

Low-level exposure to house dust mites stimulates T-cell responses during early childhood independent of atopy

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Summary

Background The immune responses which underlie the expression of allergic symptoms in childhood are believed to be initiated in infancy and early childhood. The kinetics of this response have hardly been researched.

Objective To analyse, in an environment with low house dust mite (HDM) exposure levels, the relationship between house dust mite (HDM)-specific T-cell reactivity as expressed by *in vitro* proliferation of blood mononuclear cells.

Methods The study comprised a prospective analysis of patterns of allergen-specific T-cell reactivity in a cohort of 19 children, from whom blood samples were obtained in the spring during their second and third years of life. Blood mononuclear cell cultures were established in 200 μ L AIM-V serum free medium. Crude house dust mite (HDM) and purified Der p 1 and Der p 2 extracts were used at optimal concentrations, i.e. 100 μ g/mL for HDM and 30 μ g/mL for the purified allergens. Tetanus toxoid (0.5 μ g/mL) and ovalbumin (10 μ g/mL) served as positive controls. A clinical diagnosis of allergy was verified with skin-prick tests. Dust samples were collected from a mattress and/or carpet or sofa in homes, day care centres and day care homes. Major mite allergen levels (Der p 1/Der f 1) in dust were analysed by an enzyme linked immunosorbent assay (ELISA).

Results Specific T-cell responses were seen in the majority of the children against house dust mite (crude HDM extract, Der p 1 and Der p 2). The levels of the house dust mite allergens Der p 1 and Der f 1 were low, i.e. <0.68 μ g/g fine dust in the homes of the children and the day care centres that they were attending. This indicates that doses of mite antigen well below the suggested sensitization threshold level of 2 μ g/g dust can induce mite-specific T-cell responses in young children. None of them showed clinical reactivity to house dust mites as indicated by negative skin-prick tests.

Conclusions The findings suggest that active immunological recognition of environmental allergens and the ensuing initiation of allergen-specific T-cell responses, is a normal part of the 'education' of the immune system in early childhood and can occur even at very low exposure levels. Priming *per se* does not imply clinically significant sensitivity, however.

Keywords: allergy, allergens, T-cell response, children, house dust mites, prospective

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Introduction

The immune responses which underlie the expression of allergic symptoms in childhood are believed to be initiated in infancy and early childhood [1]. Thus, IgE [2] and IgG

subclass [3] antibodies are commonly detected by the age of 1 year. In the majority of cases, the IgE component of these early allergen-specific immune responses is transient [2]; this finding, taken together with the data indicating that allergen-specific T-cell responses typically partition into either TH₁-like (normals) or TH₂-like (atopics) patterns by adulthood [4–6], suggests that these early immune

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responses involve an ongoing process of T-cell selection via cross-regulation between competing TH-cell populations [1]. The selection process is driven by normal environmental allergen exposure, and is likely to be influenced by various environmental factors, e.g. infections and pollutants [7,8]. Studies on these 'early' allergen specific T-cells in infants and young children have until recently been hampered in humans by the relatively low capacity of existing T-cell culture systems to detect responses at low precursor frequency. Recently however, improved methodology based upon serum free medium has been developed, which markedly increases the capacity to detect TH-cell responses [9], and permits reproducible demonstration of allergen-specific T-cell reactivity in a high proportion of neonates [10].

This methodology has been employed to study the development of T-cell immunity to house dust mites (HDM), which constitute a major source of indoor allergens in most climatic zones [11]. The study population are from Linköping in Sweden, which has a climate which is unfavourable to mite growth, and consequently lower levels are experienced than in more temperate areas [12]. As part of a prospective study on the kinetics of postnatal development of immune responses to allergens, we have analysed the relationship between HDM-specific T-cell reactivity and environmental HDM exposure levels of individual children, with reference to the suggested threshold level for sensitization of 2 µg/g dust [11].

