

Allergens, IgE, mediators, inflammatory mechanisms

House dust mite-induced sensitivity in mice

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Sensitivity induced by house dust mite (HDM) extract in mice was investigated in this study. Sensitized B10.RIII mice (H-2^r background) had T-cell proliferative responses to HDM extract in vitro and an HDM-specific IgE response. When mice were immunized by injection and intranasal inhalation with HDM extract, a histologic study showed eosinophils and mononuclear cell infiltration in the lung tissue and bronchial wall. Tcr α β -positive cells were also found in the cell infiltration area of the lung lesions. In the control mice that were immunized by injection or intranasal inhalation (but not both), we did not observe cell accumulation in the lung tissue or in the bronchial wall. Epitope studies suggest that T cells recognize multiple epitopes. Molecular analysis of these HDM-specific T-cell hybridoma clones suggest that T-cell receptor use is restricted to members of the V α 8 and V β 6 subfamilies. (*J Allergy Clin Immunol* 1998;101:51-9.)

Key words: HDM-induced sensitivity, T-cell receptor use, Der p 1, T-cell epitopes, eosinophilic cells, mononuclear-cell infiltration

About 15% of the world population exhibits a hypersensitivity response to common aeroallergens resulting in asthma, eczema, and rhinitis. The most frequently implicated allergens are derived from the house dust mite (HDM) including *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides microceras* (Der m), and *Dermatophagoides farinae* (Der f). Over 80% of sera from patients allergic to HDMs react with a group I allergen, which is composed of Der p 1, Der f 1, and Der m 1.^{1,2} The complementary DNA (cDNA) from this group I allergen (25 kd) has been cloned, sequenced, and shown to be similar to that of cysteine protease, papain, actinidin, cathepsin H, and cathepsin B.^{3,4} The group II (14 kd) and group III (29 kd) allergens have been extensively studied as well.⁵⁻⁷

The synthesis of IgE specific to allergens is regulated by T-cell lymphokines, in particular IL-4 and IL-13.^{8,9} In

Abbreviations used

CFA:	Complete Freund's adjuvant
HDM:	House dust mite
HEPES:	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
PBS:	Phosphate-buffered saline

spite of the low degree of similarity between groups of HDM allergens, immune cross-reactivity among groups and species of allergens (Der p and Der f) has been reported in human IgE responses.^{10,11} However, cloned Der f-specific T cells from one patient did not recognize the Der p allergen.¹⁰ These results suggest that certain B and T cells show cross-reactivity within groups and species of allergens, and some of the T cells in the immune response are allergen-species specific. Interestingly, the binding of a mouse IgG1 monoclonal antibody to Der p 1 could be inhibited by the sera of patients allergic to HDMs.¹² These data suggest that the B cells from animals, as well as human beings, recognize a very similar epitope or epitopes of Der p 1.

The participation of T cells in the allergic response is being increasingly investigated. T cells from atopic patients, but not those from control subjects, respond to the purified Der p 1 in vitro and are HLA-DR restricted.^{13,14} It has been reported that HDM-specific CD4⁺ T cells from an atopic individual dominantly express V α 3 (Tcr α V3) and V β 8 (Tcr β V8) gene segments with minimal conservation of junctional sequence.¹⁵ In animal studies it has been shown that different T-cell subsets recognize Der p 1 or Der f 1 in Der f 1-immunized H-2^b mice. On the basis of the sequences of six T-cell clones specific to Der p 1, T-cell receptor use does not appear to be restricted.¹⁶

In this study we examined HDM-induced sensitivity in a mouse model. The cellular and humoral responses, histopathologic examination of lesions, and molecular basis analysis of HDM-specific T-cell hybridoma clones have been investigated. A better understanding of immune mechanisms in the animal model will provide a window through which to understand allergic sensitivity and help to design an effective therapy to ameliorate these allergic diseases.

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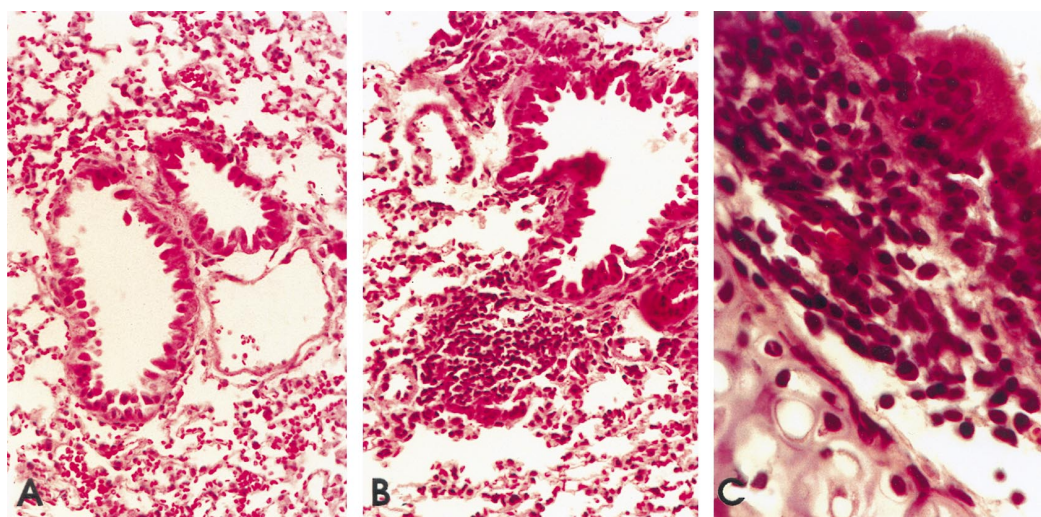


