

Analysis of human T cell responses to the group II allergen of *Dermatophagoides* species: Localization of major antigenic sites

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Background: IgE antibodies reactive with the group II allergens of *Dermatophagoides* species (house dust mite [HDM]) are a major component of the allergic immune response in HDM-allergic atopic individuals.

Methods: Here we demonstrate, with the use of overlapping synthetic peptides of the group II allergen of *Dermatophagoides pteronyssinus* (Der p II), that polyclonal T cells isolated from the majority of atopic HDM-allergic individuals and healthy nonatopic control subjects respond to Der p II and that T-cell epitopes are present in all regions of the protein.

Results: From comparison of peptide-specific T-cell proliferation in both groups of individuals, it appears that together peptides 61-86 and 78-104 are the most frequently recognized (16 of 18 individuals). We also observed that nine of the 18 individuals responded to T-cell epitopes in the region 11-50, and with Der p II-reactive T-cell clones, three distinct T-cell epitopes were mapped within the sequence 11-35. Also, with the use of T-cell clones, two additional epitopes were identified at residues 81-96 and 91-101.

Conclusions: These results suggest that T-cell epitopes located in these regions (11-50 and 61-104) are immunodominant. The value of this information in the potential application of Der p II peptides to desensitize HDM allergic responses is discussed. (*J ALLERGY CLIN IMMUNOL* 1993;92:105-13.)

Key words: Peptides, T cells, *Dermatophagoides* species, house dust mite, Der p II, desensitization

In approximately 15% of the population,¹ T-cell recognition of proteins derived from common environmental aeroallergens may result in both the induction of specific IgE synthesis and the activation of polymorphonuclear granulocytes.

Abbreviations used:

APCs: Antigen presenting cells
HDM: House dust mite
IL-2: Interleukin-2
PBMCs: Peripheral blood mononuclear cells

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Together these two effector pathways contribute to the allergic immune response.²⁻⁵ The clinical symptoms that result from the activation of these effector pathways range from extrinsic asthma and allergic rhinitis to atopic dermatitis. Evidence accumulated from clinical studies has implicated proteins from the house dust mite (HDM) *Dermatophagoides* species (*D. pteronyssinus* and *D. farinae*) as a primary cause of respiratory allergy.^{6, 7}

Multiple antigens have been identified in extracts of HDM that bind IgE antibodies present in the sera of HDM-allergic patients⁸; nevertheless,

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p II 1-15   DQVDVKDCANHEIKK
p II 1-20   DQVDVKDCANHEIKKVLVPG
p II 11-25   HEIKKVLVPGCHGSE
p II 11-35   HEIKKVLVPGCHGSEPCI IHRGKPF
p II 21-35   CHGSEPCI IHRGKPF
p II 22-35   HGSEPCI IHRGKPF
p II 22-50   HGSEPCI IHRGKPFQLEAVFEAVQNTKTA
p II 31-47   RGKPFQLEAVFEAVQNT
p II 36-60   RGKPFQLEAVFEAVQNTKTAKIEI KASIDG
p II 41-55   FEAVQNTKTAKIEI K
p II 51-65   KIEI KASIDGLEVDV
p II 51-77   KIEI KASIDGLEVDV
p II 61-75   LEVDV
p II 61-86   LEVDV

(p II 51-77) PGIDPNACHYMK
(p II 61-75) PGIDPNACHY
(p II 61-86) PGIDPNACHYMKCPLVKGQQY
p II 71-86   NACHYMKCPLVKGQQY
p II 81-96   VKGQQYDIKYTNVVPK
p II 78-104  CPLVKGQQYDIKYTNVVPKIAPKSENV
p II 91-105  TWNVPKIAPKSENVV
p II 87-112  DIKYTNVVPKIAPKSENVVTVKVMG
p II 101-115 SENVVTVKVMGDDG
p II 105-129 VVVTVKVMGDDGVLACAIATHAKIRD
p II 111-129 MGDDGVLACAIATHAKIRD

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FIG. 1. Amino acid sequences of overlapping synthetic peptides derived from *Der p II*. Residues are indicated with the single letter code.

