

Sequencing of *HLA-D* in responders and nonresponders to short ragweed allergen, *Amb a V*

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Abstract. We investigated the molecular basis for the striking association between HLA-DR2, Dw2 and human immune responsiveness to the *Ambrosia artemisiifolia* (short ragweed) pollen allergen *Amb a V* by sequencing the second exons of the *DRB* and *DQB1* genes of 17 selected ragweed-allergic Caucoid subjects. We also studied the *DQA1* allelic polymorphic regions (APRs) in these patients by dot-blotting using sequence-specific oligonucleotides (SSOs). The deduced amino acid sequences of the respective class II β and α polypeptides were compared, with particular emphasis on residues in the APRs that are implicated in antigen binding. No evidence for “new” *HLA-DRB* or *DQB* sequences unique to *Amb a V* responders were found on sequencing seven Dw2⁺ subjects. This suggests that the presence of a particular Dw2-associated class II molecule usually provides a necessary, but not always sufficient condition for responsiveness to *Amb a V*. The HLA phenotypes of three subjects suggest that they possess novel recombinant haplotypes containing either *DRB1*1501* and *DRB5*0101* (DR2.2-associated) or *DQB1*0602* (DQ1.2-associated) sequences. In these subjects, responsiveness to *Amb a V* was associated with the DR2.2 but not the DQ1.2 sequences, suggesting that DR $\alpha\beta_1$ or DR $\alpha\beta_v$ class II molecules are involved in antigen presentation. We investigated whether there may be shared *HLA-D*-encoded responder sequences present in all responders, including some exceptional DR2⁺ *Amb a V* responders. The 13 subjects producing antibody (Ab) responses to *Amb a V* [either from natural exposure and/or after ragweed immunotherapy (Rx)] possessed *DRB1*1501*, *1601*, *1602*, *0103*, *0402*, *0404*, *0801*, or *1101* sequences, which share the majority of their aa residues in the APRs 2–4. Some of these shared residues might be important for the binding

of a common *Amb a V* epitope prior to presentation of the class II *Amb a V* complex to the T-cell receptor (Tcr). An alternative postulate is that the recognition of two different *Amb a V* epitopes may be determined by the β_1 polypeptides of molecules having the DR2 and DQw3 specificities.

Introduction

Atopic allergy provides many advantages as a model for studying the mechanisms of specific immune responsiveness in humans (Marsh 1975; Marsh et al. 1988). In contrast to other immunological diseases, many of the antigens are well characterized. Natural exposure to inhaled antigens usually involves ultra-limiting, low doses (Marsh 1975), and responsiveness can readily be measured in terms of specific immunoglobulin (Ig) E and IgG serum antibody (Ab) levels (Marsh et al. 1982a, b). As in the animal models that have been studied in detail (Allen et al. 1987; Buus et al. 1987; Guillet et al. 1987), the polymorphic class II major histocompatibility complex Ia molecules almost certainly have an important function in the presentation of antigen fragments to the T-cell receptor (Tcr), initiating the immune responses associated with human allergy. Polymorphism in the human class II (*HLA-D*) region has been detected serologically (DR and DQ specificities) and by the mixed lymphocyte reaction (MLR; Dw specificities). Indeed, the striking HLA-DR and -Dw associations that have been observed with specific immune responsiveness to highly purified pollen allergens (Marsh et al. 1982a,b, 1986, 1987a; Roebber et al. 1985; Freidhoff et al. 1988; Ansari et al. 1989), as well as recent studies demonstrating the role of class II molecules in the presentation of house-dust mite

allergens to human T-cell clones (O'Hehir et al. 1987, 1988), support this hypothesis.

We have focused the present studies on *Amb a* V (formerly Ra5; Lapkoff and Goodfriend 1974; Mole et al. 1975; Marsh 1986) from *Ambrosia artemisiifolia* (short ragweed) pollen, a small allergen (M_r 5000) for which the natural exposure is less than 60 ng/year (Marsh et al. 1982a). Among 38 persons who were IgE Ab⁺ to *Amb a* V by natural exposure, 36 (95%) were found to be Dw2⁺, whereas only 39/177 IgE Ab⁻ subjects (22%) were Dw2⁺; in the same population, 85% of subjects IgG Ab⁺ to *Amb a* V were Dw2⁺ and 21% of IgG Ab⁻ subjects were Dw2⁺ ($P < 0.0001$ in both cases; Marsh et al. 1982a). This work was confirmed and extended using restriction fragment length polymorphism (RFLP) analysis; the concordant presence of DNA restriction fragments *DRB Eco* RI 6.5 kilobases (kb) and *DQB Eco* RI 2.3 kb was strongly associated both with responsiveness to *Amb a* V and with HLA-DR2, Dw2 (Zwollo et al. 1989). These marked associations suggest that a Dw2-associated gene encodes the major immune response (Ir) gene product involved in responsiveness to *Amb a* V. Based on the known linkage disequilibria for alleles of genes within *HLA-D*, this gene product is likely to be either a DR2 or a DQw1 subspecificity; no DP subspecificity is likely to be involved because of the much weaker DP-DR/Dw associations (Baur et al. 1984).

Following ragweed immunotherapy (Rx), which involves much higher doses of *Amb a* V than achieved by natural exposure, we observed that virtually all Dw2⁺ subjects make IgG Ab responses to *Amb a* V (Marsh et al. 1982b). However, there was a marked "dilution" of the Dw2 association, since some non-Dw2 subjects were found to produce (usually weak) IgG Ab responses to *Amb a* V. This may be explained by the recognition of minor class II-binding sites (agretopes; Heber-Katz et al. 1983) on the *Amb a* V molecule by class II molecules encoded by genes within other *HLA-D* haplotypes (Marsh et al. 1982b).

We will address the following questions. Do *Amb a* V responders possess unique *HLA-D* variants not normally found in *Amb a* V nonresponders, and in the Caucasoid population at large? Since only about 2% of the population (and approximately 10% of Dw2⁺ subjects) respond to *Amb a* V after natural exposure, it is possible that unique *HLA-D*-encoded aa sequence variants not detected by conventional typing may be present in such responders. If no responder-specific sequence(s) exist, can one identify "normal" *HLA-D* sequence(s) shared by all *Amb a* V responders (before and/or after Rx), independent of serological or MLR typing? Such sequences may be shared by Dw2-associated and less common non-Dw2-associated responder phenotypes, and may be a necessary, but perhaps not a sufficient condition for *Amb a* V responsiveness.

Our approach was to study the second exons of *HLA-DRB*, *-DQB1*, and *-DQA1* genes of some typical and atypical *Amb a* V responders and nonresponders, focusing particularly on responders having atypical HLA phenotypes noted in our previous serological and MLR typings (Marsh et al. 1982a,b, 1987b; Zwollo et al. 1989). We used the polymerase chain reaction (PCR; Saiki et al. 1985, 1988) to amplify the polymorphic second exons of *HLA-D* genes. We cloned the *HLA-DRB* and *-DQB1* gene segments into the M13 vector and sequenced the DNA by the dideoxy procedure; we examined the *DQA1* gene segments by dot-blotting using sequence-specific oligonucleotides (SSOs) for the different allelic polymorphic regions (APRs) of *DQA1*. Beside postulating that certain polymorphic *DRB* and *DQB1* residues may be associated with *Amb a* V responsiveness, we describe four unusual *DQB1-DQA1-DRB1-DRB5* combinations that have not previously been reported.