FIG. 1. Histopathologic examination of HDM-sensitized B10.RIII mice. Group of three B10.RIII mice were immunized by injection and intranasal inhalation of HDM extract as described in Methods. Three control mice were immunized by injection with CFA and intranasal inhalation with PBS buffer. Lung tissues from experimental and control groups of mice were removed on day 42 after immunization. **A**, Lung tissue from B10.RIII control mouse ($\times 400$). **B**, Lung tissue from immunized mouse ($\times 400$). **C**, Bronchial wall of immunized mice ($\times 1000$). Mononuclear cell infiltration is clearly observed in **B** and **C**.

METHODS

HDMs and mice

HDM crude extract (Der p allergen) was purchased from Bayer Co. (Spokane, Wash.). Mice (B10.RIII [H-2^r]) were purchased at the age of 6 to 10 weeks from the Jackson Laboratory (Bar Harbor, Me.) and bred at the University of Tennessee in Memphis. All the synthetic peptides were synthesized with a solid-phase technique on a model 430A Applied Biosystem Synthesizer (Applied Biosystem, Inc.) and purified by high pressure liquid chromatography either at the University of Tennessee at Memphis or Lucky Biotechnology (Taejon, Korea).

Culture media

Serum-free Ventrex HL-1 medium (Ventrex Laboratories, Portland, Me.) was supplemented with 1 mmol/L sodium pyruvate, 100 μ g/ml penicillin, 100 μ g/ml streptomycin, 2 mmol/L glutamine, 5×10^{-5} mol/L 2-mercaptoethanol, 20 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES; pH 7.4), and $1 \times$ nonessential amino acids. RPMI medium 1640 was supplemented with 10% fetal calf serum (Hyclone Laboratories, Logan, Utah), 1 mmol/L sodium pyruvate, 100 μ g/ml penicillin, 100 μ g/ml streptomycin, 2 mmol/L glutamine, 5×10^{-5} mol/L 2-mercaptoethanol, 20 mmol/L HEPES (pH 7.4), and $1 \times$ nonessential amino acids. RPMI medium 1640, sodium pyruvate, penicillin, streptomycin, glutamine, HEPES, and $50 \times$ nonessential amino acids were purchased from Irvine Scientific (Santa Ana, Calif.), and 2-mercaptoethanol was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Immunization of mice

Crude HDM extract was dialyzed, concentrated, and dissolved in PBS buffer (pH 7.4). HDM allergen (2 mg/ml) was emulsified with an equal volume of complete Freund's adjuvant (CFA) for immunization. For the T-cell proliferation assay in vitro and production of T-cell hybridoma clones, three mice

were immunized subcutaneously at the base of the tail (100 μ g of HDM per mouse). The mice were also given an intraperitoneal dose of 300 ng of purified pertussis toxin (List Biological Laboratories Inc., Campbell, Calif.) at 24 and 72 hours after HDM immunization. Seven days later, the mice were boosted again with the same amount of antigen in incomplete Freund's adjuvant.

In the HDM-specific IgE study (ELISA), the mice were divided into four groups. In the intranasally inhaled-immunization group, the mice were injected with CFA (without antigen) on day 1, and they were made to intranasally inhale HDM extract (10 μ g of HDM in PBS buffer) on days 7, 14, 21, 28, 35, and 42. The injection-immunized group of mice were subcutaneously immunized at the base of the tail with HDM extract and challenged with pertussis as described previously. This group of mice was also made to intranasally inhale PBS buffer on days 7, 14, 21, 28, 35, and 42. In the injection and intranasal inhalation-immunization group of mice, the mice both received injection at the base of the tail and were made to intranasally inhale HDM as described above. In the control group of mice, the mice received an injection with CFA and were made to intranasally inhale the PBS buffer.

Production of T-cell lines and T-cell hybridoma clones

In the injection-immunized group of mice, the draining lymph node cells were harvested from the inguinal, popliteal, and periaortic lymph nodes 10 days after the boost. The lymph node cells (4×10^6 cells/ml) were cultured with HDM extract (10 μ g/ml) and irradiated syngeneic spleen cells (2×10^6 cells/ml) in complete Ventrex HL-1 serum-free medium. Five days later, the resuspended T-cell blasts were expanded by IL-2 (20 U/ml; Boehringer, Indianapolis, Ind.) for 48 hours. T-cell hybridomas were generated by 50% polyethylene glycol of MW 3000-3700 (Sigma Chemical Co., St. Louis, Mo.) fusion of the expanded population of HDM-reactive T cells with equal