the major component of the humoral response appears to be directed against four major allergic proteins (groups I to IV).⁸⁻¹² Initially, although interest focused on the analysis of group I allergen-specific IgE, the importance of the group II allergens has now been substantiated by several independent studies, and it appears that the anti-group II (*Der p II*) reactive antibodies may account for between 40% and 88% of the HDM-specific IgE antibody response in HDM-allergic individuals.^{8, 11} Indeed in one report it was demonstrated that in the serum of 92% of the HDM-allergic patients investigated, IgE antibodies reactive with *Der f II* could be detected, whereas anti-*Der f I* and *Der f III* antibodies were present in the sera of only 78% and 16% of the patients, respectively.¹⁰

It is well established that CD4+ T-cell recognition of allergen is central to the induction of specific allergic immune responses.²⁻⁵ However, for many aeroallergens, including HDM, detailed information on the localization and frequency of T-cell epitopes within these proteins is limited. The identification of dominant T-cell determinants in specific allergens is relevant to understanding the functional role of antigen-specific T cells in the development of allergic inflammation and a critical initial step in the development of allergen-based immunotherapy.^{13, 14} There is evidence indicating that all of the individual group-specific proteins of HDM are antigenic for T cells

at the polyclonal level.⁴ However, on the basis of the initial serologic data, analysis of the antigen specificity of the HDM-reactive T cells has focused primarily on the response of atopic individuals to group I allergen prepared by electrophoresis and gel filtration.¹⁵ Similar to antibody responses, it is now apparent from specificity studies with T-cell clones,¹⁶ and more recently polyclonal populations,¹⁷ that a major component of the peripheral T-cell repertoire is reactive with the group II allergens. The complementary DNA sequences that encode both the group I and II genes have been cloned, and from the nucleotide sequence, the primary amino acid sequence has been derived.^{18, 19} Therefore it is now possible to investigate the T-cell epitopes in these proteins with the use of truncated recombinant proteins and synthetic peptides.

In this report to identify the T-cell epitopes present in *Der p II*, we synthesized overlapping peptides that cover the entire length of the molecule and screened them for their ability to induce proliferation of polyclonal T cells isolated from both HDM-allergic atopic individuals and normal healthy nonatopic control subjects. Using this approach, we demonstrate that both atopic and nonatopic individuals respond to *Der p II*. The proliferative responses of polyclonal T-cell populations indicate that, although T-cell epitopes are present throughout the protein, the sequences 11-50 and 61-104 contain the dominant

TABLE I. The polyclonal proliferative response of peripheral blood T cells to *Der p* II peptides

Subject	Medium	1-20	11-35	22-50	36-60	51-77	61-86	78-104	87-112	105-129	HDM
A1	1.6	5.6	13.9	12.7	7.0	3.8	54.6	35.7	17.5	7.0	61.2
A2	1.2	2.7	2.5	8.6	1.8	1.5	8.7	5.7	2.7	2.3	37.7
A3	1.0	2.2	3.2	3.5	13.4	2.0	10.0	5.9	5.5	22.9	25.5
A4	0.4	0.6	1.2	1.3	0.6	0.7	1.0	7.5	8.3	1.2	11.6
A5	3.0	5.0	6.8	4.3	4.5	4.2	8.1	3.8	4.1	4.7	33.5
A6	1.2	3.7	6.0	2.7	2.8	2.8	2.8	15.8	2.4	3.0	19.1
A7	0.9	0.9	1.5	1.6	0.9	1.5	5.9	1.7	1.5	2.2	72.7
A8	0.4	0.5	1.1	1.0	0.5	0.6	0.7	0.8	0.4	1.4	60.4
A9	1.3	2.0	2.7	3.0	2.5	3.4	2.8	3.7	2.4	1.3	36.9
NA1	2.1	4.4	4.8	9.5	2.5	2.4	22.0	14.6	2.8	5.3	71.9
NA2	0.3	0.6	0.6	0.5	0.8	4.5	4.0	3.1	0.5	0.8	6.1
NA3	1.0	4.0	3.5	8.0	10.5	10.9	9.3	13.3	3.2	3.8	13.9
NA4	0.3	0.3	0.4	0.6	0.5	2.6	3.3	1.1	0.4	4.3	8.3
NA5	1.2	1.6	3.5	2.1	4.0	2.0	2.8	5.6	2.6	1.7	56.2
NA6	0.4	0.5	0.6	0.5	0.5	1.0	1.2	1.0	1.1	0.8	18.4
NA7	0.7	0.7	1.0	0.7	0.7	0.7	0.7	0.7	0.8	1.0	11.1
NA8	0.6	0.7	0.7	0.7	0.8	0.7	0.8	1.5	0.6	0.7	31.0
NA9	1.4	1.4	3.1	1.4	1.4	1.4	3.5	2.1	1.4	1.7	11.9

PBMCs from atopic (A1 to A9) and nonatopic (NA1 to NA9) individuals were stimulated with the *Der p* II peptides over a concentration range of 0.1 to 50 $\mu\text{g/ml}$ in 7-day cultures. The maximal response for each peptide is given as counts per minute $\times 10^{-3}$ and compared with the control response of PBMCs in medium alone. SEM was less than 25% in all experiments. Responses of greater than or equal to 2.5 times background are in boldface type.