Materials and methods

Study subjects. Seventeen patients were selected from a group of Caucasoid subjects who were positive by skin test to *Amb a* pollen extract; most of these patients had been used in previous genetic studies (Marsh et al. 1982a, b, 1987b; Zwollo et al. 1989). The consanguineous, homozygous typing cell (HTC) line NOL, typing DR2, Dw2, DQw1 (a gift from A. Termijtelen, University Hospital Leiden, The Netherlands), was used for comparison. In order to address the questions raised in the Introduction concerning the *HLA-D* sequence, we focused on subjects having unusual combinations of HLA-DR, Dw, and *Amb a* V response phenotypes. Serum was available from all patients prior to ragweed Rx. Two subjects (CLi and LFI) had previously received low-dose ragweed Rx at some time in the past, but did not possess specific serum IgG or IgE Abs to *Amb a* V at the time they started receiving further ragweed Rx in our clinics. With the exception of three subjects (DHe, JKo, and ML) who possessed serum IgE and IgG Abs as a result of natural exposure to *Amb a* V, the patients were all given high-dose Rx with various ragweed antigen preparations (equivalent cumulative doses of 48–2992 µg *Amb a* V over a 2- to 10-year period), as discussed in detail elsewhere (Marsh et al. 1982b; Marsh 1987; P. Zwollo, E. E.-Kautzky, A. A. Ansari, H. A. Erlich, and D. G. Marsh, unpublished data). For patients assigned identification numbers rather than initials, the combined cumulative dosage of *Amb a* V plus *Amb p* V (a homologous antigen from western ragweed that is highly cross-reactive with *Amb a* V) was used. Serum IgG Abs to *Amb a* V were measured regularly during and after Rx.

Measurement of serum IgE and IgG Abs to *Amb a* V. The ultra-pure preparation of *Amb a* V used for the assays was identical to that used in a previous study (Marsh et al. 1982a). Serum Abs to *Amb a* V were measured by a highly sensitive double Ab radioimmunoassay capable of detecting 0.6 ng/ml of IgE Ab and 3 ng/ml of serum IgG Ab (Marsh et al. 1982a).

Nomenclature of *HLA-D* genes and encoded polypeptides. Designation of loci of *A* and *B* genes of the *DQ* and *DR* subregions within *HLA-D* will be according to the new *HLA* nomenclature (Bodmer et al. 1990). In *DR2* haplotypes, the *DRB5* locus encodes the more acidic polypeptide DR2a, and *DRB1* encodes the more basic polypeptide DR2b (Bell et al. 1987; Lee et al. 1987a; Wilkinson et al. 1988; Kawai et al. 1989). Specific alleles will be denoted by the locus designation followed by

an asterisk and the sequence number (Bodmer et al. 1990); e. g., *DRB1*1501* will be used for the *DRB1* allele that is associated with the serological typing DR2 (DRw15) and the MLR typing Dw2. Together with *DRA*, the *DRB1*1501* allele encodes the class II molecule we will refer to as DR2.2; similar designations based on DR/Dw typings will be used for other class II molecules (see Figs. 1 and 2). According to accepted usage, the polypeptides encoded by *A* and/or *B* genes will be designated by the Greek letters α and β , followed by a Roman numeral (subscript) where needed, e. g., $DQ\alpha$ and $DR\alpha_{\beta_{II}}$ are the products of *DQA1* and of *DRA/DRB3*, respectively. The abbreviation APRs will be used to describe the regions of class II polypeptide chains within which two or more allelic polymorphic residues are clustered (see Figs. 1 and 2).

HLA typing. The HLA serological and MLR typing was performed (Marsh et al. 1982a) on most study subjects in W. Bias' Immunogenetics Laboratory at the Johns Hopkins University School of Medicine (Baltimore, Maryland), as part of previous studies and as needed for the present study.

DNA purification and RFLP analysis. Genomic DNA was purified from patients' peripheral blood mononuclear cells or from the HTC line, as described elsewhere (Zwollo et al. 1989). Polymorphic DNA restriction fragments were analyzed for most subjects as part of an earlier study (Zwollo et al. 1989); the remainder were subsequently analyzed by the same procedure. Briefly, purified genomic DNA was digested with *Eco* RI and fractionated by agarose gel electrophoresis. The DNA fragments were transferred from the gel to a nylon filter (New England Nuclear, Boston, Massachusetts) by Southern blotting and allowed to hybridize with ^{32} P-labeled *DRB* or *DQB1* full-length cDNA probes. After stringent washing, the filters were exposed to X-ray film for 1–4 days.

PCR amplification of *DRB*, *DQB1*, and *DQA1* second-exon gene segments. Initial PCR studies of a few subjects were performed using the primers GH46 and GH50 (Table 1), together with the Klenow fragment of DNA polymerase I, to amplify a region encompassing nucleotides 18–238 (codons 7–79) of the second exons of *DRB* genes (Scharf et al. 1988; Erlich et al. 1989). The primer set PZ2A and PZ5A2 was subsequently designed to encompass a larger region of the *DRB* genes (codons 6–88). The design of the 5'-end primers GH46 and PZ5A2 was based on knowledge of the intron sequence immediately preceding the 5' end of *DRB1* second exons. The primer sets used for the PCR amplification of *DQB1* second-exon gene segments were selected according to available DQ typing data. Primer DGM4 was always used for the 5' end of the second exon, permitting sequencing from codon 14 of *DQB*. For the 3' end (i. e., the 5' end of the complementary strand) of the second exon of *DQB1*, the primer DGM6 was used for subjects having DQw1 specificities and DGM7 was used for subjects having non-DQw1 specificities, permitting sequencing up to codons 88 and 90, respectively. Use of DGM4 and DGM7B allowed amplification of any *DQB1* second exon that could not be amplified with the other two primer sets, but limited the sequence information obtained (only up to codon 77). The PCR using *Taq* polymerase (Saiki et al. 1988) was performed for *DRB* and *DQB1* amplification of almost all subjects, using genomic DNA (0.5 μ g) from a ragweed-allergic patient or HTC for 28 cycles of denaturation, annealing, and chain extension. The following reagents (in a 50 μ l reaction volume) were used: 50 pmoles of each of the appropriate primers (Table 1), 37.5 nmoles of each of all four dNTPs in PCR buffer (25mM KCl, 10 mM Tris-HCl at pH 8.4, 6 mM $MgCl_2$, 0.01% gelatin) and 0.5–1.0 units *Taq* polymerase (Cetus, Emeryville, California), diluted in Tris EDTA buffer, which contains ethylenediaminetetraacetate (EDTA); the final EDTA concentration was 0.02 mM. In subsequent experiments (without EDTA in the *Taq* enzyme

Table 1. PCR primers used to amplify the second exons of *HLA-DRB* and *-DQB* genes