Materials and methods

Study group and procedure

The study group comprised 26 children with a family history of allergy born between August 1991 and March 1992 after a normal pregnancy and uncomplicated delivery. For this study, blood samples were available from 19 of them. The study was designed to allow an analysis of immune responses to a seasonal allergen, i.e. birch over several years. Blood samples were therefore obtained in 1993 and 1994 during a 4-week period in May–June, i.e. during the peak of the birch pollen season. On these occasions the parents completed a questionnaire regarding the presence of symptoms and signs of allergy in their children and a physical examination was done. Furthermore, skin-prick tests were done in single using fresh cow's milk and hen's egg and standardized extracts of birch, grass and house dust mite (SQTM, ALK Hørsholm, Denmark). The diagnostic criteria were as defined previously, i.e. recurrent obstruction with wheezing at least three times and at least once verified by a physician, atopic dermatitis according to the criteria by Hanifin and Rajka and urticaria appearing at least twice in connection with a

known exposure to the offending agent [13]. Children with a confirmed clinical diagnosis and/or at least one positive skin-prick test were identified as 'atopic'. Eight children manifested eczema and/or asthma and at least one positive skin-prick test, while 11 were non-atopic.

The study was approved by the Local Ethics Committee at Linköping University.

Lymphocyte proliferation

Blood was collected into an equal volume of RPMI medium (Gibco, New York) containing preservative free heparin. Peripheral blood mononuclear cells (PBMC) were isolated on a Lymphoprep density gradient (Nycomed, Oslo, Norway) and washed three times in RPMI containing 2% fetal calf serum. The PBMC were frozen in a mixture of 10% DMSO in RPMI with 2% fetal calf serum at a concentration of 2×10^6 cells/mL. They were thawed in a 37 °C water bath. When the last ice crystals were thawing, ice cold RPMI was added dropwise. After washing the cells they were resuspended in culture medium.

Cultures were established in triplicate, comprising 2×10^5 cells in 200 µL AIM-V serum free medium (Gibco, New York), supplemented with 2×10^{-5} M 2-mercaptoethanol, in round bottom microplate wells. Crude house dust mite (HDM) and purified Der p 1 and Der p 2 extracts were gifts from Dr W.R. Thomas. They were used at optimal concentrations, i.e. 100 µg/mL for HDM and 30 µg/mL for the purified allergens. Due to an insufficient number of cells, all samples could not be tested with all the three antigens. Tetanus toxoid (0.5 µg/mL) and ovalbumin (10 µg/mL) served as positive controls.

³H-DNA synthesis was determined after 7 days in culture, adding tritiated methyl thymidine (0.5 µCi, Amersham) as labels and expressed as median counts per minute (cpm). The stimulation ratio (SR) was defined as the ratio of cpm in antigen containing cultures minus cpm in background controls. Stimulation ratios ≥ 2 , accompanied by a net response >1000 cpm were taken as indicative of positive responses.

Analysis of allergen levels

Dust samples were collected from a mattress and a carpet or sofa in homes and day care centres. All the samples were collected by one person using the same vacuum cleaner (Electrolux, 1100 W) connected to a special dust collector containing a filter (ALK). The dust sampling was standardized by time and area. Both sampling locations were vacuum cleaned for 2 min/m² with a total area of 2 m². Parents of the children were advised not to clean their houses for 3 days prior to collection of the dust samples.