T-cell epitopes. From the analysis of HDM-reactive T-cell clones a cluster of three distinct T-cell epitopes was identified within residues 11-35, and an additional two epitopes at residues 81-96 and 91-101.

METHODS

Antigens

Lyophilized unfractionated extracts of *D. pteronyssinus* were generously provided by Bencard (Brentford, Middlesex, England). Recombinant *Der p* II was cloned and expressed as previously described.¹⁹ Peptides that covered the entire *Der p* II molecule (Fig. 1) were synthesized with solid-phase techniques on an Applied Biosystems (Foster City, Calif.) peptide synthesizer as described previously.²⁰ The purity of the peptides as determined by high-performance liquid chromatography was greater than 90%.

Characterization of patients

Atopic status with HDM allergy was defined by the presence of positive prick skin test results²¹ (≥ 3 mm diameter wheal) to *D. pteronyssinus* and *D. farinae* extracts in the presence of a negative saline control. Nine HDM-allergic atopic subjects, aged 25 to 40 years, were included in the study. All of these individuals had clinical symptoms of HDM allergy (perennial rhinitis and/or asthma) and had grade III or IV serum-specific IgE for HDM as measured by RAST.

Nonatopic status was defined in a subject who was

free of symptoms by the absence of positive skin prick test results to a panel of common environmental allergens (*D. pteronyssinus*, *D. farinae*, mixed grass pollen, birch pollen, *Aspergillus fumigatus*, *Cladosporium herbarum*, plane tree, nettle) in the presence of a positive wheal response to histamine hydrochloride (1 mg/ml) of ≥ 3 mm and a negative response to a saline control. Nine nonatopic individuals participated in the study. All had normal total IgE and negative specific IgE to the skin test panel of allergens as measured by RAST. No patients were receiving oral corticosteroid therapy or had previously undergone allergen-specific desensitization.

Isolation of human HDM-reactive clones

Cloned T cells reactive with *Der p* II peptides were isolated from HDM-allergic subjects by limiting dilution cloning from established T-cell lines as published elsewhere.^{16, 22} Briefly, peripheral blood mononuclear cells (PBMCs; $10^6/\text{ml}$) were stimulated with an optimal concentration of recombinant *Der p* II (10 $\mu\text{g/ml}$) for 7 days in RPMI-1640 medium supplemented with 2 mmol/L L-glutamine, 100 IU/ml penicillin/streptomycin (Gibco, Life Technologies, Paisley, Scotland) and 5% screened, inactivated human A+ serum (Blood Transfusion Service, Edgeware, England). Lymphoblasts enriched on Ficoll-Paque (Pharmacia Biotech Ltd., Milton Keynes, England) were established as a long-term line in the presence of irradiated autologous PBMCs ($5 \times 10^5/\text{ml}$; 2500 rad), recombinant *Der p* II (20 $\mu\text{g/ml}$) and interleukin-2 (IL-2), (10% vol/vol; Lymphocult T,

