

T cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*

Richard M. O'Brien, PhD, FRACP,* Wayne R. Thomas, PhD,** and Andrew M. Wootton, PhD*** Footscray, Melbourne, and Perth, Australia

Proliferation assays were used to determine peripheral blood T cell responses to affinity-purified Der p I, Der p II, and Der f II allergens. Patients studied were sensitive to the house dust mite (HDM) either alone or in combination with other allergens. Control subjects were both nonatopic and atopic non-HDM sensitive. In general, only HDM-sensitive patients responded to the mite allergens. Mean stimulation indices (SI) were 10.2 ± 2.8 (SEM) for Der p I and 10.0 ± 2.2 for Der p II in HDM-sensitive individuals, and 2.0 ± 0.2 and 2.8 ± 0.6 for non-HDM sensitive control subjects ($p < 0.001$). Patients sensitive to HDM and other allergens had higher responses than subjects sensitive to HDM alone, and the degree of proliferation to Der p I and Der p II was well correlated in individual patients ($r = 0.71$; $p < 0.001$). Total IgE levels were elevated in the allergic patients, and values were highly correlated with the SI for both Der p I and Der p II ($p < 0.001$). Responses to Der p II and Der f II were equivalent in 82% of individuals; however, in 18%, there was a marked discordance with strong responses to Der p II only. SIs were compared between different clinical groups of patients, and patients with asthma had significantly higher values to both Der p I and Der p II than patients with rhinitis alone (17.0 ± 5.4 versus 5.7 ± 1.0 for Der p I, $p < 0.05$; 14.3 ± 6.8 versus 5.0 ± 1.0 for Der p II, $p < 0.05$). (J ALLERGY CLIN IMMUNOL 1992;89:1021-31.)

Key words: House dust mite, Der p I, Der p II, Der f II, T cell proliferation, asthma, allergy

Hypersensitivity responses to common aeroallergens frequently result in clinically significant allergic disorders, such as asthma, eczema, and rhinitis. These allergic conditions are among the most prevalent of human disorders with approximately 15% of the population being afflicted.¹ In patients with respiratory allergy, in countries in which it has been studied, the most frequently implicated allergen has been the HDM, *Dpt* or *Df*.^{2,3} Studies have indicated that between 60% to 85% of subjects with asthma are sen-

Abbreviations used

HDM:	House dust mite
SI:	Stimulation index
<i>Dpt</i> :	<i>Dermatophagoides pteronyssinus</i>
<i>DF</i> :	<i>Dermatophagoides farinae</i>
PHA:	Phytohemagglutinin
PPD:	Purified protein derivative
PBMC:	Peripheral blood mononuclear cells
MAb:	Monoclonal antibody

From *The University of Melbourne Department of Medicine, Western Hospital, Footscray; the **Western Australian Research Institute of Child Health, Princess Margaret Hospital, Perth, and the ***Biochemistry Department, Royal Melbourne Hospital, Melbourne, Australia.

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Reprint requests: R. M. O'Brien, PhD, Department of Medicine, The University of Melbourne, Western Hospital, Gordon St., Footscray, Victoria 3011, Australia.

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sitive to *Dpt* as compared with 5% to 15% in control groups,^{2,4} and there is objective evidence from avoidance studies, epidemiologic surveys, and allergen bronchial-provocation testing that HDM allergens are a major cause of bronchial hyperreactivity and asthma.^{5,6} Moreover, a recent longitudinal study from the United Kingdom has demonstrated a clear relationship between levels of exposure to HDM in early childhood and the risk of later development of asthma.⁷

The participation of IgE in allergic sensitivity⁸ and

the interaction of allergen with tissue-bound IgE resulting in release of mediators is well recognized.⁹ IgE synthesis to aeroallergens is under T-lymphocyte control, regulated in part via the T cell lymphokine, interleukin-4.¹⁰ The role of T cells in allergic disease is being increasingly investigated, both with respect to their helper function for IgE synthesis and their participation in the inflammatory response. Conflicting studies, however, exist about the responses of peripheral blood lymphocytes to allergens of the HDM.

Since 1973, studies have appeared demonstrating a specific response of lymphocytes from HDM-sensitive subjects to allergens from HDM,¹¹⁻¹⁶ whereas other studies have demonstrated no such specificity, with proliferation in non-HDM-sensitive subjects being equivalent to that in sensitive individuals.¹⁷⁻¹⁹ Generally, these studies used crude extracts from either *Df* or *Dpt*, although in two studies, partially purified group I allergens were used.^{15, 18} In these studies, however, affinity chromatography with specific MABs was not undertaken.^{20, 21} Without purified allergens, it is difficult to comment about the significance or nonsignificance of any cellular proliferative response. Moreover, in two of the studies demonstrating no statistically significant difference between control subjects and mite-allergic patients, there was a trend for greater stimulation in the sensitive individuals.^{17, 18}

The major allergens from *Dpt* have been identified, and a number have been purified and characterized. *Der p* I and *Der p* II are the two major proteins recognized by immune sera,^{22, 23} with reactivity by immunoblotting reported to be present in most mite-allergic patients tested.^{24, 25} We have isolated and cloned the cDNA sequence encoding the *Der p* I molecule, and nucleotide sequencing studies have indicated a protein structure homologous to a number of cysteine proteases, particularly in the region of the contact residues making up the active site.²⁶ More recently, the cDNA encoding *Der p* II has been identified and cloned, and studies have indicated that sera from mite-allergic individuals react with this cloned molecule in 90% of patients.^{27, 28} No functional activity has yet been ascribed to the allergen *Der p* II. Its predicted molecular weight is 14,131.

