

HLA-DQ6 and HLA-DQ8 Transgenic Mice Respond to Ragweed Allergens and Recognize a Distinct Set of Epitopes on Short and Giant Ragweed Group 5 Antigens¹

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We have investigated the genetic and molecular basis of immune responsiveness to short ragweed (SRW) (*Ambrosia artemisiifolia*) extract, and group 5 allergens from short and giant (*Ambrosia trifida*) ragweed using transgenic mice expressing DQ6 (HLA-DQA1*0103, HLA-DQB1*0601) and DQ8 (HLA-DQA1*0301, HLA-DQB1*0302) genes in class II knockout ($A\beta^0$) mice. Panels of overlapping peptides spanning the *Amb a 5* and *Amb t 5* Ags were synthesized. Mice were immunized with whole SRW extract or individual peptides s.c. and lymph node cells (LNC) were challenged in vitro. Strong T cell responses to SRW extract were measured in both HLA-DQ transgenic mice, while control, HLA-DQ6⁻/DQ8⁻/H-2A β^0 , mice were unresponsive. IL-5 and IL-10 were the primary cytokines produced by in vitro challenged LNC of SRW-primed transgenic mice. HLA-DQ6-restricted T cell responses were detected to all three peptides of *Amb t 5* and two determinants (residues 1–20 and 11–30) on *Amb a 5*. In contrast, LNC of HLA-DQ8 mice did not recognize peptide 11–30 of *Amb t 5* Ag, but recognized several *Amb a 5* determinants. The immune response in transgenic mice was dependent upon CD4⁺ T cells and was HLA-DQ restricted. Primed with purified *Amb t 5*, both transgenics recognized peptide 21–40, and an additional DQ6-restricted epitope was found within residue 1–20. SRW-immunized HLA-DQ6 mice respond to peptide 11–30 of *Amb a 5*, while HLA-DQ8 mice strongly recognize peptide 1–20. These results demonstrate the specificity of HLA class II polymorphism in allergen sensitivity and pave the way for developing antagonistic peptides for desensitization. *The Journal of Immunology*, 1998, 161: 2032–2037.

Allergy is the most widespread immunologic disorder in humans, with one in four individuals affected (1). The chief causative agent for late summer hay fever in the United States and Canada is ragweed pollen (2, 3). The two most abundant species of ragweed plants are *Ambrosia trifida* and *Ambrosia artemisiifolia*, commonly known as giant and short ragweed (SRW),⁴ respectively (2, 4, 5). SRW pollens contain 52 different Ags, but these Ags are not equally active as allergens in sensitive individuals (5–7). Ag 5 from short and giant ragweed pollens, *Amb a 5* and *Amb t 5*, for which the natural exposure is less than 60 ng/yr, are the most well characterized small allergens (m.w. = 5000 and 4400, respectively) (8–12). The genes encoding *Amb a 5* and *Amb t 5* allergens have recently been cloned and sequenced, and these proteins share 45% homology (10–12). Highly purified or recombinant proteins have been prepared and extensively used

in studies of HLA restriction and B and T cell recognition of allergens (13–23).

David Marsh and associates have shown that the presence of anti-*Amb a 5* IgE and IgG are highly associated with DR2/Dw2 [DRB1*1501] (13–15, 17–21). The HLA haplotype linked to *Amb a 5* response in the Caucasian population is DRB1*1501, DRB5*0101, DQA1*0102, and DQB1*0602 (20, 24). Immune response to *Amb t 5* was also found to be significantly associated with DRB1*1501, although the strength of this association was weaker than with *Amb a 5* (23, 25). Using serologic HLA class II typing of atopic individuals, other studies associated skin test sensitivity and specific IgE response to *Amb a 5* with HLA-DR2, and to *Amb t 5* with HLA-DRw52 [DRB3] (16). Later, the association of HLA-DQw6 in 100% of *Amb a 5*-responsive individuals was confirmed (22). The HLA-DQ8 [DQA1*0301 and DQB1*0302] molecule also could be involved in the recognition of *Amb a 5* (21).

Currently described mouse models do not fully reflect responses seen in human allergies, because MHC class II molecules are mouse derived (26). Transgenic mice, expressing individual human class II molecules in the absence of the endogenous mouse class II genes, provide a new approach to study the genetics of allergy. We have generated transgenic mice expressing human DQ6 (HLA-DQA1*0103 and HLA-DQB1*0601) and DQ8 (HLA-DQA1*0301 and HLA-DQB1*0302) genes (27–29). These mice lack endogenous class II molecules, and the only functional class II molecules on APCs are human DQ6 or DQ8 molecules.