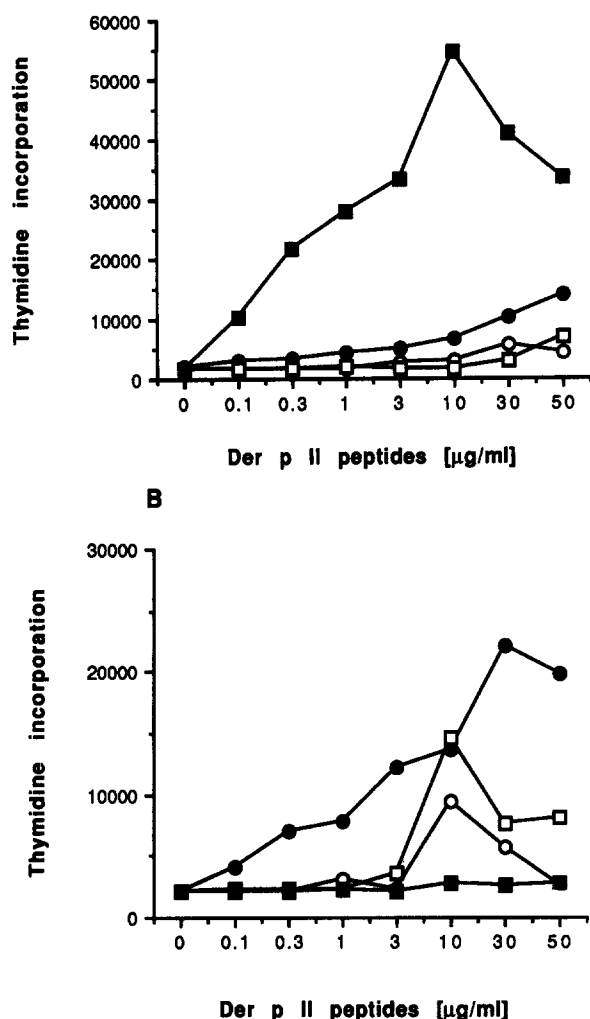


FIG. 2. The effect of peptide concentration on polyclonal T-cell proliferation to *Der p II*-derived peptides. Polyclonal T cells from an atopic individual were stimulated with different concentrations of the *Der p II* peptides 1-20 (○), 11-35 (●), 36-60 (□) and 61-86 (■) and proliferation (in counts per minute) was measured (A). In B the dose-dependent proliferation of T cells from a nonatopic individual to peptides 22-50 (○), 61-86 (●), 78-104 (□), and 87-112 (■) was measured.

Biotest Folex, Frankfurt, Germany) and subsequently cloned by limiting dilution. For cloning, viable cells (0.3 cells/well) were resuspended in supplemented medium and plated together with irradiated autologous PBMCs, *Der p II*, and IL-2. After 7 days, growing clones were expanded and maintained with IL-2 every 3 to 4 days, and irradiated autologous PBMCs and antigen were given every 7 days. Before use in proliferation assays, the clones were rested for 7 to 8 days after the last addition of antigen-presenting cells (APCs) and antigen.

Proliferation assays

Polyclonal T-cell responses. Unfractionated PBMCs (2.5×10^6 /ml) were cultured with unfraction-

ated *D. pteronyssinus* extract ($20 \mu\text{g/ml}$), recombinant *Der p II* ($10 \mu\text{g/ml}$), and *Der p II* peptides (0.1 to $50 \mu\text{g/ml}$). After 6 days' incubation, the cultures were pulsed with tritiated methyl thymidine ($1 \mu\text{Ci/well}$, Amersham International Inc., Amersham, England) and harvested 8 to 16 hours later. Proliferation as correlated with tritiated methyl thymidine incorporation was determined by liquid scintillation spectroscopy. The results were expressed as mean counts per minute for triplicate cultures with SEM less than 25% for all experiments with PBMCs.

Clonal T-cell responses. Cloned T cells (10^5 cells/ml) were stimulated with recombinant *Der p II* ($10 \mu\text{g/ml}$) or *Der p II* peptides (0.01 to $30 \mu\text{g/ml}$) in the presence of an equal number of irradiated (2500 rad) autologous PBMCs as APCs. After 60 hours of incubation, tritiated methyl thymidine was added to the cultures for 8 to 16 hours, and they were then harvested as described for the polyclonal T-cell proliferation assays. The results are expressed as mean counts per minute for triplicate cultures with SEM less than 15% for all experiments with cloned T cells. All experiments were repeated with similar results on at least three separate occasions.

RESULTS

Response of polyclonal T cells to *Der p II* peptides

To identify those regions of *Der p II* in which the major epitopes recognized by T cells are located, proliferative responses of PBMCs to unfractionated HDM extract and overlapping peptides derived from *Der p II* were compared for nine HDM-allergic atopic (donors A1 to A9, Table I) and nine nonatopic (donors NA1 to NA9, Table I) individuals. T cells from both groups of subjects proliferated to HDM extract, with responses ranging from 6.1 to 72.7×10^3 cpm, and in general, the magnitude of the response of atopic subjects exceeded that of the nonatopic subjects. The background proliferation of unstimulated T cells cultured in medium alone was in the range of 0.3 to 3×10^3 cpm (Table I). Comparison of the maximal T-cell response to each *Der p II* peptide with the medium control, for the panel of individuals investigated here, revealed that each peptide induced proliferation in at least one donor. Furthermore, when we used the criterion that proliferation equal to or greater than 2.5 times the background response of cells cultured in medium alone constitutes a positive response (highlighted in boldface type in Table I), the majority of subjects responded to one or more of the peptides. An exception was donor NA7 who failed to show a proliferative response to any of the peptides, despite a proliferative response of 10.3×10^3 cpm to the intact recombinant *Der p II*

