

Short communication

Diagnostic significance of *in vitro* T-cell proliferative responses to house-dust mite Der p 1 in children with dust-mite allergy

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The study aimed to investigate the possible diagnostic significance of T-cell proliferative responses to purified Der p 1 antigen in allergic children with and without allergic sensitization to house-dust-mite (HDM) allergens. T-cell reactivity to purified Der p 1 antigen was analyzed in 24 children with allergic sensitization to HDM demonstrated by RAST and/or positive skin prick tests. Twelve healthy young adults and 11 children with allergic sensitizations other than HDM served as controls. Of 25 HDM-allergic patients, 16 displayed strong T-cell reactivity to Der p 1 (stimulation indices >3): nine patients showed no T-cell proliferation despite the presence of specific IgE, and five showed no responses despite positive skin prick test. In two patients, a weak T-cell response to Der p 1 could be demonstrated in the absence of specific IgE and negative skin test result. The two affected subjects did not show evidence of mite allergy. No T-cell responses were observed in adult controls (stimulation indices <3). We conclude that the assessment of T-cell reactivity to Der p 1 is of little value for the diagnosis of HDM allergy. The importance of T-cell proliferative responses for the study of the pathogenesis of HDM allergy remains unchallenged.

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Children with allergic disorders such as asthma, eczema, and rhinitis frequently show allergic sensitizations to house-dust-mite (HDM) allergens. Two closely related HDM species are of major importance, *Dermatophagoides pteronyssinus* and *D. farinae*. Der p 1 and Der f 1, both of 24 kDa, have been identified as major allergens (1, 2).

Since the synthesis of IgE is partly regulated by T-cell subsets producing stimulatory cytokines such as interleukin (IL)-4, the production of specific IgE requires antigen-specific T cells (3, 4). In adult patients sensitized to HDM, peripheral blood T-cell responses to Der p 1 have been shown (5). On the clonal level, T cells specific for Der p 1 seem to belong to the TH2-subtype, secreting mainly IL-4 and IL-5 when stimulated with Der p 1 (6). Interestingly, these clones can also produce IL-2 and interferon-gamma when stimulated with polyclonal activators. Since stimulation of T-cell clones with

Der p 1 results in TH2-like responses, we asked whether T-cell reactivity to Der p 1 is restricted to patients with typical mite allergy.

As B- and T-cell epitopes on Der p 1 are not identical, we investigated whether atopic children may display T-cell responses to Der p 1 in the absence of HDM-specific IgE, or whether patients may exhibit specific IgE but no T-cell proliferation. Our results showed that lymphocyte-proliferation studies mostly confirm humoral responses and are of little or no value for routine evaluation of children with HDM allergy.

Material and methods

Patient selection

Thirty-five children with atopic diseases were investigated, of whom 29 had allergic asthma, three

had atopic eczema, and three had asthma and eczema. Twelve healthy adults without a history of atopic disease or HDM-specific IgE serum levels served as controls. Skin prick tests (SPT) with a panel of common allergens were performed in all patients with commercial extracts (ALK, Horsholm, Denmark). Positive reactions were scored from + to ++++.

Serologic studies

The total and specific IgE to HDM and other inhalant allergens relevant to the Düsseldorf area was determined by the enzyme immunoassay sorbent test (CAP-EIAST, Pharmacia).

Cell preparation and surface-marker analysis

After we had obtained parental informed consent, peripheral blood mononuclear cells (PBMC) were separated from heparinized peripheral blood by standard Ficoll-Hypaque density centrifugation, as described elsewhere (7). Analysis of lymphocyte subpopulations was performed by direct immunofluorescence with a Becton-Dickinson FACScan. Two-color staining was performed with fluorescein isothiocyanate (FITC) and phycoerythrin-labeled monoclonal antibodies to CD3, CD4, CD8, CD20, CD25, and CD29. Nonspecific murine IgG1 and IgG2 (isotype controls), as well as monoclonal antibodies, were purchased from Becton-Dickinson.