The relative importance of *Der p* I and *Der p* II as causative agents of allergic disorders is a subject of continuing research. There have been some indications, based on relative IgE levels, that *Der p* I may be more relevant in asthma and *Der p* II more relevant in eczema.²⁵ The presence and specificity of HDM-specific circulating T-lymphocytes could help in as-

certaining the importance of the different allergens. One study from O'Hehir et al.,²⁹ using *Df*, suggested that reactivity to *Der f* II was the major component of T cell recognition in an HDM-sensitive patient. However, no conclusions can be drawn from studying a single patient, and before this question can be answered more definitively, a range of HDM-sensitive individuals with different clinical manifestations of allergy will need to be studied with respect to their T cell recognition. In this particular study, we have used MAB-purified *Der p* I, *Der p* II, and *Der f* II allergens to address the question of the specificity of the peripheral blood lymphocyte response in well-characterized allergic patients with a range of manifestations.

MATERIAL AND METHODS

Patients

Adult patients referred to the clinical immunology/allergy clinics at the Royal Melbourne Hospital or Western Hospital, Footscray, were included in this study. These patients were referred for management of one or more of the following conditions: rhinitis, conjunctivitis, asthma, eczema, urticaria, and angioedema. History and clinical findings were recorded for each patient, and none of the patients studied had received previous allergen-specific desensitization.

Immediate-type hypersensitivity by skin prick testing was performed on all patients according to the method of Pepys³⁰ with extracts purchased from Dome/Hollister-Stier Laboratories (Sydney, Australia). The allergens used included house dust, HDM, (*Dpt*), cat and dog hair and danders, mold spores, including *Alternaria*, *Cladosporium*, *Aspergillus*, *Candida*, and *Trichophyton*, and a number of grass, weed, and tree pollens. In patients who were negative to the skin prick test for the HDM, an intradermal injection of an extract of the *Dpt* allergen was performed. A positive control with histamine acid phosphate (1 mg/ml) and a negative control with a buffer solution of the preservative alone were also performed on each of the subjects.

Serological studies

In all the patients studied, serum was obtained and sent to the Royal Melbourne Hospital Biochemistry Laboratory in which total IgE and RAST determinations were performed.

The total IgE levels were measured by latex-enhanced nephelometry with the Behring (Marburg, Germany) nephelometer calibrated with standards that were validated against WHO standards of the World Health Organization.³¹ Specific IgE was measured to HDM, a range of grass and tree pollens, and animal hair and danders, by RIA, Kallestad Laboratories, Inc., Austin, Texas. Patients were only included in this study when there was a concordance of the results of the skin testing and the RAST. Altogether, 13 patients were sensitive to HDM alone, 23 were sensitive to

HDM plus other allergens, 10 were sensitive to allergens apart from HDM, and eight patients were nonatopic and had no allergen sensitivity.

Cellular-proliferation assays

Ten milliliters of peripheral blood was collected into ACD tubes (Becton Dickinson, Mountain View, Calif.) and diluted with an equal volume of RPMI 1640. Mononuclear cells were aspirated from the interface after flotation on Ficoll-Hypaque, washed twice in phosphate-buffered saline, counted, and then resuspended in RPMI 1640 containing penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (2 mmol/L), and 10% pooled human AB serum. The serum had been obtained from the blood bank (Melbourne, Australia) and screened for supporting strong stimulation with minimal background. The cultures of mononuclear cells were set up in triplicate in 96-well round-bottom trays in a 200 µl volume, generally at a concentration of 5×10^5 cells per milliliter. The antigens/mitogen were added to the wells at the beginning of the cultures that were performed in a humidified incubator containing 5% CO₂.

DNA synthesis was measured by adding 0.5 µCi of [³H]thymidine during the final 16 hours of the culture. Cells were harvested with a semiautomatic multiple harvester (PHD, model 280, Cambridge Technology, Inc., Watertown, Mass.), and thymidine incorporation was assayed in a liquid scintillation counter. Results are presented either as absolute counts per minute or as an SI that is calculated as the ratio of the maximal mean (of triplicate) counts per minute of culture wells containing allergen/mitogen with that of wells containing medium alone.

Allergens, antigens, and mitogens

Native *Der p* I and *Der p* II were purified by affinity chromatography from spent mite medium (Commonwealth Serum Laboratories, Parkville, Australia) with a Sepharose 4B column (10 ml) with a number of *Der p* I-, *Der p* II-, or *Der f* II-specific MAbs chemically coupled.²⁷ Aliquots of the eluted protein were examined for purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis with Coomassie blue staining, and only a single band at either 26 kd for the *Der p* I or 15 kd for *Der p* II and *Der f* II was detected. Protein concentration was determined by optical density measurement at 280 nm. PHA (Pharmacia Diagnostics, Uppsala, Sweden), at 10 µg/ml, and PPD (Commonwealth Serum Laboratories), at 1 and 10 µg/ml, were included in each assay as positive controls.

Statistics

The Mann-Whitney U test was used to compare the SI and IgE levels between the different groups. Correlation coefficients were calculated by use of the Spearman's rank test.

Significance of differences between groups with the increasing RAST levels were determined separately for *Der p* I and *Der p* II with a one-way analysis of variance.

RESULTS

Time course and dose response of PBMC proliferation to HDM allergens

Initial studies were performed to determine the optimal day on which to harvest the T cell proliferation assays. Cultures were established with cells from mite-allergic individuals containing medium alone, *Der p* I, *Der p* II, PPD, and PHA. Cells were harvested on days 4, 6, 7, and 8. Maximal mean proliferative responses for the mite allergens and PPD, as assayed by [³H]thymidine incorporation, were evident on day 7 in all three time-course experiments performed. One representative experiment is illustrated in Fig. 1. A concentration of 3 µg/ml was used in these experiments for both *Der p* I and *Der p* II since preliminary experiments had demonstrated that this concentration was satisfactory.

