

IL-5 T-cell responses to house dust mite are associated with the development of allergen-specific IgE responses and asthma in the first 5 years of life

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Background: Allergen-specific T_H2-like cytokine responses are considered to be important in sensitization and allergic diseases.

Objective: To examine the profile of house dust mite (HDM) stimulated T-cell cytokines and their relationship to allergic disease in children over the period of the first 5 years of life. **Methods:** Subjects with a family history of asthma who were enrolled antenatally in the Childhood Asthma Prevention Study and had skin prick tests, clinical evaluation for asthma and eczema, and *in vitro* assessment of lymphocyte cytokine responses to HDM extract performed at ages 18 months (n = 281), 3 years (n = 349), and 5 years (n = 370). IL-13 at 3 and 5 years and IL-5, IL-10, and IFN- γ at 18 months, 3 years, and 5 years were measured by ELISA.

Results: House dust mite-specific cytokine responses increased with age for all cytokines except IFN- γ . HDM-specific IL-5 responses at 3 years and 5 years were significantly positively related to skin prick test positivity at 5 years. IL-5 responses at 5 years were also significantly related to asthma at 5 years.

Other HDM-specific cytokine responses were not related to

asthma or eczema at 5 years. Responses were not altered by a HDM avoidance intervention.

Conclusion: IL-5 responses to HDM, the dominant local inhalant allergen, are related to the expression of clinical illness at age 5 years.

Clinical implications: The T-cell response to HDM, as reflected in IL-5 production, is acquired over the first years of life and may play a role in the expression of allergic airways disease. (J Allergy Clin Immunol 2007;120:286-92.)

Key words: T cells, cytokines, allergens, asthma, allergy, children, eczema

One proposed mechanism by which environmental and genetic risk factors contribute to the development of atopic disease is through changes in the production of T_H1 and T_H2 cytokines. Since the delineation of type 1 and type 2 CD4⁺ cells in mice¹ and in human beings,² evidence has mounted that T_H2 lymphocytes activated by environmental allergens produce cytokines such as IL-13, IL-5, and IL-4. IL-5 is especially responsible for the cascade of eosinophil activation, and IL-13 and IL-4 for IgE production.³ IL-13 is produced at elevated levels in the asthmatic lung and blood of patients with eczema.^{4,5} These human studies, together with evidence of the effect of neutralizing antibodies in animal models, have led to the view that these cytokines are important in the pathogenesis of allergic diseases, such as asthma and eczema. However, the role individual cytokines play in the pathogenesis of allergic diseases remains unknown, and clinical trials of anti-IL-5 antibodies as therapy for asthma were disappointing.^{6,7}

Cross-sectional and some longitudinal analyses have provided important insights into the significance of allergen-specific and nonspecific cytokine responses in early life.⁸⁻¹² In general, small groups of subjects have been followed for short periods. House dust mite (HDM)-specific cytokine responses and their relationship to the development of allergic disease have not previously been examined in a large, population-based cohort over the period of the first 5 years of life. We reasoned that understanding of the acquisition of T-cell responsiveness and of the

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Abbreviations used

CAPS: Childhood Asthma Prevention Study
HDM: House dust mite
 K_w : Weighted κ value
SPT: Skin prick test

relevance of *in vitro* allergen-specific cytokine responses in the pathogenesis of allergic diseases is likely to be enhanced by prospective studies conducted in large cohorts of children followed from infancy. The Childhood Asthma Prevention Study (CAPS), a randomized controlled trial of HDM avoidance and an intervention to increase the ratio of ω -3 to ω -6 fatty acids in the diet, both implemented from birth to age 5 years in a cohort at increased risk of developing asthma, provided an excellent setting to address these questions.

METHODS

The study was conducted in Sydney, Australia, a region where HDM is the dominant local allergen.¹³ Pregnant women whose unborn children were at high risk of developing asthma were recruited from the antenatal clinics in Sydney, Australia. Subjects ($n = 616$) were randomized to active and control arms for both HDM avoidance and the dietary intervention, separately, by using a factorial design. The trial objectives and design, including details of the participants, interventions, and environmental monitoring, have been reported previously,¹⁴ as have the outcomes at ages 18 months,¹⁵ 3 years,¹⁶ and 5 years.¹⁷ Here we describe the immunologic investigations and the relevant clinical evaluations. The study was approved by research ethics committees of the South Western Sydney Area Health Service and the Children's Hospital at Westmead. Parents of the participating children gave written, informed consent.

Clinical assessment

Children were assessed at ages 18 months, 3 years, and 5 years by a nurse who was blind to the treatment group allocation. When the child was 5 years old, parents were interviewed about symptoms and diagnoses relevant to asthma and allergic disease as previously described.¹⁷ Participants were examined for the presence of flexural eczema. Spirometric lung function was measured as previously described.¹⁷ We defined asthma at the age of 5 years if there was a parental report of any wheeze in the previous 12 months at age 5 years and either a parental report of diagnosed asthma at ages 18 months, 3 years, or 5 years or a $>12\%$ increase in FEV₁ after bronchodilator at age 5 years. Eczema was defined as the presence of flexural eczema on inspection or a parental report of a history of itchy rash coming and going for a period of 3 months or more accompanying a history of seeking medical care for eczema and/or using steroid or emollient creams in the last 12 months. We assessed allergen-specific IgE responses by using skin prick tests (SPTs) to the ingested and inhalant allergens as previously described.¹⁷ Wheal sizes that were ≥ 2 mm and were also $>$ negative control were classified as positive.