PCR primer	Gene Region	Restriction site	Oligonucleotide sequence (5'–3')*
GH46 [†]	<i>DRB</i> 5'	<i>Bam</i> HI	ccggatccTTCGTGTCCCCACAG ¹⁴ CACG ¹⁷
GH50 [†]	<i>DRB</i> 3'	<i>Pst</i> I	²⁶⁶ CTCCCCAACCCCGTAGTTGTGTCTGCA(G) ²⁴⁰ (239)
PZ5A2	<i>DRB</i> 5'	<i>Hind</i> III	aaaagcttACCGGATCGTTCCTTGTCCCCCAG ¹⁴ CA ¹⁵
PZ2A	<i>DRB</i> 3'	<i>Bam</i> HI	ccggatcctt ²⁸² TCGCCGCTGCACTGTGAA ²⁶⁵
DGM4	<i>DQB</i> 5'	<i>Bam</i> HI	ttggatcc ¹⁴ AGGATTTCTGTGTATCCAGTTTAAAG ³⁷
DGM6 [‡]	<i>DQB</i> 3'	<i>Hind</i> III	ttaagctt ²⁸² TCTCCTCTGCAG/AGATCCC ²⁶⁵
DGM7 [‡]	<i>DQB</i> 3'	<i>Hind</i> III	ttaagcttCTCACC ²⁸² TCGCCGCTGCA ²⁷²
DGM7B [‡]	<i>DQB</i> 3'	<i>Hind</i> III	ttaagctt ²⁴⁹ GTAGTTGTGTCTGCACAC ²³²
GH26	<i>DQA</i> 5'	<i>Pst</i> I	gtgctgca ³⁴ GGTGTAACCTTGTACCAG ⁵¹
GH27	<i>DQA</i> 3'	<i>Bam</i> HI	cacggatcc ²⁵⁸ GGTAGCAGCGGTAGAGTTG ²⁴⁰

* The second exons of *DRB*, *DQB*, and *DQA* are comprised of nucleotide residues 14–282 (cDNA sequence numbering). The plus-strand sequence is given in the case of the 5'-end primers, and the minus-strand sequence for the 3'-end primers. Nucleotides shown in capitals constitute actual primer sequence; nucleotides in lower case (not underlined) are necessary for cloning purposes; restriction sites are underlined; regions covering intron sequences are in italics. Primer pairs GH46 and GH50, and GH26 and GH27 have been described previously (Scharf et al. 1988; Erlich et al. 1989); the sequences of other primers are based on information previously published (Andersson et al. 1987; Todd et al. 1987; Lee et al. 1987b; Jonsson et al. 1987; Kappes and Strominger 1988; Horn et al. 1988). Nucleotides shown in outline font are known polymorphic intron residues; the nucleotides used at these positions are found in known *DRB1* sequences (Andersson et al. 1987; G. Horn and H. A. Erlich, unpublished data).

[†] Primers GH46 and GH50 were used for the amplification of *DRB* second exons of NOL and subjects #3, #238, #41224, WE, and CLi. The 3' end of the GH50 primer covers five of six nucleotides (240–244) of a *Pst* I site in a conserved region of *DRB* (nucleotides 239–244), which provides a restriction site for cloning into M13 vectors. The GH46 and GH50 primers were generally used for the determination of *DRB3* sequences (encoding DRw52 specificities), since primers PZ5A2 and PZ2A gave a low degree of amplification of these sequences.

[‡] For amplification of *DQB1* second exons, DGM4 was used in combination with DGM6 (for patients typing DQw1), with DGM7 (non-DQw1 specificities), and with DGM7B (any DQw specificity); see text. The following 3'-end primers and study subjects were used for *DQB1* amplification: DGM6 (subjects DHe, #3, DM, #41224, #403, #218, and CLo); DGM7 (subjects CLo, JKo, and ML); DGM7B (subjects CSz, #218, WE, JKo, LFl, LL, WL, and JM).

diluent), 2.0–2.5 mM Mg^{2+} was found to be optimal using the stated primer and dNTP concentrations. For *DRB* amplification, the DNA samples were initially denatured for 10 min at 95 °C before the *Taq* polymerase was added. Subsequent denaturation was performed for 2 min at 95 °C, primer annealing was carried out for 2 min at 55 °C, and chain extension was allowed to proceed for 1 min at 72 °C. Annealing was performed at 55 °C to provide high stringency and minimize amplification of gene segments from *DRB2* pseudogenes (see Results). For *DQB1* amplification, the same conditions were used except that annealing was performed at 50 °C (2 min); no detectable amplification of *DQB2* (*DXB*) sequences was observed at this temperature. The incubation temperatures were manipulated by transferring the samples between heating blocks set at the respective temperatures, or by using an automated temperature-cycling apparatus. The degree of *DRB* or *DQB* amplification was monitored by agarose gel electrophoresis and the specificity of amplification was studied by Southern blotting, followed by hybridization with ^{32}P -labeled full-length *DRB* or *DQB1* cDNA probes, under hybridization conditions that permitted detection of any *DRB* or *DQB* second exon, respectively (Zwollo et al. 1989). Amplification of *DQA1* second exons was performed using *Taq* polymerase with primers GH26 and GH27 in accordance with the published procedure (Scharf et al. 1988), which uses an approach similar to that described above for *DRB* and *DQB1*.

M13 cloning of *DRB*- and *DQB1*-amplified products and dideoxy sequencing. Ten percent of the *DRB*- or *DQB1*-amplified products was allowed to incubate for 3 h at 37 °C with 40 units each of *Hind* III and *Bam* HI (*Bam* HI and *Pst* I for primers GH46 and GH50). Digested DNA was purified using a GeneClean kit (BIO-101, La Jolla, California) as suggested by the manufacturer. Twenty-five percent of the digested DNA was then ligated into the M13mp10 vector for 16 h at 15 °C in the presence of T4 ligase (Bethesda Research Laboratories, Bethesda, Maryland). Recombinant vectors were transfected into the *Escherichia coli* host strain JM103. Recombinants were screened for *HLA-DRB* or *DQB* inserts by hybridization with a *DRB* or *DRQB1* full-length cDNA probe (Zwollo et al. 1989). The single-stranded phage DNA was isolated and sequenced by the dideoxy primer extension method using the Sequenase kit, as suggested by the manufacturer (US Biochemicals, Cleveland, Ohio). DNA sequences with dyad symmetries containing dG and dC residues were also sequenced using dITP in the place of dGTP in order to resolve GC-rich regions. Several clones from each allele of *DRB* or *DQB1*-amplified products were sequenced. The resulting consensus sequences were determined using the BIONET computer analysis system.

Dot-blot analysis of PCR-amplified *DQA1* segments. Dot-blot analysis of all *DQA1*-amplified gene segments was performed using SSOs derived from the APRs of *DQA1* alleles, in which the SSOs were labeled with biotin-psoralen (Bugawan et al. 1988). The subtypes of *DQA4*, now known as *DQA1*0401*, and *0601*, were not differentiated in these analyses.

Results

The first half of Table 2 shows the serum IgE and IgG Ab levels to *Amb a V* prior to ragweed Rx and the maximal IgG Ab levels attained during or after Rx of the 17 ragweed-allergic study subjects, together with the presence or absence of the RFLP *Eco* RI fragments that are associated with DR2 and Dw2 typings. For previously nonresponder patients who received Rx, the cumulative *Amb V* dosages did not differ significantly between moderate-to-high responders (>100 ng/ml IgG Ab to

Amb a V) and weak or negative responders (<100 ng/ml); geometrical mean cumulative *Amb V* doses were 260 ng vs 294 ng (ranges 48–2992 ng vs 71–2455 ng), respectively. The relationship between dose, Ab response, and HLA type is discussed in detail elsewhere (Marsh et al. 1982b; 1987b; D. Marsh, unpublished data). The *DRB*, *DQB1* and *DQA1* second exons of these subjects were amplified using PCR technology. Dideoxy sequencing of *DRB* and *DQB1* and SSO dot-blotting of *DQA1* yielded the information summarized in Table 2. Analysis of the *DRB1*, *DRB3*, *DRB4*, *DRB5*, and *DQB1* sequences allowed us to deduce the majority of the sequences of the polymorphic membrane-distal (β_1) domains of the encoded DR β and DQ β polypeptides, as shown in Figures 1 and 2, respectively.

