

HLA-DQ8 Transgenic Mice Lacking Endogenous Class II Molecules Respond to House Dust Allergens

Identification of Antigenic Epitopes¹

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We have introduced HLA-DQ8 (*HLADQB*0302* and *HLA-DQA*0301*) genes into $\text{A}\beta^0$ knockout mice. Transgenic animals were immunized with a whole body extract of *Dermatophagoides pteronyssinus* (*Der p*), one of the causative agents of house dust mite allergy. Transgenic mice expressing HLA-DQ8 genes elicited HLA-DQ8-restricted responses driven by CD4^+ T cells. Synthetic overlapping peptides representing a major allergen of house dust mite (*Der p* 2) were synthesized and used as immunogens. HLA-DQ8⁺ mice responded to three peptides: 8 (residues 61–80), 11 (residues 91–110), and 13 (residues 111–129). The mice produced IL-2, IL-4, and IL-6 response to *Der p* challenge, suggesting a mixed Th1/Th2 response. These mice represent a new model for studies of the immune basis of allergy. *The Journal of Immunology*, 1996, 156: 3191–3195.

It has been estimated that nearly 20% of the general population is genetically predisposed to developing hypersensitivity to various environmental stimuli (1, 2). House dust mites (HDM)⁵ are a significant source of indoor allergens, causing symptoms ranging from atopic dermatitis and extrinsic asthma to allergic rhinitis in 10% of individuals. The mite, *Dermatophagoides pteronyssinus* (*Der p*), one of the causative agents of HDM, is the most frequently implicated allergen in patients with respiratory allergy (2). Approximately 40 to 88% of the IgE response in HDM allergic patients is directed against the group 2 allergens, while immune responses to group 1 allergens are less pronounced (3, 4). It has been determined that there are 32 IgE binding proteins comprising the HDM allergens. Recently, the major group 2 allergen, *Der p* 2, has been identified by Western blotting, cloned, and sequenced (5, 6). Recombinant *Der p* 2 is a potent inducer of immediate hypersensitivity reactions in HDM allergic patients. Four distinct T cell epitopes on the *Der p* 2 molecule have been identified and demonstrated to be restricted by HLA-DQB1*0301 or HLA-DRB1*1101 molecules (4, 7–9).

Determining HLA-DQ/DR restriction elements using human lymphoid tissue is complicated due in part to the heterozygosity of the human population and the difficulties and expense of obtaining and maintaining typing reagents. Attempting to circumvent this obstacle, human class II genes have been introduced into mice.

Although studies have reported that human class II genes can function in transgenic animals (10, 11), demonstration of human class II-restricted immune responses has been far less successful (12, 13). The failure to generate reliable human class II-restricted responses has been ascribed to poor interactions between mouse CD4 and human class II molecules (14). The application of gene targeting to render mice deficient for class II expression has opened new possibilities for expressing human class II molecules at levels sufficient to generate human class II-restricted responses. We have introduced *HLA-DQB1*0302* and *HLA-DQA1*0301* (HLA-DQ8) genes into mouse class II-deficient H-2A β^0 mice (15). Mice expressing HLA-DQ8 genes in the absence of endogenous class II molecules are capable of generating HLA-DQ8-restricted responses against peptide Ags (15) and developing arthritis following immunization with type II collagen (16). Here we demonstrate that HLA-DQ8/A β^0 transgenic mice are capable of responding to *Der p* Ags and represent a new tool for investigating immune responses to allergens and for developing antagonist peptides for immunointervention.

Materials and Methods

Mice

Cosmids H11A and X10A, containing the *HLA-DQA*0301* and *HLA-DQB*0302* genes, respectively, were introduced into (B10 $\times$ SWR) mice and transferred by selective breeding into mouse class II-deficient H-2A β^0 (A β^0) mice as described previously (15).