For lymphocyte-function assays, PBMC were carefully washed and cultured in RPMI 1640 (Gibco) supplemented with penicillin (100 mg/ml), streptomycin (50 mmol/l), glutamine (2 mmol/l), and 10% pooled AB serum at 1×10^6 cells/ml for 7 days at 37°C in a 5% CO₂ humidified atmosphere. T-cell reactivity to the major HDM allergen Der p 1 was measured by ³H-labeled thymidine incorporation, as described by Burmester et al. (7). Tetanus toxoid served as a control antigen to establish antigen-specific lymphocyte reactivity. Experimental conditions had previously been optimized with respect to time course and dose response to Der p 1 with cells of two HDM-allergic adults and one child. For stimulation, Der p 1 was used at concentrations of 0.01, 0.1, 1, 10, and 100 µg per ml. To define the optimal culture period, we cultured cells for 6, 7, or 8 days and then harvested them. Maximum mean stimulation values were obtained on day 7 with either 1 or 10 µg/ml of the Der p 1 antigen. Subsequent analyses were thus performed by stimulation of PBMC with 1 and 10 µg/ml Der p 1 for 7 days, and optimum stimulation rates were used for calculations. Tetanus toxoid was used at dilutions of 1 : 1000 and 1 : 10 000. The stimulation

increment (Δ cpm) of triplicates was calculated as the difference between cultures with maximal antigen stimulation and cultures with medium alone. The stimulation index was calculated by dividing the results of stimulated cultures by those of medium controls.

Der p 1 was purified from whole-mite culture of *D. pteronyssinus*. The purification was performed by hydrophobic interaction chromatography, essentially as described by Lind (8). As judged by SDS-PAGE stained for protein content, the purified preparation contained only minor contaminants, none of which were allergens.

Results

RAST and skin prick test (SPT)

In the 12 healthy adult controls, only CAP-EIAST analyses were performed. All 35 children were studied by SPT and CAP-EIAST. There was no evidence of HDM sensitization in 10 children with atopic asthma and one with atopic eczema. Of the 24 children with positive CAP-EIAST to Der p, 23 also reacted to Der f. No patient had IgE to Der f alone. Of the 24 patients with positive CAP-EIAST, 20 also had positive SPT to Der p and Der f. No patient with negative CAP-EIAST had positive SPT.

Patient population and characterization

According to CAP-EIAST results with Der p, the children were divided into three groups (Table 1). *Group I* comprised 11 atopic children with negative CAP-EIAST and negative SPT to Der p or Der f. *Group II* comprised seven children with weak sensitization to HDM (CAP-EIAST classes 1–3 for Der p), of whom 5/7 had positive SPT, all had allergic asthma, and all but one were sensitized to various inhalant allergens, as demonstrated by CAP-EIAST and/or SPT. *Group III* comprised 17 children (13 with asthma, three with asthma and eczema, and one with eczema alone) with a high degree of sensitization to HDM (CAP-EIAST classes 4–6 to Der p). Sixteen children also had specific IgE to Der f (CAP-EIAST classes 3–5). Except for two patients, SPT was positive to both Der p and Der f.

T-cell studies

There was no difference in the relative distribution of lymphocytes in the peripheral blood with regard to CD3, CD4, or CD8 expression. In addition, the relative proportion of activated T cells, as determined by CD25-positive T cells, did not differ in

Table 1.

	M/F	Age mean $\pm$ SD	Total IgE (kU/l)	CAP-RAST class (Der p)
Group I	7/4	6.4 $\pm$ 2.9	56 $\pm$ 61	0
Group II	5/2	10.8 $\pm$ 3.5	262 $\pm$ 358	1–3
Group III	12/5	7.5 $\pm$ 2.9	632 $\pm$ 557	4–6

Table 2.