Sequences associated with DR2, Dw2, and *Amb a V* responsiveness. The DR2.2⁺ HTC NOL and seven subjects who were typed as DR2.2⁺ were studied by RFLP mapping (Zwollo et al. 1989 and this study). They were all found to have *DRB Eco* RI 6.5 kb and *DQB Eco* RI 2.3 kb fragments usually associated with the DR2.2 phenotype (Table 2, first eight rows). RFLP analysis of one additional subject (#218), who consistently typed Dw2⁺ with one HTC and Dw2[−] with another (referred to as Dw2[±]), had previously been shown to have a *DRB Eco* RI 6.5 kb, but not a *DQB Eco* RI 2.3 kb fragment (Table 2). Two of the Dw2⁺ subjects (DHe and #3) and the Dw2[±] subject #218 had produced specific IgE and IgG Abs to *Amb a V* as a result of natural pollen exposure; three Dw2⁺ subjects (GD, #41224, and CSz) produced IgG Ab (but no IgE Ab) only after ragweed Rx and the remaining Dw2⁺ subjects (#403 and #238) produced very little or no IgG Ab to *Amb a V* after Rx.

The PCR-amplified second exons of these eight subjects (and the Dw2⁺ cell line NOL) were sequenced in order to investigate the possibility that *Amb a V* responder-specific sequences were present in the Dw2⁺ and Dw2[±] responders to *Amb a V* (before and/or after Rx), but absent in the Dw2⁺ nonresponders. All eight subjects were found to have *DRB1*1501* and *DRB5*0101* (Dw2-associated) second-exon sequences in common, independent of their *Amb a V* response phenotypes. Their *DRB1* and *DRB5* sequences were also identical to those published for the DR2.2⁺ cell line PGF (references for *DRB* sequences are cited in the footnote to Figure 1). The *DRB1* and *DRB3/B4* sequences from the alternate chromosomes of all of these subjects showed no unusual features.

Five of the six Dw2⁺ subjects who were sequenced for *DQB* were shown to have the expected *DQB1*0602* sequences identical to published sequences. (The seventh Dw2⁺ subject, whose *DQB1* genes were not sequenced, also appears to possess this typical Dw2-associated sequence; see footnote to Table 2.) However, an exceptional Dw2⁺ subject (CSz) was found to possess the

Table 2. Overview of the *Amb a V* response phenotypes, RFLP analyses, and *HLA-D* sequences of the study subjects.

Patient or HTC*	Age	Sex	<i>Amb a V</i> Ab (ng/ml)			<i>DRB</i> <i>Eco</i> RI 6.5 kb	<i>DQB</i> <i>Eco</i> RI 2.3 kb	Sequences of the expressed genes					SSO dot- blot data
			IgE Rx ⁻	IgG Rx ⁻	IgG Rx ⁺⁺			<i>DRB1</i>	<i>DRB3</i>	<i>DRB4</i>	<i>DRB5</i>	<i>DQB1</i>	<i>DQA1</i>
NOL (HTC)													
DHe	56	F	25	338	No Rx	+	+	1501			0101	ND*	0102
#3	42	M	38	525	18 300	+	+	1501, 0401		0101	0101	0602, ND	0102, 0301
GD	45	M	≤0.6	≤3	1 730	+	+	1501, 0701		0101	0101	0602, ND	0102, 0201
#41224	33	M	≤0.6	≤3	310	+	+	1501, 0301	0101		0101	0602, ND	0102, 0501
CSz	31	F	≤0.6	≤3	3 580	+	+	1501, 0701		0101	0101	0603, 0201	0102, 0201
#403	50	M	≤0.6	≤3	16	+	+	1501, 0404		0101	0101	0602, ND	0102, 0301
#238	47	M	≤0.6	≤3	≤3	+	+	1501, 0301	0101		0101	ND*	0102, 0501
#218	44	M	3.4	69	4 900	+	-	1501, 0701		0101	0101	0502, 0201	0102, 0201
JMc	27	M	≤0.6	≤3	≤3	-	+	0301, 0404	0101	0101		0602, 0302	0102, 0301
CLo	36	M	≤0.6	≤3	1 660	+	-	1601, 0301	0201		0201	0502, 0201	0102, 0501
WE	38	M	≤0.6	≤3	129	+	-	1602, 0301	0101		0201	0502, 0201	0102, 0501
CLi	55	M	≤0.6	≤3	10	+	-	1601, 0101			0201	ND [§]	0102, 0101
JKo	50	M	74	496	2 200	-	-	0901, 0801		0101		0303, 0401	0301, "04"
ML	63	F	10	~60	No Rx	-	-	0404, 0301	0101	0101		0302, 0201	0301, 0501
WL	31	M	≤0.6	≤3	291	-	-	0901, 1101	0201	0101		0303, 0301	0301, 0501
LFI	44	M	≤0.6	≤3	75	-	-	(0102) [°] , 0402		0101		0501, 0302	0101, 0301
LL	50	M	≤0.6	≤3	142	-	-	0101, 0103				0501, 0301	0101, 0501

* In order to facilitate cross-referencing with our other studies, the patients' initials are used in the case of the local clinic patients and identifications numbers are used for Westinghouse Electric Corporation ((Hunt Valley, MD) volunteers.

† Maximun IgG Ab levels toward *Amb a V* observed during or after ragweed Rx (Marsh et al. 1982b; P. Zwollo, E. E. Kautzky, A. A. Ansari, H. A. Erlich, and D. G. Marsh, unpublished data).

‡ Subsequent SSO dot-blot analyses of *DQ* APRs are consistent with these two DNA preparations containing typical *DQB1**0602 sequences (P. Zwollo and D. G. Marsh, unpublished data).

|| The subtypes of *DQA4*, now known as *DQA1**0401, and 0601 were not differentiated.

§ Subsequent SSO dot-blot analyses of *DQ* APRs are consistent with this DNA preparation containing a typical *DQB1**0502 sequence (P. Zwollo and D. G. Marsh, unpublished data).

° Subsequently found by SSO dot-blot; see Discussion.

ND, not done.

*DQB1**0603 sequence and the Dw2[±] subject (#218) to possess the *DQB1**0502 sequence. Like *DQB1**0602, the 0603 sequence is associated with the serological specificity DQw6 (Bell et al. 1987), but differs notably at codon 30 [0602 has TAC (Tyr) and 0603 has CAC (His); Fig. 2]. The *DQβ* 0602 polypeptide sequence differs markedly from *DQβ* 0502 (patient 218); again, there is a Tyr/His difference at residue 30 (Fig. 2). In order to rule out the possibility of a *Taq* polymerase misreading artifact (Saiki et al. 1988) during *DQB1* second-exon amplification of subject CSz, a second, independent PCR amplification and further sequence analyses were performed, which confirmed the *DQB1* sequences.