Antigens

Hollister Stier *Der p* extract (2000 allergy units (AU)/ml; $\sim 63 \mu\text{g}/\text{ml}$) was a gift from the Allergic Diseases Research Laboratory at the Mayo Clinic (Rochester, MN). Synthetic overlapping peptides comprising the 129 residues of the *Der p* 2 allergen were prepared at the Peptide Core Facility (Mayo Clinic). A total of 12 overlapping peptides were synthesized. Each peptide was 20 amino acids long, with a 10-residue overlap with the previous peptide in the series (see Table I).

Immunizations and in vitro culture

Mice were immunized s.c. into the tails and footpads with 3000 AU of *Der p* whole body extract emulsified in CFA. Seven days after injection, the draining lymph node cells were removed and prepared for cell culture (17). Lymph node cells (1×10^6) were challenged with varying amounts of *Der p* extract (200–0.02 AU), peptide (100–0.01 μg), or human thyroglobulin

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⁵ Abbreviations used in this paper: HDM, house dust mites; AU, allergy units; *Der p*, *Dermatophagoides pteronyssinus* (house dust mite); *Der p* 2, *Dermatophagoides pteronyssinus* house dust mite major allergen.

FIGURE 1. HLA-DQ8⁺/A β ⁰ mice display a dose-dependent response to *Der p* extract. Data represent the means of triplicate cultures from a representative experiment. The mean negative control (human thyroglobulin) value was 1761 $\pm$ 105.

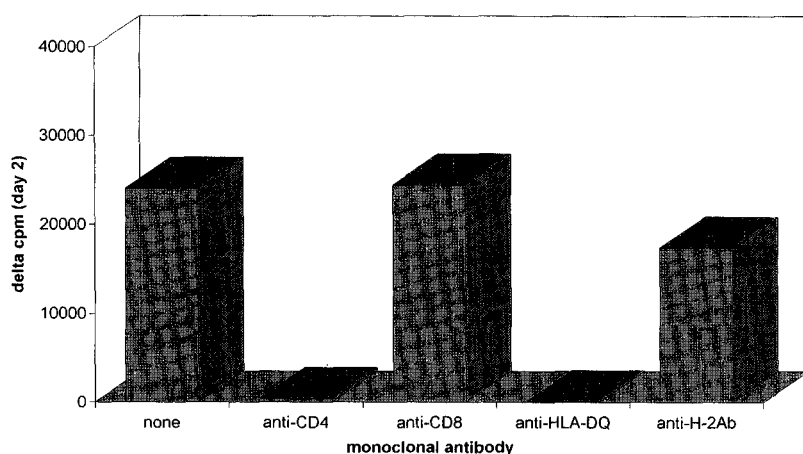
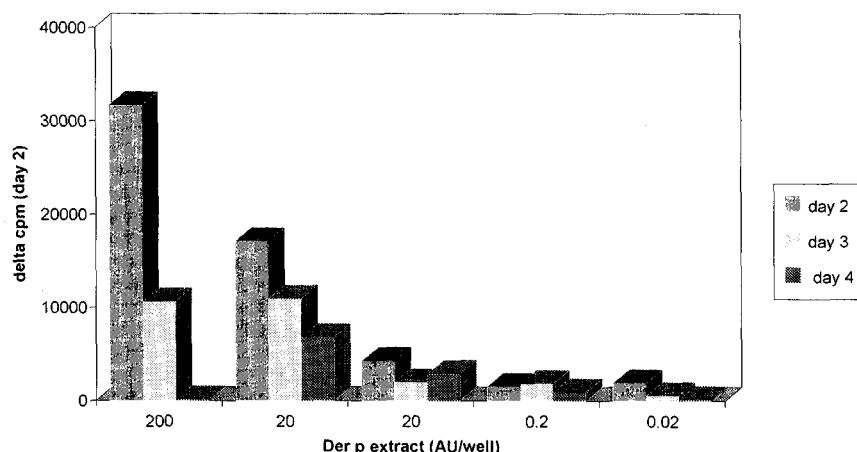


FIGURE 2. The response to *Der p* Ags is mediated by CD4⁺ cells and is HLA-DQ8 restricted. Lymph node cells were challenged with *Der p* in the presence of the indicated mAbs. The mean negative control (human thyroglobulin) value was 6707 $\pm$ 292.