Immunologic evaluation

Blood was collected into lithium heparin-containing tubes at ages 18 months, 3 years, and 5 years. PBMCs were isolated and stimulated at 10^6 viable cells/mL in serum-free lymphocyte medium (AIM-V medium; GIBCO Invitrogen Corp, Carlsbad, Calif) with phytohemagglutinin (PHA-mucoprotein; Sigma-Aldrich Co, St Louis, Mo) at

10 μ g/mL, aqueous sonicated extract of *Dermatophagoides pteronyssinus* (CSL Bioscience, Melbourne, Australia) at 50 μ g/mL total protein, or medium alone. The concentration of Der p 1 allergen in the *D pteronyssinus* extract was 16.6 μ g/mL. After incubation at 37°C in 5% CO₂ for 48 hours, the supernatants were collected and stored at -70°C .¹⁸ The cytokines IL-5, IL-10, and IFN- γ were assayed by ELISA by using commercially available antibody sets (BD OptEIA Human Sets; BD Biosciences Pharmingen, North Ryde, New South Wales). For specimens collected at ages 3 and 5 years, IL-13 was also measured (IL-13 Module Set; Bender MedSystems, Vienna, Austria). The cytokine standards were measured in triplicate and samples in duplicate. The limits of cytokine detection were established as the mean of the lowest measurable calibration point for each assay. The cytokine response in phytohemagglutinin-stimulated culture was treated as the positive control and medium-only culture as the negative control. Cytokine levels from the HDM cultures were adjusted for background by deducting either the cytokine level from the corresponding negative control culture or the detection limit, depending on which was greater. Results for samples in which levels of all the cytokines in the phytohemagglutinin culture were less than 3 times the corresponding cytokine level in the negative control culture (or the detection limit for that cytokine, whichever was greater) were considered invalid for all cytokines for that sample. Cytokine concentrations of IL-5, IL-13, and IL-10 in the HDM-stimulated PBMC were classed as nonresponders (<10 pg/mL), low responders (10–50 pg/mL), and high responders (>50 pg/mL). IFN- γ responses were classed as nonresponders (<50 pg/mL) or responders (>50 pg/mL) because the lower limit of detection for this assay was higher than for the other cytokines.

Data analysis

Weighted κ values (K_w)¹⁹ were calculated to estimate agreement between successive occasions (18 months vs 3 years and 3 years vs 5 years) of measurement of cytokine responses to HDM, classified as nonresponders (<10 pg/mL), low responders (10–50 pg/mL), or high responders (>50 pg/mL).

The effects of age and intervention group status on the level of HDM-specific cytokine responses, expressed as continuous variable and adjusted for negative control values, were tested by using generalized estimating equations with an autoregressive error structure.²⁰ A negative binomial error structure was used because of the highly skewed distribution with many 0 values.²¹ The exponents of the relevant coefficients from this model are rate ratios. For age, the rate ratio is the ratio of cytokine levels at 3 years compared with 18 months and at 5 years compared with 3 years. For intervention group, the rate ratio is the ratio of cytokine levels in the active group compared with the control group.

The independent effects of cytokine responses on SPT positivity and clinical disease outcomes were tested by logistic regression. Cytokine responses were classified as an ordinal variable (nonresponders, low and high responders) with randomization status on the 2 interventions and sex included as covariates. For each cytokine and for each outcome, models were constructed to include cytokine responses at each of the 3 time points. Hence, the significance of each cytokine at each time point was tested after adjustment for response of that cytokine at other time points.

The strength of association between IL-5 responder status at 3 years and 5 years, classified as nonresponder and low and high responder, and the outcomes at age 5 years were quantified as relative risk. This was estimated by log-linear model fitted using a log link and a binomial error structure. Separate models were fitted for cytokines at 3 years and 5 years and for each outcome. Each model included response status on all 4 cytokines so that relative risks for IL-5 were adjusted for the effect of response status for other cytokines at that age.