The *DQA1* SSO dot-blotting data indicate the *DQB1**0102 sequences normally associated with all *DR2* subtypes (Table 2). Furthermore, none of the *DQB1* sequences determined for the above subjects differed from those previously reported (references for *DQB1* sequences are cited in the footnote to Fig. 2). However, the phenotypic combinations of *DQ*-*DR* possessed by patients CSz(*DQB1**0603, *DQA1**0102, *DRB1**1501, *DRB5**0101 / *DQB1**0201, *DQA1**0201, *DRB1**0701, *DRB4**0101)

and #218 (*DQB1**0502, *DQA1**0102, *DRB1**1501, *DRB5**0101 / *DQB1**0201, *DQA1**0201, *DRB1**0701, *DRB4**0101) are unusual; they suggest the presence of *DR2*-associated haplotypes (underlined) not previously reported, based on the known linkage disequilibrium between alleles encoding *DR7* and *DQw2* and the associations usually observed between the allelic variants encoding *DR2* and *DQw1* (Baur et al. 1984). Since both CSz and #218 are *Amb a V* responders, these findings further suggest that a *DRB1**1501 or *DRB5**0101 (*DR2*-associated) gene product or a *DQA1**0102 (*DQw1*-associated) gene product may be involved in this responsiveness; the involvement of a *DQB1**0602 (*DQw1*-associated) gene product is less probable.

We studied another Dw2⁻ subject (JMc), who was a nonresponder to *Amb a V* after prolonged, high-dose ragweed Rx (Marsh et al. 1982b). This individual possessed the *DQB* *Eco* RI 2.3 kb restriction fragment characteristic of *DQB1**0602 and 0603, but did not have the *DRB* *Eco* RI 6.5 kb fragment associated with *DR2*. His serological typing was *DR3*, 4, *DQw1*, 3, which suggests the possibility of the *DR3*, *DQw1* haplotype (relatively

DR.Dw		Encoded aas		APR 1																APR 2																													
HLA allele	Assoc	1																20																30															
DRB1*1501		G	D	T	R	P	R	F	L	W	Q	P	K	R	E	C	H	F	F	N	G	T	E	R	V	R	F	L	D	R	Y																		
DRB1*1501	2.2 (w15)						(-)																																										
DRB1*1601	2.21 (w16)						(-)																																										
DRB1*1602	2.22 (w16)																																																
DRB1*0101	1.1						(-)					L	F														L	E	C																				
DRB1*0103	1.3											L	F														L	E	C																				
DRB1*0301	3.3 (w17)						(-)			E	Y	S	T	S													Y																						
DRB1*0401	4.4								E	E	V	H																																					
DRB1*0402	4.10								E	E	V	H																																					
DRB1*0404	4.14								E	E	V	H																																					
DRB1*0701	7						(-)				G	Y	K													Q		E	L																				
DRB1*0801	8.1								E	Y	S	T	G			Y																																	
DRB1*0901	9								K		D	F															Y	H	G																				
DRB1*1101	5.5 (w11)								E	Y	S	T	S																																				
DRB3*0101	w52a						(-)		E	L	R	S															Y																						
DRB3*0201	w52b								E	L	R	S																	E	H																			
DRB4*0101	w53a								E	L	R	S															W	N	I	D																			
DRB5*0101	2.2 (w15)						(-)		O	A	Y																		H	D																			
DRB5*0201†	2.21 (w16)						(C)		Q	D	Y																		H	G																			

APR 2 (cont.)																APR 3																																															
31																40																50																60															
DRB1*1501		F	Y	N	Q	E	E	S	V	R	F	D	S	D	V	G	E	F	R	A	V	T	E	L	G	R	P	D	A	E	Y																																
DRB1*1501	2.2 (w15)																																																														
DRB1*1601	2.21 (w16)																	Y																																													
DRB1*1602	2.22 (w16)																	Y																																													
DRB1*0101	1.1	I																Y																																													
DRB1*0103	1.3	I																Y																																													
DRB1*0301	3.3 (w17)		H					N																																																							
DRB1*0401	4.4		H					Y										Y																																													
DRB1*0402	4.10		H					Y										Y																																													
DRB1*0404	4.14		H					Y										Y																																													
DRB1*0701	7							F										Y										V	S																																		
DRB1*0801	8.1							Y										Y										S	S																																		
DRB1*0901	9	I						N										Y										V	S																																		
DRB1*1101	5.5 (w11)							Y										Y											E	S																																	
DRB3*0101	w52a		H					F	L									Y										V	S																																		
DRB3*0201	w52b		H					Y	A									Y			R																																										
DRB4*0101	w53a	I						Y	A	Y	N		L				Y	Q																																													
DRB5*0101	2.2 (w15)	I						D	L								Y																																														
DRB5*0201†	2.21 (w16)	I						N									Y																																														

APR 4																61	70	80	90	94																																																											
61																70																80																90																94															
DRB1*1501		W	N	S	Q	K	D	I	L	E	Q	A	R	A	A	V	D	T	Y	C	R	H	N	Y	G	V	V	E	S	F	T	V	Q	R	R																																												
DRB1*1501	2.2 (w15)																																																																														
DRB1*1601	2.21 (w16)							F			D	R																																																																			
DRB1*1602	2.22 (w16)							L			D	R																																																																			
DRB1*0101	1.1							L			R																																																																				
DRB1*0103	1.3										D	E																																																																			
DRB1*0301	3.3 (w17)							L			K	G	R			N																																																															
DRB1*0401	4.4							L			K																																																																				
DRB1*0402	4.10										D	E																																																																			
DRB1*0404	4.14							L			R																																																																				
DRB1*0701	7										D	R	G	Q		V																																																															
DRB1*0801	8.1							F			D	R		L																																																																	
DRB1*0901	9							F			R	R		E		V																																																															
DRB1*1101	5.5 (w11)							F			D	R																																																																			
DRB3*0101	w52a							L			K	G	R		N																																																																
DRB3*0201	w52b							L			K	G																																																																			
DRB4*0101	w53a							L			R	R		E																																																																	
DRB5*0101	2.2 (w15)							F			D	R																																																																			
DRB5*0201†	2.21 (w16)																																																																														

†Identical to DRB5*0202 (DR2.22) in the second exon.

Fig. 1. Summary of *HLA-DRB* sequences determined for the study subjects (see also Table 2). APRs are shown in *bold type*. The sequence segments shown in *parentheses* were not determined in subjects #3, #238, #41224, WE, and CLi, where only the PCR primer set GH46 and GH50 was used (see Materials and methods). In all cases at least two (and usually three or more) concordant sequences were obtained for different clones. Previously published references for sequences: Treber et al. (1987); Todd et al. (1987); Horn et al. (1988); Liu et al. (1988); Scharf et al. (1988); Kappes and Strominger (1988); Bidwell et al. (1989); Gregersen et al. (1989).

rare in Caucasoids). Sequencing of the *DQB1* second exons revealed the presence of *DQB1*0602*. Our data suggest that JMc possesses the haplotypes *DQB1*0602*, *DQA1*0102*, *DRB1*0301*, *DRB3*0101* / *DQB1*0302*, *DQA1*0301*, *DRB1*0404*, *DRB4*0101*, based on *DQ-DR* linkage disequilibria. Thus, patient JMc appears to be the converse of CSz and #218 in that he possesses *DQB1*0602* (but not *DRB1*1501* and *DRB5*0101*) and does not respond to *Amb a V*. Taken together, the se-

quence data for *Amb a V* responders CSz and #218 and nonresponder JMc suggest that the principal class II molecule involved in the presentation of *Amb a V* is either *DRα/DRβ₁* 1501 or *DRα/DRβ₃* 0101, but probably not *DQα* 0102/*DQβ* 0602.