(HTg; negative control) for 48 h. Eighteen hours before harvesting the cultures, 10 μ l of [³H]thymidine solution (180 μ Ci/ml) was added to the microtiter wells. Results are expressed as Δ counts per minute and are calculated as: Δ cpm = (mean cpm of triplicate cultures containing Ag) – (mean cpm of triplicate cultures containing human thyroglobulin). The mean number of count per minute of cells cocultured with human thyroglobulin was invariably <8000 and was indistinguishable from the mean values of cells cultured with medium alone.

Monoclonal antibodies

In some experiments, mAbs (20 μ l of culture supernatant) were added to microtiter wells. The culture supernatants were prepared using the following cell lines: anti-CD4 (GK1.5), anti-CD8 (53-6.72), anti-HLA-DQ8 (IVD12), anti-H-2A^b (25-5-16), and anti-HLA-DR (L227) (18). There was no cross-reactivity between IVD12 and mouse class II molecules or between 25-5-16 and HLA-DQ8 molecules (15).

PCR analyses of lymphokine production

Lymph node cells cocultured with 200 AU *Der p* for 7 h were harvested and centrifuged. RNA was isolated from the cell pellets using the RNeasy B kit (Tel-Test, Friendswood, TX). Chloroform was added to the lysate and centrifuged. The aqueous phase was removed, and isopropanol was added to effect precipitation of RNA. The RNA-containing material was washed using 75% ethanol, centrifuged, and air-dried. Upon performing a reverse transcription reaction (42°C for 1 h), PCR was initiated using specific primers for actin, IL-2, IL-4, IL-6, γ -IFN, TGF- β , and MIP- α . The resulting products were subjected to PAGE (500 V for 10 min). Relative band densities were analyzed using AMBIS.

Results

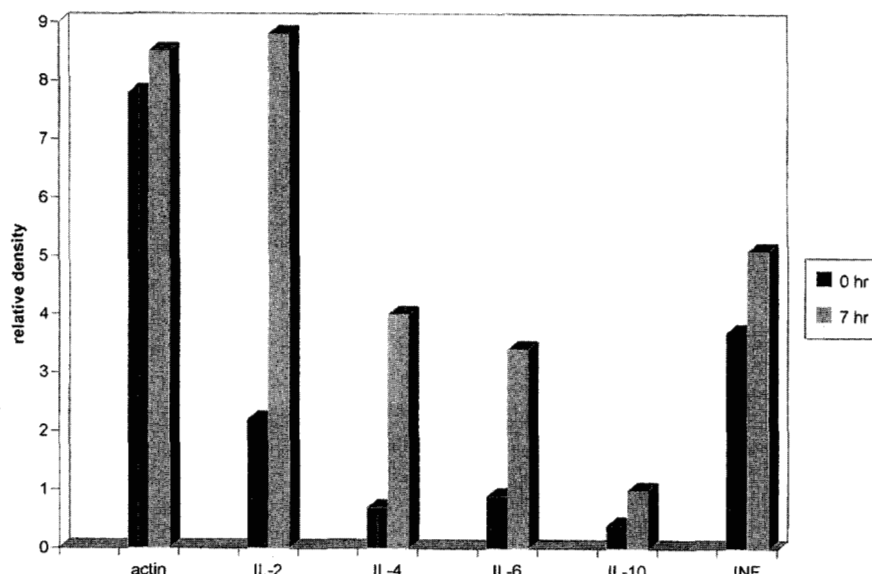
HLA-DQ8⁺/A β ⁰ lymph node cells display a dose-dependent response to *in vitro* challenge using *Der p* extract

HLA-DQ8⁺/A β ⁰ mice (DQ8⁺) were immunized using 2000 AU of *Der p* extract, and the draining lymph node cells were challenged *in vitro* (Fig. 1). When 1×10^6 lymph node cells were challenged with 200 AU of *Der p* extract, a strong response (Δ cpm, 31,653) was detected. As the DQ8⁺ cells were challenged with progressively less *Der p* extract (20–0.02 AU), there was a corresponding loss (from Δ cpm, 17,104 to Δ cpm, 1,907) of immune reactivity. At all *in vitro* challenge doses, maximal T cell reactivities were measured after 48 h of culture. Under the same conditions, HLA-DQ8[–]/A β ⁰ littermates were unresponsive (Δ cpm, <1,000).

