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## Class II major histocompatibility complex restriction of human T cell responses to short ragweed allergen, *Amb a* V\*

Although T cells are known to play a crucial role in the induction of IgE synthesis, the class II major histocompatibility complex (MHC) restriction of aeroallergen-induced T cell responses in humans is incompletely defined. We have previously shown that, in allergic Caucasoid individuals, HLA-DR2 and Dw2 (DR2.2) is strongly associated with specific IgE and IgG antibody responses to highly purified *Ambrosia* (ragweed) allergen, *Amb a* V, from the *artemisiifolia* (short) species. For example, 95% of IgE antibody responders to *Amb a* V were typed as DR2.2. In a novel study of the genetic control of T cell responses to the *Amb a* V allergen, we have investigated the MHC class II restriction specificity of three CD4, *Amb a* V-specific T cell clones derived from a DR2.2<sup>+</sup> atopic patient, and a polyclonal *Amb a* V-reactive T cell line from another DR2.2<sup>+</sup> patient. We observed proliferative responses of all three clones to *Amb a* V only when either HLA-DR2.2 or DR2, Dw12 (DR2.12; found on Mongoloid populations) was present on the antigen-presenting cells, regardless of the HLA-DQ phenotype of the cells. Moreover, the responses of T cell line and clones were abolished by anti-DR but not by anti-DQ nor by anti-DP monoclonal antibodies, and, significantly, anti-DR $\alpha$ / $\beta$ <sub>1</sub>2 (anti-DR $\alpha$ / $\beta$ <sub>1</sub>w15/w16; anti-“DR2b”) monoclonal antibody blocked, in a dose-dependent manner, the cloned T cell responses to *Amb a* V. These findings demonstrate that DR $\alpha$ / $\beta$ <sub>1</sub>2.2 (DR $\alpha$ / $\beta$ <sub>1</sub>1501) and DR $\alpha$ / $\beta$ <sub>1</sub>2.12 (DR $\alpha$ / $\beta$ <sub>1</sub>1502) are functional in the restriction of the T cell recognition of *Amb a* V. These findings also illustrate the power of the allergy model for definitive investigation of the molecular basis of the human immune response.

### 1 Introduction

The polymorphic MHC class II molecules on APC play a key role in restricting Ag-specific T cell activation [1–3]. This activation event is crucial in the induction of Ab synthesis, including IgE [4], as well as in the regulation of chronic allergic inflammation [5]. In humans, the class II molecules are encoded by at least three class II gene families, HLA-DR, -DQ, and -DP, all of which appear to be functional in controlling the T cell responses [6–8]. Furthermore, the DR region on most haplotypes contains a single expressed A gene and two expressed B genes which encode two heterodimeric  $\alpha$ / $\beta$  products [9, 10]. For example, in DR2 haplotypes there are two expressed B genes encoding  $\beta$ <sub>1</sub> and  $\beta$ <sub>2</sub> polypeptides associated with a single  $\alpha$  chain [11–13].

While the functional role of class II molecules, primarily from the animal studies, has been well documented, the restriction specificity of allergen-induced T cell responses in humans is ill defined. Earlier analysis of the T cell responses to a major allergen of *Dermatophagoides pteronyssinus* (house dust mite) suggested DR restriction of such re-

sponses [14]. More recently, studies of the T cell responses to the related *D. farinae* species by O’Hehir et al. [15] and Lamb et al. [16] have suggested that the DRw52 and DRw53 supertypic specificities encode the predominant restriction elements. These studies are limited in that the specific allergens remain incompletely characterized; but they provide the first evidence for the involvement of class II molecules in the presentation of allergens to T cells. In more extensive studies of atopic Caucasoid populations, striking HLA associations with specific immune responsiveness to several well-defined allergens have been established, suggesting an important role of MHC-linked *I*r genes in the regulation of T cell responses to allergens (reviewed in Marsh [17]). In this study, we have focused on a well-characterized allergen, *Amb a* V ( $M_r$  = 5000; [18]), and have investigated the molecular basis of specific immune responsiveness to this allergen.