The DR2⁺, Dw2⁻ phenotype and Amb a V responsiveness. The next question we addressed related to the possible role of DR and DQ in *Amb a V* responsiveness

HLA allele	DQ Assocn.	Encoded aas	APR 1										APR 2																			
			10										20										30									
DQB1*0602			R	D	S	P	E	D	F	V	F	Q	F	K	G	M	C	Y	F	T	N	G	T	E	R	V	R	L	V	T	R	Y
DQB1*0602	1.2 (w6)																															
DQB1*0603	1.18 (w6)																															
DQB1*0501	1.1 (w5)																															
DQB1*0502	1.21 (w5)																															
DQB1*0201	w2																															
DQB1*0301	3.1 (w7)																															
DQB1*0302	3.2 (w8)																															
DQB1*0303	3.3 (w9)																															
DQB1*0401	4.15 (w4)																															

HLA allele	DQ Assocn.	Encoded aas	APR 2 (cont.)										APR 3																										
			31										40										50										60						
DQB1*0602			I	Y	N	R	E	E	Y	A	R	F	D	S	D	V	G	V	Y	R	A	V	T	P	Q	G	R	P	D	A	E	Y							
DQB1*0602	1.2 (w6)																																						
DQB1*0603	1.18 (w6)																																						
DQB1*0501	1.1 (w5)																																						
DQB1*0502	1.21 (w5)																																						
DQB1*0201	w2																																						
DQB1*0301	3.1 (w7)																																						
DQB1*0302	3.2 (w8)																																						
DQB1*0303	3.3 (w9)																																						
DQB1*0401	4.15 (w4)																																						

HLA allele	DQ Assocn.	Encoded aas	APR 4										APR 5																										
			70										80										90										94						
DQB1*0602			W	N	S	Q	K	E	V	L	E	G	T	R	A	E	L	D	T	V	C	R	H	N	Y	E	V	A	F	R	G	I	L	Q	R	R			
DQB1*0602	1.2 (w6)																																						
DQB1*0603	1.18 (w6)																																						
DQB1*0501	1.1 (w5)																																						
DQB1*0502	1.21 (w5)																																						
DQB1*0201	w2																																						
DQB1*0301	3.1 (w7)																																						
DQB1*0302	3.2 (w8)																																						
DQB1*0303	3.3 (w9)																																						
DQB1*0401	4.15 (w4)																																						

Fig. 2. Summary of HLA-DQB1 sequencing results (see also Table 2). APRs are shown in *bold type*. The sequence segments shown in *parentheses* were not determined in subjects JMc, WE, WL, LFI, and LL, where only the PCR primer set DGM4 and DGM7B was used (see Materials and methods). In all cases at least two (and usually three or more) concordant sequences were obtained for different clones. Previously published sequences: Todd et al. (1987); Lee et al. (1987); Kappes and Strominger (1988); Horn et al. (1988); Scharf et al. (1988); Bidwell et al. (1989).

for individuals who were typed as DR2⁺ but Dw2⁻. As reported previously (Marsh et al. 1982b), none of six such individuals responded to *Amb a V* by natural exposure. Three DR2⁺, Dw2⁻ patients (CLo, WE, and CLi) selected for sequencing in this study produced specific IgG Ab after high-dose ragweed Rx (Table 2). Subject CLo, who responded well to *Amb a V* after Rx, was DR2⁻, Dw2⁻ from serological and MLR typings, but possessed an RFLP typing consistent with DR2⁺, Dw2⁻. (CLo had originally been classified as DR2⁻ since only one of eight DR2 typing sera exhibited positive cytotoxicity.) Patients WE and CLi, who responded much more weakly to *Amb a V*, were shown to be DR2⁺, Dw2⁻ from serological, MLR, and RFLP typings (Marsh et al. 1982a; Zwollo et al. 1989).

Patients CLi and CLo both had a normal *DRB1*1601* (Dw21-associated) sequence, identical to that of AZH (Lee et al. 1987a). Subject WE had a second-exon sequence identical to that of (Dw22-associated) *DRB1*1602* found in cell line LD-5a (Liu et al. 1988), in which the T at nucleotide position 199 (in Dw21) is replaced by a C (in Dw22), resulting in the substitution of Phe by Leu at codon 67. This result was confirmed by sequence data from two independent PCR amplifications.

Subjects CLo, WE, and CLi were all found to possess normal *DRB5*0201/0202* second-exon sequences identical to those in both Dw21⁺ and Dw22⁺ HTCs (Todd et al. 1987; Horn et al. 1988; Liu et al. 1988), which share

with *DRB5*0101* several encoded APR residues (Fig. 2). The *DRB* sequences from the other chromosomes of these three subjects were identical to sequences that have previously been published (Todd et al. 1987; Horn et al. 1988; Kappes and Strominger 1988). The *DQB1* second exons of subject CLo showed typical *DQB1*0502* and *DQB1*0201* sequences (Todd et al. 1987; Lee et al. 1987b; Jonsson et al. 1987; Horn et al. 1988; Kappes and Strominger 1988); the *DQB1* sequences of WE were *DQB1*0502* (Dw21-associated) and *DQB1*0201*; those of subject CLi were not determined. All three of these patients possessed the expected DQA1*0102 phenotype according to SSO dot-blotting.

Two rare DR2⁻, Dw2⁻ *Amb a V* responders following natural exposure to *Amb a V*. We studied two highly atypical subjects who were DR2⁻ by serological and RFLP typings and Dw2⁻ by MLR and RFLP typings (Marsh et al. 1982b; Zwollo et al. 1989), but made IgE and IgG Abs to *Amb a V* as a result of natural exposure to ragweed pollen. These are the only such individuals we have ever observed among over 50 "natural" *Amb a V* responders studied (Marsh et al. 1982a, b, 1987b; Zwollo et al. 1989; P. Zwollo and D. G. Marsh, unpublished data). Subject JKO was found to possess *DRB1*0801* and *0901*, *DRB4*0101*, *DQB1*0401* and *0303*, and *DQA1*0301* and "04" sequences; subject ML was shown to have *DRB1*0301* and *0404*, *DRB3*0101*,

*DRB4*0101*, *DQB1*0201* and *0302*, and *DQA1*0301* and *0501* sequences (Figs 1 and 2; Table 2). The combination of *DRB1*0801* and *DQB1*0401* in JKO (which are presumably on the same chromosome) has not previously been reported. *DQB1*0402*, which differs by a single nucleotide from *0401* (at codon 23), has previously been found to be associated with *DRB1*0801*.

DR2⁻ and *Amb a* V responsiveness after Rx. Three selected subjects (WL, LFL, and LL; Table 2) possessing no detectable serum IgE or IgG Abs to *Amb a* V before ragweed Rx all produced moderate-to-low amounts of IgG Ab, but no IgE Ab, after receiving extensive Rx. Subject WL was found to possess *DRB1*0901* and *1101*, *DRB4*0101*, *DRB3*0201*, and *DQB1*0303*, *0301* sequences, patient LFL to have *DRB1*0402*, *DRB4*0101*, and *DQB1*0302*, and *0501*, and subject LL to possess *DRB1*0101*, and *0103* and *DQB1*0501*, and *0301*; all of these are identical to published sequences (Todd et al. 1987; Lee et al. 1987b; Jonsson et al. 1987; Horn et al. 1988; Kappes and Strominger 1988; Scharf et al. 1988; Liu et al. 1988; Gregersen et al. 1989; Bidwell et al. 1989). The DQA phenotypes of these patients (Table 2) were as anticipated from the known Caucasoid *DR-DQ* sequence combinations. Subject LL probably possesses the relatively rare *DR1.3*, *DQw3.1* haplotype (Bidwell et al. 1989). A second *DRB1* allele of subject LFL was not detected after the sequencing of 51 DRB clones (19 clones having the *DRB1*0402* sequence, 13 having *DRB4*0101*, and 19 having the DRw53-associated pseudogene sequence were found). The serotype of LFL was found to be DR1, DRw4; DQw1, DQw3, which suggests that a *DRB1*01* sequence was not adequately amplified using primers PZ5A2 and PZ2A.