The *in vitro* response is mediated by CD4⁺ cells and is HLA-DQ8 restricted

Lymph node cells from HLA-DQ8⁺/A β ⁰ mice were challenged with 20 AU of *Der p* extract 7 days postimmunization. At the time of initiation of cultures, mAbs were added to the wells (Fig. 2). Under conditions whether *Der p* extract elicited a strong *in vitro* response (Δ cpm, 24,033), the addition of anti-CD4 (GK1.5) mAb resulted in complete inhibition of the response (Δ cpm, 401). The addition of anti-CD8 (53-6.72) mAb was without effect (Δ cpm, 24,440). Anti-HLA-DQ (IVD12) mAb inhibited the *in vitro* response (Δ cpm, 0), while anti-H-2A^b (25-5-16) mAb had only a

FIGURE 3. HLA-DQ8⁺ transgenic mice produce IL-2, IL-4, and IL-6 in response to immunization with *Der p*. RNA was isolated from in vitro challenged lymph node cells, expanded by PCR, and hybridized using the indicated IL-specific probes. Cells challenged with negative control Ag (human thyroglobulin) did not hybridize strongly with the IL probes.



marginal inhibitory effect (Δ cpm, 17,395). These results are consistent with the in vitro response to *Der p* extract being mediated by CD4⁺, HLA-DQ8-restricted T cells.

Lymphokine profiles from challenged cells suggest the existence of both Th1 and Th2 responses

After 7 h of culture with 20 AU of *Der p* extract, RNA was isolated from the challenged cells and analyzed for the presence of lymphokine mRNA (Fig. 3). Appreciable hybridization was measured using IL-2- and IFN- γ -specific probes. Slightly less intense bands were detected using IL-4 and IL-6 probes. Bands representing IL-10 were barely measurable. Virtually no lymphokine production was detected when the cells were challenged with human thyroglobulin (not shown). Since both IL-2 and IL-4 mRNAs were isolated from cultures challenged using *Der p* extract, it can be concluded that both Th1 and Th2 responses may be elicited in HLA-DQ8⁺ transgenics.

*HLA-DQ8⁺/A β ⁰ mice respond to several determinants on the major allergen, *Der p* 2*

Transgenic animals were immunized with *Der p* extract, and the draining lymph node cells were challenged in vitro with either *Der p* extract (Δ cpm, 28,307) or overlapping synthetic peptides (Table I) representing the 129-amino acid major HDM allergens (*Der p* 2). Maximal responses to 3 of the 12 peptides were detected (Fig. 4). HLA-DQ8⁺ lymph node cells responded maximally to peptides 8 (residues 61–80; Δ cpm, 5,455), 11 (residues 91–110; Δ cpm, 8,409), and 13 (residues 111–129; Δ cpm, 8839). The reduced responses to peptides 7, 9, 10, and 12 (Δ cpm, <3,500) were probably to the presence of excess nonstimulatory flanking residues. The amino-terminus (residues 1–60) of the *Der p* 2 molecule (represented by peptides 1–6) was not immunostimulatory (Δ cpm, <500). Transgene negative littermates were unresponsive to all peptides (data not shown).