*Amb a* V, a minor allergen from short ragweed pollen, is a cystine-rich protein with known amino acid sequence [18]. Approximately 10% of the ragweed-allergic population develop IgE Ab to *Amb a* V following natural exposure [19]. The importance of MHC class II molecules in the initiation of specific immune responses to *Amb a* V has been implicated from our previous molecular and genetic studies, demonstrating that specific IgE and IgG Ab responses to *Amb a* V are strongly associated with DR2.2 in Caucasoid populations [19, 20]; for example, 36/38 (95%) of IgE Ab responders were DR2.2. In this communication, we have extended the study by investigating the MHC class II restriction of T cell responses to *Amb a* V, and provided further evidence for a critical role of  $\alpha$ / $\beta$ <sub>1</sub> heterodimers in both DR2.2 and DR2.12 haplotypes in the presentation of *Amb a* V to the T cells.

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## 2 Materials and methods

### 2.1 Antigens

*Ambrosia* (ragweed) allergens, *Amb a* V, *Amb t* V and *Amb p* V were isolated and purified from short, giant and Western ragweed pollens, respectively, as described [19, 21, 22], *Lol p* III was isolated and purified from rye grass pollen [23].

### 2.2 Generation of *Amb a* V-specific T cell lines and clones

*Amb a* V-specific T cells were derived from PBMC from two ragweed-allergic individuals following periodic *Amb a* V stimulation. Both patients were receiving low-dose immunotherapy with ragweed pollen extract. Serum anti-*Amb a* V titers determined as described [19, 20] for patient AP were, before immunotherapy, IgE, 256 ng/ml, and IgG, 297 ng/ml; for patient BE, before immunotherapy, IgE, 48 ng/ml and IgG, 100 ng/ml. During immunotherapy IgG titers rose in both patients. The T cell cloning procedure was a modification of that described by O'Hehir et al. [15]. This was performed by successive stimulation of peripheral blood T cells *in vitro* with purified *Amb a* V and rIL 2, followed by cloning and subcloning using the limiting dilution technique. These T cell clones were derived from two independent T cell lines at different occasions. For proliferation assays, T cells ( $5 \times 10^4$  cells/ml) were stimulated in triplicate, with or without highly purified *Amb a* V antigen or control Ag, *Amb t* V, *Amb p* V, and *Lol p* III, in the presence of irradiated (500 rad) autologous (patient AP) or other patients' PBMC ( $5 \times 10^5$  cells/ml) for 3 days. The concentration of all Ag used was usually 1 µg/ml or as otherwise indicated. The cell cultures were then pulsed with 1 µCi = 37 kBq of [ $^3$ H]dThd (DuPont, Wilmington, DE), harvested 18 h later, and counted.

### 2.3 Inhibition of T cell responses to *Amb a* V by anti-class II MHC mAb

T cell clones (AP1.2, AP6 and AP9 from patient AP;  $5 \times 10^4$  cells/ml), or a T cell line (from patient BE;  $5 \times 10^4$  cells/ml), were incubated in triplicate with or without *Amb a* V (0.5 µg/ml) in the presence of autologous APC ( $5 \times 10^5$  cells/ml). Anti-human MHC class II mAb was added, at a series of concentrations, at the initiation of the culture. After 3 days in culture, cells were pulsed with [ $^3$ H]dThd, harvested 18 h later and counted. mAb used: anti-DR (L243), anti-DQ (Leu-10), and anti-DP (B7/21) were purchased from Becton Dickinson (Mountain View, CA); mAb Hu30 [24, 25] (anti-DR $\alpha$ / $\beta$ <sub>1</sub>w15/w16, anti-DR2b, [26]) was a gift of Drs. A. Wakisaka and M. Aizawa; control ascites (mAb H5A4, anti-CD11b; [27]) was kindly provided by Dr. B. Bochner.

## 3 Results

### 3.1 General remarks

*Amb a* V is present in very low concentration in short ragweed pollen (ca. 0.03%, [28]). Isolation of *Amb a* V-

specific T cell clones represents a difficult task, because of their low frequency *in vivo* as a result of the limiting immunization with inhaled *Amb a* V (<60 ng/year, [29]). To optimize our chances of cloning specific T cells, we selected highly *Amb a* V-sensitive, DR2.2<sup>+</sup> patients who had received short ragweed immunotherapy (Rx) and had high levels of serum Ab before and after Rx, determined as previously described [19, 20]. Following extensive effort, we obtained a polyclonal T cell line reactive to *Amb a* V from subject BE, and from patient AP, we generated three *Amb a* V-specific T cell clones, AP1.2, AP6 and AP9.