The response of transgenic mice to the whole SRW extract was examined first. Strong T cell responses were seen in both HLA-DQ6 and HLA-DQ8 mice, while transgene-negative class II knockout mice were unresponsive. After stimulation with whole SRW extract, LNC of both HLA-DQ transgenic mice secreted primarily IL-5 and IL-10, indicating a Th2-type response. In order to

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⁴ Abbreviations used in this paper: SRW, short ragweed antigen for *Ambrosia artemisiifolia*; *Amb a 5*, *Ambrosia artemisiifolia* antigen 5; *Amb t 5*, giant ragweed for *Ambrosia trifida* antigen 5; LNC, lymph node cells; *Der p*, *Dermatophagoides pteronyssinus*; TMB, tetramethyl benzyldine.

identify the T cell epitopes present in *Amb a 5* and *Amb t 5*, we synthesized overlapping 20-mer peptides that cover the entire length of the molecules and screened them for their ability to induce proliferation of LNC isolated from extract- or peptide-primed HLA-DQ6⁺/H-2A β ⁰ and HLA-DQ8⁺/H-2A β ⁰ transgenic mice. Using this approach, we have demonstrated heterogeneity in HLA-DQ restriction specificity of responses to *Amb a 5* and *Amb t 5* Ags.

The information derived from these studies is critical for our understanding of the role of particular HLA-DQ molecules in ragweed allergic response and for the development of peptide-based therapy.

Materials and Methods

Mice

The production and characterization of transgenic mice expressing HLA-DQ8 (HLA-DQA1*0301 and HLA-DQB1*0302) or HLA-DQ6 (HLA-DQA1*0103 and HLA-DQB1*0601) genes in mice deficient in endogenous class II molecules (H-2A β ⁰) have been described in detail (27–29). Expression of HLA-DQ and H-2 class II molecules on the surface of peripheral blood leukocytes was analyzed by flow cytometry (28, 29). Neither H-2A α ^b nor H-2E β ^b molecules were detected on DQ6 or DQ8 peripheral blood leukocytes. The surface expression of hybrid molecules formed by H-2A α ^b and DQ β chains was not observed in either HLA-DQ6 or HLA-DQ8 mice. Expression of the HLA-DQ molecule in H-2A β ⁰ mice induces the selection of CD4⁺ V β TCR⁺ cells and restores CD4⁺ T cell population in the periphery to a substantial level (5.0–9.3%) (28, 29). Mice were bred and maintained in the pathogen-free Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, MN).

Antigens

SRW (*A. artemisiifolia*; *Amb a*) extract was purchased from the Bayer Corporation (Elkhart, IL). Purified Ag 5 (*Amb t 5*) from giant ragweed (*A. trifida*) was provided by Dr. Marsh (Johns Hopkins Asthma and Allergy Center, Johns Hopkins University School of Medicine, Baltimore, MD). Overlapping peptides representing short (*Amb a 5*) and giant (*Amb t 5*) ragweed allergen 5 were prepared at the Peptide Core Facility of the Mayo Clinic. These peptides were 20 amino acid residues long, and 10 amino acid residues overlap with the previous peptide in the panel (see Table I).

Monoclonal Abs

Culture supernatant from the cell lines producing mAb specific for HLA-DQ α -chain (IVD 12), HLA-DR (L227), HLA-DQ6 β -chain (61.11.1), H-2A α ^b (7-16-17), H-2A β ^b (25-5-16), H-2E α / β ^b (Y-17), CD4 (GK1.5), CD8 (53-6.72), and anti-HLA-A, B, C (MB40) were prepared in our laboratory.

Immunization and in vitro culture

Mice were injected s.c. with 20 μ g of SRW extract, 20 μ g of *Amb t 5* allergen, or 200 μ g of synthetic peptide emulsified in CFA into the tails and footpads. Seven days postinjection the draining lymph nodes were removed and cells were prepared for in vitro cultures as described previously (30, 31). Lymphocyte proliferation was assessed using [³H]thymidine incorporation. Results are expressed as the change (Δ) in cpm and are calculated as Δ cpm = (mean cpm of triplicate cultures containing Ag) – (mean cpm of triplicate cultures containing medium).