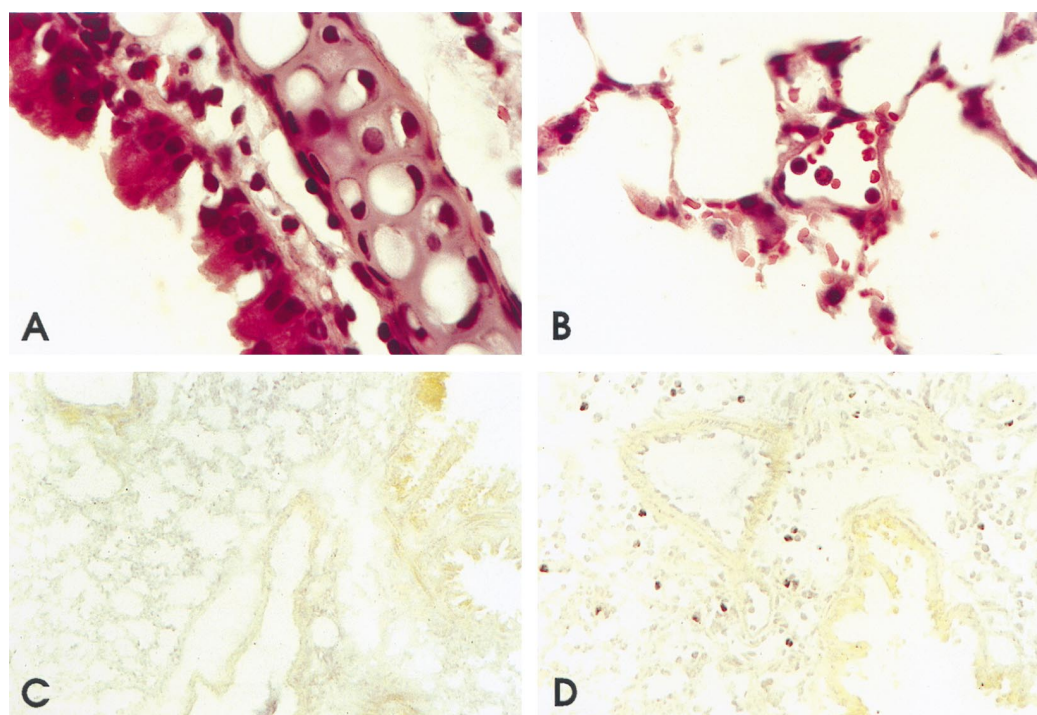


FIG. 2. Eosinophils and T cells among mononuclear cell-infiltrated lesions. Lesions from lungs and bronchial walls of HDM-sensitized mice in Fig. 1 were stained for eosinophils and T cells. **A**, Bronchial wall of immunized mice ($\times 1000$). Eosinophils were observed at mononuclear cell-infiltrated lesion. **B**, Vessel of lung lesion of immunized mice ($\times 400$). Eosinophils were observed at mononuclear cell-infiltrated lesions. **C**, Lung tissue of control mice ($\times 1000$). Negative control for T-cell monoclonal antibody staining. **D**, Lung tissue of immunized mice ($\times 200$). T cells (brown color) were observed at mononuclear cell-infiltrated lesions.

numbers of the hypoxanthine guanine phosphoribosyl transferase-deficient BW α - β - cell line. Hybrids were selected through HAT (100 μ mol/L hypoxanthine, 0.4 μ mol/L aminopterin, and 16 μ mol/L thymidine) and HT medium (100 μ mol/L hypoxanthine and 16 μ mol/L thymidine) and cloned at 0.3 cells/well. The HDM specificity of these T-cell hybridoma clones was demonstrated by antigen-specific proliferation and cytokine production assays.

T-cell proliferation assay (in vitro)

For T-cell proliferation assays, 1×10^6 HDM-specific T cells were cultured for 4 days with the crude extract of HDM and a serial dilution of allergen (range, 0.7 to 50 μ g/ml) in the presence of 4×10^5 syngeneic irradiated spleen cells, and then pulsed for 18 hours with 1 μ Ci of tritiated methyl-thymidine (Du Pont, Wilmington, Del.) per well in 96-well plates. The incorporation of [^{3} H]-methyl-thymidine was measured by liquid scintillation counting. Values were expressed in counts per minute as follows: Counts per minute with antigen – Counts per minute without antigen. Each sample was run in triplicate.

Determination of HDM-specific IgE

The blood from four groups of mice (immunization by injection, immunization by inhalation, immunization by injection and inhalation, and control) was collected on days 0, 14, 28, and 42 after immunization. The red blood cells were separated from sera by centrifugation. The HDM-specific IgE levels were determined by ELISA as follows. One hundred microliters of HDM extract (5 μ g/ml in 0.1 mol/L carbonate buffer, pH 9.6)

was dispensed in each well of a polystyrene microtiter plate (Costa, Cambridge, Mass.) and incubated overnight at 4° C. At the same time, mouse sera were incubated in anti-mouse whole-molecule IgG antibody-coated 96-well plates (Sigma, St. Louis, Mo.) at 4° C for 4 hours to eliminate the HDM-specific IgG. The HDM-coated plates were washed three times in 0.05% PBS-Tween buffer and incubated with IgG depleted sera from the immunized and control groups overnight at 4° C. The plates were washed five times with 0.05% PBS-Tween buffer and incubated overnight with ϵ -chain-specific anti-mouse IgE antibody (Bioscience Resource Project, Kennebunk, Me.) at 4° C. The plates were washed five times (0.05% PBS-Tween buffer) before the addition of citric acid-phosphate buffer (pH 5.0) containing 0.15 mg/ml of o-phenylenediamine (Sigma Co., St Louis, Mo.). The color was developed at room temperature and the reaction was stopped by 2.5 mol/L sulfuric acid. The color was measured at 492 nm (Bio-Rad, Richmond, Calif.).