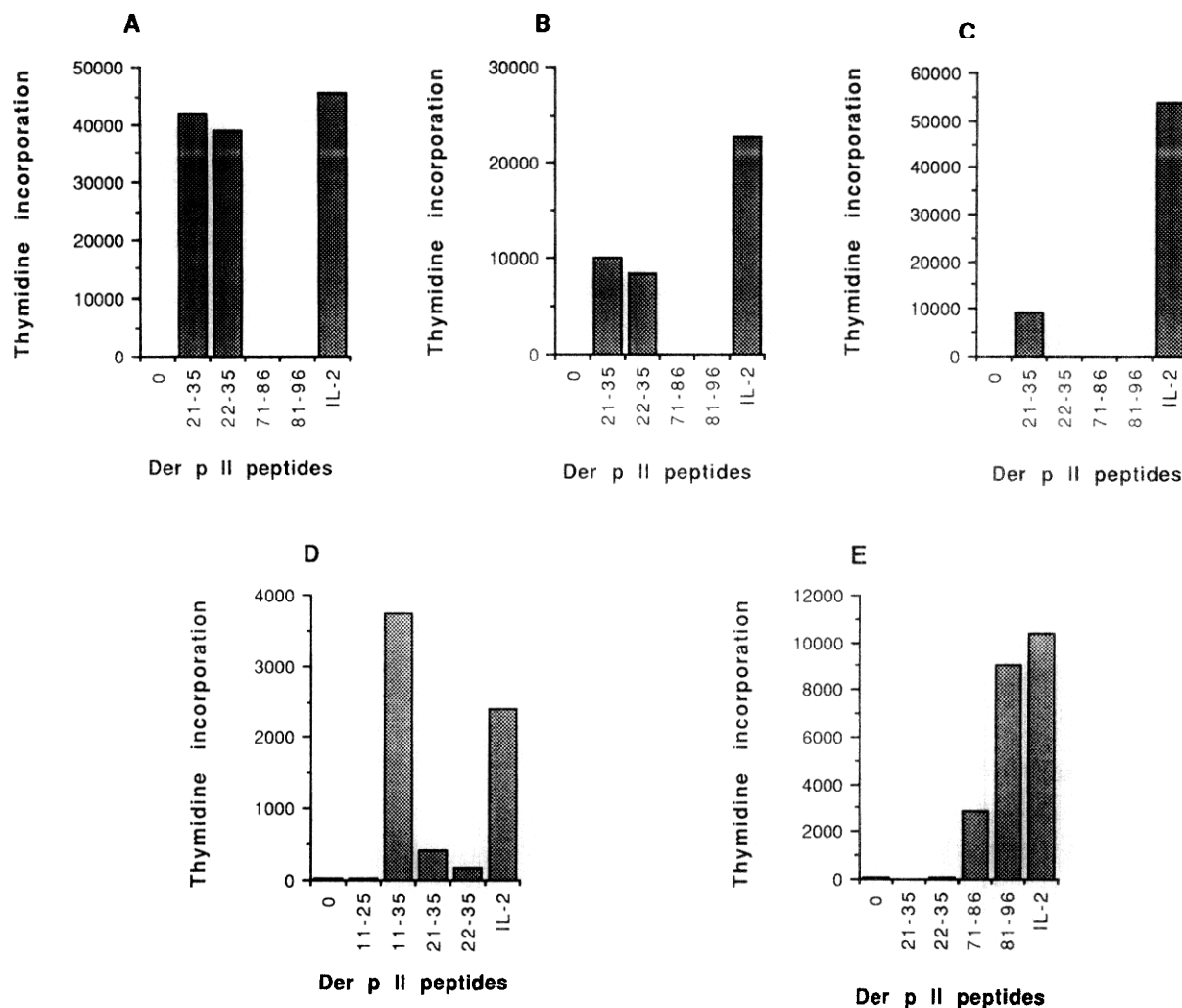


FIG. 3. Antigen specificity of *Der p II*-reactive T-cell clones. Cloned T cells from an HDM-atopic individual were stimulated with *Der p II* peptides in the presence of APCs, and proliferation was measured.