Cytokine determination

For detection of in vitro cytokine productions, LNC were cultured at a concentration of 10×10^6 cells/ml in 0.5-ml volume in 24-well plates (Costar, Cambridge, MA), with 0.5 ml of culture medium, or 5 μ g/0.5 ml of Con A, or 100 μ g/0.5 ml of SRW extract in the analogous culture medium with supplements that were used for proliferation assays. After 24, 48, 72, and 96 h of incubation at 37°C, cultures were harvested from each well and centrifuged, and supernatants were collected and immediately stored at –70°C upon cytokine determination.

Cytokine secretions by in vitro-challenged LNC were measured by sandwich ELISA using mini-kits for IL-2, IL-4, and IFN- γ from Duoset, Genzyme Diagnostics (Cambridge, MA) (catalog nos. 80-3573-00, 80-3537-00, and 80-3931-00, respectively) according to instructions provided. IL-5 secretion was measured using a mini-kit from Endogen (Cambridge, MA) (catalog no. KM-IL5). The “Intertest-6X” ELISA kit was used for

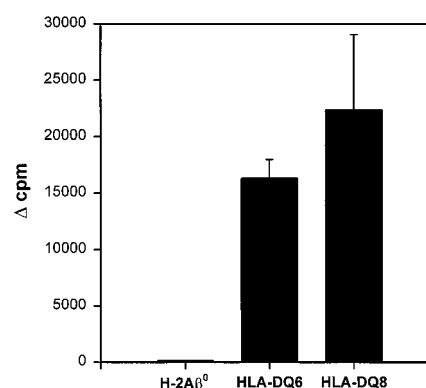


FIGURE 1. HLA-DQ6 and HLA-DQ8 LNC respond to in vitro challenge using short ragweed, *A. artemisiifolia*, extract. Data represent the means of [³H]thymidine incorporation for triplicate cultures $\pm$ SD. At the same conditions, HLA-DQ6[–]/DQ8[–]/H-2A β ⁰ mice were unresponsive to any dose of extract at any point in time. The mean negative control (human thyroglobulin) value was 146 ± 124 .

quantification of mouse IL-6 (Genzyme Diagnostics) (catalog no. 80-3748-21-00). Purified anti-IL-10 mAb (2 μ g/ml, catalog no. 18141D), the corresponding biotinylated anti-IL-10 mAb (2 μ g/ml, catalog no. 18152D), and mouse rIL-10 standard (catalog no. 19281V) were purchased from PharMingen (San Diego, CA). The horseradish peroxidase-conjugated avidin (500 ng/ml) (Jackson ImmunoResearch Laboratories, West Grove, PA) was utilized as a detection reagent, and the corresponding substrate was TMB (Sigma, St. Louis, MO). All ELISA assays were performed in 96-well flat-bottom microtiter plates (Immulon 2, Dynatech Laboratories, Chantilly, VA). Plates were read in a microtiter autoreader (Bio-Rad, Pleasanton, CA) at 450 nm. Supernatant cytokine levels were quantified by comparing them with standards added to each plate, using the Microplate Manager software for the Macintosh computer (Bio-Rad).

Results

Response of HLA-DQ6 and HLA-DQ8 to whole SRW extract

Orientation experiments involving priming and challenging transgenic mice with varying doses of SRW revealed that an in vivo priming dose of 20 μ g/mouse and an in vitro challenge dose of 20 μ g/well elicited maximum [³H]thymidine incorporation at 48 h of culture (data not shown). Under these conditions, both HLA-DQ6⁺/H-2A β ⁰ and HLA-DQ8⁺/H-2A β ⁰ transgenic mice responded strongly to SRW (Fig. 1). No in vitro proliferation was measured in transgene-negative control mice (Fig. 1). No response was seen when LNC were stimulated with a control protein, human thyroglobulin (Δ cpm, 124 ± 146) (data not shown).