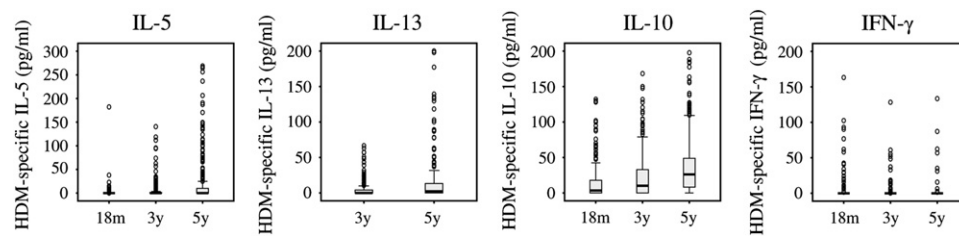


FIG 1. Box and whiskers plots showing the distribution of IL-5, IL-10, and IFN- γ responses at 18 months, 3 years, and 5 years and IL-13 responses at 3 years and 5 years. The following numbers of observations representing extreme outliers are not shown: 2 for IL-5 at 5 years, 3 for IL-13 at 5 years, 2 for IL-10 at 18 months, 2 for IL-10 at 3 years; 1 for IFN- γ at 18 months, and 3 for IFN- γ at 3 years.

TABLE I. Effect of age and the HDM avoidance intervention on HDM-specific cytokine responses

	IL-5	IL-13†	IFN- γ	IL-10
Age (18 mo vs 3 y vs 5 y)	4.54* (2.84-7.26)	3.54 (2.55-4.92)	0.50 (0.34-0.74)	1.52 (1.34-1.73)
HDM avoidance vs control	1.90 (0.94-3.82)	0.92 (0.60-1.41)	1.92 (0.76-4.87)	1.23 (1.00-1.53)

*Values are rate ratios (95% CIs) for predictors of cytokine responses, mutually adjusted for age and intervention group using generalized estimating equations.

†IL-13 was not measured at 18 months.

TABLE II. HDM-specific cytokine responses: number of subjects with different levels of cytokine responses at 18 months, 3 years, and 5 years and within-subject repeatability

	IL-5			IL-13†			IL-10			IFN- γ	
	<10*	10-50	>50	<10	10-50	>50	<10	10-50	>50	<50	>50
18 mo	263	6	1	—	—	—	173	80	22	252	9
K _w ‡	0.18 (−0.07 to 0.43)			—			0.10 (−0.01 to 0.21)			−0.03 (−0.04 to 0.00)	
3 y	297	33	8	246	33	6	168	122	47	330	7
K _w ‡	0.24 (0.14 to 0.35)			0.20 (0.08 to 0.33)			0.09 (0.00 to 0.19)			−0.02 (−0.03 to 0.00)	
5 y	268	50	40	187	60	23	93	167	82	246	4

*Levels of response (pg/mL).

†IL-13 responses were not measured at age 18 months.

‡K_w weighted κ values (95% CIs) for within-subject comparison of cytokine responses between 18 months and 3 years and between 3 years and 5 years.

Data were acquired in a Microsoft Access database and exported for analysis in SAS System for Windows version 9.1 (SAS Institute, Cary, NC). Graphics were created in SPSS Version 15.0 (SPSS Schweiz AG, Zurich, Switzerland).

RESULTS

Characteristics of the population

Valid data for 1 or more cytokine responses were available for 485 subjects, of whom 244 (50.3%) were male subjects and 242 (49.9%) had been randomly allocated to the active HDM avoidance group. There were 281 subjects with valid data for 1 or more cytokines at 18 months, 349 at 3 years, and 370 at 5 years (see this article's Table E1 in the Online Repository at www.jacionline.org). The proportion of subjects with positive SPTs to any allergen increased with age, and the proportion of these who had a positive response to HDM allergen also increased with age (Table E2). At age 5 years, the prevalence of eczema was 22% and the prevalence of asthma was 21%.

House dust mite-specific IL-5 and IL-13 responses increased with age during early childhood. IL-10

responses also increased, but to a lesser extent (Fig 1 and Table I). There were few children with HDM-specific IFN- γ responses at all ages (Fig 1), and these responses decreased with age (Table I). The allergen avoidance intervention did not have a significant effect on HDM-specific cytokine responses (Table I). At age 5 years, subjects who were IL-5 responders were more commonly also IL-13 and IL-10 responders ($P < .0001$; see this article's Table E3 in the Online Repository at www.jacionline.org).

Longitudinal relationship among HDM-specific cytokine responses in early life

T-cell responsiveness to HDM, as reflected in IL-5 production, was rarely detectable at 18 months of age (3% of subjects). However, it was progressively acquired in the preschool years with 12% of subjects at 3 years of age and 25% of subjects at 5 years of age having levels > 10 pg/mL (Table II). Between 3 and 5 years of age, the prevalence of IL-13 responses increased from 14% to 31%. A similar increase was observed for IL-10 responses at the following ages: 37% at 18 months, 50% at 3 years, and 73% by 5 years (Table II).