		Der p 1		Tetanus toxoid	
		Δ cpm	Stimulation index	Δ cpm	Stimulation index
Controls	n = 12	646 $\pm$ 375	2.1 $\pm$ 0.8	14 030 $\pm$ 15 303	21.5 $\pm$ 14.2
RAST 0	n = 11	413 $\pm$ 428	1.4 $\pm$ 0.6	6980 $\pm$ 7082	8.1 $\pm$ 7.7
RAST 1–3	n = 7	4458 $\pm$ 5127	3.3 $\pm$ 2.6	22 633 $\pm$ 9635	16.8 $\pm$ 9.9
RAST 4–6	n = 17	4297 $\pm$ 4763	6.7 $\pm$ 5.1	4090 $\pm$ 4763	12.4 $\pm$ 24.8

allergic children from healthy adults. The number of CD20-positive B cells was relatively higher in children, while the memory subset of T-helper cells (CD29-expressing subset of CD4-positive cells) in the allergic children did not reach the levels of healthy adults. No differences in surface-marker expression were found in HDM-sensitized children with demonstrable T-cell reactivity compared to those without (data not shown).

T-cell reactivity was tested in 35 atopic children and 12 healthy adults. Tetanus toxoid (TT) responses were measured in all but two children and all adults to assess antigen-specific T-cell reactivity in general. Although all children had been immunized with TT during the first 2 years, only 23/33 tested showed significant *in vitro* response to TT, 5/10 in group I, 7/7 in group II, and 11/16 in group III. Reactivity to TT tended to correlate positively with age, whereas the opposite seemed to be the case for reactivity to Der p 1 (data not shown).

In control groups consisting of 12 healthy adults without demonstrable specific IgE, there were only insignificant responses to Der p 1 (Δ cpm < 1500, SI < 3), as shown in Table 2. Data obtained from healthy adults were used to calculate a normal range of mean plus one standard deviation for the increment of proliferation (Δ cpm 1021) and for the stimulation indices (SI 2.9).

Stimulation indices (SI) in group I patients (controls) were 1.2 $\pm$ 0.4 (1 μ g Der p 1/ml) and 1.9 $\pm$ 1.6 (10 μ g Der p 1/ml). In group I, 2/11 children showed significant T-cell responses to Der p 1, patient 1 with Δ cpm of 6450 and SI of 6.6, and patient 2 with Δ cpm of 3800 and SI of 2.8, despite negative RAST and SPT. Of the seven children in

group II with weak HDM sensitization, three showed significant T-cell reactivity to Der p 1 *in vitro*, with Δ cpms of 2727, 9887, and 13 199 and SIs of 2.2, 7.5, and 6.7, respectively. Of the 17 highly sensitized group III patients, 11 showed significant T-cell reactivity (Δ cpm up to 13 480, SI up to 20.3), while in six patients, only weak T-cell responses were obtained. In total, 10/24 patients in groups II and III showed no significant T-cell reactivity, although all had positive RAST results and all but four had positive SPT (Fig. 1).

Discussion

The following major points emerged from our study:

- 1) There was significant T-cell reactivity to the Der p 1 antigen in most Der p-allergic children.
- 2) Strong *in vitro* T-cell responses were positively correlated with the degree of allergic sensitization assessed by SPT and RAST.
- 3) In the control groups with negative RAST or SPT, only a few weak T-cell reactions were observed *in vitro* in subjects without evidence of disease.

Because synthesis of allergen-specific IgE requires cognate interaction of antigen-presenting cells with T cells, one would expect T-cell reactivity to HDM antigens to be common in allergic patients. Interestingly, only 60% of our HDM-allergic children showed detectable T-cell response to Der p 1, a finding which is in accordance with previous findings in adults (8, 9).

Although specific IgE was measured with whole-mite extracts and the T-cell response to purified major allergen, B- and T-cell responses correlated well. Quantitatively, a high degree of sensitization by SPT or RAST correlated with high T-cell responses to Der p 1. In the control group of atopic children without allergic sensitization to HDM, there were only two children with some T-cell proliferation *in vitro* in the absence of specific IgE in serum. This finding may reflect the fact that T cells reactive to Der p allergens are common even in normal subjects; however, their frequency in blood is lower than in atopic patients. This observation is in accordance with O'Brien & Thomas (10), who demonstrated T-cell proliferative responses to Der p 1 and Der p 2 in all patients with specific IgE, but also in some subjects without demonstrable specific IgE reactivity. In addition, with serum-free medium, it has been shown that significant T-cell proliferative response to Der p 1 and Der p 2 can be demonstrated in adults with and without specific IgE (6).