In vitro activation of LNC from ragweed-immunized transgenic mice is mediated by CD4⁺ T cells and is HLA-DQ restricted

To identify the subpopulation of T cells necessary for proliferative responses to SRW in transgenic mice, anti-CD4 (GK1.5) or –CD8 (53-6.72) mAbs were added to the wells. Proliferation was inhibited by 93.9% (Δ cpm from $11,090 \pm 1,794$ to Δ cpm 677 ± 345 ; HLA-DQ6 mice) or completely eliminated (Δ cpm, 0; HLA-DQ8 mice) in cultures with anti-CD4 mAb. The anti-CD8 mAb did not markedly alter the responses (Fig. 2). The addition of IVD12 mAb (reactive with HLA-DQ8 molecules) inhibited 94.89% of in vitro response using LNC from HLA-DQ8 mice (from Δ cpm $13,481 \pm 1,195$ to Δ cpm 689 ± 696). Likewise, the addition of mAb reactive with HLA-DQ6 molecules (61.11.1) resulted in a 69.29% inhibition (to Δ cpm, 3406 ± 1715). No significant inhibition was observed in cultures containing control mAb specific for mouse H-2A α ^b, H-2A β ^b, H-2E α / β ^b, or for human MHC class I molecules (Fig. 2). Thus, the in vitro response in HLA-DQ6 and HLA-DQ8

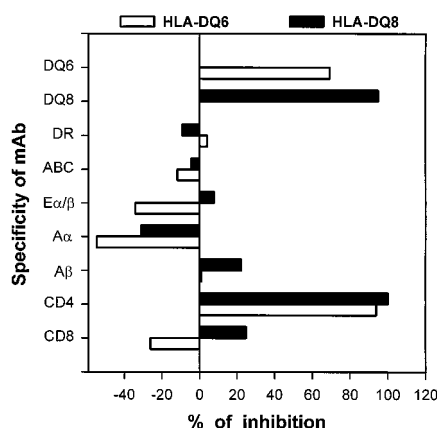


FIGURE 2. The in vitro response to SRW extract is mediated by CD4⁺ T cells and is DQ restricted. LNC were challenged with ragweed extract in the presence of the indicated mAbs. The data represent the percentage of inhibition of [³H]thymidine incorporation in cultures with SRW in five (HLA-DQ6 mice) and three (HLA-DQ8 mice) representative experiments.

transgenic mice to SRW extract is mediated by CD4-positive HLA-DQ-restricted T cells.

Th2 responses are present in HLA-DQ6 and HLA-DQ8 mice to SRW extract

Culture supernatants from LNC of HLA-DQ6 and HLA-DQ8 transgenic mice were assayed for the presence of IFN- γ , IL-2, IL-4, IL-5, IL-6, and IL-10 cytokines. In vitro culture of the LNC of HLA-DQ6 mice with medium alone showed a trace amount of IFN- γ secretion (Fig. 3E). No IFN- γ was seen in either transgenic mice after in vitro challenge with allergen. Minimal amounts of IL-2 were found following SRW stimulation of LNC (Fig. 3D). IL-10 was expressed at high levels after the treatment with SRW of LNC from either DQ6 or DQ8 mice (Fig. 3A), while the IL-5 level was much higher in DQ8 mice compared with DQ6 (Fig. 3B). IL-5 production in response to SRW stimulation occurred early with LNC from the HLA-DQ8 mice (24 h), and reached a peak level at 72 h. HLA-DQ8 mice also had higher IL-6 upon stimulation with SRW than HLA-DQ6 mice (Fig. 3C). No IL-4 was detected throughout the 4-day cultures (not shown). Thus, the Th2-type cytokines, IL-5 and IL-10, are the primary cytokines produced by in vitro challenged LNC from both HLA-DQ transgenic mice. However, the levels and kinetics of cytokines were not similar for HLA-DQ6 and HLA-DQ8 transgenic mice.

T cell epitopes on SRW allergen Amb a 5 recognized by HLA-DQ6 or HLA-DQ8 transgenic mice

To determine which of the epitopes representing *Amb a 5* could be processed for presentation by APC of ragweed-primed HLA-DQ6 or HLA-DQ8 mice, transgenic animals were immunized with whole extract and the draining LNC were challenged in vitro with either SRW extract or synthetic overlapping peptides representing a component allergen *Amb a 5* (Table I). All of the HLA-DQ8 mice tested recognized a single peptide of *Amb a 5* within residue 1–20 (Δ cpm, 8676 $\pm$ 2175) (Fig. 4A). In contrast, HLA-DQ6 mice primed with extract showed in vitro proliferative response to peptide 11–30 (Δ cpm, 5927 $\pm$ 572) (Fig. 4A). Thus, a naturally processed SRW epitope for DQ8 can conceivably reside within residue 1–10 or, alternatively, between residues 8–20, while for DQ6 it can be within residue 10–20 or, alternatively, between residues 18–30.

