

Cytokine responses to Der p 1 and Der p 7: house dust mite allergens with different IgE-binding activities

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Summary

Background There is very limited information comparing T-cell responses to different house dust mite (HDM) allergens even though T cells are essential in the initiation and regulation of immunoglobulin (Ig) E synthesis and eosinophilia.

Objective To compare the level of T-cell proliferation and cytokine production to the group 1 and group 7 HDM allergens which are known to have different IgE-binding capabilities.

Methods Freshly isolated peripheral blood mononuclear cells (PBMC) from HDM-allergic and HDM-nonallergic donors were stimulated with the group 1 and group 7 allergens of *Dermatophagoides pteronyssinus* and the level of proliferation as well as IL-5 and IFN γ production were measured.

Results The proliferative and IL-5 production to the group 1 and group 7 allergens were equivalent despite the group 7 allergen's lower frequency of IgE-binding. However more IFN γ was produced to Der p 7 than to Der p 1, particularly for the nonallergic donors. As expected IL-5 production was much higher for PBMC from the allergic donors than for cells from nonallergics; however, there was no difference in the level of T-cell proliferation between the donor groups.

Conclusion The relative importance of the individual HDM allergens is normally determined by measuring the frequency of IgE-binding to the allergen in sera from an allergic population. The equivalent increased IL-5 response of PBMC from allergic people to the group 1 and group 7 allergens despite the different IgE-inducing activity indicates that these allergens may be equally capable of contributing to an asthmatic response by inducing eosinophilia.

Keywords: house dust mite, *Dermatophagoides pteronyssinus*, Der p 1, Der p 7, T cells, cytokines, IgE.

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Introduction

At least 15 different HDM allergens have been identified and the majority have been characterized by cDNA cloning. The relative importance of these individual allergens has traditionally been assessed by their ability to bind to IgE

from the sera of allergic patients. Few studies have compared T-cell responses to the individual HDM allergens even though it is well established that T cells are essential in the initiation and regulation of IgE synthesis and induction and maintenance of eosinophilia. Moreover, individuals who contain allergen-specific IgE in their sera (allergics) have a bias in generating T helper 2 (Th2) cytokines (IL-4 and IL-5) [1–4], and the production of these cytokines *per se* can be a primary cause of allergic inflammation in allergic individuals, particularly via IL-5 which is essential

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for the selective terminal differentiation of eosinophil precursor cells and their survival in inflamed tissues [5,6,8].

Information on T-cell responses to individual HDM allergens other than the major group 1 and group 2 allergens is limited. The group 1 and 2 allergens are the most extensively studied allergens and are presumably the most important because they frequently bind IgE in allergic sera (80–100%) at high titre, and are major components in house dust [9–11]. Allergen groups 3, 9, 10 and 11 also bind IgE at a high frequency (70–100%) and titre [12–16]; and groups 5 and 7 react with IgE in 50% of sera at high titre [17–19]. The binding by the group 6 allergen is moderate [15–20] and the degree of IgE-binding to groups 4, 8, 12, and 13 has not been quantified in relation to the other allergens. There have only been two studies comparing T-cell responses to individual affinity purified HDM allergens, namely between the group 1 and group 2 allergens, and the level of T-cell proliferation to these two allergens was generally well correlated in individual subjects [21,22]. Although many of the studies have shown that the magnitude of T-cell recognition to an allergen is generally higher in allergic than nonallergic groups [7,21–28], it is now clear that both groups contain allergen-specific T cells in their peripheral immune populations [29–35]. This is supported by the finding that all subjects have IgG antibody to mite allergens [36,37].

In this study, we compared the level of T-cell proliferation and the cytokine profile (Th1/Th2) to two different HDM allergens which induce IgE at different frequencies. The aim was to determine whether the group 7 allergens, which only induce IgE in 50% of HDM-allergic patients, stimulated T cells to release Th2 cytokines at a lower frequency and magnitude than the group 1 allergens, as might be expected from the IgE-binding data, particularly in HDM-allergic subjects who did not produce anti-Der p 7 IgE. Despite the reported difference in IgE reactivity, the proliferative and IL-5 responses to the group 1 and group 7 allergens were about the same for all individuals and some of the HDM-allergic donors who did not contain IgE against Der p 7 in their sera still produced high levels of IL-5. A difference in responses was however, noted for IFN γ production. PBMC, especially for the nonallergic donors, produced higher levels of IFN γ to Der p 7. These results indicate that allergens which bind IgE at a lower frequency than the high IgE-binding allergens may have the potential to contribute equally well in allergic inflammation via non-IgE-mediated mechanisms such as cytokine release from T cells.

Methods

Study population

Peripheral blood was obtained fresh from 26 donors (18 females, eight males; age range 21–50) recruited from

laboratory staff and the general population. Serum was extracted for measurement of specific IgE to house dust mite and rye pollen using the Pharmacia CAPS system (a RAST score ≥ 2 on a scale of 1–6 was considered positive), and for the measurement of specific IgE against Der p 7 by dot-blot analysis. The RAST analysis was kindly done by Dr Richard Loh of the Immunology Department at Princess Margaret Hospital for Children (Perth, Western Australia). Fifteen of the 26 subjects had no symptoms of allergic disease (asthma, rhinitis or dermatitis) and were RAST-negative to dust mite and pollen. The remaining 11 subjects were RAST-positive to the HDM allergen and displayed one or more allergic symptoms.

Culture Medium

Cells were cultured using AIM-V lymphocyte medium (Gibco, New York, USA). This medium contains DMEM and F12 media, supplemented with unspecified amounts of human albumin, insulin, transferrin, lipids, L-glutamine, streptomycin, gentamycin, and HEPES buffer. The addition of 2-mercaptoethanol (4×10^{-5} M; BDH) was done in house.