To study the dose response in more detail, assays were initiated with peripheral blood mononuclear cells (PBMC) from mite-allergic individuals with concentrations of *Der p* I from 0.1 to 30 µg/ml and *Der p* II from 0.01 to 5 µg/ml. Five allergic individuals were studied, and proliferative responses were assayed on day 7. A representative study from an individual sensitive to HDM and other allergens is illustrated in Fig. 2. For *Der p* I, maximal proliferation was achieved with concentrations of 3 and 10 µg/ml; however, reasonable proliferation was also evident with 1 and 30 µg/ml. For *Der p* II, differences were not pronounced. Maximal responses were evident at 0.2 µg/ml. Included in the assay as a specificity control was the glutathione-S-transferase protein. No response was evident to this irrelevant antigen. In the experiments that follow, a minimum of three concentrations were used for both *Der p* I and *Der p* II with the optimal concentrations, established in these experiments, being included. The result presented is that from the allergen concentration eliciting the highest mean proliferation for cells from both allergic and nonallergic control individuals.

SIs of PBMCs from mite-sensitive and insensitive individuals to *Der p* I and *Der p* II

T cell proliferation studies were performed in groups of individuals demonstrating sensitivity to HDM alone, HDM plus other allergens, in atopic patients with sensitivities other than to HDM, and in nonatopic control subjects. Because background proliferation varied significantly between individuals, the responses were presented as an SI calculated as the ratio of the maximal mean counts per minute of the triplicate assays from the wells containing differing

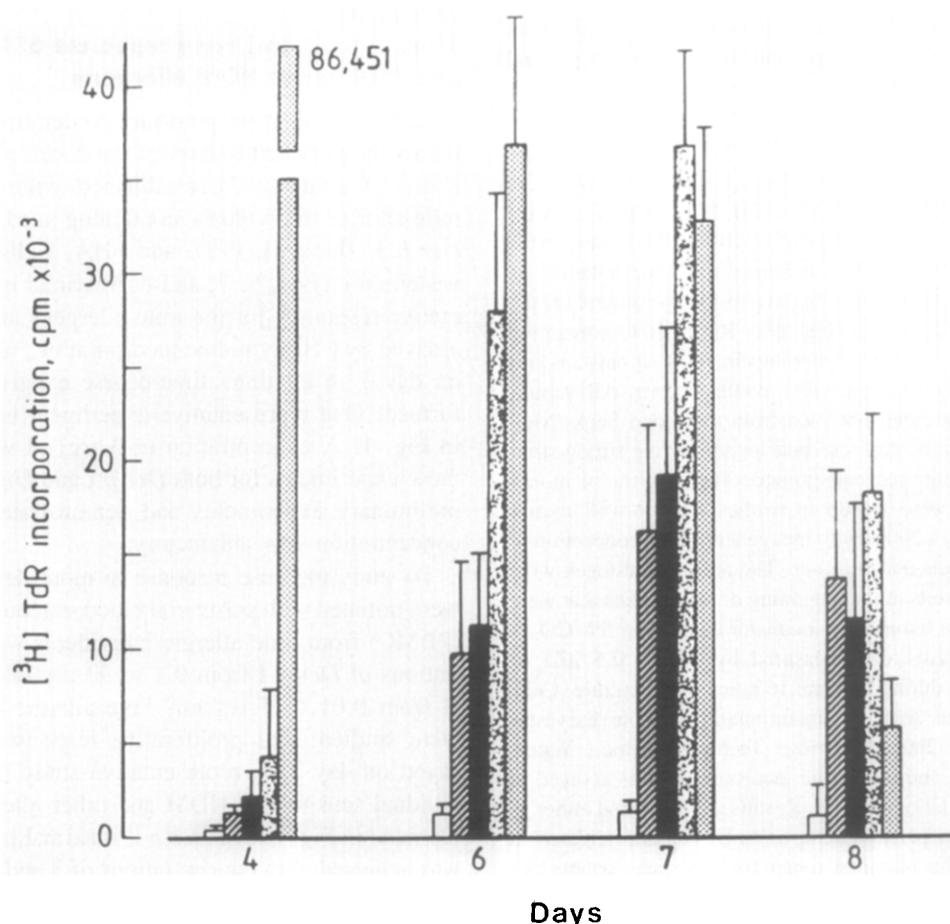


FIG. 1. Time course of proliferative responses of PBMCs from HDM-sensitive individual cultured with Nil (□), *Der p I* (▨), *Der p II* (■), PPD (▩), and PHA (▤). Results, means of triplicate assays; Bars, SD.

allergen concentrations to the maximal mean counts per minute of the wells containing medium alone. The upper limit of normal was arbitrarily chosen as the mean plus 2 SD of the SI of cells from the eight nonatopic control subjects to *Der p I* and *Der p II*.

The results are illustrated in Fig. 3, A and B, for the nonatopic and non-HDM-sensitive control subjects, the patients sensitive to HDM alone, and for patients sensitive to a number of allergens, including the HDM, after stimulation with *Der p I* and *Der p II*. A summary of these results, along with the mean proliferative responses to *Der f II* and PPD, is presented in Table I. Importantly, the proliferative responses to PPD were almost identical in atopic and nonatopic groups. There was also evidence of little difference in proliferation between the nonatopic control subjects and the non-HDM-sensitive atopic subjects to either *Der p I* or *Der p II*.