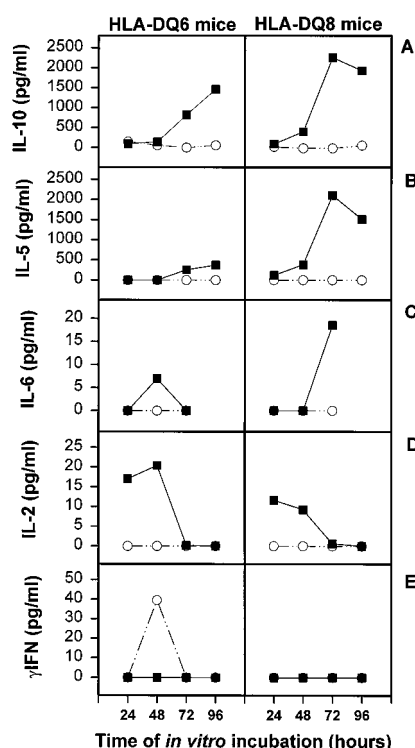


FIGURE 3. Cytokine production by LNC of HLA-DQ6 and HLA-DQ8 transgenic mice in response to immunization with SRW. LNC were challenged in vitro with medium alone (○) or ragweed extract (■) and harvested at 24, 48, 72, and 96 h of culture. Cytokine profiles were measured by ELISA. Results are the mean of cytokine concentration for one of two representative experiments.

These results suggest that different SRW determinants may be recognized by HLA-DQ6 and HLA-DQ8 molecules. As a means of exploring such a possibility, transgenic mice were immunized with 200 μ g of individual peptide, and LNC were challenged in vitro with 200 μ g/ml of the relevant peptide. Certain concentrations of peptide for immunization and in vitro challenge were chosen on the basis of the results from previous experiments. HLA-DQ6 mice responded to two peptides of *Amb a 5* (amino acid residues 1–20 and 11–30) while responses to the peptides localized at the C terminus were undetectable (Fig. 4B). HLA-DQ8 mice responded to three (1–20, 11–30, and 21–40) of the four peptides representing *Amb a 5* (Fig. 4B). No response was detected to the last peptide 31–45. LNC cultures with Con A positive control, gave strong in vitro responses measured by [³H]thymidine incorporation in all experiments (data not shown).

It can be inferred from these results that at least two HLA-DQ8-restricted determinants are localized within the region 1–40, while at least one HLA-DQ6-restricted determinant lies within residues 1–30. The most antigenic portion of *Amb a 5* allergen is the N-terminal region for both DQ6- and DQ8-restricted T cell epitopes.

HLA-DQ6 and HLA-DQ8 mice respond to peptides representing Ag 5 of giant ragweed, Amb t 5

To localize the T cell epitopes recognized by the HLA-DQ molecules following immunization with purified *Amb t 5* protein, mice were primed with 20 μ g of *Amb t 5*, and LNC were challenged with 20 μ g/ml of protein (Δ cpm of 28,689 $\pm$ 6,400 for HLA-DQ6 mice, and 35,776 $\pm$ 6,889 for HLA-DQ8 mice) or 200 μ g/ml of synthetic peptides (Table I). The proliferative responses of the LNC population of HLA-DQ6 mice, primed with HPLC-pure *Amb*

Table I. Sequences of synthetic overlapping peptides used in this study

Allergen	Position	Sequence
<i>Amb a 5</i>	1–20	LVPCAWAGNVCGEKRAYCCS
	11–30	CGEKRAYCCSDPGRYCPWQV
	21–40	DGRYCPWQVVVCYESSEICS
	31–45	VCYESSEICSSKKCGK
<i>Amb t 5</i>	1–20	DDGLCYEGTNCCKVGVKGYCCS
	11–30	CGKVGKYCCSPIGKYVCYD
	21–40	PIGKYVCYDLSKAICNKNCT

t 5, indicate that, although T cell epitopes are present throughout the protein, the sequences 1–20 and 21–40 contain the dominant DQ6-restricted epitopes (Fig. 5A). LNC from *Amb t 5*-primed HLA-DQ8 mice proliferated to in vitro challenge using peptide 21–40 (Δ cpm, 35,671 $\pm$ 9,744). Low responses were detected to the two other *Amb t 5* peptides (Fig. 5A). Therefore, the naturally processed *Amb t 5* HLA-DQ6-restricted epitopes are also within residues 1–20 and 21–40, while a strong HLA-DQ8-restricted epitope lies within the 21–40 sequence.