Allergens

Natural Der p 1 and Der p 7 were purified by affinity chromatography from spent mite medium using the 4C1 mAb against Der p 1 and the WH9 mAb against Der p 7 using the method described in Chua *et al.* [38]. The Der p 7 preparation showed major bands at 31 and 30 kDa by Coomassie-stained SDS-PAGE and a less prominent band at 26 kDa in keeping with results of previous studies showing multiple glycosylation products. Der p 1 ran at 25 kDa. Tuberculin purified protein derivative (PPD) and dried HDM were purchased from CSL Limited (Melbourne, Australia) and were included in each tissue-culture assay as positive controls at 30 μ g/mL, respectively. Preliminary work found the optimal T-cell proliferation and cytokine release was obtained at 30 μ g/mL for each allergen so this concentration was used.

Cell extraction and proliferation assays

Blood was collected and diluted with an equal volume of RPMI containing preservative free heparin. PBMC were isolated on a density gradient (Lymphoprep, Nycomed, Norway), washed three times in RPMI, and resuspended in AIM-V culture medium. The cells from 10-allergic and 15-nonallergic donors were seeded at 2×10^5 cells/well in 96-well round-bottom trays in a volume of 200 μ L. The allergens, PPD and recombinant glutathione-S-transferase (GST) from *Shistosoma japonicum* were added in triplicate

to the cell cultures at 30 µg/mL, and control wells contained no antigen. Following incubation at 37 °C in 5% CO₂ for 5 days, DNA synthesis was measured by adding ³H thymidine (0.5 µCi; DuPont). Thymidine uptake was assayed by harvesting the cultures onto glassfibre filter mats 16–18 h later and counting thymidine incorporation using a Packard Matrix 9600 beta counter. The allergens were considered to be causing proliferation if the stimulation index (SI) ≥ 3.0, where SI = mean of wells containing antigen/mean of control wells.

Cytokine assays

The cells from 11-allergic and 14-nonallergic donors were seeded at 3 × 10⁶ cells/well in 24-well plates in a 2-mL volume for the measurement of IL-5 and IFNγ production. The allergens and PPD were added to the cells individually at 30 µg/mL. Supernatant was harvested on day 4 and day 6 for the measurement of IL-5 and IFNγ, respectively. IL-5 was measured using the BAF-BO3 bioassay as described previously [39] which was sensitive to 1 pg/mL. IFNγ was measured using the Pelikine ELISA kit (CLB, Amsterdam, the Netherlands) and the sensitivity of the kit was approximately 10 pg/mL.

Dot-blot analysis

IgE against Der p 7 was measured by dot-blot analysis using enhanced chemiluminescence (ECL; Amersham Life Sciences Ltd, Little Chalfont, England). Briefly, 2 µg of Der p 7 and 0.2 µg of hyper-IgE sera were dotted onto Hybond-PVDF (Amersham Life Sciences Ltd), the membrane was blocked for 1 h in 5% skim milk/0.1% Tween 20/PBS at room temperature, then the membrane was incubated overnight at 4 °C in serum diluted 1:1 in 5% skim milk/0.1% Tween 20/PBS after washing in 0.1% Tween 20/PBS (PBS-T). The 2-µg dot of Der p 7 was used based on titrations showing that maximum IgE-binding of high titre sera was reached at 1 µg. The membrane was incubated for 1 h in a secondary mouse antihuman IgE antibody [38], followed with 1 h incubation in a tertiary antimouse Ig horseradish peroxidase antibody (Amersham Life Sciences Ltd, Little Chalfont, England), with washing in PBS-T between the steps. The antibodies were diluted in PBS-T and the optimal dilutions were determined in preliminary experiments. The membrane was finally incubated with ECL detection reagent which emits light as a signal which can be detected by exposing the membrane to autoradiography film. For each sera, IgE was dotted onto the membrane as a positive standard and used to normalize the IgE-binding. The IgE gave a linear signal from 1 ng to over 200 ng/dot. Depletion of IgG was performed by passing 2 mL of a 1/2 dilution of serum through a 5-mL protein G Hi

Trap column with 20 mg/mL IgG-binding capacity (Amersham Pharmacia Biotech UK Ltd).

Statistical analysis

Differences between experimental groups were assessed by either the paired Wilcoxon signed rank test or by the unpaired Mann–Whitney *U*-test. Probabilities less than or equal to 0.05 using the Instat statistical package (GraphPad Software, CA, USA) were considered significant.

Results

Dose-response of T-cell proliferation to the group 7 allergen

Preliminary dose–response experiments were performed to determine the optimal allergen concentration for the use in the proliferation assays and to assess whether the group 1 and group 7 allergens activated the cells in a similar dose–response manner. PBMCs from six donors were induced with Der p 1 and Der p 7 at concentrations from 0.1 to 30 µg/mL and the cells were assayed at day 6. Table 1 shows the mean response of the six donors to the various Der p 1 and Der p 7 concentrations. Maximal proliferation was induced with either 10 or 30 µg/mL for the group 1 and group 7 allergens. For the subsequent experiments, 30 µg/mL was used for each allergen.

Proliferative T-cell responses to the group 1 and group 7 HDM allergens

PBMCs from 10-allergic and 15-nonallergic donors were stimulated with 30 µg/mL of affinity purified Der p 1 and Der p 7. The responses are presented as a stimulation index (SI) (Fig. 1). The proliferation to the group 1 and 7 allergen groups was the same and almost 100% of the cells from the allergic and nonallergic donor groups reacted strongly. There was a high correlation ($r = 0.634$; $P < 0.001$) between the responses to Der p 1 and Der p 7 (Fig. 2). Several subjects did not show significant responses even though they responded to PPD showing the allergens are not mitogens.