Immunohistologic study of lung and trachea tissues

The lung and trachea tissues from the experimental (immunized, intranasal inhalation, or both groups) and control groups of mice were removed after the final intranasal inhalation (42 days after immunization). They were fixed with periodate-lysine-paraformaldehyde solution for 24 hours at 4° C. The specimens were rinsed with 0.01 mol/L of PBS (pH 7.4) containing 10% to 20% sucrose for 36 hours at 4° C, embedded in OCT compound (Miles Laboratories Inc., Elkhart, Ind.), and immediately frozen. The tracheal tissue

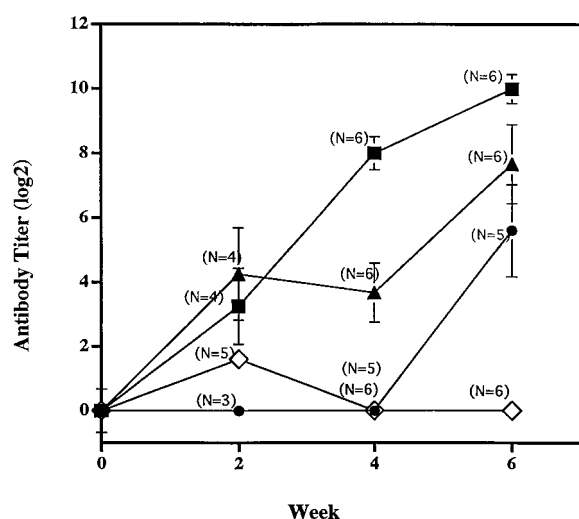


FIG. 3. HDM-specific IgE production in sensitized B10.RIII mice. B10.RIII (H-2^d) mice were immunized by injection, intranasal inhalation, or both with HDM extract as described in Methods. Control group of mice were immunized by injection with CFA and intranasal inhalation with PBS buffer. HDM-specific IgE was determined by ELISA assay. *Filled boxes* represent sera from mice immunized by injection and intranasal inhalation with HDM. *Filled triangles* represent sera from mice immunized by injection with HDM. *Filled circles* represent sera from mice immunized by intranasal inhalation with HDM. *Open diamonds* represent sera from control mice. Mean of antibody titer and standard error are indicated.

was immersed in 10% ethylenediamine tetraacetic acid and decalcified for 10 days at 4° C. Frozen sections cut 4 to 6 μ m in thickness were rehydrated and rinsed in cold PBS. The endogenous pseudoperoxidase was blocked with absolute methanol containing 0.5% hydrogen peroxide for 20 minutes at room temperature. The sections were treated with 10% normal goat serum in PBS to reduce the nonspecific binding. Biotin conjugated hamster anti-mouse TCR $\alpha\beta$ (Tcrab) monoclonal antibody (Pharmingen, San Diego, Calif.) diluted to 1:200 in PBS containing 0.5% bovine serum albumin was applied to the sections and incubated overnight at 4° C. After rinsing, the sections were incubated with avidin-biotin peroxidase complexes (Vectastain Elite ABC Kit; Vector Laboratories Inc., Burlingame, Calif.) for 30 minutes at room temperature and rinsed sufficiently with PBS. The reaction was developed with 0.02% 3,3'-diaminobenzidine in 0.05 mol/L of Tris buffer (pH 7.6) with 0.005% hydrogen peroxidase for 7 minutes. The counterstained sections were dehydrated, cleared in xylene, and mounted. PBS and normal hamster serum were used in place of the primary antibody on some sections as a control for the specificity of antibody staining.

HDM-specific T-cell receptor α -chain (Tcr α) and β -chain (Tcr β) sequences

Total messenger RNA (mRNA) was prepared by using Ultraspec extraction solution (Biotecx Lab., Houston, Tex.). Briefly, 1×10^7 cells were suspended in 1 ml of the Ultraspec extraction solution. After 5 minutes incubation in ice, 0.1 ml of chloroform was added and incubated on ice for an additional 5

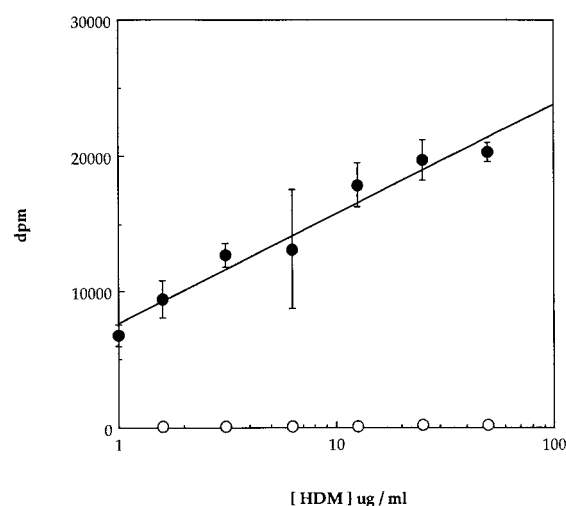


FIG. 4. In vitro T-cell stimulation by HDM antigen. A pool of lymphocytes was taken from the lymph nodes of three HDM-sensitized or control B10.RIII mice. The HDM concentration in T-cell stimulation ranged from 0.7 to 50 μ g/ml. Cell concentration in this assay was 1×10^6 per well, and the incubation time was 96 hours. [³H]-methyl thymidine was added during the last 18 hours of incubation. *Filled circles* represent T cells from HDM-immunized mice. *Open circles* represent T cells from naive mice.