A modest but highly significant increase in the SI to both allergens is evident in subjects sensitive to HDM alone ($p < 0.005$ for HDM alone versus either

nonatopic or nonmite sensitive, for both *Der p I* and *Der p II*), whereas a very substantial proliferative response is present in more broadly allergic subjects ($p < 0.0001$ for HDM plus other allergens versus either nonatopic or nonmite sensitive, for both *Der p I* and *Der p II*). The differences between the responses observed in the individuals sensitive to HDM alone and individuals sensitive to HDM plus other allergens were not significant at the 5% level by the Mann-Whitney U test ($0.1 > p > 0.05$, for both *Der p I* and *Der p II*). To demonstrate that the low SI in the nonmite-sensitive individuals was due to low experimental counts and not due to high background counts, we have presented in the legend of Fig. 3 the mean background and total counts for each of the four groups stimulated by either *Der p I* or *Der p II*.

Responses to *Der p I* and *Der p II* generally paralleled one another in magnitude, and to determine the degree of correlation between these responses, we compared the maximal SI to these allergens in individual HDM-allergic patients (Fig. 4). Overall, there

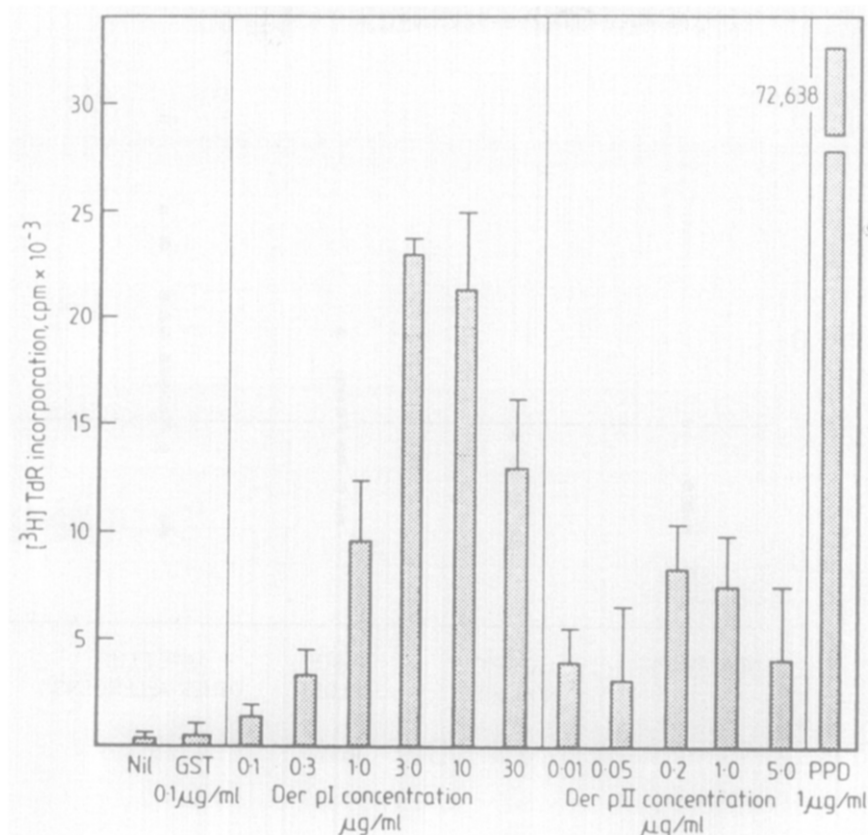


FIG. 2. Dose-response study of PBMCs from HDM-sensitive individual to *Der p I* and *Der p II*. PPD included as positive control; glutathione-S-transferase protein, irrelevant antigen; results, means of triplicate assays; Bars, SD.

was a highly significant correlation ($r = 0.71$; $p < 0.001$).

Comparison of responses to *Der p II* and *Der f II*

To explore the possibility of detecting species-specific variation in T cell recognition, in 11 of the proliferation experiments, parallel cultures containing *Der f II* were also established at antigen concentrations from 0.01 to 5 $\mu\text{g/ml}$. The results of the SIs for *Der p II* and *Der f II* are presented in Table II. In nine of these experiments, a similar degree of proliferation was evident to both *Der p II* and *Der f II*; however, in two of the 11 experiments (R. S. and G. A.), the SI to *Der f II* was very substantially lower than that to *Der p II*. In none of the remaining nine experiments was the SI to *Der f II* significantly higher than that to *Der p II*.

Relationship between total and specific IgE levels and cellular proliferative responses

Total IgE levels were ascertained to determine if a correlation existed between these concentrations and the T cell proliferative responses. We first compared

the mean IgE levels for the following groups: non-atopic control subjects, atopic nonmite-sensitive subjects, atopic individuals sensitive to HDM alone, and atopic individuals sensitive to a number of allergens, including HDM. These results are presented in Table III. Mean IgE levels were highest in those patients sensitive to the HDM plus other allergens, whereas those subjects sensitive to HDM alone had a mean level only minimally elevated above the level of that of the nonatopic control subjects. The mean IgE level for individuals sensitive to allergens apart from HDM was in between these two levels. Comparisons were then sought at an individual level between total IgE and the T cell proliferative response to both *Der p I* and *Der p II*. A highly significant correlation ($r = 0.45$ and $p < 0.01$ for *Der p I*; $r = 0.43$ and $p < 0.01$ for *Der p II*) was detected for both (Fig. 5, A and B).