Cytokine responses to the group 1 and group 7 allergens

PBMCs from 11-allergic and 14-nonallergic donors were stimulated with the group 1 and group 7 allergens of *D. pteronyssinus*. Supernatant was collected on day 4 and day 6 for the measurement of IL-5 and IFNγ, respectively. Previous experiments had shown that it was not possible to detect IL-4 by the Pelikine ELISA kit (sensitivity < 2 pg/mL). A summary of the level of T-cell proliferation and

Table 1. Dose–response study of PBMCs from six donors to Der p 1 and Der p 7

	Donor						Mean $\pm$ SEM
	A	B	C	D	E	F	
Der p 1							
0	2812	1298	2505	3967	4440	3712	3122 $\pm$ 469
0.1	3128	1552	5610	8092	7170	5940	5248 $\pm$ 1009
0.3	4065	1515	4605	7740	7380	6622	5321 $\pm$ 972
1	3570	1890	4770	6510	9428	5872	5340 $\pm$ 1060
3	3705	1972	10485	14768	9758	9728	8402 $\pm$ 1930
10	14175	4350	16522	14692	12578	23505	14304 $\pm$ 2529
30	22612	4118	31748	24098	31568	21308	22575 $\pm$ 4121
Der p 7							
0	2812	1298	2505	3967	4440	3712	3122 $\pm$ 469
0.1	6112	1988	5872	13305	6450	7680	6901 $\pm$ 1502
0.3	4590	2565	9450	10208	4642	7372	6471 $\pm$ 1235
1	11962	2948	20062	8370	4140	24278	11960 $\pm$ 3524
3	37822	8040	22252	15922	15368	20062	19911 $\pm$ 4098
10	44812	49928	32880	26250	25538	32918	35387 $\pm$ 4054
30	47235	35100	39015	52972	32295	32865	39914 $\pm$ 3444

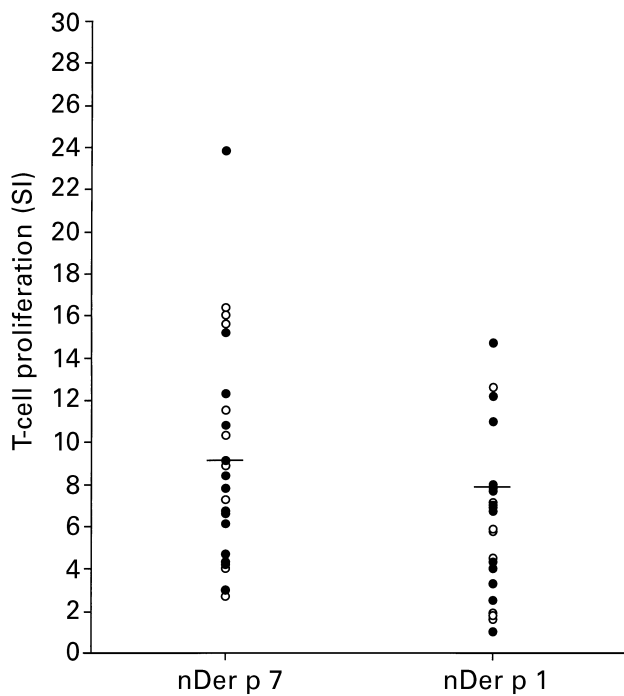


Fig. 1. T-cell proliferation of allergic (○) and nonallergic (●) donors to Der p 1 and Der p 7. No difference was found between stimulation induced by Der p 1 or Der p 7 or between the proliferation responses of PBMCs from allergic and nonallergic donors. T-cell proliferation is given as the stimulation index (SI) which is calculated by dividing the mean of the wells containing antigen with the mean of the control wells. The bar represents the mean SI for all donors to the individual HDM allergens.

cytokine production to the individual allergens is listed in Table 2. The production of IL-5 was significantly higher ($P < 0.005$) for the allergic donors than for the nonallergic donors to both allergens, as was the ratio of IFN γ /IL-5 production for the nonallergic groups. The nonallergic

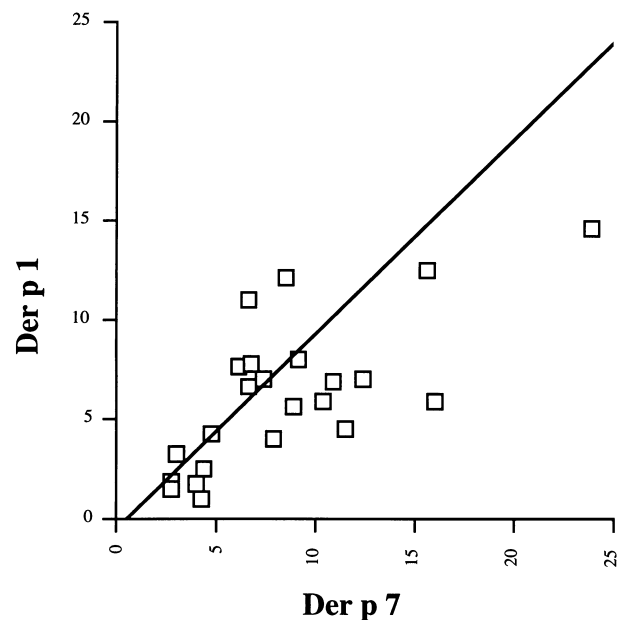


Fig. 2. Correlation of SIs of PBMCs in parallel assays to Der p 1 and Der p 7 ($r = 0.634$; $P < 0.001$).