minutes. Total mRNA was separated from DNA and proteins by centrifugation at 12,000 rpm for 15 minutes. Total mRNA in the aqueous phase was precipitated with an equal volume of isopropanol, followed by two washes with 70% ethanol. One microgram of mRNA was converted to first-strand cDNA with the Perkin-Elmer RT/PCR kit (Perkin-Elmer Co., Branchburg, N.J.). The Tcr α and Tcr β gene segments were amplified from the cDNA. The primers for Tcr α chain amplification are T-cell receptor α -chain constant region (Tcr α C) at position 54 (5'-AGA GGG TGC TGT CCT GAG AC-3') paired with a mixture of Tcr α degenerate primers (A: 5'-CCT TAT CTG TTC TGG TAT GTG CA-3', B: 5'-TAC TAC TTC CCT TGG TAC AAG CA-3', C: 5'-AAG TAC TTC TAG TGG TAC CGA CAG-3', D: 5'-ACC CAG CTG CAG TGG TTT TAC CAA-3'). Primer A primes to Tcr α V 3, 4, 8, and 13 subfamilies at positions 148, 148, 157, and 163, respectively. Primer B primes to Tcr α V 2, 5, 7, 15.1, 16, 18, and 19 subfamilies at positions 148, 106, 154, 156, 131, 112, and 184, respectively. Primer C primes to Tcr α V 1, 6, 11, 12, 14, and 15.2 subfamilies at positions 151, 139, 94, 139, 179, and 228, respectively. Primer D primes to Tcr α V 9 and 10 subfamilies at positions 139 and 169, respectively. The Tcr β gene segment was amplified from cDNA with T-cell receptor β -chain constant region (Tcr β C) at position 78 (5'-GCC AAG CAC ACG AGG GTA GCC-3') paired with a Tcr β V degenerate primer (5'-GAG CTG GAA TTC TGT ACT GG TAT CAA CAG-3'). This Tcr β V degenerate primer primes to Tcr β V1 through Tcr β V17 subfamilies at position 90 of the Tcr β V1 subfamily. Thermal cycling conditions were 35 cycles of 1 minute at 94° C, 1 minute at 45° C, and 1 minute at 72° C. The amplified product was ligated into the TA cloning vector (pCRII) overnight at 12° C and then transformed into One Shot cells (Invitrogen, San Diego, Calif.). Colonies containing inserts were selected by blue/white screening and grown in large quantities for DNA preparation. The se-