All three T cell clones were found to be CD3<sup>+</sup>CD4<sup>+</sup> by FCM analysis (data not shown). In addition, all three clones were *Amb a* V specific, since they were incapable of responding to the *Amb a* V homologues, *Amb t* V and *Amb p* V, and an additional control Ag, *Lol p* III, even at ten times higher concentration (data not shown).

### 3.2 Determination of the MHC class II restriction element controlling T cell responses to *Amb a* V

The MHC class II restriction specificity was examined first by testing the ability of various histocompatible or histoincompatible APC to present *Amb a* V to the T cell clones. Panels of APC, including irradiated PBMC of individual patients and EBV-transformed homozygous typing cell lines, were selected to include the different DR2 variants (Dw2, Dw12, Dw21 and Dw22), as well as DR4 (Dw4, Dw10 and Dw14), DR5 (DRw11), DRw8 and DR9. Certain subjects having the specified DR2.2<sup>-</sup> typings develop serum Ab to *Amb a* V after high-dose ragweed Rx, and exceptionally before Rx [19, 20, 30, 31]. Only APC possessing DR2.2 and DR2.12 were able to present *Amb a* V to the cloned T cells (Table 1). In particular, APC from certain atypical *Amb a* V responder patients (#218 and CSz; [31]) who have DR2.2, but not DQ1.2 (the usual DQ specificity associated with DR2.2) were capable of presenting *Amb a* V to all three T cell clones; in contrast, the APC of an atypical nonresponder patient (JMc) who was DQ1.2<sup>+</sup>, but DR2.2<sup>-</sup> were not able to present the Ag. Interestingly, the APC from an unusual DR2.2<sup>+</sup> subject (MB), who was highly atopic but produced no Ab to *Amb a* V even after high-dose ragweed Rx, were able to present *Amb a* V to the cloned T cells, this suggests no Ir gene "defect" in her APC. Thirteen HTC representing the ten different DR/Dw specificities stated above, including four known DR2 variants, were similarly used in Ag presentation experiments with clone AP1.2. Again, only DR2.2<sup>+</sup> and DR2.12<sup>+</sup> cell lines were able to present *Amb a* V to the cloned T cells (data not shown).

These results suggest that a DR gene product ( $\alpha/\beta_1$  or  $\alpha/\beta_V$ ) of both DR2.2 and DR2.12, but not the other DR2 subtype (DR2.21 and DR2.22), can present *Amb a* V to the T cell clones of patient AP. The results are consistent with our sequencing and dot blot analysis showing that homologous sequences exist in both B1 and B5 genes of DR2.2 and DR2.12 haplotypes (see discussion; [31] and this study; see also [30]). Of interest is the close sequence similarity of the respective DR  $\beta_1$  polypeptides, which differ only at position 86 (valine vs. glycine).

To define further the restriction specificity of *Amb a* V-specific T cell responses, the blocking effects of various