Peptide 8 (residues 61–80) was selected for further study. Mice were immunized with 100 μ g of peptide, and the draining lymph node cells were challenged 7 days later in vitro. Dose-response and kinetic analyses indicated that a maximal response (Δ cpm, 17,566) was obtained after 48 h of in vitro culture with 20 μ g of peptide (our unpublished observations). The addition of anti-CD4

Table I. Sequences of peptides representing *Der p* 2

Peptide	Position	Sequence
1	1–20	D Q V D V K D C A N H E I K K V L V P G
2	11–30	H E I K K V L V P G C H G S E P C I I H
3	21–40	C H G S E P C I I H R G K P F Q L E A V
4	31–50	R G K P F Q L E A V F E A V Q N T K T A
6	41–60	F E A V Q N T K T A K I E I K A S I D G
7	51–70	K I E I K A S I D G L E V D V P G I D P
8	61–80	L E V D V P G I D P N A C H Y M K C P L
9	71–90	H A C H Y M K C P L V K G Q Y D I D K Y
10	81–100	V K G Q Q Y D I K Y T W N V P K I A P K
11	91–110	T W N V P K I A P K S E N V V V T V K V
12	101–120	S E N V V V T V K V M G D D G V L A C A
13	111–129	M G D D G V L A C A I A T H A K I R D

(GK1.5) or anti-HLA-DQ8 (IVD12) mAbs completely inhibited (Δ cpm 0 value) the response to peptide 8 (Fig. 5). In contrast, mAbs (53-6.72 and L227) reactive with CD8 (Δ cpm, 19,606) or HLA-DR (Δ cpm, 22,273) molecules only slightly inhibited the anti-peptide response.

Discussion

Susceptibility to HDM-induced allergy has been linked to certain HLA class II molecules (1, 7, 8). Although the number of HDM allergens is large, the *Der p* 2 allergen probably represents a major antigenic component of the dust mite *D. pteronyssinus* (3–6). *Der p* 2 is a 129-amino acid-long protein with a molecular mass of approximately 14 kDa (1). *Der p* 2 functions as a lysozyme. T cells from HDM sensitive individuals respond to in vitro challenge using *Der p* 2, and four T cell determinants have been identified (4, 7–9). The A β ⁰ class II negative mice used in these studies also have a nonfunctional H-2E α gene and, therefore, do not express H-2A or H-2E molecules. Moreover, hybrid A α E β , A α DQ β , or DQ α E β molecules are not expressed in these mice (15). Therefore, the anti-*Der p* responses detected in these mice are entirely a consequence of HLA-DQ8 recognition.

HLA-DQB1*0301 has been associated with responsiveness to residues 16–31, 22–40, 82–105, and 111–129 on *Der p* 2 (1, 8). HLA-DRB1*1101-mediated recognition of residues 22–40, 82–105,

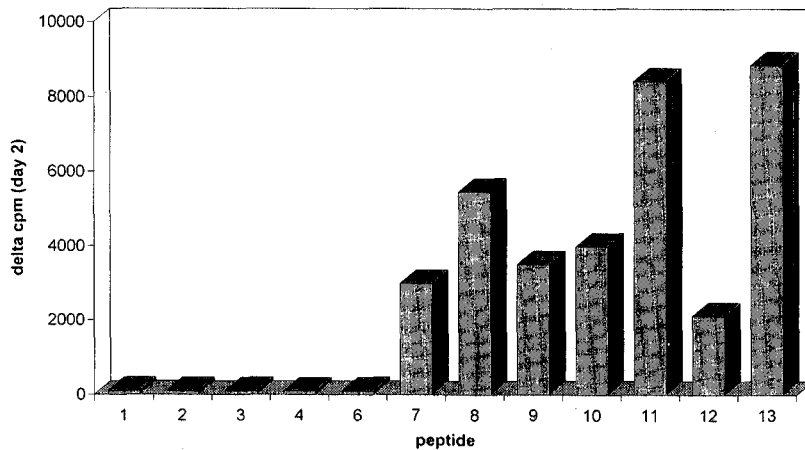
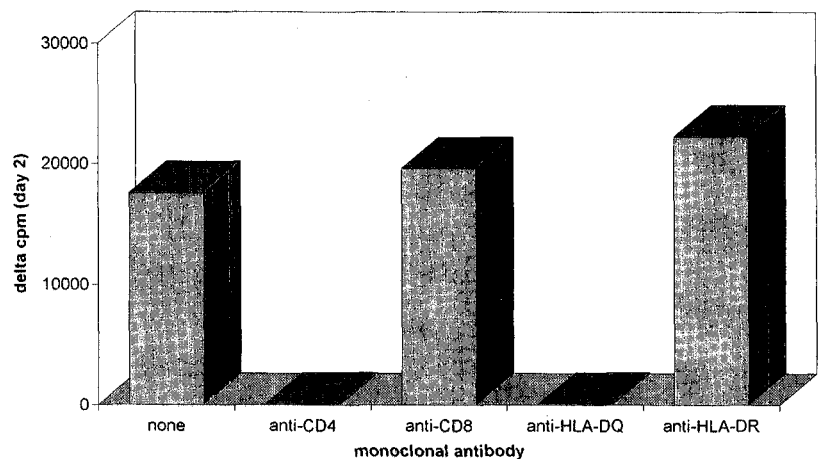