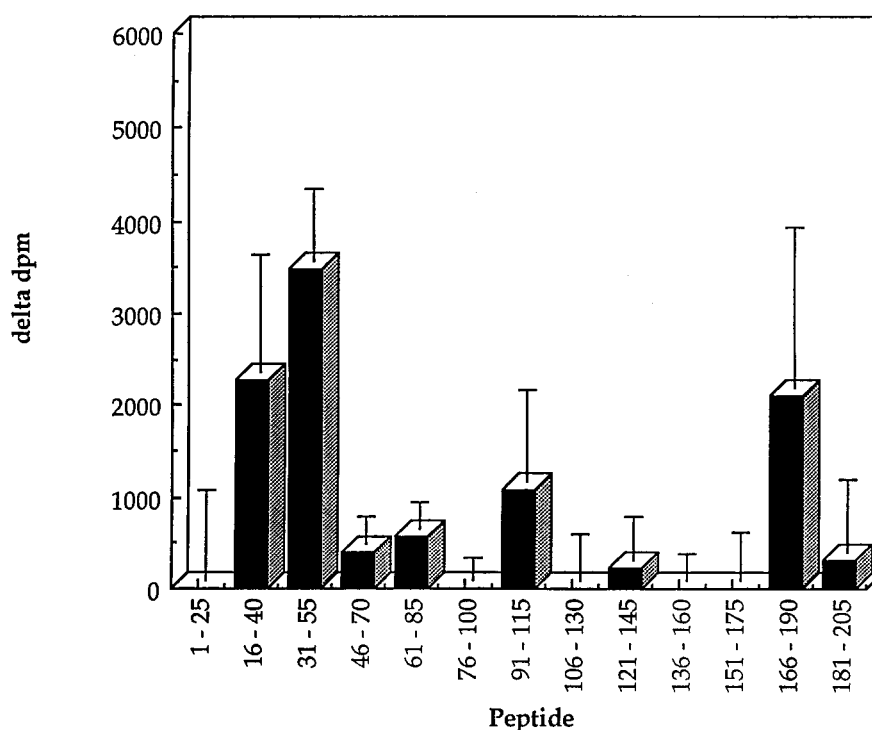


FIG. 5. T-cell epitopes study. A pool of lymphocytes was taken from lymph nodes of three sensitized B10.RIII mice. Synthetic peptide concentration in T-cell stimulation assay was fixed at 10 μ mol/L. T-cell concentrations and procedures in this assay were the same as described in Fig. 4. Synthetic peptides of Der p 1 (sequence is derived from reference 3) are as follows (N' to C'): 1-25, TNACSGINGNAPAEIDLQMRVTPTI; 16-40, LRQM-RTVTP IRMQGGCGSCWAFSGV; 31-55, CGSCWAFSGVAATE SAYLAHRNOSL; 46-70, AYLAHRNOSLDLAE-QELVDCASQHG; 61-85, ELVDC ASQHGCHGDTIPRGIEYIQH; 76-100, IPRGIEYIQHNGVVQESYYRYVARE; 91-115, ESYRYVAREQSCRRPNQAQRFGISN; 106-130, PNAQRFGISNYCQIYP PNANKIREA; 121-145, PPNANKIREALAQ PQRYCRHYWTIK; 136-160, RYCRHYWTIKDLDAF RHYDGRITIQ; 151-175, RHYDGRITIQRDNGYQPNY-HAVNIV; 166-190, QPNY HAVNIVGYSNAQGVYDWIVRN; L, 181-205, QGVYDWIVRNSWDTNWDNGYGYFA.

quences of the clones were obtained by Sequenase DNA polymerase (USB, Cleveland, Ohio). The sequences were assigned to known Tcr α V and Tcr β V families.

RESULTS

Immunohistologic study of lung and trachea tissue

High specific IgE titer and mononuclear cell infiltration in lung lesions have been reported in allergic sensitivity. Thus we investigated whether cell infiltration was observed in HDM-immunized mice. When the B10.RIII mice underwent immunization by injection and intranasal inhalation with HDM, eosinophilic and mononuclear cell infiltration was observed in the lungs and the bronchial walls of the immunized group (Fig. 1, *B* and *C*, and Fig. 2, *A* and *B*). This cell infiltration was not observed in the control mice that were injected with CFA and inhaled the PBS buffer, that were immunized by injection alone, or that were immunized by inhalation alone with HDM extract (Fig. 1, *A*). Moreover, in the immunohistologic examination, Tcr α -positive cells were identified in the mononuclear cell infiltration area of the lung lesions (Fig. 2, *D*). These

phenomena were not observed in the control groups of mice (Fig. 2, *C*). This evidence suggests that sensitivity and inflammation occur in the lung and bronchial lesions of these sensitized mice.

HDM-specific IgE responses in immunized and/or intranasal inhalationally immunized mice

One of the important factors in allergic sensitivity is the production of allergen-specific IgE. Therefore we have investigated the production of HDM-specific IgE in the sensitized B10.RIII mice. At 6 weeks after immunization, the HDM intranasally immunized group of B10.RIII mice showed HDM-specific IgE responses approximately 32 times higher than those of the control group of mice. The group of mice immunized by HDM injection showed IgE responses approximately 120 times higher than those of the control mice. This response began from the first week after immunization and persisted until 6 weeks after immunization at the end of this study. The mice immunized by intranasal inhalation and HDM injection showed specific IgE responses approximately 10^3 times higher than those in the control group.

TABLE I. Summary of Tcra and Tcrb genes usage of HDM-specific T-cell hybridoma clones

Clone		Tcra										
1411	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	AGT	GGA	ATA	GTG	<u>CAG</u>		TcraJ32
		Y	C	A	L	S	G	I	V	Q		
1455	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	AGT	GGA	ATA	GTG	<u>CAG</u>		TcraJ32
		Y	C	A	L	S	G	I	V	Q		
2052	TcraV19	<u>CTC</u>	<u>TGT</u>	<u>GCA</u>	CCA	GGC	ATG	GGC	AAT	<u>AAC</u>		TcraJ24
		L	C	A	P	G	S	G	N	N		
2053	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	ACC	CAC	ACA	AAT	<u>GCT</u>		TcraJ23
		Y	C	A	L	T	H	T	N	A		
2140	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	AGT	GGA	ATA	GTG	<u>CAG</u>		TcraJ32
		Y	C	A	L	S	G	I	V	Q		
2202	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	AGT	GGA	ATA	GTG	<u>CAG</u>		TcraJ32
		Y	C	A	L	S	G	I	V	Q		
3201	TcraV19	<u>CTC</u>	<u>TGT</u>	<u>GCA</u>	CCA	GGC	ATG	GGC	AAT	<u>AAC</u>		TcraJ24
		L	C	A	P	G	M	G	N	N		
3212	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	AGT	GGA	ATA	GTG	<u>CAG</u>		TcraJ32
		Y	C	A	L	S	G	I	V	Q		
3904	TcraV3	<u>TGC</u>	<u>TGT</u>	<u>GAG</u>	CGG	GCT	GGA	GGC	AAT	<u>AAT</u>		TcraJ47
		C	C	E	R	A	G	G	N	N		
3907	TcraV3	<u>TGC</u>	<u>TGT</u>	<u>GAG</u>	CGC	GTT	AAT	ACA	GGA	<u>AAC</u>		TcraJ33
		C	C	E	R	V	N	T	G	N		
4145	TcraV8	<u>TAC</u>	<u>TGT</u>	<u>GCT</u>	CTG	AGG	GGA	AGC	TTA			TcraJ18
		Y	C	A	L	R	G	S	L			

ND, Not determined.

*Southern blot analysis.

This strong specific IgE response started from a week after immunization and continued steadily until 6 weeks after immunization. The control group showed no specific IgE responses to HDM during this 6-week period (Fig. 3).