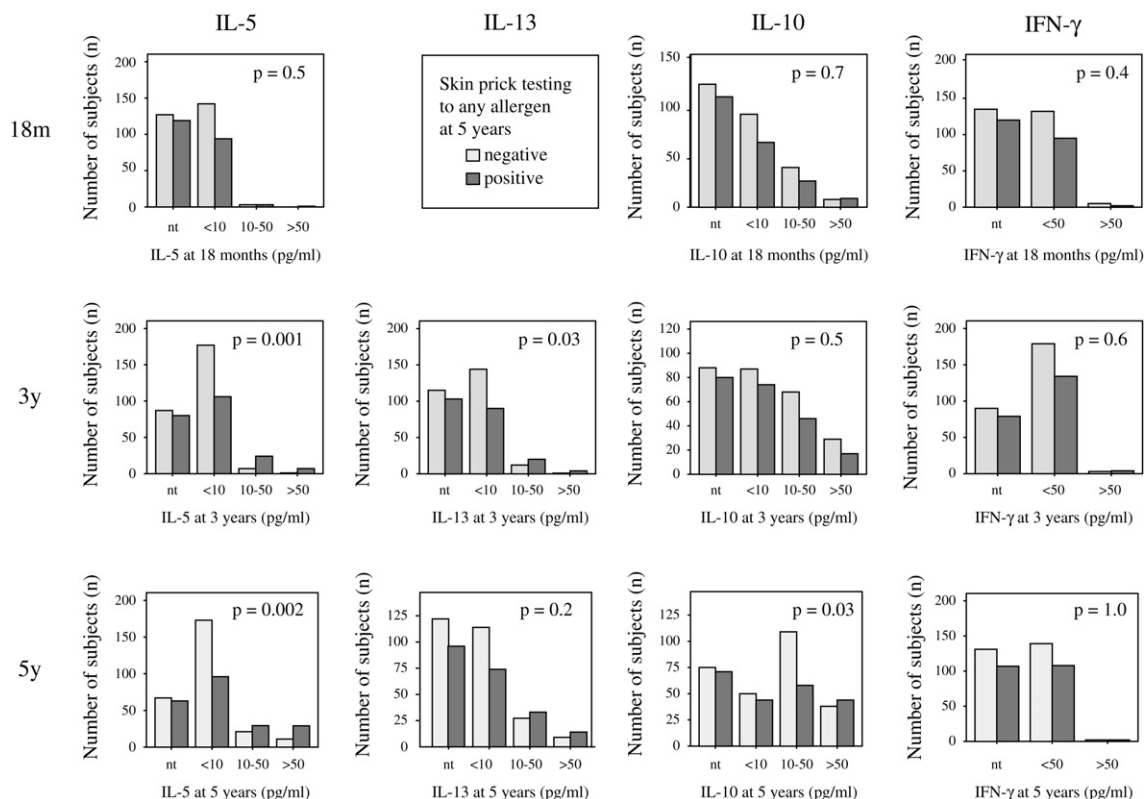


FIG 2. Prevalence of IL-5, IL-13, IL-10, and IFN- γ responses at 18 months, 3 years, and 5 years in relation to allergic sensitization (any SPT wheal ≥ 2 mm) at 5 years. Subjects are classified as nonresponders (<10 pg/mL), low responders (10-50 pg/mL), or high responders (>50 pg/mL) for IL-5, IL-13, and IL-10, and as nonresponders (<50 pg/mL) or responders (>50 pg/mL) for IFN- γ . *P* values are derived from logistic regression models testing HDM-specific cytokine responses at 18 months, 3 years, and 5 years in a single model for each cytokine and represent the significance of the cytokine at the specified time point, after adjustment for response of that cytokine at other time points. *Error bars* represent 95% CIs. *nt*, No valid cytokine result.

The κ values quantifying repeatability of HDM-specific cytokine responses at 18 months and 3 years were not significantly different from 0 (Table II), indicating that these responses were not stable at 18 months of age. At age 3 years, IL-5 and IL-13 responses, but not IL-10 or IFN- γ responses, were weakly related to those determined at age 5 years (Table II).

House dust mite-specific cytokine responses as predictors for outcomes at 5 years

We assessed HDM-specific cytokine responses at ages 18 months, 3 years, and 5 years in a single model for each cytokine and for each of the 3 outcomes: sensitization to any allergen, asthma, and eczema at age 5 years. HDM-specific IL-5 responses at ages 3 years and 5 years were independently related to sensitization at 5 years of age (Fig 2), and IL-5 responses at age 5 years were also related to the presence of asthma but not eczema (see this article's Fig E1 and E2 in the Online Repository at www.jacionline.org). HDM-specific IL-5 responses at ages 3 years and 5 years were also significantly associated with specific sensitization to HDM at age 5 years ($P = .0002$ and $P < .0001$, respectively; see this article's

Fig E3 in the Online Repository at www.jacionline.org). The higher relative risk for SPT positivity and for asthma associated with strongly positive (>50 pg/mL) HDM-specific IL-5 responses is evidence of a consistent dose-response relationship (Fig 3). HDM-specific IL-13 responses at 3 years of age were significantly positively related to sensitization and to eczema at age 5 years, but the associations with IL-13 responses at 5 years were not significant after adjusting for the 3 year responses (Fig 2 and Fig E2). IL-13 responses were not related to the presence of asthma at age 5 years. No consistent relation was observed between other cytokine responses and outcomes (Fig 2).