To determine if there was also an association between specific-IgE antibody levels to HDM allergens and T cell proliferation to *Der p I* and *Der p II*, allergic individuals were categorized into one of four groups (1+, 2+, 3+, and 4+) according to the magnitude of their RAST determination. Mean SI for each group

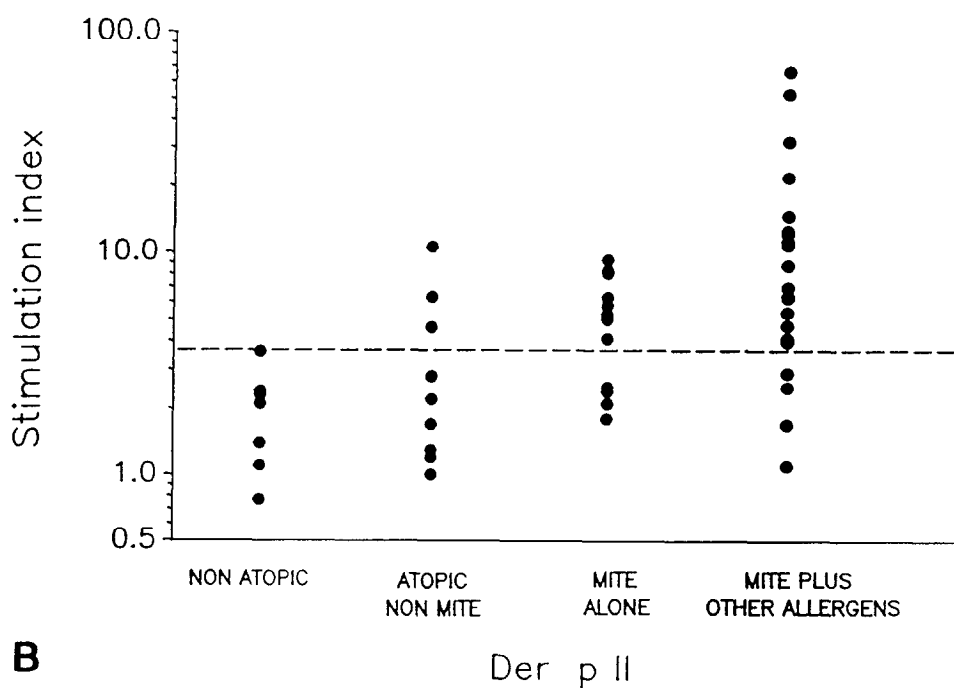
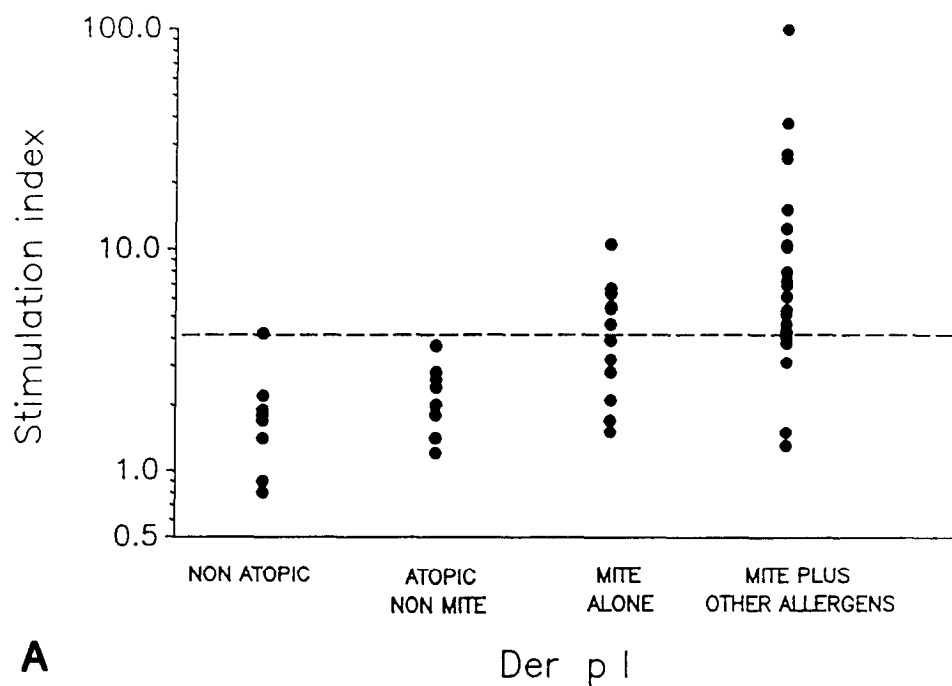


FIG. 3. Maximal SIs of PBMCs from HDM-sensitive and insensitive individuals. **A**, To *Der p I*. **B**, To *Der p II*; (---), mean + 2 SD of eight nonatopic control subjects to *Der p I* (**A**) and *Der p II* (**B**). Mean background incorporation ($\pm$ SD) for each of four groups: nonatopic, 433 ± 78 ; atopic nonmite sensitive, 449 ± 114 ; mite sensitive alone, 415 ± 218 ; mite plus other allergens, 484 ± 173 . Mean maximal counts for same groups: nonatopic, *Der p I*, 518 ± 104 ; *Der p II*, 472 ± 63 ; atopic nonmite sensitive, *Der p I*, 634 ± 217 ; *Der p II*, 783 ± 385 ; mite alone, *Der p I*, 3441 ± 2083 ; *Der p II*, 4175 ± 3112 ; mite plus other allergens, *Der p I*, 7189 ± 5444 ; *Der p II*, 5867 ± 4611 .