The three *DR2⁻* subjects who responded to *Amb a* V after ragweed Rx all have subtypes of DQw3 as do the two unusual patients, JKO and ML, who had serum IgE and IgG Abs to *Amb a* V prior to Rx. Most of the APRs encoded by *DQB1* genes associated with the three different DQw3 subtypes found in these five subjects are shared (Table 2, Fig. 2), but the *DQA1* allele of LFL differs from the others.

Pseudogenes. Use of the GH46 and GH50 primer set for NOL (*DR2.2⁺*) and for patients #3, #41224, CLi, and WE (various subtypes of *DR2*) led to be efficient amplification of an additional gene segment which represents the *DRB2* sequence (Erlich et al. 1989), a probable pseudogene; this sequence was identical in NOL and all *DR2⁺* patients studied. Using the same primers, a similar, but not identical gene segment was found in association with DR1 in both subjects #3 and CLi (P. Zwollo and D. G. Marsh, unpublished data). While no detectable amplification of these *DRB2* pseudogenes was observed using primers PZ5A2 and PZ2A, this primer set

permitted amplification of the second exon of the DRw53-associated pseudogene (Andersson et al. 1987) in people possessing *DRB1*04*, *0701*, or *0901* (with *DRB4*0101*), namely, subjects #218, 403, CSz, DHe, GD, JKO, JMc, LFL, ML, and WL. According to the number of pseudogene vs *DRB1*04*, *0701*, or *0901* clones, the amplification efficiencies for this pseudogene and its associated *DRB1* sequences appear to be similar.

Discussion

As previously noted (Marsh et al. 1982a, b, 1987b; Marsh 1986; Coulter et al. 1987; Zwollo et al. 1989), *Amb a* V responsiveness is strongly associated with HLA-*DR2* and *Dw2* (*DR2.2*). We found that *DR2.2⁺* subjects possessed *DRB1*, *DRB5*, and *DQB1* second-exon sequences identical to those previously published for haplotypes that encode *DR2.2*, irrespective of the person's *Amb a* V phenotype [responder before ragweed Rx, responder after Rx, or the rare nonresponder after Rx (Table 2)]. The *DQA1* DNA typings obtained by SSO dot-blotting gave no unexpected results. Also, the second-exon sequences of *Amb a* V responders (before or after Rx) having certain non-*DR2.2* phenotypes all correspond to previously published "normal" sequences for the respective phenotypes. Thus, no unusual *HLA-DRB* or *-DQB* sequences (and no unusual *DQA* typings) appear to be present uniquely among *Amb a* V responders. In this respect, our data are analogous to the findings for patients with autoimmune diseases (e. g., Todd et al. 1987; Horn et al. 1988; Scharf et al. 1988). The *DR2.2⁺* nonresponder (#238) and weak responder subjects (after Rx), the *DR2.2⁺* patients who respond only after Rx, and the *DR2.2⁺* subjects who respond before Rx (Table 2) may differ from each other in terms of their expressed Tcr repertoires, their idiotype priming, and other factors such as antigenic exposure (Marsh 1986; Coulter et al. 1987); all of these possibilities will be explored in subsequent work.

Recombination between certain haplotypes that encode *DR2*, *DR3*, and *DRw6* is suggested by three "new" *DQ-DR* combinations found in our studies; namely, *DQB1*0502*, *DQA1*0102*, *DRB1*1501*, *DRB5*0101* (#218, responder before Rx), *DQB1*0603*, *DQA1*0102*, *DRB1*1501*, *DRB5*0101* (CSz, responder after Rx) and *DQB1*0602*, *DQA1*0102*, *DRB1*0301*, *DRB3*0101* (JMc, nonresponder after Rx). The pattern of *Amb a* V responsiveness for the three new *DR-DQ* recombinants points to either *DRαβ₁* 1501 or *DRαβ_v* 0101, rather than *DQα0102 β0602*, as being the major *Amb a* V Ir gene product. This interesting postulate, which could not previously be made from our published DR, Dw, and RFLP typing data (Marsh et al. 1982a, b; Zwollo et al. 1989), has recently been confirmed by showing that

DR-specific, but not DQ- or DP-specific monoclonal Abs (mAbs) inhibit the stimulation by *Amb a* V of DR2.2-restricted, *Amb a* V-specific human T-cell clones (Huang and Marsh 1990; S.-K. Huang, P. Zwollo, D. G. Marsh, unpublished data). These authors have also shown that mAb Hu30 (anti-DR $\alpha\beta_2$) inhibits T-cell proliferation in this system, which implicates DR $\alpha\beta_1$ 1501, not DR $\alpha\beta_1$ 1501, as the antigen-presenting class II molecule.

An additional "new" haplotype, and possibly a new *DRB1* sequence, is implicated for patient WE (responder after Rx), who possesses the second-exon sequences *DRB1**1602 (Dw22-associated), along with *DRB5**0201/0202 (identical for the second exon in Dw21/22), *DQB1**0502 (Dw21-associated), and *DQA1**0102 (found in DR2s). *DRB1**1602 and *DRB5**0202 (together with DQw3-associated sequences) have been reported to be present in a primitive South American Indian tribe (Dw22 cell line LD-5a; Liu et al. 1988). Because the second-exon sequences of *DRB1*, *DRB5*, and *DQB1* in subject WE differ from Dw21-associated sequences only at nucleotide 199 (codon 67) of *DRB1*, this is more likely to be the result of a point mutation in the *DRB1* sequence of a Dw21 phenotype, rather than a recombinational event between *Dw21* and *Dw22* haplotypes that are found in divergent racial groups.

Our failure to amplify the expected *DRB1**01 sequence of LFI is of interest; this may result from polymorphism within the intron sequence immediately prior to the second exon, which is incompatible with the sequence of the PZ5A2 primer. Recently we found this sequence to be *DRB1**0102 (N. Shinomiya and D. G. Marsh, unpublished data).

We will next examine whether there may be shared aa residues in postulated antigen-binding regions (desetopes) encoded by the *HLA-D* alleles of subjects who respond to *Amb a* V (before or after Rx). A shared (or similar) desotope may allow a single *Amb a* V agretope (or two agretopes having very similar motifs) to bind to related class II molecules. We postulate the existence of two classes of *Amb a* V agretope, a major agretope which binds to certain class II molecules associated with DR2, and a minor agretope which interacts with certain class II molecules found (much less commonly) in DR2⁻ responder subjects. Of relevance to this hypothesis, some amino acid sequence comparisons of DR β and DQ β APR residues that are postulated to point into the antigen-binding groove, according to the model of Brown and co-workers (1988), are shown in Figure 3.