We also assessed the relation between cytokine responses to HDM at age 5 years and outcomes at age 5 years in a single model for each outcome. This confirmed that the relation of IL-5 to sensitization status ($P = .0004$) and to asthma ($P = .0002$) was independent from the effect of other cytokines. However, the effect of IL-13 responses at age 3 years on sensitization at age 5 years was no longer significant ($P = .66$) after adjusting for the concurrent effect of IL-5 ($P = .0002$) and other cytokines at this age.

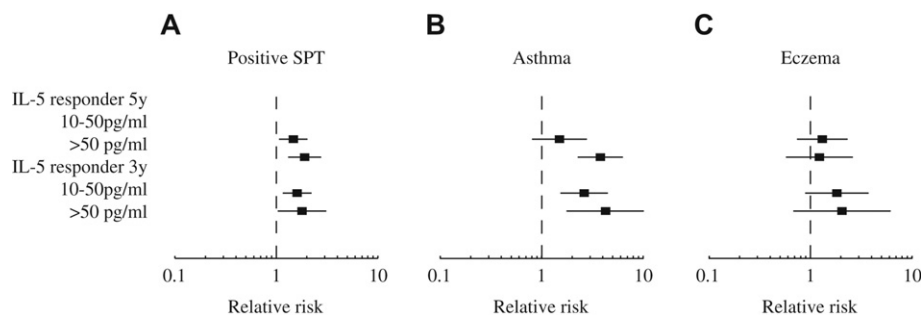


FIG 3. The relation between HDM-specific IL-5 responses at 3 years and 5 years and the presence of allergic sensitization, asthma, and eczema at 5 years. Relative risks for low responders (10–50 pg/mL) and high responders (>50 pg/mL) compared with nonresponders (<10 pg/mL) are shown with 95% CIs. Relative risks are adjusted for other cytokines measured at the same age.

DISCUSSION

House dust mite–specific IL-5 responses at ages 3 and 5 years were associated with allergic sensitization at 5 years, and IL-5 responses at 5 years were associated with asthma at that age. IL-5 responses were not related to the presence of eczema at this age. In contrast IL-13, IL-10, and IFN- γ responses to HDM were not independently related to asthma, eczema, or sensitization at age 5 years. HDM-specific IL-5, IL-13, and IL-10 increased over the first 5 years of life. HDM-specific IFN- γ responses were uncommonly detected throughout the first 5 years of life. Those that were present were not fixed and were unrelated to the presence of allergic disease at 5 years.

A particular strength of this study is the large study population and the longitudinal nature of the study design. This has enabled analysis of changes in HDM-specific cytokine responses during the first 5 years of life and has also allowed us to assess the consequences, at age 5 years, of HDM-specific cytokine responses in earlier life. The subjects had an increased risk of asthma and other allergic illness because 1 or both parents or an older sibling had a history of asthma or wheezing illness. Hence, the prevalence of HDM allergy, eczema, wheeze, and asthma are likely to be higher than in the general population, and the prevalence of HDM-specific T-cell responses may also be higher. This has enhanced the power of this study to detect associations between HDM-specific cytokine responses and clinical and allergic outcomes. Although the study's interventions (HDM avoidance and dietary fatty acid modification) did not prevent the onset of asthma, eczema, or atopy,¹⁷ we adjusted for randomization status in analyzing the association between cytokine responses and age.

We have shown that HDM-specific cytokine responses changed between the ages of 18 months and 3 years and also between 3 years and 5 years. Cytokine responses at 18 months are not fixed and are not related to cytokine responses at later ages. At age 3 years, IL-5 and IL-13 responses, but not IL-10 or IFN- γ responses, are weakly related to those at age 5 years. Given this lack of stability, it is unsurprising that responses at age 18 months were not predictive of clinical and allergic outcomes at age 5 years. The allergen-specific responses to cytokines in early life,

including the IFN- γ responses, warrant further exploration in large cohorts. This study however, clearly demonstrates that T-cell responsiveness to HDM as measured by IL-5 production, although uncommon at 18 months (3%), is progressively acquired until the age of 5 years (25%). This implies that the early life patterning of immune responses is not necessarily restricted to a limited window of opportunity in very early life. The predictive effect of cytokine responses at age 5 years will be determined by a follow-up of this cohort when the subjects are 8 years old.

House dust mite–specific T-cell responses in this study were associated with asthma at 5 years, but not with eczema. This might suggest that T-cell responses to HDM may be more relevant in the clinical expression of asthma than eczema.

The clinical relevance of skin test responses to HDM in school-age children has been established for decades.^{22,23} The onset of sensitization before the age of 8 to 10 years also has important prognostic implications for persistence or subsequent onset of asthma and asthmalike symptoms.^{24,25} Nevertheless, the meaning of SPT results in younger children is not well established. Among participants in the German Multicenter Cohort Study who were sensitized to food or inhalant allergens at age 2 years, only 99 (55%) remained sensitized at age 7 years.²⁶ Those with transient early life sensitization had no higher risk of asthma than those who were persistently nonsensitized. On the other hand, among those who were sensitized at age 7 years, those who had been sensitized at age 2 years had a higher risk of asthma than those who had first developed sensitization after age 2 years. Interestingly, the subgroup of subjects who were sensitized to inhalant allergens at age 2 years did not have an increased risk of asthma at age 7 years. Therefore, the population of subjects who are sensitized in early childhood includes some who are destined to have persisting sensitization, with its attendant clinical consequences, and others who do not have an increased risk for allergic illness. The longer-term consequences of HDM allergy at 5 years remains to be established with further follow-up of this cohort.