protein. The sequence 1-20 was the least antigenic of the peptides tested, in that for each of the subjects there were at least two peptides that stimulated greater proliferation; nevertheless, in donors A1, A6, and NA3 *Der p II* residues 1-20 induced stimulation indices of 3.5, 3.1, and 4, respectively (Table I). T-cell recognition of the *Der p II* peptides varied among individuals, and no pattern of proliferation that was unique to either the atopic or the nonatopic groups was observed (Table I). However, it appeared that both the atopic and nonatopic groups recognize predominantly T-cell determinants located within the region of 61-104 of *Der p II*, in that 16 of the 18 individuals had strong responses to peptides 61-86 and/or 78-104. Nine of the 18 subjects also responded to peptides spanning the region 11-50 (peptides 11-35 and 22-50). The sensitivity of the polyclonal T cells to different concentrations of

peptide as illustrated in the dose-response curves of two individuals, one atopic subject (donor A1; Fig. 2, A) and one nonatopic subject (donor NA1; Fig. 2, B) may result from a number of different effects such as variation in T-cell receptor affinities (Fig. 2). The induction of T-cell nonresponsiveness may account for the reduction in proliferation observed at higher concentrations of peptide as observed in the response of donor A1 to peptide 61-86, for example (Fig. 2, A). The influence of peptide concentration on proliferation, as illustrated in Fig. 2, reinforces the need to assay peptides over a wide range of concentrations to determine whether or not the T cells of a given individual are reactive. The failure of any one of the *Der p II* peptides to induce proliferation in all individuals indicates that these peptides do not have the properties of antigen-nonspecific mitogens.

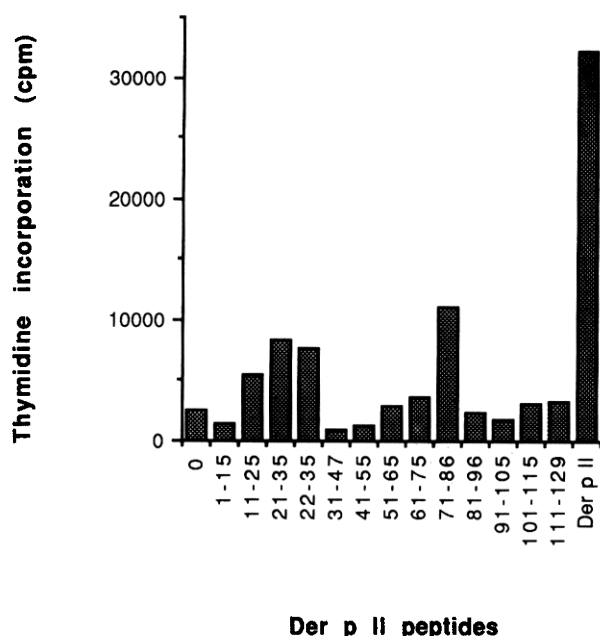


FIG. 4. Polyclonal T-cell response to *Der p II* peptides by HDM-allergic atopic individual from whom the T-cell clones shown in Fig. 3 were derived.

Isolation and characterization of *Der p II* reactive T-cell clones

In order to identify those regions of *Der p II* that constitute the major sites of T-cell recognition, PBMCs from an HDM-allergic atopic individual were stimulated with recombinant *Der p II* under optimal conditions and cloned by limiting dilution. Four of the *Der p II*-induced T-cell clones responded to epitopes at the amino terminal end of the molecule located between residues 11 and 35 (Fig. 3, A to C). Two of the T-cell clones proliferated to peptides 21-35 and 22-35 (Fig. 3, A and B), whereas one T-cell clone responded to only peptide 21-35 and not the sequence 22-35 (Fig. 3, C). The fourth T-cell clone was reactive with the N-terminus of *Der p II*, recognizing residues 11-35 but with considerably reduced proliferation to the peptide 21-35 (Fig. 3, D). An additional T-cell clone that responded strongly to the peptide containing residues 81-96 but less well to 71-86 was isolated. However, the ability of both peptides to induce proliferation of the T-cell clone suggests that the core residues of the epitope are associated with amino acids 81-86 (Fig. 3, E). In accordance with these results, the polyclonal T cells of this donor responded to recombinant *Der p II* and to the *Der p II* peptides 11-25 and 21-35, with the dominant response directed to residues 71-86 (Fig. 4). In contrast, proliferation to residues 81-96 was more limited (Fig. 4).