**Table 1.** Analysis of MHC restriction of *Amba* V-specific T cell proliferation using patients' APC

| Patients <sup>a)</sup> | APC                     |                   | [ <sup>3</sup> H]dThd uptake (mean cpm ± SE) |                 |           |                 |           |                 |
|------------------------|-------------------------|-------------------|----------------------------------------------|-----------------|-----------|-----------------|-----------|-----------------|
|                        | DR <sup>b)</sup>        | DQ <sup>b)</sup>  | Clone AP1.2                                  |                 | Clone AP6 |                 | Clone AP9 |                 |
|                        |                         |                   | No Ag                                        | + <i>Amba</i> V | No Ag     | + <i>Amba</i> V | No Ag     | + <i>Amba</i> V |
| AP                     | 2.2, 6a                 | 1.2 <sup>c)</sup> | 383 ± 102                                    | 39 721 ± 2592   | 833 ± 125 | 22 908 ± 2055   | 601 ± 113 | 25 135 ± 655    |
| WR                     | 2.2, 7                  | 1.2, 2            | 291 ± 32                                     | 16 468 ± 835    | 786 ± 249 | 5 806 ± 601     | 424 ± 4   | 13 969 ± 386    |
| MB                     | 2.2, 17                 | 1.2, 2            | 291 ± 60                                     | 29 818 ± 1394   | 744 ± 35  | 18 220 ± 1436   | 593 ± 193 | 17 940 ± 288    |
| #218                   | 1.1, 7                  | 1.21, 2           | 166 ± 74                                     | 11 887 ± 564    | 629 ± 228 | 5 455 ± 520     | 178 ± 26  | 9 549 ± 313     |
| CSz                    | 2.2, 7                  | 1.18, 2           | 293 ± 15                                     | 36 147 ± 1601   | 588 ± 91  | 24 097 ± 3014   | 686 ± 69  | 23 024 ± 1693   |
| TK                     | 2.12, 9                 | 1.12, 3.3         | 230 ± 29                                     | 16 107 ± 1636   | ND        | ND              | ND        | ND              |
| CLo                    | 2.21, 17                | 1.21, 2           | 522 ± 182                                    | 772 ± 147       | 204 ± 48  | 154 ± 13        | 306 ± 42  | 335 ± 29        |
| WE                     | 2.22 <sup>d)</sup> , 17 | 1.21, 2           | 574 ± 227                                    | 394 ± 225       | 148 ± 29  | 130 ± 42        | 220 ± 80  | 292 ± 22        |
| JHo                    | 4.4, 1.1                | 3.1, 1.1          | 79 ± 25                                      | 152 ± 43        | 97 ± 7    | 71 ± 24         | 264 ± 81  | 274 ± 194       |
| MS                     | 4.10, 7                 | 3.2, 2            | 128 ± 27                                     | 287 ± 86        | 324 ± 52  | 319 ± 57        | 230 ± 75  | 404 ± 27        |
| JMc                    | 4.14, 3                 | 3.2, 1.2          | 269 ± 103                                    | 233 ± 45        | 176 ± 31  | 130 ± 13        | 304 ± 55  | 224 ± 33        |
| JSt                    | 4.14, 7                 | 3.2, 3.3          | 187 ± 85                                     | 391 ± 60        | 538 ± 76  | 197 ± 76        | 350 ± 69  | 328 ± 31        |

a) All patients except MB and JMc were *Amba* V responders. All were Caucasoids, except for AP (Negroid) and TK (Mongoloid).

b) The HLA-DR and DQ types were determined serologically and cellularly. Allelic subtypings were also performed as follows: the second exons were amplified by the polymerase chain reaction. The resulting gene segments were cloned into M13 and sequenced and/or were analyzed by dot-blotting using sequence-specific oligonucleotides (SSO) corresponding to specific allelic polymorphic regions of the various DRB and DQB alleles [31] and this study, patients AP and TK).

c) Sequencing and SSO dot-blot analysis suggested that AP is homozygous for the DQB1 allele.

d) The second exon of the DRB1 gene as DRB1\*1602 (2.22) sequence, which differs from DRB1\*1601 (2.21) only at codon 199. The remaining exons have not yet been sequenced.

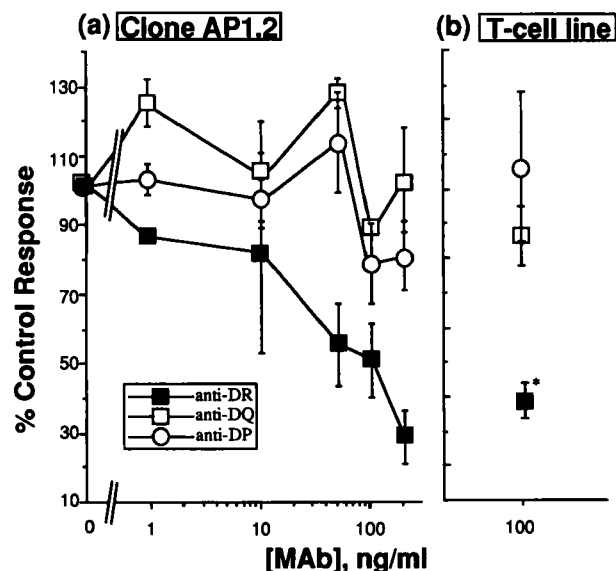


**Figure 1.** Blocking effect of murine mAb against human MHC class II nonpolymorphic determinants on the proliferative responses of clone AP1.2 (from patient AP) and a T cell line (from patient BE) to *Amba* V. Closed squares, anti-DR (L243); open squares, anti-DQ (Leu-10); open circles, anti-DP (B7/21). The mean cpm of cultures in the absence of *Amba* V and mAb were 2018 ± 282 (SE) for clone AP1.2, and 5020 ± 380 for T cell line; in the presence of *Amba* V the mean cpm were 37 553 ± 2862 and 12 118 ± 1908, for clone AP1.2 and T cell line, respectively; \* indicates a significant ( $p < 0.005$ ) between the proliferative response of a T cell line to *Amba* V in the presence and absence of anti-DR mAb (100 ng/ml) as determined by Student's *t*-test.