One limitation inherent in the study of asthma and allergic disease in young children is the difficulty in defining robustly clinical outcomes at these ages. In

particular, wheezing illness in preschool age children is a heterogeneous entity²⁷ reflecting a range of underlying causes and uncertain prognosis. In this study, parental reports of both wheeze and diagnosed asthma, which reflect the medical attendants' interpretation of wheeze and other respiratory symptoms, were used as indicators of asthmatic-like symptoms at this stage of life.

House dust mite is a common allergen in the Sydney area and, as this study confirms, is the dominant target for specific inhalant allergen sensitization as measured by SPTs. HDM was therefore the most suitable allergen to test as the specific stimulus for lymphocyte production of cytokines in this region. We chose to use an extract of HDMs, rather than a single purified allergen, because this may reflect naturally occurring sensitization better. Furthermore, there are numerous relevant antigens in HDMs,^{28,29} and selecting a single 1 of these for use in culture potentially excludes the effect of other important antigens. It is possible that HDM extract might contain nonallergenic substances, such as endotoxin, that could nonspecifically stimulate PBMCs to secrete cytokines. However, the absence of such a response in the majority of samples, even though they responded to the mitogenic stimulus phytohemagglutinin, is evidence against such a nonspecific effect.

There is no clearly established level of cytokine production that can be regarded as a threshold for a positive response. For this reason, we have examined the actual level of each cytokine response, after subtraction of the control value. Because the distributions were skewed and included many 0 (or not detectable) responses, we classified subjects as nonresponders (<10 pg/mL), low responders (10–50 pg/mL), and high responders (>50 pg/mL) to IL-5, IL-10, and IL-13 and as nonresponders (<50 pg/mL) or responders (>50 pg/mL) to IFN- γ and examined the relation of this classification to SPT positivity, asthma, and eczema at age 5 years.

The findings of this study are consistent with previous studies in supporting a link between T_H2-like cytokine production in response to allergen-specific stimulation and the clinical manifestations of allergic disease. In a study of 5-year-old children in Perth, Western Australia, IL-4 and IL-5 responses to HDM were greater in 20 children with HDM allergy compared with 20 children without HDM allergy, whereas IFN- γ responses were increased in both to a similar extent.³⁰ Children who had either elevated allergen-specific IgE or other allergic manifestations at age 2 years ($n = 18$) had lower levels of allergen-specific IL-4, IL-13, and IFN- γ production at birth, measured as cytokine or as mRNA, than children who were not atopic at age 2 years ($n = 13$).⁹ Measured at 6-month intervals, these differences declined over time because of a fall in T_H2-like cytokine responses in nonatopic subjects and an increase in all 3 cytokine responses in atopic subjects. By age 6 years, HDM-specific IL-5 and IL-13 responses were positively associated with HDM sensitization among 59 members of a birth cohort.⁸ Taken together, these findings were interpreted as evidence that atopic children have a persisting T_H2-like

response whereas nonatopic children develop deviation to T_H1-like responses.⁹

This study has shown a very low prevalence of T_H1-like (IFN- γ) cytokine responses to HDM stimulation at ages 18 months, 3 years, and 5 years, and these responses were unrelated to the presence of sensitization. Other studies in younger and older children have found detectable IFN- γ responses to HDM stimulation,³¹ but the associations with atopic sensitization have been variable.^{32,33}

There was a high prevalence of HDM-specific IL-10 responses in this study population but, in contrast with the findings of Van der Velden et al³¹ in 1-year-old children, we did not find that HDM-specific IL-10 responses were lower in atopic subjects. IL-10 is an anti-inflammatory cytokine that mediates its effects by reducing macrophage activation and inhibiting T_H1-like immune responses, both through inhibition of IL-12 production and reduced antigen presentation to T cells, as well as by inhibition of IgE production and switching to IgG₄ production.^{34,35} It is possible that the observed IL-10 responses reflect non-specific IL-10 production or release by allergen-specific regulatory T cells.³⁶

In summary, detectable *in vitro* cytokine responses to stimulation with HDM allergen, the dominant local environmental allergen in our region, are not fixed in early life and have a very low prevalence in infancy but increase with age. At ages 3 and 5 years, IL-5 responses were associated with positive SPT responses and with asthma in a dose-dependent manner. This implies that IL-5 responses to common allergens may be particularly relevant to the development of the atopic state and clinical airways disease. Although the HDM avoidance intervention implemented here had no effect on the cytokine responses, it remains to be established whether these maturing T-cell responses can be modified by other environmental interventions and whether this modification has lasting effects on allergic disease.