From the peripheral blood of a second HDM-allergic atopic individual, T-cell clones were induced with recombinant *Der p II*. Analysis of the antigen specificity of the cloned T cells isolated from this atopic subject revealed that they respond to peptide 91-105 but not to residues 81-96 or 101-115, suggesting that the epitope maps to residues 91-101 (Fig. 5). At the polyclonal level the T cells from this donor proliferated strongly to peptides containing residues 51-77, 61-86, and 78-104 (Fig. 6). Thus the presence of major T-cell determinants suggested by analysis of the polyclonal response are reflected at the clonal level.

DISCUSSION

Although initially the major allergenic determinants were thought to reside in the group I allergens of HDM, recent analyses of specific antibody⁸⁻¹² and T-cell responses^{16, 17} indicate that immune recognition of the group II allergens is an equally important component of the allergic response to HDM. In this study, designed to investigate the specificity of human T-cell responses to the group II allergen of *D. pteronyssinus* at the epitope level, we demonstrate, using overlapping peptides, the presence of multiple T-cell epitopes located in all regions of the protein capable of stimulating T cells from both atopic and non-atopic individuals. Furthermore, we observed that the majority of both atopic and nonatopic individuals examined here respond to T-cell epitopes located within regions 11-50 and 61-104.

The analysis of the proliferative response of polyclonal T-cell populations to intact *Der p II* expressed as a recombinant protein and synthetic peptides indicated that components of the T-cell repertoire of both atopic and nonatopic individuals are reactive with *Der p II*, although quantitatively the responses may differ. One nonatopic donor, however, although able to recognize the full-length *Der p II* molecule, failed to respond to any of the peptides tested. Differences in the amino acid sequences of "naturally processed" T-cell epitopes as compared with the peptides that were synthesized may account for this observation. The findings reported here support our previous observations that HDM-reactive T cells are present in the peripheral T-cell pool of both atopic and nonatopic individuals.¹⁶ In contrast, it has been reported that peripheral T cells from nonatopic individuals fail to proliferate to *Der p II*,¹⁷ as well as *Der p I*.^{15, 17} Furthermore, in the studies described here, it appears that the polyclonal T cells from both groups of subjects proliferate more vigorously to sites located between 61

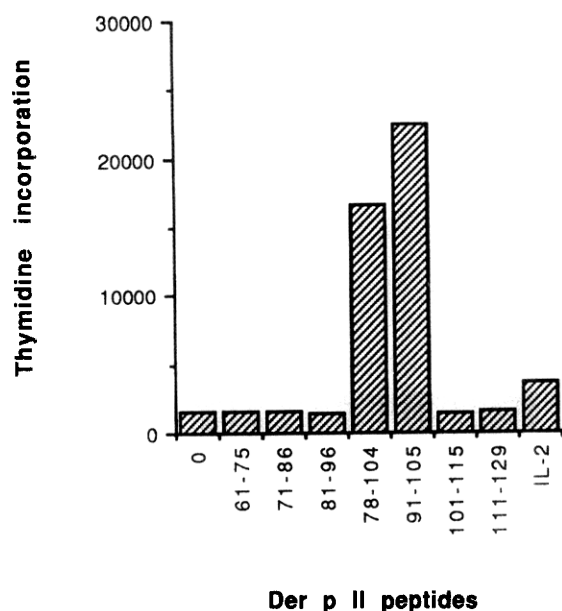


FIG. 5. Antigen specificity of *Der p II*-reactive T-cell clones. Cloned T cells from a HDM-atopic individual were stimulated with *Der p II* peptides in the presence of APCs.

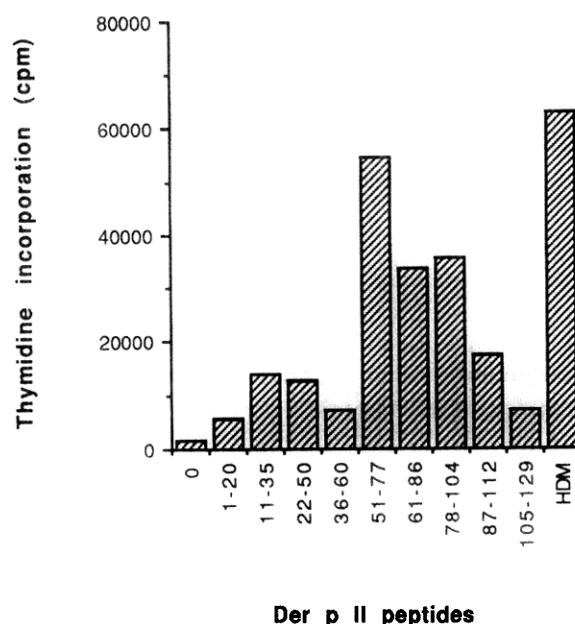


FIG. 6. Polyclonal T-cell response to *Der p II* peptides by HDM-allergic atopic individual from whom the T-cell clones shown in Fig. 5 were derived.