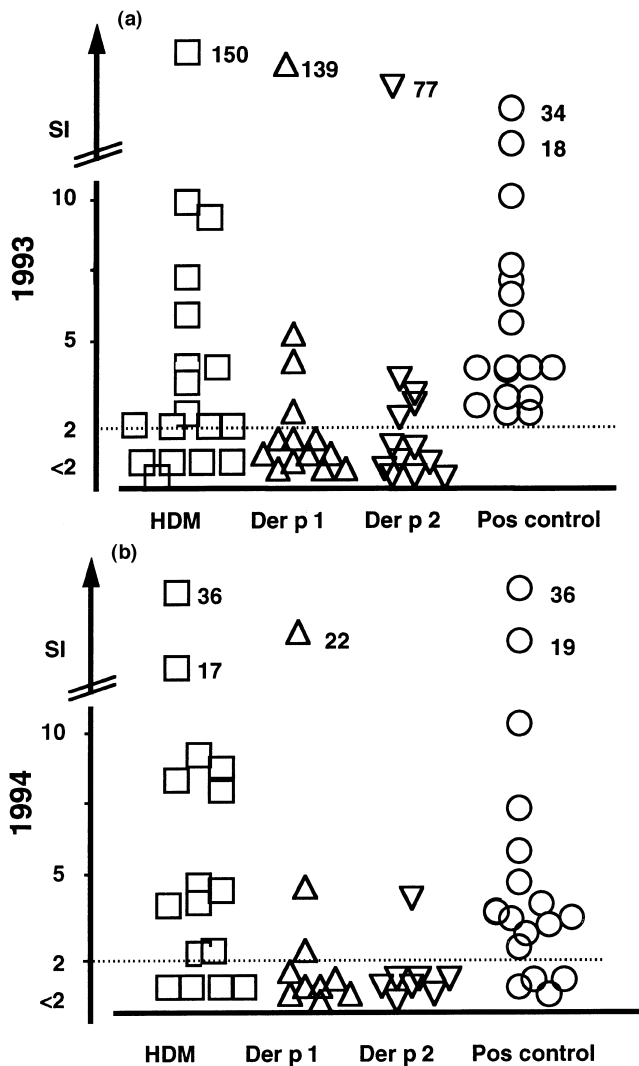


Fig. 1. Proliferative responses (expressed as stimulation indices, SI) to crude house dust mite extract (100 $\mu\text{g/mL}$) and purified Der p 1 and Der p 2 (30 $\mu\text{g/mL}$) by blood mononuclear cells in 1993 and 1994. Tetanus toxoid (0.5 $\mu\text{g/mL}$) and ovalbumin (10 $\mu\text{g/mL}$) served as positive controls. (a) 1993. (b) 1994.

In three day care centres no carpet or sofa was present. Thus, out of an ideal number of 72 samples from the homes and day care centres, 69 samples were available for analysis. Aqueous extracts of house dust samples were prepared by using an extraction ratio of 1:20 (w/v) by dissolving the dust sample in Ammonium bicarbonate buffer (pH 7.8) during constant rotation at +4 °C, overnight [12]. The extracts were stored frozen at -20 °C until analysis was done.

Major mite allergen levels (Der p 1/Der f 1) in dust were analysed by an enzyme linked immunosorbent assay (ELISA). The ELISA kits were obtained from ALK. The analysis was performed as described previously [14].

Results

Twenty-one blood samples from 14 children were tested in parallel with crude and purified Der p 1 and Der p 2 (Table 1, Fig. 1). In five children there was not a sufficient number of cells to allow an analysis with all the three antigens. In 15 children two blood samples were analysed. Employing the cut-off levels described above, positive blood mononuclear cell responses to HDM were present in at least one of the annual samples in all of them, as well as in three of the four children who were analysed only once (Table 1). The proliferative responses to Der p 1 and Der p 2 were generally smaller than against the crude HDM extract, however, their presence confirmed that the responses to HDM were not non-specific. The results were similar in the eight children who developed eczema or recurrent wheezing and in the 11 who did not. None of them were SPT-positive to HDM (Table 1).

The allergen levels at home and in the day care centres were generally low (Table 1). The highest level recorded was 0.68 $\mu\text{g/g}$ dust in one of the homes.

Discussion

The results cannot be explained by the presence of mitogenic activity in the HDM extract. Of the 25 tested samples that were positive for HDM, 19 were tested in parallel against Der p 1 and/or Der p 2, and 10/19 responded to one or both purified allergens. Furthermore, of the nine blood samples that were negative to the HDM extract six were also tested against Der p 1 and/or Der p 2 and they were all negative to the purified antigens. All subjects who responded to either of the purified mite allergens also responded to crude HDM.

On the basis of a wide body of data presented at an international workshop [11], the suggested threshold exposure levels for 'sensitization' to HDM have been set at 2 μg Der p 1 or Der p 2/g dust. The definition of 'sensitization' in this context relates to the presence of mite specific IgE in serum and positive SPT reactivity. The present study focuses on the earliest phase of this process, viz the initial priming of the T-cell system for T-helper cell responses to HDM allergens. The results indicate that T-cell priming can occur at exposure levels which are 10–50-fold lower than the 2 $\mu\text{g/g}$ threshold. This phase of T-cell priming clearly precedes the IgE antibody formation and clinical sensitivity, evidenced by the present finding that none of the subjects in this study were HDM responsive on the basis of SPT or serum IgE. These results are consistent with recent reports that T-cell responses to many environmental antigens may be initiated *in utero* [8,15–18], a process which must involve extremely low-level exposure.