Table 2. A summary of the level of T-cell proliferation (SI) and cytokine responses* to the individual group 1 and group 7 allergens of *D. pteronyssinus* from HDM-allergic and nonallergic donors

	Proliferation (SI)		IL-5 (pg/mL)		IFN γ (pg/mL)		IFN γ /IL-5 ratio	
	Allergic	Nonallergic	Allergic	Nonallergic	Allergic	Nonallergic	Allergic	Nonallergic
Der p 1	8.02 $\pm$ 3.0	8.62 $\pm$ 1.9	55 $\pm$ 12.7 [†]	3.6 $\pm$ 1.0	352 $\pm$ 218	806 $\pm$ 384	18 $\pm$ 8.9	240 $\pm$ 87 [§]
Der p 7	9.53 $\pm$ 1.7	8.64 $\pm$ 1.4	54 $\pm$ 9.5 [†]	10.9 $\pm$ 3.6	595 $\pm$ 305 [‡]	1276 $\pm$ 379 [‡]	26 $\pm$ 14	279 $\pm$ 123 [§]

*Results are presented as means ($\pm$ SEM) of the level of proliferation and cytokine production to the individual HDM allergens. [†]The level of IL-5 production was significantly higher for the allergic donors than for the nonallergic donors. [‡]The level of IFN γ production to Der p 7 was higher than what was found for Der p 1 for both donor groups. [§]The IFN γ /IL-5 ratio was significantly higher for the nonallergic group than for the allergic donor group.

group included two subjects allergic to allergens other than mite and expectedly they did not make elevated levels of IL-5. Of particular importance the cells of the allergic donors produced similar amounts of IL-5 to the Der p 1 and Der p 7 allergens even though Der p 7 is reported to bind to IgE at a lower frequency than the group 1 allergen. Moreover, the correlation between the level of IL-5 production to the group 1 and group 7 allergens was extremely strong ($r=0.959$; $P<0.001$) (Fig. 3). Although the production of IFN γ *per se* appeared higher for the nonallergic than for the allergic group, this did not reach statistical significance. The correlation of IL-5 and IFN γ production for the individual donors is shown in Fig. 4. The cells from both the allergic and nonallergic donor groups produced significantly higher

levels of IFN γ to Der p 7 than to Der p 1 ($P<0.05$). This was particularly noticeable with the PBMC from nonallergics in Fig. 4.

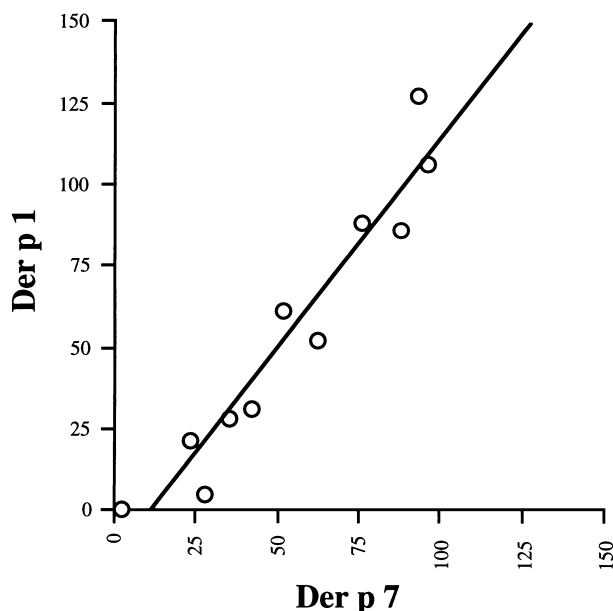
A distinct pattern emerged when a comparison between the level of proliferation (SI) and the level of cytokine production (IL-5 and IFN γ) to the allergens was carried out. There was a positive correlation between the SI and level of IFN γ production, and no correlation between the SI and the level of IL-5 production when the cells from the individual donors were stimulated with the natural allergens as shown for Der p 1 in Fig. 5.

IgE-binding to the group 7 allergen

To compare the level of IgE-binding and IL-5 production to Der p 7 for the individual HDM-sensitized donors, the ratio of Der p 7-specific IgE for nine of the 11 donors was measured by dot-blot analysis. This analysis was also the first to use the natural group 7 allergen. The previously reported studies used the recombinant (GST fusion) allergen. The percentage of binding of the individual sera was assessed by comparing the degree of binding to the individual allergens with the strength of signal of reactivity to hyper-IgE. The results of this analysis found no correlation between the level of IgE binding and the production of IL-5 to Der p 7 (Fig. 6). Individuals with no or almost undetectable anti-Der p 7 IgE produced high levels of IL-5. There was also no correlation (or inverse correlation) with IFN γ . Two serum samples with very low IgE-binding were depleted of IgG with a protein G column and re-assayed. The IgE-binding in both cases was only marginally increased by 4%.

Discussion

The elevated secretion of IL-5 from T cells has been proposed as a primary cause of allergic inflammation in

**Fig. 3.** Correlation of IL-5 production of PBMCs from HDM-allergic individuals to Der p 1 and Der p 7 ($r=0.959$; $P<0.001$).