The fact that normal subjects with T-cell recognition of Der p 1 do not synthesize IgE may be

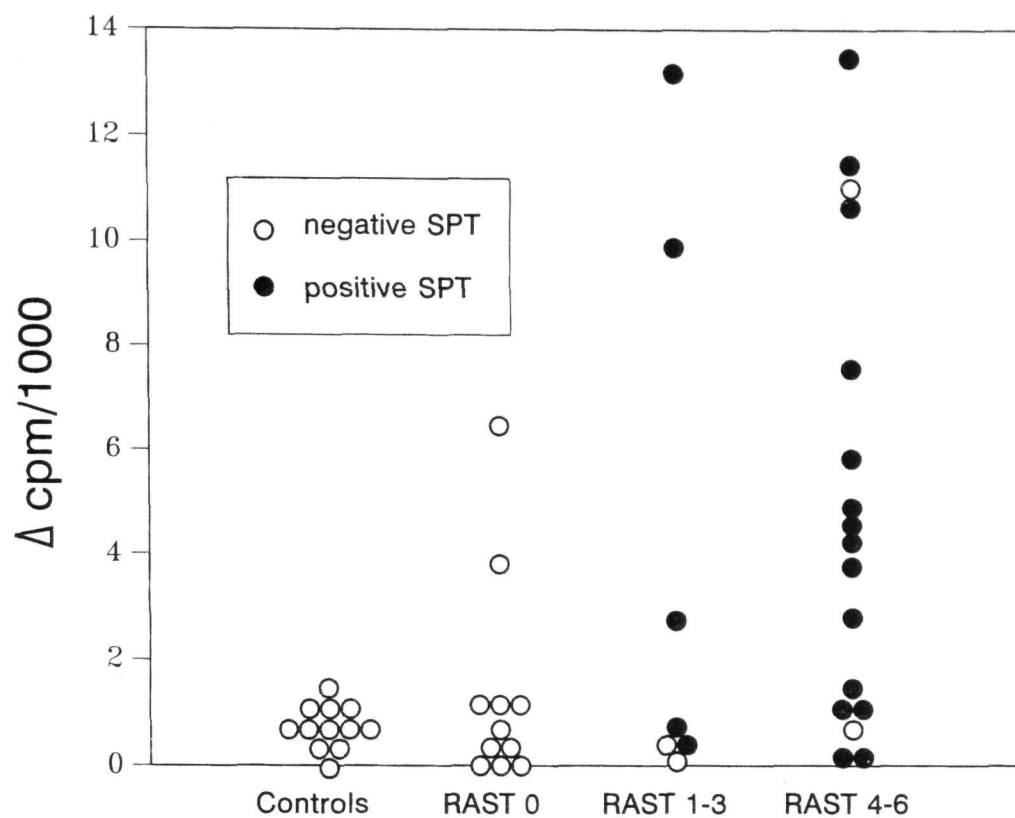


Fig. 1. Specific T-cell response to purified Der p 1 antigen determined by ^3H -labeled thymidine incorporation. Stimulation increment (Δcpm) was calculated as difference between stimulated cultures and cultures with medium alone.

linked to the subset (TH1 or TH2) of helper T cells involved in allergen recognition (11). A recent study of T-cell clones found that most of the HDM-specific clones from atopic patients exhibited a TH2 cytokine production profile. In contrast, HDM-reactive clones from a nonallergic control were of the TH1 type (12). This TH2 cytokine production profile may result in higher frequencies of IgE-producing B-cell precursors in atopic patients than in nonatopic subjects (13). Currently, studies are underway in our laboratory to analyze precursor frequencies specific for Der p 1 and characterize T-helper clones for their cytokine profile.

From our results, we conclude that the analysis of T-cell reactivity to Der p 1 antigen is of minor importance for the diagnosis of HDM allergy. However, comparative analyses of T-cell proliferative responses to Der p 1 in atopic and nonatopic subjects may be essential to understand why, despite identical environmental conditions, one person develops HDM allergy while another remains unaffected.

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