In vitro T-cell stimulation

Because T cells play a pivotal role in immune responses, we wanted to examine the HDM-specific T-cell response in the sensitized B10.RIII mice. The T cells from B10.RIII mice immunized by HDM extract showed a linear stimulation when the concentration of HDM ranged from 0.5 μ g/ml to 50 μ g/ml. T cells harvested from the naive mice failed to be stimulated at this range of allergen concentration (Fig. 4). These results suggest that T-cell stimulation by allergen is HDM-specific and not caused by a mitogen effect.

T-cell epitope study

Multiple T-cell epitopes from Der p 1 or Der p 2 have been identified in H-2^b, H-2^k, and H-2^d background mice, and the T-cell epitopes of Der p 1 have been examined in patients allergic to HDM.^{16,17} We have investigated whether T cells from H-2^r mice recognize multiple epitopes. In this study a series of overlapping peptides from Der p 1 were synthesized and used for T-cell stimulation in vitro (Fig. 5). At a peptide concentration of 10 μ mol/L, T cells from H-2^r mice immunized by HDM were stimulated by four peptides comprising residues 16-40, 31-55, 91-115, and 166-190. The peptides at residues 16-40 and 31-55 could be sharing a single T-cell epitope in the region 31-40. Further experiments

are needed to test this possibility. The epitopes at residues 16-55 provided the best in vitro T-cell stimulation. Epitopes at residues 166-190 showed a moderate T-cell stimulation. Epitope residues 91-115 provided the least T-cell stimulation.

Tcra and Tcrb usage in the HDM-specific T-cell hybridoma clones

We have made 11 HDM-specific T-cell hybridoma clones. We have completed 11 Tcra and four Tcrb sequences of these HDM-specific T-cell hybridoma clones. The results suggested that TcraV and TcrbV usage in these clones is not random. It was predominantly restricted to the TcraV8 (64%) and TcrbV6 (91%) subfamilies. Three minor subfamilies (TcraV3, 18%; TcraV19, 18%; and TcrbV1, 9%) were also observed (Table I). These TcraV gene segments joined with less restricted TcraJ gene segments (TcraJ18, 9%; TcraJ23, 9%; TcraJ24, 18%; TcraJ32, 46%; TcraJ33, 9%; and TcraJ47, 9%). Of these 11 hybridoma clones, seven clones expressed TcraV8 subfamily and five clones (1411, 1455, 2140, 2202, and 3212) encoded identical nucleotide sequences. Clone 2053 expressed TcraV8 and joined to TcraJ23, whereas clone 4145 expressed TcraV8 and joined to TcraJ18. The N-region, between the variable (V) and joint (J) region, also showed a restrictive insert of CTG (Leu). On average, there were seven nucleotides (range, 3 to 10 nucleotides) added per N-region. We have completed four TcrbV sequences of these HDM-specific T-cell hybridoma clones. Hybridoma clones of 1411, 3907, and 3212 expressed the TcrbV6 subfamily and joined to the TcrbJ2.3 gene

Tcrb													
TcrbD2													
1411	TcrbV6	<u>GCC</u>	<u>AGC</u>	<u>AGG</u>	<u>AGA</u>	<u>CTG</u>	<u>GGA</u>	<u>GGC</u>	<u>GCA</u>	<u>GAA</u>	<u>ACG</u>	<u>CTG</u>	TcrbJ2.3
		A	S	R	R	L	G	G	A	E	T	L	
1455	TcrbV6* (SB)												
2052	TcrbV6* (SB)												
2053	ND												
2140	TcrbV6* (SB)												
2202	TcrbV6* (SB)												
TcrbD1													
3201	TcrbV1	<u>GCC</u>	<u>AGC</u>	<u>AGC</u>	<u>ACT</u>	<u>CCG</u>	<u>GAC</u>	<u>ACC</u>	<u>TAT</u>	<u>GCT</u>	<u>GAG</u>	<u>CAG</u>	TcrbJ2.1
		A	S	S	T	P	D	T	Y	A	E	Q	
TcrbD1													
3212	TcrbV6	<u>GCC</u>	<u>AGC</u>	<u>AGG</u>	<u>AGA</u>	<u>CTG</u>	<u>GGA</u>	<u>GGG</u>	<u>GGG</u>	<u>GAA</u>	<u>ACG</u>	<u>CTG</u>	TcrbJ2.3
		A	S	R	R	L	G	G	G	E	T	L	
3904	TcrbV6* (SB)												
TcrbD2													
3907	TcrbV6	<u>TGT</u>	<u>GCC</u>	<u>AGC</u>	<u>AGG</u>	<u>AGA</u>	<u>CTG</u>	<u>GGG</u>	<u>AGT</u>	<u>GCA</u>	<u>GAA</u>	<u>ACG</u>	TcrbJ2.3
		C	A	S	R	R	L	G	S	A	E	T	
4145	TcrbV6* (SB)												

segment with different TcrbD gene segments (TcrbD2 for clones 1411 and 3907 and TcrbD1 for clone 3212, respectively). Clone 3201 expressed TcrbV1, TcrbD1, and TcrbJ2.1 gene segments. Although clones 1411 and 3907 have the same Tcrb gene rearrangement, the N regions between V and diversity (D), and D and J, as well as TcrbV, TcrbJ gene segments are different. These results suggest that clone 1411 and 3907 are different clones.