In order to identify those regions of giant ragweed Ag 5, *Amb t 5*, that constitute the major sites of recognition for HLA-DQ6- or HLA-DQ8-restricted T cells, mice were primed s.c. and LNC were challenged in vitro with an individual peptide representing *Amb t 5* molecule (Table I). All three peptides (residues 1–20, 11–30, and 21–40) representing *Amb t 5* elicited vigorous in vitro responses (Δ cpm of 34,551 $\pm$ 11,036; 54,927 $\pm$ 12,764; and 50,483 $\pm$ 10,811, respectively) using HLA-DQ6 LNC (Fig. 5B). HLA-DQ8

mice responded to peptides 1–20 (Δ cpm, 57,353 $\pm$ 2,134) and 21–40 (Δ cpm, 97,315 $\pm$ 6,513) but not to peptide 11–30 (Δ cpm, 784 $\pm$ 783) (Fig. 5B). These results suggest that both the N and C termini harbor major HLA-DQ8- and HLA-DQ6-restricted antigenic determinants. Thus, there are at least two HLA-DQ6- and HLA-DQ8-restricted epitopes within residue 1–40 of *Amb t 5* allergen. For HLA-DQ8, these epitopes can be limited to residues 1–10 and 30–40.

The responses to immunodominant peptides of Amb a 5 and Amb t 5 are dependent upon CD4⁺ T cells and are HLA-DQ restricted

Peptides covering *Amb a 5* or *Amb t 5* Ag that contain HLA-DQ6- or HLA-DQ8-restricted T cell epitopes were selected for further study. In some experiments, 20 μ l of culture supernatant anti-mouse-CD4 (GK1.5), anti-mouse-CD8 (53-6.72), anti-HLA-DQ (IVD12), and anti-HLA-DR (L227) mAbs were added

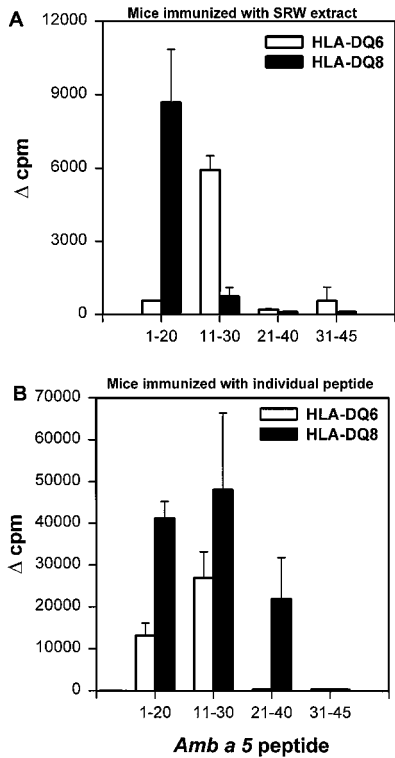


FIGURE 4. Localization of T cell epitopes on *Amb a 5* allergen recognized by HLA-DQ6 and HLA-DQ8 mice. Mice were immunized with whole SRW extract (A) or with individual peptides representing *Amb a 5* (B), and LNC were challenged with individual peptides. The data represent the mean of the responses $\pm$ SD for the triplicate cultures of three representative experiments. Under the same conditions, HLA-DQ6[−]/DQ8[−]/H-2A β ⁰ mice were unresponsive to any peptide.