and 104 than to other sequences in *Der p II*. The reason that this region of the protein is immunodominant has not been defined but may reflect the presence of peptides capable of binding with high affinity to a wide range of major histocompatibility complex class II molecules. The majority of individuals responded to two or more peptides, and multiple T-cell epitopes appear to be distributed throughout the protein, as has been observed for *Der p I*,²³ with no apparent immunologically silent regions. Furthermore, no characteristic patterns of proliferation that allowed atopic and nonatopic subjects to be differentiated were observed, which may reflect differences in the HLA haplotype of the different individuals studied.

The presence of T-cell epitopes in *Der p II* was examined further by analyzing the antigen specificity of *Der p II*-induced T-cell clones isolated from the peripheral blood of atopic individuals. In the first of the atopic individuals (whose polyclonal T cells responded to residues 11-25, 21-35, and 71-86), four T-cell clones that recognize determinants at the amino terminus of the molecule were isolated. From the analysis of the fine antigen specificity of these T-cell clones, three closely related epitopes within residues 11-35 were identified. Another T-cell clone that was isolated from the same individual responded to stimulation with residues 81-96, even though the polyclonal T-cell response to this peptide was weak. With overlapping peptides from this region of *Der p II*, it was

observed that peptide 71-86, which was immunodominant at the polyclonal level, did not contain the optimal T-cell epitope recognized by the cloned T cells. For another atopic donor, from whom *Der p II*-specific T cells were cloned, their specificity for residues 97-105 overlapped with one of the regions identified at the polyclonal level. However, the most marked proliferative response of the unfractionated T cells was to residues 51-77. In both donors the antigen specificity of the T-cell clones and the polyclonal response overlapped, indicating that T-cell clones may provide a valid reflection of the T-cell repertoire as a whole. When synthetic peptides were used to probe the specificity of *Der p II*-reactive IgE, a B-cell epitope in the same region (residues 65-78) of the molecule was mapped.²⁴ Although T cells and B cells tend to recognize distinct epitopes within a protein,²⁵ there are instances in which it has been demonstrated that they overlap.²⁶ However, the majority of the *Der p II* peptides failed to bind IgE, which may reflect an alteration in the conformation of B-cell sites as compared with the intact protein.²⁷

The identification of the major T-cell determinants in allergens has practical applications in the design of immunotherapeutic agents for clinical desensitization, and considerable interest has been directed to the use of peptides for redirecting²⁸ or downregulating allergic immune responses.²⁹ Although we observed that the region

encompassed by residues 61-104 (peptides 61-86 and 78-104) is recognized by 89% of the individuals tested in this study, the overall heterogeneity of the antigen specificity of the *Der p* II-reactive T-cell repertoire suggests that peptide-based hyposensitization may be difficult to achieve. This will be further complicated by the differential sensitivity of polyclonal T cells to different concentrations of particular peptides. Furthermore, the region of the protein encompassing residues 61-104 contains an epitope that binds *Der p* II-specific IgE,²⁴ and therefore therapy based on peptides that contain the dominant T-cell epitopes in this instance may induce adverse reactions in patients with serum antibodies of this specificity. Even if nonresponsiveness were induced to dominant epitopes, it is possible that other minor regions may trigger allergic immune responses.³⁰ In the murine model of experimental allergic encephalomyelitis, during the induction phase of the disease, the T-cell response is directed toward an immunodominant site at the amino terminus of myelin basic protein.³¹ However, as the disease becomes chronic, or if encephalomyelitis is induced with the amino terminal peptide alone, additional determinants that are cryptic after the initial immunization then become immunogenic.³² This suggests that desensitization with polypeptide fragments of *Der p* II, in which the B-cell sites have been silenced, may offer an alternative approach to inducing nonresponsiveness to *Der p* II.

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