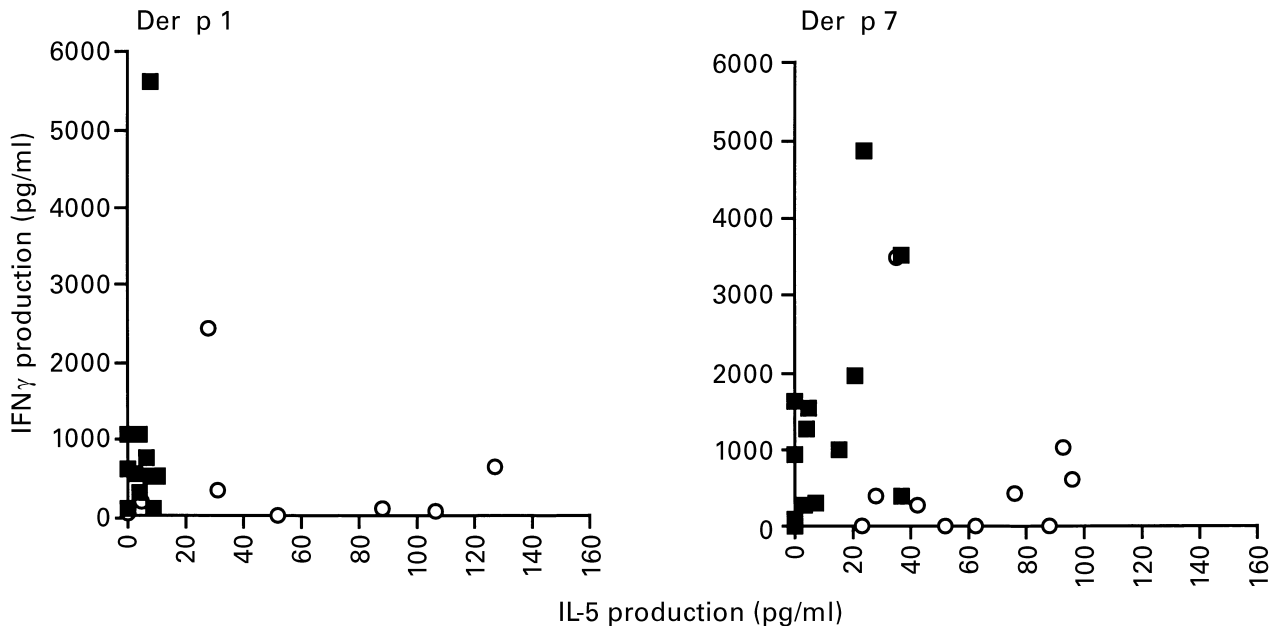


Fig. 4. The level of IL-5 and IFN γ production of PBMCs from 11 atopic (○) and 14 nonatopic (■) donors to the group 1 and group 7 allergens of *D. pteronyssinus*. The cells were stimulated with 30 μ g/ml of each allergen and the supernatants were harvested at day 4 and day 6 for the respective measurement of IL-5 and IFN γ .

asthma. Several *in vivo* and *in vitro* studies have strongly indicated that the eosinophil as the major cell type involved in mild and severe asthma and in the production of seasonal rhinitis. Patients show selectively elevated levels of eosinophils in the bronchial mucosa, and bronchial and nasal lavage [40–47]. Moreover, T cells from bronchoalveolar lavage and bronchial biopsies express elevated levels of mRNA for IL-4

and IL-5 [48,49]. Asthma is associated with high IgE-binding to HDM extracts but only about 30% of subjects with high IgE antibody levels develop disease and the risk of developing disease is similar over a wide range of IgE anti-HDM titre [50]. A study by Chang showed that IgE-binding was not directly related to the ability of the patients T cells to produce IL-5, but the production of IL-5 was related to asthma [7].

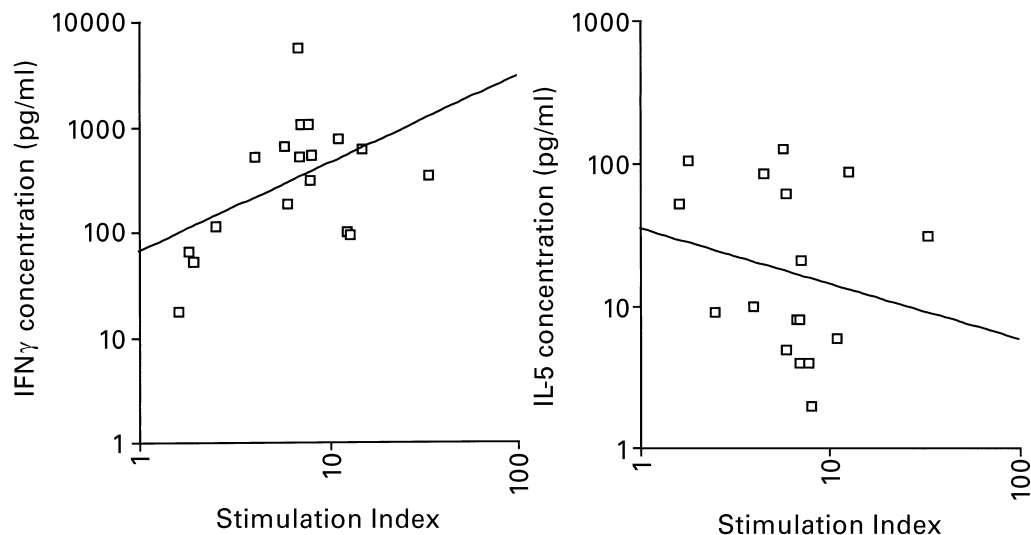


Fig. 5. The correlation between the level of T-cell proliferation and IFN γ production to Der p 1 was positive ($r = 0.485$; $P < 0.05$) whereas a negative correlation was found between the level of T-cell proliferation and IL-5 production ($r = 0.207$).

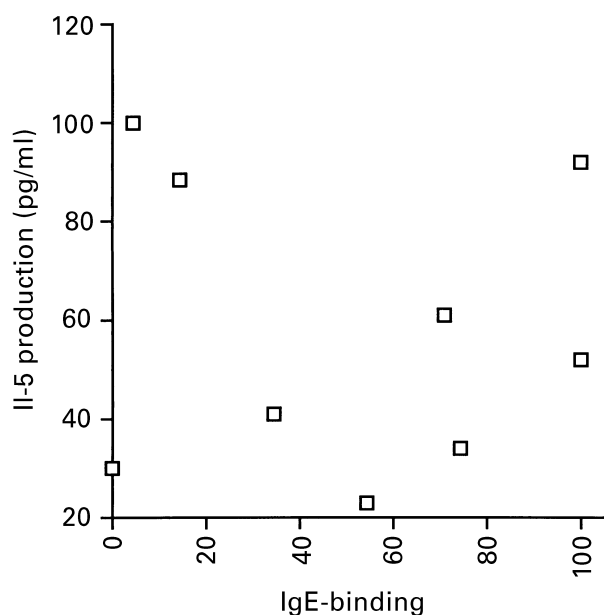


Fig. 6. There was no correlation ($r = 0.068$) between the level of IL-5 production and the degree of IgE-binding to the natural Der p 7 allergen for the allergic donor group.