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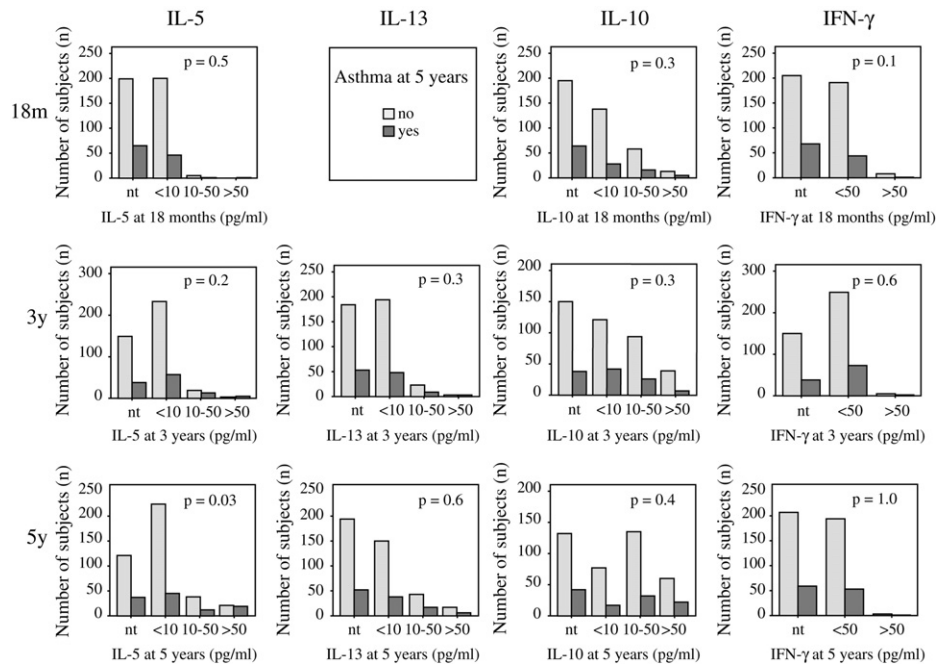


FIG E1. Prevalence of IL-5, IL-13, IL-10, and IFN- γ responses at 18 months, 3 years, and 5 years in relation to asthma at 5 years. Subjects are classified as nonresponders (<10 pg/mL), low responders (10-50 pg/mL), or high responders (>50 pg/mL) for IL-5, IL-13, and IL-10 and as nonresponders (<50 pg/mL) or responders (>50 pg/mL) for IFN- γ . *P* values are derived from logistic regression models testing HDM-specific cytokine responses at 18 months, 3 years, and 5 years in a single model for each cytokine and represent the significance of the cytokine at the specified time point after adjustment for response of that cytokine at other time points. Error bars represent 95% CIs. *nt*, No valid cytokine result.