Table 1. T-cell proliferative responses to house dust mite antigens in 19 children and the highest levels of house dust mite allergens (Der p 1 and Der f 1, $\mu\text{g/g}$ dust) detected in either their homes or day care centres that they were regularly attending. Some cell samples were too small to allow stimulation with all the three antigens. Skin-prick test (SPT) results and allergic manifestations (E = eczema, A = asthma) are also shown. ND. Denotes not done due to insufficient cell numbers

Child birthdate	Year	SPT	Symptom	House dust mite		Der p 1		Der p 2		Allergen level, $\mu\text{g/g}$	
				SR	$\Delta\text{-cpm}$	SR	$\Delta\text{-cpm}$	SR	$\Delta\text{-cpm}$	Home	Daycare
MB	1993	–	E	<2		<2		<2			
26.08.1991	1994			8.7	4600	4.5	2100	<2		0.21	0.07
RM	1993	Egg	E	3.8	12 900	ND		ND		0.08	<0.02
15.10.1991	1994			4.0	2700	<2		<2			
HE	1993	Egg	E	4.1	4100	<2		3.9	3900	0.18	0.18
03.10.1991	1994			4.6	4100	<2		<2			
AO	1993	–	A	2.0	1900	<2		<2		0.68	<0.02
09.10.1991	1994			<2		ND		ND			
JH	1993	Birch	–	2.0	4400	<2		<2		0.04	0.06
30.07.1991	1994			8.3	6300	2.6	1400	4.1	2700		
JE	1993	–	–	<2		ND		ND		0.18	0.08
28.02.1992	1994			4.6	5300	<2		<2			
FH	1993	Milk	E	2	6100	ND		ND		0.27	<0.02
25.02.1992	1994			<2		ND		ND			
GR	1993	–	–	9.7	11 700	5.5	6100	3.0	2800	0.35	<0.02
04.01.1992	1994			7.7	12 500	ND		ND			
JC	1993	–	A, E	<2		<2		<2		0.16	0.16
01.10.1991	1994			4.2	14 000	<2		ND			
LP	1993	–	E	6.2	7200	2.5	2100	<2		0.33	0.08
16.03.1992	1994			2.0	2800	<2		<2			
AL	1993	Birch	–	4.4	9200	<2		2.8	5000	0.05	0.08
24.03.1992	1994			<2		<2		<2			
EJ	1993	Birch	–	2.0	6100	<2		<2		0.10	<0.02
13.08.1991	1994			9.2	17 800	2.0	2100	<2			
TB	1993	–	–	<2		<2		<2		0.09	<0.02
17.07.1991	1994			17.1	7000	ND	ND				
AE	1993	–	–	150	2700	139	2500	77	1400	0.08	0.10
06.07.1991	1994			36	12 400	22	7500	<2			
SA	1993	–	–	<2		<2		ND		0.11	0.09
24.02.1992	1994			2.2	3800	ND		ND			
TEK	1993	Birch, timothy	E	7.5	9800	ND		ND		0.12	0.05
11.08.1991											
GH	1993	–	–	2, 7	3900	<2		<2		0.20	<0.02
08.03.1992											
EK	1993	–	–	10.0	5800	4.5	2200	ND		0.06	<0.02
01.08.1991											
PR	1994	–	–	<2		<2		ND		0.07	0.07
07.01.1992											

The available epidemiological data indicate that clinical sensitization to HDM in both adults and children is of low prevalence throughout most of Scandinavia [12]. The present results, indicating high prevalence of T-cell reactivity to the same allergens in Scandinavian infants, imply that T-cell 'recognition' of mite allergens does not per se carry

inherent risk for development of allergy. Instead, these findings are consistent with the general notion that immune recognition of environmental allergens is virtually universal amongst humans [1], and that qualitative aspects of the relevant TH-cell responses determine the nature of individual host reactivity [4–6]. It has been argued that

these TH-cell responses are ultimately shaped by a process of allergen-driven T-cell selection, during infancy and early childhood [1]. In the context of these Scandinavian HDM responses, it thus seems likely that where the low levels of HDM exposure are sufficient for initial T-cell 'priming', they are generally insufficient to drive the process towards TH₂ selection, which appears to be the key to the development of clinical sensitization [4–6]. We do not currently have direct information on the cytokine patterns associated with HDM reactivity in infants or adults in this population, so it is not yet known whether these responses in infancy wane, or develop towards TH₁; these possibilities will be tested in the future studies.

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