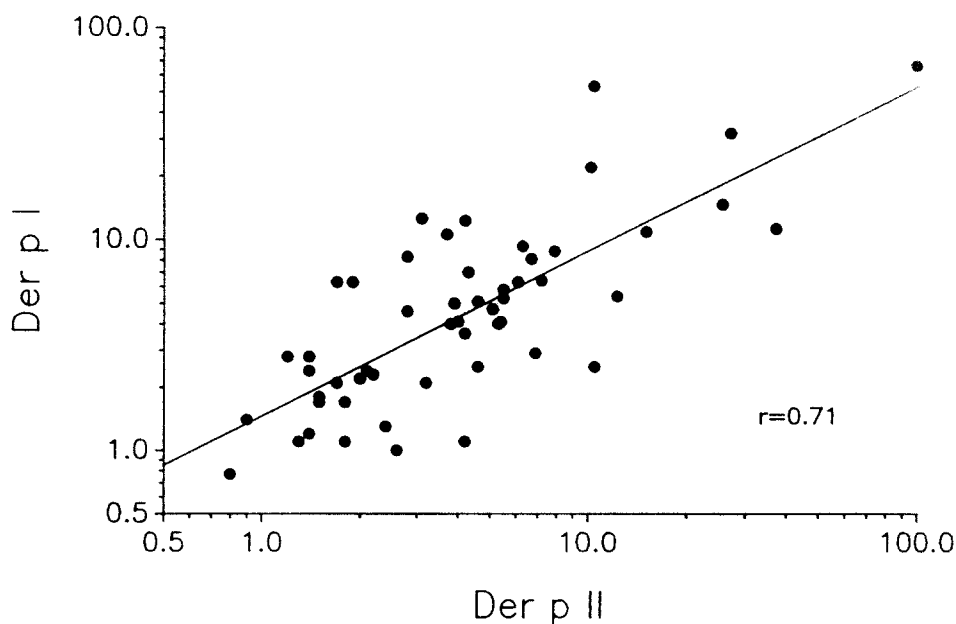


FIG. 4. Correlation of maximal SIs of PBMCs in parallel assays to optimal concentrations of *Der p I* and *Der p II*.

TABLE I. Summary of cellular proliferative responses* from HDM-sensitive and insensitive individuals to *Der p I*, *Der p II*, *Der f II*, and PPD

Clinical sensitivity	<i>Der p I</i>	<i>Der p II</i>	<i>Der f II</i>	PPD
HDM alone	4.59 ± 0.69 (13)†	5.08 ± 0.68 (13)	4.30 ± 0.94 (6)	103.06 ± 43.33 (13)
HDM plus other allergens	13.32 ± 4.32 (23)	12.85 ± 3.46 (23)	5.10 ± 3.16 (5)	105.50 ± 14.48 (23)
HDM total‡	10.17 ± 2.84 (36)	10.04 ± 2.24 (36)	4.66 ± 0.79 (11)	104.62 ± 17.80 (36)
Atopic but non-HDM	2.12 ± 0.24 (10)	3.45 ± 0.95 (10)	—	96.57 ± 10.74 (10)
Nonatopic	1.86 ± 0.43 (8)	1.95 ± 0.04 (8)	—	89.53 ± 13.71 (5)
Non-HDM§	2.01 ± 0.22 (18)	2.83 ± 0.60 (18)	—	93.91 ± 18.32 (15)

*Results are means (±SEM) of the maximal SI from individuals in the groups as indicated.

†Numbers of individuals in each group is listed in parentheses.

‡HDM total is a combination of HDM alone and HDM plus other allergens.

§Non-HDM is a combination of atopic non-HDM and nonatopic individuals.

was then ascertained for both *Der p I* and *Der p II*. These data are presented in Table IV and reveal a highly significant association between T cell proliferation and the mite-specific IgE level. (The RAST groups 1+ to 4+ had significantly different SI by analysis of variance, $p < 0.01$ for *Der p I* and $p < 0.05$ for *Der p II*.)

Allergic symptoms, SI, and total IgE levels

Correlations were sought between T cell proliferation to *Der p I* and *Der p II* and the clinical features of the allergic individuals. The patients were divided into three groups: (1) patients with rhinitis alone, (2) patients with asthma, with or without rhinitis, but without eczema, and (3) pa-

