Dw24	6,2	*Amb t* V+*Amb a* V
9	2(w15),Dw2,5(w11),Dw24,Dw25	6,7	*Amb t* V+*Amb a* V
10	2(w15),Dw2,3(w17),Dw24	6,2	*Amb t* V+*Amb a* V
11	2(w15),Dw2,7,53	6,2	*Amb t* V+*Amb a* V
12	2(w15),Dw2,7,53	6,2	*Amb a* V
13	2(w15),Dw2,7,53	6,2	*Amb a* V
14	2(w15),Dw2,1	6,5	*Amb a* V
15	2(w15),Dw2,5(w11),Dw25	6,7	*Amb a* V
16	2(w15),Dw2,3(w17),Dw24	6,2	*Amb a* V
17	2(w15),Dw2,4,53	6,7	*Amb a* V
18	2(w15),Dw2,1	6,5	*Amb a* V
19	2(w15),Dw2,4,53	6,7	*Amb a* V
20	w6(w13),Dw19,Dw26,7/9*,53	6,9	*Amb a* V
21	2(w15),Dw12,w6(w14),Dw9,Dw25	6,5	*Amb a* V
22	4,53	7,8	negative
23	1,4,53	5,8	negative
24	4,7/9*,53	8,9	negative
25	1,4,53	5,7	negative
26	4,7,53	2,7	negative
27	4,53,3(w17),Dw24	8,2	negative
28	7,7/9*,53	2,9	negative
29	1,4,53	5,7	negative
30	3(w17),Dw24,2(w16),Dw21	2,5	negative
31	4,3(w17),53,Dw25	8,2	negative
32	2(w15),Dw2,5(w11),Dw25 5(w11),w6(w14),Dw16,Dw24,Dw2	6,7	negative
33	5	7,7	negative
34	2(w15),Dw2	6	negative

* DR7 and DR9 cannot be differentiated by the RFLPs used in this study, when associated with DQw9.

Results and Discussion

HLA class II RFLP typing (Table 1) confirmed the serologic association of *Amb t* V responsiveness with HLA-DRw52 (10/11; 90%) compared to *Amb t* V and *Amb a* V non-responsive patients (5/13; 38%) (p=0.01, RR 16) and of *Amb a* V responsiveness with the HLA-DR2(DRw15), Dw2, DQw6(DQw1) haplotype (14/16; 88%) versus 2/13 (15%) of *Amb t* V, *Amb a* V-nonresponsive patients (p=0.0005, RR 38.5) (Table 2). For *Amb t* V, no one particular DRw52 subtype (4 Dw24(DRw52a), 5 Dw25(DRw52b), 1 Dw26 (DRw52c) and 1 DRw8 associated DRw52) was responsible for the HLA-DRw52 association. The molecular basis for the supertypic DRw52 serological specificity is not fully understood (18–20). Serologically, HLA-DRw52 is expressed along with DRB1-encoded DR3, DR5, DRw6 and DRw8 molecules. Sequence homologies found among DR3,DR5 and DRw6 alleles suggest that DRw52-bearing haplotypes constitute a "gene family" evolutionarily distinct from other haplotypes (21–23) and bearing 2 DRB genes, DRB1 and DRB3. The HLA-DRw8 haplotype appears to have evolved from this DRw52 group, with a large deletion encompassing the 3′ untranslated portion of DRB1 through the coding sequences of DRB3 (24). The result is that the HLA-DRw8 haplotype bears only one DRB gene, which is a fusion gene composed of the DRB1 coding sequence that shares homology with DR3, DR5 and DRw6, and the 3′ untranslated portion of the DRB3 gene (21, 25). Although we found only one DRw8 positive *Amb t* V-responsive patient (patient 4), this suggests that the DRw52 DRB3 coding sequences on DR3 and DR5 haplotypes are less likely to underly the reactivity to *Amb t* V, as this one DRw8-positive patient should have only DRB1 and no DRB3 coding sequences. We suggest that it is more likely the linked DRB1 alleles in these haplotypes (DR5, DR3 and DRw8 in these cases) are actually the common denominator underlying *Amb t* V reactivity. In fact, the DRB1-encoded first hypervariable region amino acids at positions 9–13 (Glu-Tyr-Ser-Thr-Ser) are shared by DR3, DR5 and DRw6, with DRw8 differing only at position 13