anti-HLA-D mAb were tested on *Amba* V-induced proliferation of the three T cell clones and an *Amba* V-reactive T cell line from patient BE (Figs. 1 and 2). Of three anti-HLA-D nonpolymorphic region mAb (anti-DR, anti-DQ and anti-DP), only anti-DR mAb effectively blocked the responses of cloned T cells to *Amba* V (Figs. 1a, 2c and 2d). The inhibition was directly dependent on the dose of anti-DR mAb. Significant inhibition of a polyclonal T cell line from patient BE to *Amba* V by anti-DR mAb was also observed ( $p < 0.005$ ; Fig. 1b). More importantly, mAb Hu30 [anti-DR $\alpha$ /β<sub>1</sub>w15/w16 (anti-DR2b)] blocked, in a dose-dependent fashion, the responses of cloned T cells to *Amba* V in the presence of autologous APC (Figs. 2a, c, d). Similar inhibition data were also obtained using DR2.12<sup>+</sup> APC (Fig. 2b). The blocking effects of both anti-DR and mAb Hu30 were specific, since the control ascites fluid at an equivalent dilution was not inhibitory. Furthermore, the mitogenic responses of all three clones to PHA were not inhibited by either mAb at the highest concentrations used in the blocking experiments (data not shown). Consistent with a previous report [26], mAb Hu30 did not inhibit the one-way MLR between DR2.12<sup>+</sup> responder and DR2.2<sup>+</sup> stimulator cells (data not shown), which share almost identical DRβ<sub>1</sub> sequences.

A direct *Amba* V presentation experiment using L-cell transfectant cell line (LDR2b; [26]) expressing DR $\alpha$ /β<sub>1</sub>2.2 was, however, unsuccessful since such cell line failed to induce significant proliferative responses of cloned T cells to *Amba* V even at ten times higher concentration of *Amba* V (data not shown). The failure of DR $\alpha$ /β<sub>1</sub>2.2 transfectant cell line to induce *Amba* V-specific T cell responses may reflect the improper processing of complex antigen, such as *Amba* V, or the lack of essential accessory molecules on L cells, which have been suggested elsewhere in both murine and human system [26, 32].