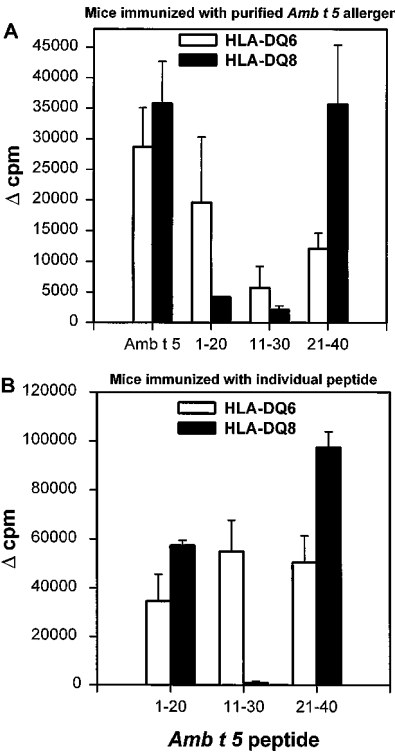


FIGURE 5. The in vitro responses of HLA-DQ6 and HLA-DQ8 mice to overlapping peptides of *Amb t 5* Ag. Mice were immunized with HPLC-pure *Amb t 5* Ag (A) or with individual peptide representing *Amb t 5* (B), and LNC were challenged with either protein or relevant peptide. The data represent the means of Δ cpm $\pm$ SD for triplicates in three representative experiments.

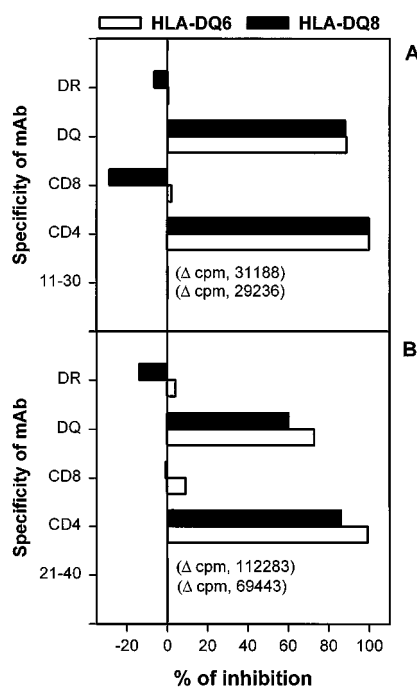


FIGURE 6. The responses of transgenic mice to immunodominant determinants on *Amb a 5* (A) and *Amb t 5* (B) are dependent upon CD4⁺ T cells and are HLA-DQ restricted. Mice were primed with individual peptides, and LNC were challenged in vitro in the presence of the indicated mAbs. Results are shown as percentage of inhibition of [³H]thymidine incorporation for triplicate cultures of LNC with peptide and mAb compared with cultures with peptide alone. Δ cpm values for cultures with peptide alone (0% inhibition) are indicated.

to the microtiter wells containing LNC and peptide. The addition of anti-CD4 mAbs completely inhibited (Δ cpm, 0 value) the responses of HLA-DQ6 and HLA-DQ8 LNC to all immunodominant peptides of *Amb a 5* protein, while anti-HLA-DQ mAb inhibited the responses by 90% (in Fig. 6A, results of in vitro depletion studies of HLA-DQ8 and HLA-DQ6 response to peptide 11–30 are presented). Both anti-CD4 and anti-HLA-DQ mAbs showed significant inhibition of proliferation when LNC from either HLA-DQ6 or HLA-DQ8 mice primed with peptide 21–40 of *Amb t 5* were restimulated with this peptide in vitro (Fig. 6B). Anti-HLA-DR (L227), as well as anti-CD8 mAbs, did not show any inhibition effect in experiments performed (Fig. 6). Thus, the response of HLA-DQ transgenic mice to immunodominant peptides as well as to whole SRW extract is also dependent upon CD4⁺ T cells and is HLA-DQ restricted.

Discussion

In this study, the HLA-DQ6- and HLA-DQ8-restricted T cell responses to SRW and epitope mapping of ragweed allergens *Amb a 5* and *Amb t 5* are reported. We used transgenic mice expressing human DQ6 (HLA-DQA1*0103 and HLA-DQB1*0601) and DQ8 (HLA-DQA1*0301 and HLA-DQB1*0302) genes in class II knockout mice. The important finding in this study is that HLA-DQ transgenic mice are valuable in identifying T cell determinants on allergens. Previous work from this laboratory has demonstrated that HLA-DQ8 mice are capable of responding to *Der p* (house dust mite, *Dermatophagoides pteronyssinus*) allergen, and four distinct DQ8-restricted T cell epitopes on the major Ag *Der p* 2 have been identified (31).