Table 2.
Frequencies of selected restriction fragment length polymorphism-determined HLA class II specificities among 34 caucasian patients with ragweed allergy

HLA class II specificity	*Amb a* V responsive patients* (n=16)	*Amb t* V responsive patients* (n=11)	*Amb a* V, *Amb t* V non-responsive patients (n=13)
DR2(DRw15),Dw2	14 (88%)†	7 (64%)	2 (15%)
DRw52	9 (56)	10 (90)‡	5 (38)
Dw24	4 (25)	4 (36)	3 (23)
Dw25	4 (25)	5 (45)	3 (23)
Dw26	2 (13)	1 (9)	0
DR3	3 (19)	3 (27)	3 (23)
DR5(w11)	2 (13)	4 (36)	2 (15)
DRw6(w13)	1 (6)	1 (9)	0
DRw6(w14)	2 (13)	1 (9)	1 (8)
DRw8	0	1 (9)	0
DQw6	16 (100)§	7 (64)	2 (15)

* 6 patients were responsive to both *Amb a* V and *Amb t* V.
† $p=0.0005$, RR 38.5 versus *Amb a* V, *Amb t* V non-responsive patients.
‡ $p=0.01$, RR 16 versus *Amb a* V, *Amb t* V non-responsive patients.
§ $p=2.3\times10^{-6}$, RR > 83 versus *Amb a* V, *Amb t* V non-responsive patients.

(18, 26, 27). No other known class II alleles share this homology. However, this is not proven by the present data, and is therefore only a theoretical hypothesis. The DRB3 alleles and/or DRw52 serologic specificity remain alternative candidates for underlying *Amb t* V responsiveness.

Of interest, this same gene segment has been recently implicated in immune responsiveness to a grass pollen allergen (Rye III) (6) and in the anti-Jo-1 autoantibody response in inflammatory myositis (17). Transposition of this DRB1 sequence onto the postulated 3-dimensional structure of the MHC class II molecule (28) maps it to the floor of the peptide binding groove. Here this sequence would be available for a physical association with antigen, in this case *Amb t* V, and be involved in promoting the immune response to this antigen. Nonetheless, as mentioned above, the DRw52 serologic specificity itself remains an alternative candidate for underlying the immune response to *Amb t* V. There is, however, controversy in this area, with another study finding an HLA-DR2, Dw2 association with immune responsiveness to *Amb t* V as well as *Amb a* V (4). The reason for these conflicting results is not clear; however, possible explanations include differences in antigen used for atopy testing and/or differences in ethnic genetic background of the subjects studied, as in previous studies ethnic background was not mentioned.