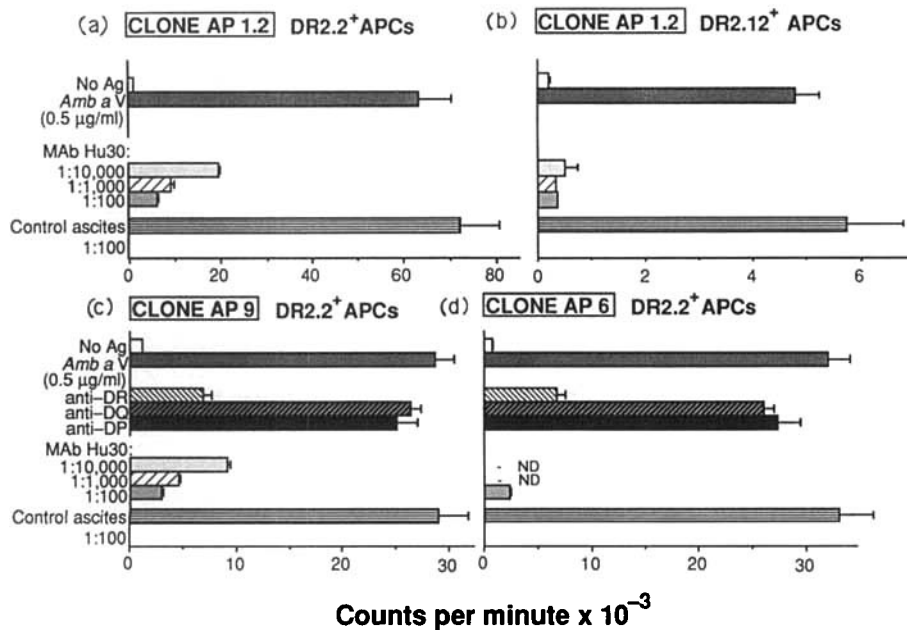


Figure 2. Inhibition of *Amb a V*-induced T cell proliferation by anti-HLA-D mAb. The cultures were set up the same as described in Sect. 2.3. For clones AP6 and AP9, autologous APC were used. mAb concentrations used: 1 µg/ml of each purified anti-DR (L243), anti-DQ (Leu-10), and anti-DP (B7/21) in clone AP6 and AP9 experiments; Hu30, 1:100–1:10 000 dilutions of ascites fluid; control ascites (H5A4), 1:100 dilution.

#### 4 Discussion

Allergen-induced T cell responses have only recently been studied at the clonal level [16, 33]. We demonstrate the derivation of human T cell clones specific for a well-defined allergen, *Amb a V*, from a DR2.2<sup>+</sup> atopic subject, and of an *Amb a V*-reactive T cell line from an additional DR2.2<sup>+</sup> atopic subject, despite their extremely low frequency in peripheral blood. Investigation of the MHC class II restriction specificity of T cell responses to *Amb a V* using the T cell line and clones show that first only DR2.2<sup>+</sup> and DR2.12<sup>+</sup> APC are capable of presenting *Amb a V* to the T cells, and second, anti-DR and anti-DRα/β<sub>1</sub>w15/w16 mAb blocked the proliferative responses of T cells to *Amb a V*.

These data, together with the findings of the marked association of DR2.2 with responsiveness to *Amb a V* seen in Caucasoid populations [19, 20, 31], suggest that DRα/β<sub>1</sub>2.2 (DRα/β<sub>1</sub>1501) determines specific immune recognition of the major *Amb a V* MHC class II binding site. It would be desirable to extend these findings to further DR2.2 subjects who comprise 95% of IgE Ab responders. However, when the peripheral blood T cells were tested, almost all of the Ab responder subjects exhibited very low stimulation indices to *Amb a V* (SI = 1.5–2), because of the extremely low frequency of specific T cells in peripheral blood. In an effort to generate polyclonal T cell lines by repeated Ag stimulation *in vitro*, we were confronted with the finding that “autoreactive” T cells predominate over Ag-specific T cells in culture, which prevented us from performing further extensive screening and cloning of specific T cells from additional allergic individuals.

It appears that the DRα/β<sub>1</sub>2.12 heterodimer commonly found in Mongoloids can substitute for the almost identical DRα/β<sub>1</sub>2.2 heterodimer. These data are completely consistent with the β<sub>1</sub> sequences of these two specificities. The β<sub>1</sub> chains of DR2.2 and DR2.12 exhibit only a Gly-Val difference at residue 86, which is at the less polymorphic “far left-hand end” of the Ag-binding groove of HLA-DR

[34]. Conversely, the β<sub>V</sub> chains of these specificities show three amino acid residue differences (at positions 30, 37 and 38) including important charge differences at positions 30 (Asp-Gly) and 37 (Asp-Asn), which lie within the polymorphic “right-hand end” of the Ag-binding groove.

Cells possessing either DR2.21 or DR2.22 [29, 31], were not able to present *Amb a V* to the DRα/β<sub>1</sub>2.2-restricted T cells. These differences may reflect the polymorphic residues (particularly the charge differences at positions 70 and 71) in the third allelic hypervariable region of their β<sub>1</sub> polypeptides; or alternatively, the polymorphic residues in the first and second hypervariable regions of their β<sub>V</sub> polypeptides. According to the Brown et al. [34] model, the first hypervariable region is located on the α-helical region and point into the Ag-binding groove, and the first and second hypervariable regions are positioned on the β-pleated sheets in the Ag-binding groove. The polymorphic residues seen in DR2.21 and DR2.22 may influence the recognition of the binding to the TcR or *Amb a V* peptide. Our present data do not enable us to discriminate between these possibilities, but provide a useful model for determining the molecular nature of the trimolecular interactions between MHC class II molecules, Ag peptides and TcR in humans.

Taken together, these studies validate the use of allergy as a model for exploring the molecular basis of human immune responsiveness and its causal relationship to an immunological disease. The highly purified, well-defined *Amb a V* allergen provides an optimal system for such studies.

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