The region on class II molecules critical for interaction with CD4 molecules lies within sequence 110–140 of the second do-

main (32, 33). This region is identical between human DQβ and mouse Aβ (28). Thus, human DQ molecules in transgenic mice lacking endogenous Ab gene interact efficiently with mouse CD4 molecules to restore the CD4⁺ T cell population and shape the T cell repertoire (positive selection of various TCRs) (28). Human DR molecules interact less efficiently with mouse CD4 molecules (34, 35).

We have demonstrated that HLA-DQ6 and HLA-DQ8 transgenic mice elicit strong in vitro responses to ragweed Ags that are dependent upon HLA-DQ-restricted CD4⁺ T cells. It should be noted that the H-2^b strain of mice was unable to recognize any epitopes on *Amb a 5* and *Amb t 5* ragweed proteins (26). Studies in HLA-DQ transgenic mice identified several epitopes on these Ags. Therefore, the responses in our transgenic mice reflect human class II recognition of ragweed epitopes. Although we have been unable to detect measurable levels of IL-4 in culture supernatants following in vitro challenge of LNC with SRW, varying amounts of other Th2-type cytokines (IL-5, IL-6, and IL-10) were produced by HLA-DQ6 and HLA-DQ8 transgenic mice. Based on in vitro and in vivo observations, the role of IL-5 as a stimulator of the growth, differentiation, activation, and prolongation of eosinophil survival is very well established. IL-10 is known for suppressing Th1 response and for suppression of MHC class I and costimulatory molecule B7 (reviewed in Ref. 36). IL-6 is produced by a wide spectrum of cells, including CD4⁺ Th2 cells and APCs (37, 38). IL-6 cytokine is a key factor in differentiation of CD4⁺ T cells to Th2 phenotype (39) and is implicated in the genesis of allergic reaction (40–42). Interestingly, LNC of HLA-DQ8 transgenic mice also expressed a high level of IL-6 mRNA in response to *Der p* challenge (31). In contrast, in response to in vitro challenge with non-allergen-associated Ag such as bovine type 2 collagen, LNC of HLA-DQ8 transgenic mice have shown Th1-type cytokine profile (high level of IFN-γ and low IL-4) (43).

In humans, HLA class II alleles are associated with the response to several allergens (reviewed in Refs. 23 and 25), including short and giant ragweed Ag 5, *Amb a 5* and *Amb t 5* (13–25). It has been reported that HLA-DQ6(DQA1*0102 and DQB1*0602) is associated with immune responsiveness to Ag 5 of SRW (22). Our results demonstrate that DQA1*0103 and DQB1*0601 confer responsiveness to SRW and overlapping peptides representing Ag 5. Our transgenes differ from DQA1*0102 and DQB1*0602 by two and seven amino acid residues in the α- and β-chain of encoded molecules, respectively. Nevertheless, the transgenes are homologous enough that responsiveness to *Amb a 5* is retained. Most probably there are multiple DQ and DR alleles capable of conferring responsiveness to SRW Ags in the human population. Our transgenic mice express one of these susceptible molecules.

The T cell epitopes on some mite-, grass pollen-, tree pollen-, cat-, bee venom-, and chicken-derived allergens have been identified and published (reviewed in Ref. 44). Formal analyses of T cell determinants on the *Amb a 5* and *Amb t 5* molecules in humans have not been published. Three *Amb a 5*-specific DRα/β 2.2-restricted T cell clones from atopic patients have been reported to recognize C-terminal residue (18). Identification of DR- and DQ-restricted allergen epitopes in humans is very difficult due to the heterogeneity of the human population. While studies with T cell clones isolated from atopic patients might suggest potential epitopes, they do not provide a comprehensive picture of important epitopes for all class II molecules. To that end, our HLA-DQ mice, as well as our more recently developed HLA-DR transgenic mice, provide a powerful model for an exhaustive characterization and identification of the antigenic determinants on allergens.

The use of peptides in immunotherapy is a very attractive and promising avenue for future management of ongoing allergic diseases (45–47). Using synthetic peptides, we demonstrated the presence of multiple T cell epitopes, even on small molecules such as *Amb a 5* and *Amb t 5* proteins (45 and 40 amino acid residues long, respectively). Truncation analysis is needed to precisely localize critical residues. The set of transgenic mice we have generated should aid in developing an *in vivo* model for ragweed allergy, which is currently in progress in our laboratory. The epitopes identified in this study could be tested for their efficacy for desensitization in the *in vivo* model.

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