For *Amb a* V-responsive individuals, although a strong association with the HLA-DR2(DRw15), Dw2,DQw6 haplotype is confirmed by molecular techniques, not all *Amb a* V-reactive subjects are explained by this association. In particular, 2 *Amb a* V-responsive subjects (patients 20 and 21, Table 1) do not bear this HLA haplotype, but rather type as HLA-DRw6(w13), Dw19, Dw26, DQw6, DR7/DR9, DRw53, DQw9 and HLA-DR2(DRw15), Dw12, DQw6, DRw6(w14), Dw9, Dw25, DQw5. Therefore, DQw6 was found in all 16 *Amb a* V-responsive patients, compared to 2 out of 13 (15%) of *Amb a*, *Amb t* V-nonresponsive patients (2.3×10^{-6}, RR > 83), with this being the strongest association in this group of patients (Table 2). RFLP HLA class II typing can provide only indirect evidence of specific alleles present; however, the most common allele that would be expected to be found by direct DQA1 allele typing in *Amb a* V-sensitive patients is DQA1*0102 (DQA1.2) (29) (15/16 or 94%) compared to 4 out of 18 (22%) of non-*Amb a* V-responsive atopic patients ($p=1.2\times10^{-4}$, RR 53) (Table 3). The DQA1*0102 allele codes for the DQα 1.2 chain as defined by 2-dimensional protein gel electrophoresis (9). The DQA1*0102 allele is generally found on all HLA-DR2(DRw15),Dw2 haplotypes, and on the HLA-DRw6(w13),Dw19 haplotype found in 1 of the 2 non-DR2,Dw2 *Amb a* V-responsive exceptions (patient 20, Table 1). The remaining exception (patient 21, Table 1) would be expected to have 2 DQA1 alleles, namely DQA1*0101 (DQA1.9) and DQA1*0103 (DQA1.18), which are known to differ from DQA1*0102 by coding sequences for only 1 and 2 amino acids, respectively (30). Admittedly, only 2 *Amb a* V-responsive, HLA-DR2 (DRw15) Dw2-negative patients were found in this study, and larger numbers of such patients will be necessary to further test our suggestions. No other HLA class II α or β chain is predicted by the RFLP data to be common to ≥94% of the *Amb a* V-reactive patients. We therefore suggest that the strong association of *Amb a* V sensitivity with HLA-DQw6 is possibly a reflection of an association with DQA*0102. We stress, however, that the association with DQA*0102 is only theoretical, as we have not provided direct evidence for this. HLA class II genotyping of these patients to confirm these suggestions is underway in our laboratory.

This DQα 1.2 chain is associated with a subset of DQw6 and DQw5 haplotypes (old DQw1) which has previously been associated with *Amb a* V sensitivity. It is of interest to note that it has recently been suggested that it is the DQα chain which denotes the DQw1 serologic specificity (31). Studies of other immune diseases such as multiple sclerosis (32) and insulin-dependent diabetes mellitus (33), have implicated the DQα chain in involvement in disease susceptibility.