Traditionally the importance of allergens has been judged by IgE-binding characteristics. Little work has been done comparing T-cell reactivity to different allergens, particularly with respect to cytokine production. It was therefore of interest to compare the cytokine response induced by the major Der p 1 allergen which induces IgE in almost all allergic subjects and Der p 7, an allergen which only binds IgE in 50% of sera from mite-allergic subjects. The results showed that not only did the T cells from every individual respond to Der p 7, but the amount of proliferation and IL-5 production was the same as the major allergen Der p 1. It had previously been determined that only about 50% of HDM-allergic subjects had IgE to Der p 7 and it is shown here that individuals without detectable serum anti-Der p 7 IgE produced high levels of IL-5 to this allergen. The association with IL-4 would be interesting but it could not be measured in primary PBMC cultures. Serum depleted of IgG by protein G did not show a marked increase in IgE-binding so interference of IgG antibodies in the assays was not a significant factor.

The proliferation to Der p 1 and Der p 7 was the same for cells from allergic and control subjects but the responses of allergic subjects were clearly distinguished by their high IL-5 production. Cells from nonallergic subjects tended to produce more IFN γ than cells from allergic subjects which was, although not significantly different by itself, seen when expressed as a ratio of IFN γ to IL-5.

The finding that the PBMC from nonallergic subjects responded as well as allergic subjects in proliferation assays

has been observed previously. Some reports do show an increase proliferation for PBMC from allergic subjects but this is not universal and in most studies there is considerable overlap [21–28,33–51]. The most clear increase in T-cell responses to allergens found by O'Brien was for patients with multiple allergies and high IgE immunoglobulin levels [21]. Patients only allergic to mite had a modest elevation in response. It is clear that in efficient culture systems, where the PBMC from all the allergic subjects have significant stimulation, that the sensitization of cells from nonallergic subjects can readily be demonstrated. This is in keeping with their ability to produce IgG antibody [36,37].

The T-cell responses to Der p 1 and Der p 7 differed in only a single aspect. Der p 7 induced more IFN γ release than Der p 1 which was significant for the results from allergic and nonallergic groups but was most noticeable for the nonallergic donors. This could be related in some way to the decreased frequency of IgE production to Der p 7 but not directly because the individuals without IgE didn't make higher quantities of IFN γ than subjects with IgE. Since allergens are presumably inhaled as a mixture, the IFN γ induced to allergens like Der p 7 could also be important in the regulation of responses to other allergens. Similarly, the ability to produce more IFN γ to Der p 7, and possibly other allergens, suggests that cross-regulation of cytokines could be important in immunotherapy and that the major allergens may not be the best targets. The difference in the IFN γ response between Der p 1 and Der p 7 also shows the T-cell responses measured here are characteristic for each allergen.

An interesting aspect of the anti-IgE antibody production to Der p 7 is that while only 50% of HDM-allergic people have antibody, the titre of the responders are as high as those to the major allergens. Given that this is not because they are nonresponders to the allergen, there must be some tight regulatory mechanisms or threshold influence on the response. In the few studies performed on T-cell responses to different allergens the results have shown an excellent correlation. This has been found in comparison of the proliferation induced by the major allergens to Der p 1 and Der p 2 [21] and by comparing the major allergens Der p 2 and Mag3 [27]. We show a correlation between the proliferation to Der p 1 and Der p 7. When comparing the correlation of cytokine production and T-cell proliferation it was clear that this was most apparent for proliferation and IFN γ , but not for IL-5. This is in keeping with the ability of Th1 clones to produce more IL-2 than Th2 clones [1,53].

The report of Würtzen comparing the responses to a major grass extract and the responses to the major allergen Phl p 5 has similarities to the present study [28]. Both allergic and nonallergic populations had strong T-cell stimulatory responses to grass extract and Phl p 5 although the cells from the allergic group were statistically higher. Several patients with IgE antibody to grass extract had

T-cell responses to the major allergen Phl p 5 in the absence of anti-Lol p 5 IgE antibody. Previous studies which compared T-cell proliferation and specific IgE antibodies to Der p 1 and Der p 2 showed a similar result in that some HDM-allergic patients produced T-cell stimulation to Der p 1 or 2 but did not produce IgE [22]. The cells from nearly all the grass-allergic patients produced IL-5 to Phl p 5 but there was no correlation with IL-5 and IgE antibody levels. The pure Phl p 5 allergen did not induce high IFN γ titre but the whole Phl p extract did. This has similarities to the major allergens Der p 1's inability to induce IFN γ compared to the lesser allergens Der p 7 in our study.

The role of the allergen Der p 7 in sensitization and disease needs to be considered. It clearly can sensitize T cells to produce IL-5 to the same degree as Der p 1 and this activity could make it an important allergen for all HDM-allergic patients. The increased induction of IFN γ compared to Der p 1 could be an ameliorating factor. It is possible that different thresholds of allergen are required to cause sensitization and to induce sufficient quantities of IL-5 to produce disease, but this is not known. Der p 7 is present in 40-fold less concentration than Der p 1 in extracts but there are no measurements of its concentration accumulated in the environment or the amount inhaled after exposure to mites. Extracts show a significant amount of degradation products of Der p 7 so the standard techniques used to measure the amount of allergen per gram of dust extract (usually made after storage) may not be an accurate reflection of the amount inhaled from the exposure to live mites. In any case, even using 1/40 of the Der p 1 level as an estimate, it still can achieve high levels in dust in areas of infestation where levels of 100 μ g of Der p 1 per gram of dust are not uncommon. The level of <1 μ g/mL in extracts however, is not a sufficient dose to induce *in vitro* T-cell responses or to contribute significantly to the assessment of IgE anti-HDM extract antibody titre. It may therefore be useful to develop allergen preparations with more of this allergen.