FIGURE 4. HLA-DQ8⁺ transgenic mice respond to three peptides from a panel representing Der p 2 allergen. Mice were challenged with *Der p* Ag extract. Cells were challenged in vitro using whole *Der p* extract or individual peptides of Der p 2 Ag. The mean negative control (human thyroglobulin) value was 7763 ± 139 .

FIGURE 5. The response to peptide 8 (residues 61–80) is dependent upon CD4⁺ cells and is HLA-DQ8 restricted. Mice were primed with peptide 8 and challenged in vitro in the presence of the indicated mAbs. The mean negative control (human thyroglobulin) value was 6656 ± 676 .



and 111–129 has also been demonstrated (1, 7, 8). Results from these limited studies suggest that in addition to the existence of HLA class II-specific epitopes there may exist immunodominant determinants capable of binding to more than one HLA class II molecule. Systematic analyses of all 129 residues of Der p 2 using a panel of overlapping peptides in HLA-DQ8⁺ (*HLADQB*0302*) transgenic mice revealed reactivity to peptides 8 (residues 61–80), 11 (residues 91–110), and 13 (residues 111–129). Responsiveness to residues 91 to 110 (peptide 11) and 111 to 129 (peptide 13) is similar to data obtained using peripheral blood lymphocytes from *HLA-DQB*0301* HDM-sensitive patients. However, HLA-DQ8⁺ transgenic mice were not responsive to amino-terminus (residues 1–40) residues (peptides 1–4) of *Der p*, which have been reported to be antigenic in some *HLA-DQB*0301* individuals (1, 7, 8). In contrast we were able to measure responsiveness to a new determinant within residues 61–80 (peptide 8). There are several possible explanations for these results.

*HLA-DQB*0302* and *HLA-DQB*0301* differ at four residues (positions 13, 26, 45, and 57) within the first domain of the molecule (19). Our results suggest that despite the allelic polymorphisms between *HLA-DQB*0302* and *HLA-DQB*0301*, there is recognition of a subset of common determinants on Der p 2. In addition, there may exist *HLA-DQB*0302*-unique determinants. It is believed that T cell reactivity to Der p 2 leads to subsequent development of IgE Ab responses. Der p 2 elicits T cell responses in atopic individuals and binds IgE Abs (9). Moreover, both IL-2 and IL-4 lymphokines are secreted in vitro in response to in vitro

challenge using Der p 2. HLA-DQ8⁺ mice produced IL-2, IL-4, and IL-6 in response to *Der p* challenge. The elicited lymphokine responses suggest a mixed Th1/Th2 response, as has been reported in HDM patients (20). However, our PCR results are only semi-quantitative and need to be more rigorously addressed. Additional analyses using antigenic peptides may result in the identification of Th1- and Th2-specific epitopes. Similarly, the Ab response in HLA-DQ8⁺ transgenics may shed insight on the relationship of T cell and B cell epitopes.

The studies described here represent a new animal model for investigating the immunopathogenesis of allergy. These humanized transgenic mice may be useful in studies aimed at determining the efficacy of peptide-based immunotherapies. Toward that end, we are attempting to induce Der p 2-specific IgE responses and airway hyper-responsiveness following the inhalation of allergen.

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