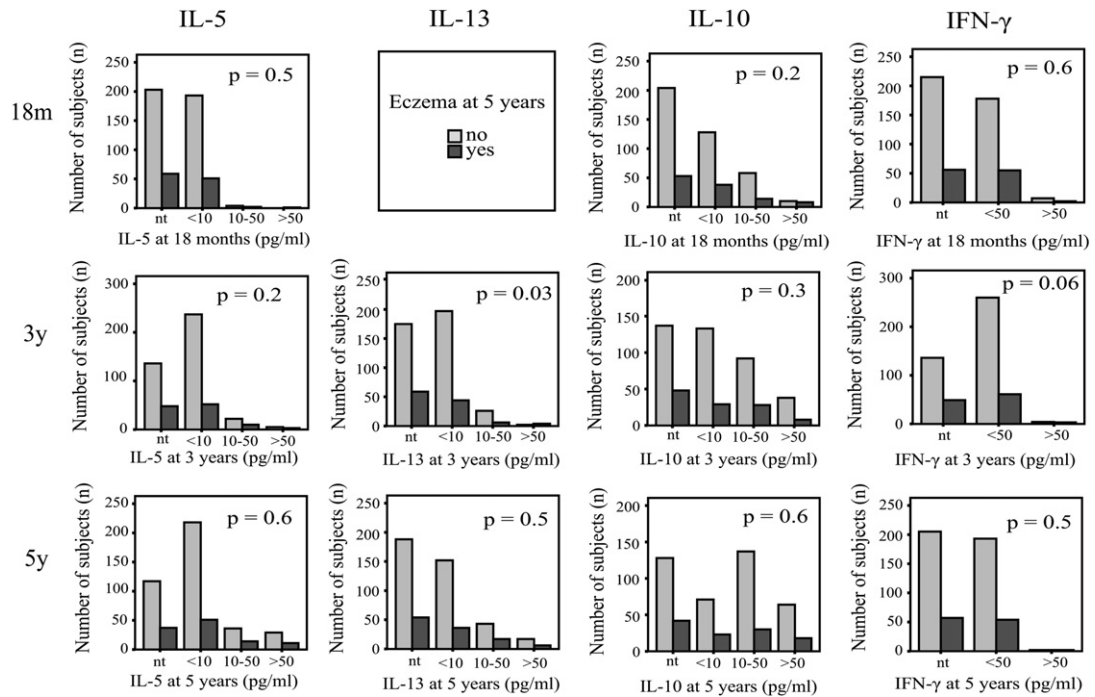


FIG E2. Prevalence of IL-5, IL-13, IL-10, and IFN- γ responses at 18 months, 3 years, and 5 years in relation to eczema at 5 years. Subjects are classified as nonresponders (<10 pg/mL), low responders (10-50 pg/mL), or high responders (>50 pg/mL) for IL-5, IL-13, and IL-10 and as nonresponders (<50 pg/mL) or responders (>50 pg/mL) for IFN- γ . *P* values are derived from logistic regression models testing HDM-specific cytokine responses at 18 months, 3 years, and 5 years in a single model for each cytokine and represent the significance of the cytokine at the specified time point after adjustment for response of that cytokine at other time points. Error bars represent 95% CIs. *nt*, No valid cytokine result.

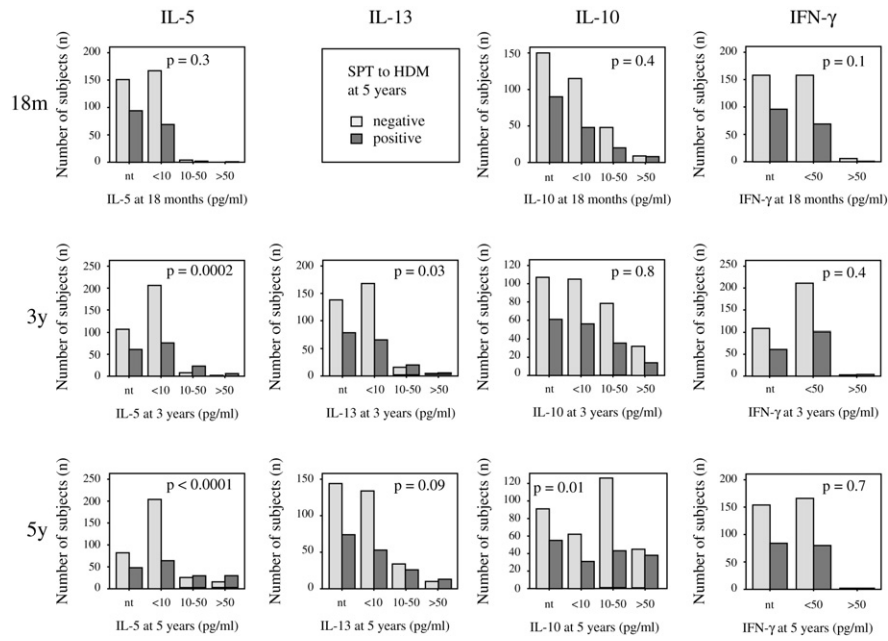


FIG E3. Prevalence of IL-5, IL-13, IL-10, and IFN- γ responses at 18 months, 3 years, and 5 years in relation to HDM allergy at 5 years. Subjects are classified as nonresponders (<10 pg/mL), low responders (10-50 pg/mL), or high responders (>50 pg/mL) for IL-5, IL-13, and IL-10 and as nonresponders (<50 pg/mL) or responders (>50 pg/mL) for IFN- γ . *P* values are derived from logistic regression models testing HDM-specific cytokine responses at 18 months, 3 years, and 5 years in a single model for each cytokine and represent the significance of the cytokine at the specified time point after adjustment for response of that cytokine at other time points. Error bars represent 95% CIs. *nt*, No valid cytokine result.

TABLE E1. Number of valid measures of cytokine responses at each age

	IL-5	IL-13	IL-10	IFN- γ
18 mo	270	—	275	261
3 y	338	285*	337	337
5 y	358	270†	342	250†

*Cell culture supernatants for some subjects assessed early in the study were not assayed for IL-13.

†Technical difficulties led to failures in some IL-13 and IFN- γ assays performed on samples collected at 5 years.

TABLE E2. Outcomes at age 5 years in participants with valid cytokine responses at 18 months, 3 years, and 5 years

	18 mo	3 y	5 y
HDM sensitization*	28/280 (10%)	76/349 (22%)	120/370 (32%)
Any sensitization*	62/280 (22%)	103/349 (30%)	158/370 (43%)

*SPT response $\geq$ 2 mm.

TABLE E3. Relation between IL-5 responder status and IL-13, IL-10, and IFN- γ responder status at age 5 years

IL-5	IL-13**			IL-10**			IFN- γ ***	
	<10*	10-50	>50	<10	10-50	>50	<50	>50
<10*	164	30	1	79	128	48	187	1
10-50	11	20	5	8	27	13	36	0
>50	1	9	17	5	12	21	22	2

*pg/mL; ** $P < .0001$; *** $P < .01$ by χ^2 test.