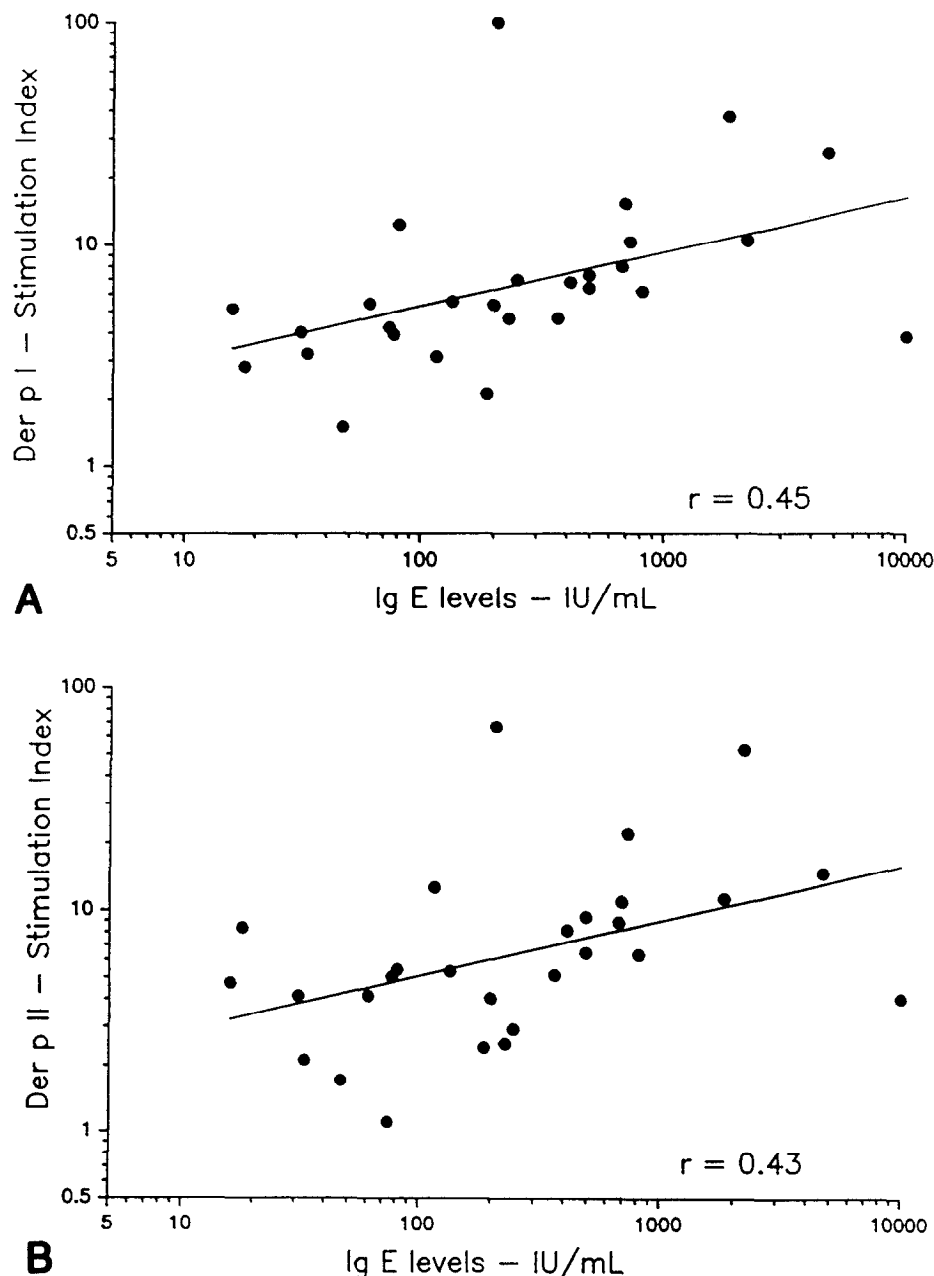


FIG. 5. Correlation of total IgE levels with maximal SIs of PBMCs from HDM-sensitive individuals to *Der p I* (A) and *Der p II* (B).

tients with eczema, irrespective of other clinical features.

The means of the maximal SI are presented in Table V. The highest SIs were evident in patients with asthma, and the lowest, in patients with rhinitis alone, for both *Der p I* and *Der p II*. In patients with eczema, the SI for *Der p I* and *Der p II* was in between the two clinical groups, although the mean response to *Der p II* was higher than that to *Der p I*.

Total IgE levels were also determined for these three

groups, and these mean data are also presented in Table V. The levels here are lowest in patients with rhinitis alone, are somewhat higher in patients with asthma, but are very substantially raised in patients with eczema as a part of their allergic illness.

DISCUSSION

The major finding documented in this study is that in a large group of well characterized atopic and non-atopic patients, T cell stimulation by the affinity-pu-

TABLE II. Cellular proliferative responses* to *Der p* II and *Der f* II

Patient	<i>Der p</i> II	<i>Der f</i> II
W. S.	5.1	8.3
D. W.	2.4	1.5
S. D.	4.1	4.1
S. J.	5.8	3.5
B. H.	6.3	5.5
R. S.†	8.3	2.9
N. S.	2.5	1.8
T. L.	2.9	1.9
G. R.	4.7	2.7
G. A.†	14.7	1.4
T. A.	13.7	17.7

*Results presented are maximal SI for the patients presented.

†There was a strong response to *Der p* II and a minimal response to *Der f* II in these patients.

rified major mite allergens, *Der p* I and *Der p* II, is present far more frequently and is of a greater magnitude in HDM-sensitive individuals than in atopic subjects sensitive to allergens other than the HDM or in nonatopic control subjects. Patients sensitive to the mite alone had modest proliferative responses to *Der p* I and *Der p* II, whereas patients sensitive to a range of allergens, including the HDM, had a greater response (Table I). Interestingly, the proliferative responses to *Der p* I paralleled that to *Der p* II, suggesting they were coordinately regulated and that acquisition of reactivity to one allergen was accompanied by acquisition of similar reactivity to the other mite allergen (Fig. 4). This concordance of lymphocyte proliferative responses is also supported by previous data demonstrating similar levels of elevation of the *Der p* I- and *Der p* II-specific IgE levels.³²

Our finding, that patients with a higher number of allergen sensitivities had greater T cell proliferative responses, was paralleled by the results we obtained for the total IgE levels, in that patients sensitive to mite alone had only mildly elevated levels, whereas patients with multiple sensitivities had far higher total IgE values (Table III). That a correlation existed between the mite-specific IgE antibody titer and the SI of lymphocytes to both a crude HDM extract and to antigen P₁ has been previously demonstrated.^{13, 15} We have confirmed and extended these findings in demonstrating a significant association between the mite-specific IgE level and the T cell responses to both *Der p* I and *Der p* II. Correlations between total IgE levels and the number of allergen sensitivities had not been documented in these studies, although in a study from Black and Marsh,³³ a weak but significant correlation

TABLE III. Total IgE levels and allergic sensitivities in HDM-sensitive individuals and control subjects

Allergic sensitivity	No. of patients*	Mean total IgE† (IU/ml)
Control nonatopic	56	88.93 ± 15.16
Atopic, nonmite-sensitive	8	549.25 ± 201.05
Mite alone	9	198.11 ± 59.88
Mite plus other allergens	19	1228.37 ± 551.07

*The control nonatopic patients included the eight studied for cellular proliferative responses and 48 other nonallergic healthy individuals. The patients in the other categories were only patients studied in the cellular assays.