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References

- 1 Kapsenberg ML, Wierenga EA, Bos JD, Jansen HM. Functional subsets of allergen-reactive human CD4+ T cells. *Immunol Today* 1991; 12:392–5.
- 2 Yssel H, De Waal Malefyt R, Roncarolo MG, Abrams JS, Lahesmaa R, Spits H, de Vries JE. IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. *J Immunol* 1992; 149:2378–84.
- 3 Byron KA, O'Brien RM, Varigos GA, Wootton AM. *Dermatophagoides pteronyssinus* IL-induced interleukin-4 and interferon-gamma expression by freshly isolated lymphocytes of atopic individuals. *Clin Exp Allergy* 1994; 24:878–83.
- 4 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17:138–46.
- 5 Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989; 73:1504–12.
- 6 Parronchi P, Manetti R, Simonelli C, Rugiu FS, Piccinini MP, Maggi E, Romagnani S. Cytokine production by allergen (Der p 1) -specific CD4+ T cell clones derived from a patient with severe atopic disease. *Int J Clin Laboratory Research* 1991; 21:186–9.
- 7 Chang JH, Chan H, Quirce S, Green T, Noertjojo K, Lam S, Frew A, Keown P, Chan-Yeung M. *In vitro* T-lymphocyte response and house dust mite-induced bronchoconstriction. *J Allergy Clin Immunol* 1996; 98:922–31.
- 8 Till S, Dickason R, Huston D, Humbert M, Robinson D, Larche M, Durham S, Kay AB, Corrigan C. IL-5 secretion by allergen-stimulated CD4+ T cells in primary culture: relationship to expression of allergic disease. *J Allergy Clin Immunol* 1997a; 99:563–9.
- 9 Chapman MD, Platts-Mills TA. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980; 125:587–92.
- 10 Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985b; 76:753–61.
- 11 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TA. Antigenic and structural analysis of group II allergens (Der f II and Der, p. II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; 83:1055–67.
- 12 Stewart GA, Thompson PJ, Simpson RJ. Protease antigens from house dust mite. *Lancet* 1989; 2:154–5.
- 13 Ando T, Homma R, Ino Y, Ito G, Miyahara A, Yanagihara T, Kimura H, Ikeda S, Yamakawa H, Iwaki M. Trypsin-like protease of mites: purification and characterisation of trypsin-like protease from mite faecal extract *Dermatophagoides farinae*. Relationship between trypsin-like protease and Der f III. *Clin Exp Allergy* 1993; 23:777–84.
- 14 Aki T, Kodama T, Fujikawa A, Miura K, Shigeta S, Wada T, Jyo T, Murooka Y, Oka S, Ono K. Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *Dermatophagoides farinae*. *J Allergy Clin Immunol* 1995; 96:74–83.
- 15 King C, Simpson RJ, Moritz RL, Reed GE, Thompson PJ, Stewart GA. The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1996; 98:739–47.
- 16 Tsai LC, Chao PL, Shen HD, Tang RB, Chang TC, Chang ZN, Hung MW, Lee L, Chua KY. Isolation and characterization of a novel 98-Kd *Dermatophagoides*. *J Allergy Clin Immunol* 1998; 102:295–303.