We also performed a Southern blot to examine the remaining TcrbV use in these clones. All of the clones (1455, 2052, 2140, 2202, 3904, and 4145) hybridized to the TcrbV6 probe on digestion with the restriction enzymes EcoRI and HindIII (data not shown). The expression of the TcrbV gene segment in clone 2053 has not been identified. All the EcoRI and Hind III digested DNA from these clones (1455, 2052, 2140, 2202, 3904, and 4145) failed to hybridize to the TcrbV1 probe (data not shown).

DISCUSSION

The importance of T cells in allergic disorders has been discussed elsewhere. However, the restrictive use of allergen-specific T cells is controversial. This is due to a number of factors, among them the heterogeneous human genetic background and the variety of environmental factors affecting the population. In this study we have tried to examine the immune mechanism in HDM-induced sensitivity in an animal model. This animal model could lead to a better understanding of the immune mechanism and to the design of experimental therapies. To maintain maximum resemblance to the natural antigen, we have chosen to use a crude extract of HDM rather than individual purified protein antigens. Because the life span of mice is short

and the incidence of environmentally induced allergic sensitivity is low, we used immunization by injection to rapidly induce the immune response. To boost the IgE response, we challenged the immunized mice with pertussis. The injection immunization process, with accompanying pertussis toxin injection, produces an enhanced IgE response to antigen, and has been established in other animal studies.¹⁸ Finally, we administered allergen intranasally to mimic the natural pathway of antigen exposure to intranasal and lung tissue.

It is well documented that the presence of allergen-specific IgE is one of the major indications of an allergic disorder. In this study the sensitized B10.RIII mice showed the HDM-specific IgE response and specific T-cell response to HDM in vitro. It has been reported that mice receiving alum-absorbed Der p 1 immunization produce a better IgE response than do mice challenged without alum.¹⁹ Our results suggest that mice sensitized to HDM extract without alum that receive pertussis challenge also produce a high HDM-specific IgE response. Immunization by injection and inhalation produced the best HDM-specific IgE response. These results suggest that the process of intranasal inhalation of allergen after immunization by injection enhances the immune response to the allergen. Regular exposure of aerosolized antigen or cryptic T-cell epitope to rodents that had a poor IgE response induced tolerance in allergen-specific IgE responses. However, this intranasal immunization failed to induce tolerance in rates with high IgE response.^{19, 20} Obviously, the mechanism by which intranasal inhalation enhances allergen sensitivity or induces tolerance needs to be carefully interpreted. It has been reported that HDM-specific IgE persisted

for up to 4 months after immunization.^{19, 21} The production of allergen-specific IgE beyond 6 weeks after primary immunization was not determined in this study.

The Der p 1 T-cell epitope and the expression of certain HLA genes in the HDM-hypersensitive patients have been examined. Multiple epitopes with HLA-restricted expressions were identified.^{16, 17} In an animal study, multiple T-cell epitopes have been identified in Der p 1 or Der p 2 mice immunized by injection.¹⁷ In our T-cell epitope study, the results suggest that T cells from B10.RIII mice immunized with HDM recognize multiple epitopes (Der p 1-derived peptides comprising residues 16-40, 31-55, 91-115, and 166-190). It is of interest to note that one of the epitopes (residues 91-115) is similar to a human T-cell epitope, despite the difference in the major histocompatibility complex background. It is not clear whether this cross-recognition of T-cell epitopes between mice and human beings is a coincidence.

When the mice were immunized with HDM by injection and intranasal inhalation, eosinophils and mononuclear cell infiltration was observed in the lungs and bronchial walls. Tcrab cells were observed among the eosinophils and mononuclear cell infiltration area. These phenomena were not observed in the control mice. The evidence clearly suggests that sensitivity and inflammation occur in these HDM-sensitized mice. It is not clear whether the same subsets of T cells are present both in the lung lesions and in the T-cell hybridoma clones.

It is of interest to note that Tcrav or Tcrbv gene segment usage of these HDM-specific T-cell hybridoma clones is restricted to Tcrav8 or Tcrbv6 subfamilies, respectively. Because the majority of these HDM-specific T-cell hybridoma clones expressed a Tcrav8 gene segment and shared an identical sequence, it is possible that a dominance of a certain T-cell clone is involved in HDM recognition. It is of interest to note that a Tcrbv6 gene segment could form a functional TCR with either a Tcrav3 or Tcrav8 subfamily. The Tcrav6 gene segment expressed in clone 3201 joined with Tcrav19 to form a functional TCR. The restrictive usage of T cells in this study offers a possible method to apply monoclonal antibodies to abolish sensitivity in vivo. The immune mechanism involved in HDM-atopic disease in human beings is much more complicated than in the animal study we presented here. However, several lines of evidence discussed in this article are consistent with phenomena or symptoms observed in patients with allergy.

In conclusion, our study of immune response to HDM in a mouse model demonstrates five things. First, HDM-specific IgE could be produced by injection, intranasal inhalation immunization, or both with allergen in CFA and challenge with pertussis. Second, the allergic immune response could be enhanced by intranasal inhalation immunization of allergen after injection immunization. Third, infiltration of eosinophils and mononuclear

cells, including Tcrab-positive cells, was observed in the lung and bronchial wall of HDM-sensitized mice. Fourth, HDM-specific T cells recognized multiple epitopes in the Der p 1 allergen. Fifth, TCR gene segment usage was restricted in HDM-specific T-cell hybridoma clones.

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