†Results are means ± SEM.

TABLE IV. Correlation of RAST values with mean SIs to *Der p* I and *Der p* II

RAST value	No.*	Mean SI†	
		<i>Der p</i> I	<i>Der p</i> II
1+	6	3.3 ± 1.8‡	3.0 ± 1.2
2+	5	5.3 ± 1.1	5.2 ± 0.9
3+	5	8.8 ± 4.1	8.4 ± 1.6
4+	7	12.6 ± 6.2	10.9 ± 7.3

*Number of individuals in each group.

†The RAST groups 1+ to 4+ had significantly different SI by analysis of variance; $p < 0.01$ for *Der p* I and $p < 0.05$ for *Der p* II.

‡Mean SI of the group ± SD.

had been demonstrated between the lymphocyte response to ragweed antigen E and the total IgE levels. Moreover, a correlation has been demonstrated between the degree of skin sensitivity to an array of inhalant allergens and the total IgE level.³⁴ Our results suggest that T cell reactivity may be regulated by the same or linked genetic element(s) that determine the total IgE levels, that is, the putative IgE-regulator gene.^{35, 36}

SIs to the group II allergen from *Df*, *Der f* II, were also determined in 11 HDM-sensitive individuals, and generally, these responses paralleled SIs to *Der p* II, although in two cases a very much lower response was evident (Table II). This reduced proliferative response was not associated with a reduced response to PPD and was somewhat unexpected because *Der p* II and *Der f* II share extensive cross-reactivity serologically, and cDNA sequencing has demonstrated 88% homology.^{27, 37, 38} Nevertheless, differences at the T cell level were revealed in previous studies with T cell clones in which a number reactive with *Der f* II did

TABLE V. Cellular proliferative responses to *Der p* I and *Der p* II and total IgE levels in HDM-sensitive individuals with differing clinical manifestations

Clinical manifestation*	SIT		Total IgE level† (IU/ml)
	<i>Der p</i> I	<i>Der p</i> II	
Rhinitis alone (13)‡	5.73 ± 0.95	5.03 ± 1.03	226.4 ± 80.0
Asthma (14)	17.00 ± 5.36	14.34 ± 6.75	400.5 ± 178.2
Eczema (9)	6.59 ± 1.46	10.13 ± 4.19	2592.6 ± 1370.6

*See RESULTS section for description of the clinical manifestation categories.

†Results are means ± SEM.

‡The number of patients in each group is presented in parentheses.

not cross-react with *Der p* II.²⁹ This finding strongly suggests that (at least) some of the T cell response to *Der p* II is species specific, a point with important implications regarding the potential allergens used in immunotherapy.

The concept of differing degrees of cellular immunologic reactivity being an important discriminative factor between individuals with different clinical allergic manifestations has been studied by previous authors in the hope of gaining insight into the etio-pathogenesis of the different allergic disorders. Rawle et al.¹⁵ (1984) compared T cell responses to *Der p* I in different patients' groups and found low values in patients with rhinitis, higher levels in patients with asthma, and the greatest responses in patients with eczema. In contrast, Cavaillon et al.¹⁸ (1987) were unable to find any difference in lymphocyte transformation indices comparing patients with rhinitis or asthma. Our results further support the low level of stimulation present in patients with rhinitis alone; however, our findings indicate very significantly higher responses in patients with asthma (with or without rhinitis) and somewhat less T cell responses among patients with eczema. The presence of a greater allergic "response" in patients with asthma versus patients with rhinitis alone has been well documented in relationship to skin test positivity and (specific) IgE levels.^{3, 39-41} Our detailing of a higher T cell proliferative response may well be an expression of the same excess response at the cellular level.

The lower cellular response in patients with eczema, in the presence of a substantially higher total IgE level, may reflect an additional and separate impairment of T cell immunity, known to be present in patients with atopic dermatitis and possibly related to the presence of suppressor or feedback effects.⁴²⁻⁴⁴ To address further this dilemma, we have undertaken proliferation studies with CD4 (helper T-lymphocyte)- and CD8 (cytotoxic/suppressor T-lymphocyte)-depleted T cell populations in both eczematous and non-eczematous HDM-sensitive patients. In preliminary

studies we have found that there is, generally, an enhancement of proliferation in the CD8-depleted cultures and that this occurs more frequently and is of a greater magnitude in the patients with eczema (data not presented). These results do support the concept of a specific "suppressor"-cell population in eczematous individuals.

Although the tendency was not statistically significant, there was a tendency for higher mean SI to *Der p* II than to *Der p* I in patients with atopic dermatitis. In fact, a higher degree of sensitization to the mite-body allergen, *Der p* II, has previously been reported in eczema with respect to specific IgE levels. Thompson et al.²⁵ (1988) reported that with immunoblotting in serum of patients with atopic dermatitis, IgE antibodies specific for *Der p* I were present in only 50% whereas *Der p* II reactivity was present in 100%. In patients with asthma, there was a more equivalent IgE response to *Der p* I and *Der p* II, also as observed for cellular responses in this study.

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