Most DR2.2⁺ Caucasian allergic subjects respond well to *Amb a* V as a result of natural pollen exposure and/or after ragweed Rx, whereas DR2.2⁻ Caucasians patients either do not respond or respond only after Rx (Marsh et al. 1982a,b, 1987b; Zwollo et al. 1989 and this paper). The relationship between the DR2-associated sequences encoded by *DRB1* alleles found in *Amb a* V

HLA ALLELE	DR TYPE	ENCODED AMINO ACID RESIDUE															
		9	11	13	28	30	37	38	57	60	67	70	71	74	86		
←Beta-pleated sheet→ ←Alpha-helix→																	
1) <i>DRB1</i> *1501	2.2	W	P	R	D	Y	S	V	D	Y	I	Q	A	A	V		
<i>DRB1</i> *1502	2.12	W	P	R	D	Y	S	V	D	Y	I	Q	A	A	G		
<i>DRB1</i> *1601	2.21	W	P	R	D	Y	S	V	D	Y	F	D	R	A	G		
<i>DRB1</i> *1602	2.22	W	P	R	D	Y	S	V	D	Y	L	D	R	A	G		
2) <i>DRB5</i> *0101	2.2	Q	D	Y	H	D	D	L	D	Y	F	D	R	A	G		
<i>DRB5</i> *0102	2.12	Q	D	Y	H	G	N	V	D	Y	F	D	R	A	G		
<i>DRB5</i> *0201/02	2.21/2.22	Q	D	Y	H	G	N	V	D	Y	I	D	A	A	V		
3) <i>DRB1</i> *1501	2.2	W	P	R	D	Y	S	V	D	Y	I	Q	A	A	V		
<i>DRB1</i> *1502	2.12	W	P	R	D	Y	S	V	D	Y	I	Q	A	A	G		
<i>DRB5</i> *0201/02	2.21/2.22	Q	D	Y	H	G	N	V	D	Y	I	D	A	A	V		
4) <i>DRB5</i> *0101	2.2	Q	D	Y	H	D	D	L	D	Y	F	D	R	A	G		
<i>DRB5</i> *0102	2.12	Q	D	Y	H	G	N	V	D	Y	F	D	R	A	G		
<i>DRB1</i> *1601	2.21	W	P	R	D	Y	S	V	D	Y	F	D	R	A	G		
<i>DRB1</i> *1602	2.22	W	P	R	D	Y	S	V	D	Y	L	D	R	A	G		
5) <i>DRB1</i> *1501	2.2	W	P	R	D	Y	S	V	D	Y	I	Q	A	A	V		
<i>DRB1</i> *1601/02	2.21/2.22	W	P	R	D	Y	S	V	D	Y	F/L	D	R	A	G		
<i>DRB1</i> *0103	1.3	W	L	F	E	C	S	V	D	Y	I	D	E	A	G		
<i>DRB1</i> *0402	4.10	E	V	H	D	Y	Y	V	D	Y	I	D	E	A	V		
<i>DRB1</i> *0404	4.14	E	V	H	D	Y	Y	V	D	Y	L	Q	R	A	V		
<i>DRB1</i> *0801	8.1	E	S	G	D	Y	Y	V	S	Y	I	D	R	L	G		
<i>DRB1</i> *1101	5.5	E	S	S	D	Y	Y	V	D	Y	I	D	R	A	G		
DQ TYPE																	
6) <i>DQB1</i> *0301	3.1	Y	F	A	T	Y	Y	A	D	Y	V	R	T	E	E		
<i>DQB1</i> *0302	3.2	Y	F	G	T	Y	Y	A	A	Y	V	R	T	E	E		
<i>DQB1</i> *0303	3.3	Y	F	G	T	Y	Y	A	D	Y	V	R	T	E	E		

Fig. 3. Comparisons of selected polymorphic class II aa residues that are postulated to point into the antigen-binding groove (Brown et al. 1988) and may be implicated in binding to hypothesized *Amb a* V agretope(s). With the exception of residue 86 (which has two variants), only residues known to possess three or more variants in different class II β chains are shown. Comparison 1–4 show various combinations of *DRB1* and/or *DRB5*-encoded residues associated with the different DR2 subtypes; they relate to hypotheses concerning DR2 class II molecules involved in binding to a major *Amb a* V agretope. Comparison 1 relates to the preferred DR2 hypothesis (see text). Comparisons 5 and 6 relate to *Amb a* V responsiveness in DR2⁻ subjects. The residues in APRs 2–4 of certain DR β_1 polypeptides may be involved in binding to a major *Amb a* V agretope, in view of the similarity of these residues in DR2⁺ and DR2⁻ responder subjects (comparison 5); alternatively, the sharing of most APR residues by DQw3 β chains found in all DR2⁻ responders suggests that these closely related structures may be involved in binding to a minor *Amb a* V agretope (comparison 6).

responders (Fig. 3, comparison 1) suggests that the common APR residues pointing into the antigen-binding groove may be important in binding to the major *Amb a* V agretope. [This figure also includes DR2.12-associated sequences for comparison purposes, because our further studies (Huang and Marsh 1990; S.-K. Huang, P. Zwollo, D. G. Marsh, unpublished data) suggest that this haplotype, which is common in Orientals, is also important in presentation of the major *Amb a* V agretope.] The differences seen at residues 67, 70, 71, and perhaps 86 (particularly the changes from neutral to charged residues at positions 70 and 71) may explain differences in the degree of responsiveness seen between DR2.2⁺ (high-responder) and DR2.21/22⁺ (low-responder) subjects. Comparisons 2–4 show alternative possibilities, where the residues of the β polypeptides of DR2 haplotypes that are encoded by *HLA-DRB5*, or combinations of *DRB1*/B5

alleles may be involved in *Amb a* V presentation. There are a larger number of aa differences between DR2.2 vs DR2.21/22 shown in these comparisons than in comparison 1. Moreover, our further data using *Amb a* V-specific T-cell clones clearly implicate DR β_1 , and not DR β_V , for DR2.2 and 2.12 in *Amb a* V presentation, and the same is likely to hold true for DR2.21 and 2.22.

The two exceptional *Amb a* V responders prior to ragweed Rx (JKo and ML) and the three moderate-to-weak IgG Ab responders after Rx (WL, LFI, and LL) possess *DRB1**0801, 0404, 1101, 0402, and 0103 sequences; the respective β_1 polypeptides show considerable homology with DR2 β_1 polypeptides in APRs 2-4 (Fig. 3, comparison 5). This suggests the possibility that the related sequences may be involved in the presentation of the same (or a closely similar) *Amb a* V agretope. All five of these subjects share very similar DQw3-associated APR sequence in DQ β (Fig. 3, comparison 6), but not DQ α (subject LFI is exceptional; Table 2); these findings suggest the alternative possibility that DQ β 303 polypeptides may be involved in the presentation of an *Amb a* V agretope probably distinct from that recognized by the quite different DR2 molecules. Four of these five subjects also possess the DRw53 β_{IV} polypeptide, which shows a lower degree of homology with DR2 (data not shown). However, in view of the low prevalence of *Amb a* V immune responsiveness in the population, it is improbable that the very common Dw53 specificity is involved in *Amb a* V presentation.

It should be emphasized that these postulates need to be examined further on a larger number of patients. The *DRB*, *DQB*, and *DQA* gene segments are being studied using SSO dot-blot technology and further antigen-presentation studies using *Amb a* V-specific T-cell clones are being employed to test the validity of these hypotheses.

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