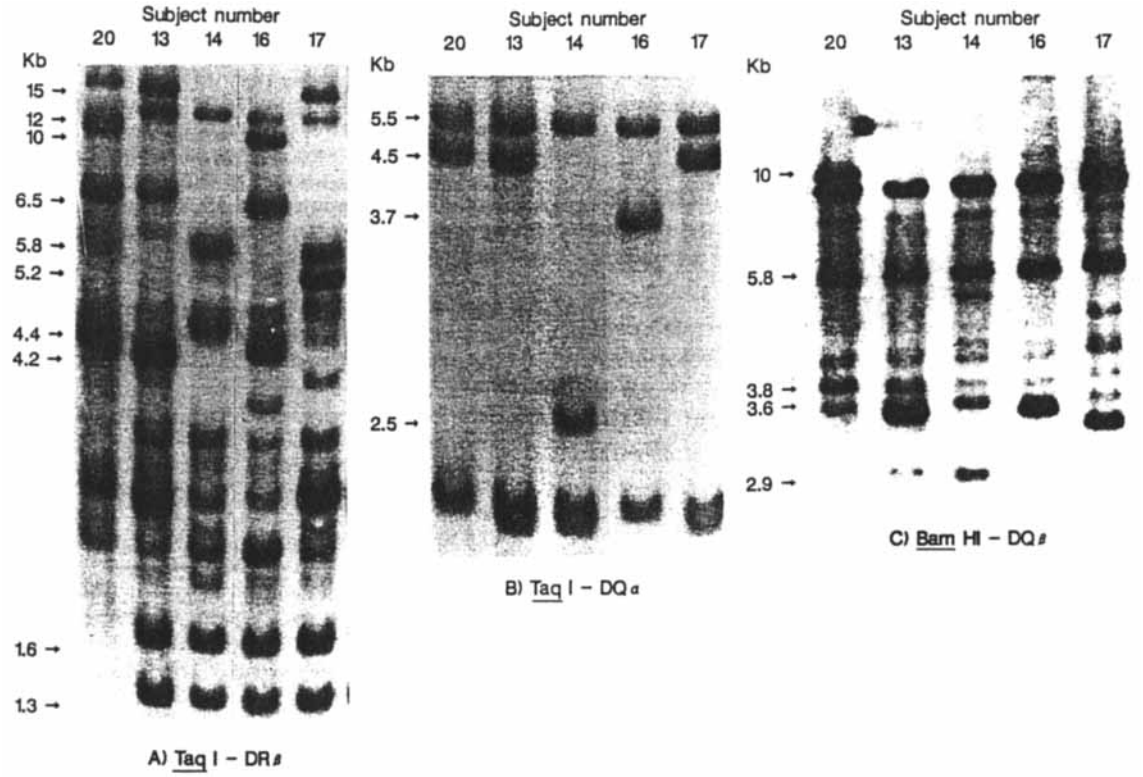

Figure 1. Example of Southern blot analysis of restriction fragment length polymorphisms used for HLA class II typing. A) *Taq* I-digested genomic DNA hybridized to the HLA-DRβ probe. B) *Taq* I-digested genomic DNA hybridized to the HLA-DQα probe, and C) *Bam* HI-digested genomic DNA hybridized to the HLA-DQβ probe. Subject identifications are presented at the top of the figure. See Table I for corresponding HLA-DR and DQw types for these subjects. The patterns used to assign DR and DQw specificities are according to the Tenth International Histocompatibility Testing Workshop and our previous studies (8,17). For example, subject 20 types as HLA-DR7 or DR9 (15 and 4.4 kb *Taq* I DRβ bands), and DRw6 (Dw19,Dw26) (10 and 6.5 kb *Taq* I DRβ bands, 5.5 kb *Taq* I DQα band), DQw6 (5.8 kb *Bam* HI DQβ band) and DQw9 (10 kb *Bam* HI DQβ band).

This study has implicated the HLA-DRB1 gene first hypervariable region in responsiveness to the *Amb t* V component of giant ragweed pollen in atopic individuals. In contrast, responsiveness to *Amb a* V component of short ragweed pollen is associated with the HLA-DR2(DRw15), Dw2, DQw6 haplotype, and most strongly with HLA-DQw6 by RFLP. Although not proven by our data, we suggest an association of *Amb a* V responsiveness with DQA*0102. This study has confirmed and strengthened previously reported associations of *Amb t* responsiveness and HLA-DRw52, and of *Amb a* V responsiveness with the HLA-DR2, Dw2, DQw6 haplotype (1–5). The strongest association of *Amb a* V responsiveness with DQw6, and the suggestion that this may involve the DQA*0102 allele has not, to our knowledge, been described before. Examination of the known amino acid sequences of *Amb a* V (34) and *Amb t* V (1) with DRB1 and DQA amino acid sequences, respectively, show no homologous regions. Further studies aimed at establishing the regions of these class II molecules involved, and the inheritability of these susceptibilities in families, are underway. These studies may allow us to understand this specific human immune response in order to predict individuals at risk for Ra5 ragweed sensitivity and to design therapy aimed at blocking the sites of the HLA class II molecule that are involved in the *Amb t* V and *Amb a* V sensitivity.

Table 3.
Frequencies of HLA-DR2(DRw15),Dw2,DQw6-associated HLA class II alleles predicted from RFLP data in *Amb a* V-responsive ragweed allergy patients

HLA class II allele	*Amb a* V responsive patients (n=16)	*Amb a* V non-responsive patients* (n=18)	p	RR
DQB1*0602	14 (88%)	3 (17%)	1.6×10^{-4}	35
DQA1*0102	15 (94)	4 (22)	1.2×10^{-4}	53
DRB1*1501	14 (88)	3 (17)	1.6×10^{-4}	35

* includes 5 *Amb t* V-responsive patients.

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