- 17 Shen HD, Chua KY, Lin KL, Hsieh KH, Thomas WR. Molecular cloning of a house dust mite allergen with common antibody binding specificities with multiple components in mite extracts. *Clin Exp Allergy* 1993; 23:934–40.
- 18 Lin KL, Hsieh KH, Thomas WR, Chiang BL, Chua KY. Characterization of Der p V allergen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein. *J Allergy Clin Immunol* 1994; 94:989–96.
- 19 Lynch NR, Thomas WR, Garcia NM, Di Prisco MC, Puccio FA, L'Opez RI, Hazell LA, Shen HD, Lin KL, Chua KY. Biological activity of recombinant Der p 2, Der p 5 and Der p 7 allergens of the house-dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 1997; 114:59–67.
- 20 Yasueda H, Mita H, Akiyama K, Shida T, Ando T, Sugiyama S, Yamakawa H. Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993; 23:384–90.
- 21 O'Brien RM, Thomas WR, Wootton AMT. Cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1992; 89:1021–31.
- 22 O'Brien RM, Thomas WR. Immune reactivity to Der p I and Der p II in house dust mite sensitive patients attending paediatric and adult allergy clinics. *Clin Exp Allergy* 1994a; 24:737–42.
- 23 Kimura JY, Ohta N, Ishii A, Nagano T, Usui M. Functional characterization of lymphocyte response to fractionated house dust mite antigens (*Dermatophagoides pteronyssinus*) in atopic and non-atopic individuals. *Immunology* 1990; 70:385–90.
- 24 McHugh SM, Lachmann PJ, Ewan PW. Peripheral blood mononuclear cells from house dust mite allergic patients produce IL-2 in response to specific allergen challenge. *Clin Exp Allergy* 1993; 23:137–44.
- 25 Okano M, Nagano T, Kino K, Yasueda H, Baba Y, Saito C, Masuda Y, Ohta N. Population analysis of cellular responses to synthetic peptides of Der p II, a major allergen molecule of *Dermatophagoides pteronyssinus*, in allergic and nonallergic subjects. *Allergy* 1994; 49:436–41.
- 26 Horneff G, Schou C, Wahn V. Diagnostic significance of *in vitro* T-cell proliferative responses to house-dust mite Der p I in children with dust-mite allergy. *Allergy: European J Allergy Clin Immunology* 1996; 51:842–6.
- 27 Fujii S, Ono K, Shigeta S, Jyo T, Yamashita U. Human T cell responses to recombinant mite antigens of *Dermatophagoides farinae*. *Clin Exp Immunology* 1997; 108:284–8.
- 28 Wurtzen PA, van Neerven RJ, Arved J, Ipsen H, Sparholt SH. Dissection of the grass allergen-specific immune response in patients with allergies and control subjects: T-cell proliferation in patients does not correlate with specific serum IgE and skin reactivity. *J Allergy Clin Immunol* 1998; 101:241–9.
- 29 Halvorsen R, Bosnes V, Thorsby E. T cell responses to a *Dermatophagoides farinae* allergen preparation in allergics and healthy controls. *Int Arch Allergy Appl Immunology* 1986; 80:62–9.
- 30 Cavaillon JM, Fitting C, Guinnee MT, Rassemont R, David B. Lymphocyte proliferative responses to the purified *Dermatophagoides farinae* major allergen in untreated and hyposensitized atopic patients. *Allergy: European J Allergy Clin Immunology* 1988; 43:146–51.
- 31 O'Hehir RE, Bal V, Quint D, Moqbel R, Kay AB, Zanders ED, Lamb JR. An *in vitro* model of allergen-dependent IgE synthesis by human B lymphocytes: comparison of the response of an atopic and a non-atopic individual to *Dermatophagoides* spp. (house dust mite). *Immunology* 1989; 66:499–504.
- 32 Wierenga EA, Snoek M, Bos JD, Jansen HM, Kapsenberg ML. Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. *European J Immunology* 1990a; 20:1519–26.
- 33 O'Hehir RE, Verhoef A, Panagiotopoulou E, Keswani S, Hayball JD, Thomas WR, Lamb JR. Analysis of human T cell responses to the group II allergen of *Dermatophagoides* species: localization of major antigenic sites. *J Allergy Clin Immunol* 1993b; 92:105–13.
- 34 Upham JW, Holt BJ, Baronhay MJ, Yabuhara A, Hales BJ, Thomas WR, Loh RKS, Okeeffe PT, Palmer L, Lesouef PN. Inhalant allergen-specific T-cell reactivity is detectable in close to 100-percent of atopic and normal individuals – covert responses are unmasked by serum-free medium. *Clin Exp Allergy* 1995; 25:634–42.
- 35 Slunt JB, Taketomi EA, Platts-Mills TA. Human T-cell responses to *Trichophyton tonsurans*: inhibition using the serum free medium Aim V. *Clin Exp Allergy* 1997; 27:1184–92.
- 36 Tame A, Sakiyama Y, Kobayashi I, Terai I, Kobayashi K. Differences in titres of IgE, IgG4 and other IgG subclass anti-Der p 2 antibodies in allergic and non-allergic patients measured with recombinant allergen. *Clin Exp Allergy* 1996; 26:43–9.
- 37 Smith AM, Yamaguchi H, Platts-Mills TAE, Fu SM. Prevalence of IgG anti-Der p 2 antibodies in children from high and low antigen exposure groups – relationship of IgG and subclass antibody responses to exposure and allergic symptoms. *Clin Immunol Immunopathology* 1998; 86:102–9.
- 38 Chua KY, Dilworth RJ, Thomas WR. Expression of *Dermatophagoides pteronyssinus* allergen, Der p II, in *Escherichia coli* and the binding studies with human IgE. *Int Arch Allergy Appl Immunology* 1990a; 91:124–9.
- 39 Hales BJ, Thomas WR. T-cell sensitization to epitopes from the house dust mites *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. *Clin Exp Allergy* 1997; 27:868–75.
- 40 Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy* 1985; 15:411–8.
- 41 Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respiratory Dis* 1989; 139:806–17.
- 42 Bousquet J, Chanet P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P. Eosinophilic inflammation in asthma. *New England J Med* 1990; 323:1033–9.
- 43 Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twyman OP, Howarth RH, Holgate ST. Mucosal inflammation in asthma. *Am Rev Respiratory Dis* 1990; 142:434–57.

- 44 Azzawi M, Johnston PW, Majumdar S, Kay AB, Jeffery PKT. Lymphocytes and activated eosinophils in airway mucosa in fatal asthma and cystic fibrosis. *Am Rev Respiratory Dis* 1992; 145:1477–82.
- 45 Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, Irani AM, Kay AB, Durham SR. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992a; 89:877–83.
- 46 de Graaf-i, n t Veld C, Garrelds IM, Koenders S, Gerth van Wijk R. Relationship between nasal hyperreactivity, mediators and eosinophils in patients with perennial allergic rhinitis and controls. *Clin Exp Allergy* 1996; 26:903–8.
- 47 Di Lorenzo G, Mansueto P, Melluso M, Candore G, Colombo A, Pellitteri ME, Drago A, Potestio M, Caruso C. Allergic rhinitis to grass pollen: measurement of inflammatory mediators of mast cell and eosinophils in native nasal fluid lavage and in serum out of and during pollen season. *J Allergy Clin Immunol* 1997; 100:832–7.
- 48 Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *New England J Med* 1992; 326:298–304.
- 49 Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am J Respiratory Cell Mol Biol* 1995; 12:477–87.
- 50 Shibasaki M, Tajima K, Morikawa A, Mitsuhashi M, Sumazaki R, Tokuyama K. Relation between frequency of asthma and IgE antibody levels against *Dermatophagoides farinae* and total serum IgE levels in schoolchildren. *J Allergy Clin Immunol* 1988; 82:86–94.
- 51 Rawle FC, Mitchell EB, Platts-Mills TAT. Cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P1: comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 1984; 133:195–201.
- 52 Romano MF, Valerio G, Turco MC, Spadaro G, Venuta S, Formisano S. Defect of CD2- and CD3-mediated activation pathways in T cells of atopic patients: role of interleukin 2. *Cellular Immunol* 1992; 139:91–7.
- 53 Jung T, Lack G, Schauer U, Uberuck W, Renz H, Gelfand EW, Rieger CH. Decreased frequency of interferon-gamma- and interleukin-2-producing cells in patients with atopic diseases measured at the single cell level. *J Allergy Clin Immunol* 1995b; 96:515–27.