

Toward improved prediction of risk for atopy and asthma among preschoolers: A prospective cohort study

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Background: Atopy and asthma are commonly initiated during early life, and there is increasing interest in the development of preventive treatments for at-risk children. However, effective methods for assessing the level of risk in individual children are lacking.

Objective: We sought to identify clinical and laboratory biomarkers in 2-year-olds that are predictive of the risk for persistent atopy and wheeze at age 5 years.

Methods: We prospectively studied 198 atopic family history–positive children to age 5 years. Clinical and laboratory assessments related to asthma history and atopy status were undertaken annually; episodes of acute respiratory illness were assessed and classified throughout and graded by severity.

Results: Aeroallergen-specific IgE titers cycled continuously within the low range in nonatopic subjects. Atopic subjects displayed similar cycling in infancy but eventually locked into a stable pattern of upwardly trending antibody production and T_H2-polarized cellular immunity. The latter was associated with stable expression of IL-4 receptor in allergen-specific T_H2 memory responses, which was absent from responses during infancy. Risk for persistent wheeze was strongly linked to early sensitization and in turn to early infection. Integration of these data by means of logistic regression revealed that attaining mite-specific IgE titers of greater than 0.20 kU/L by age 2 years was associated with a 12.7% risk of persistent wheeze, increasing progressively to an 87.2% risk with increasing numbers of severe lower respiratory tract illnesses experienced.

Conclusion: The risk for development of persistent wheeze in children can be quantified by means of integration of measures related to early sensitization and early infections. Follow-up studies along similar lines in larger unselected populations to refine this approach are warranted. (*J Allergy Clin Immunol* 2010;125:653–9.)

Key words: IgE, respiratory tract infection, wheeze, asthma, infancy

Sensitization to inhalant allergens is a major risk factor for the development of persistent wheeze during childhood,¹ particularly if sensitization occurs during the first few years of life.^{2–5} The risk for development of persistent wheeze is amplified if early sensitization is accompanied by severe lower respiratory tract infection during the same period.^{3,6} The latter and a range of complementary findings are consistent with the hypothesis that interactions between inflammatory pathways triggered by exposure to aeroallergens and respiratory pathogens perturb the normal growth and differentiation of lung and airway tissues, which is maximal during early life, leading to phenotypic changes that predispose to subsequent development of persistent wheeze.^{1,7}

The precise nature of these interactions remains to be defined, but the strength of the epidemiologic evidence regarding their sequelae relating to respiratory functions justifies targeting this axis between infection and atopy for asthma prevention.^{1,7,8} A key feature of future intervention strategies will be the capacity to accurately identify high-risk children at an early stage of disease because evidence from a variety of avenues suggests that reversibility decreases over time. The present study was designed to gain information relevant to that aim and to further elucidate underlying pathogenic mechanisms. Thus we have recruited a cohort of children at high risk of atopy and asthma on the basis of family history and followed them to age 5 years, including annual well visits, collecting data on their clinical and immunologic phenotypes and infection history.

Information on the cohort has already been published relating to infection in early infancy,⁹ the immunologic mechanisms associated with susceptibility to infections,¹⁰ the nature of the allergen-specific T-cell priming process during infancy,¹¹ and the chronology of infections and sensitization in relation to the risk for development of persistent wheeze.³ This study extends the immunologic analyses relating to sensitization to aeroallergens to age 5 years and in particular addresses issues related to the potential use of immunologic profiling data on preschoolers to predict the persistence of early sensitization and wheeze.

METHODS

Patients and sample collection

Subjects in this study were part of an ongoing prospective birth cohort ($n = 198$), as previously described.^{3,9} All subjects were enrolled antenatally and classified as having an atopic family history based on a standard questionnaire³ and a positive doctor's diagnosis of asthma, hay fever, or atopic dermatitis for 1 or both parents. Blood was collected at birth and at 0.5, 1, 2, 3, 4, and 5 years, and children were assessed by one of the authors for the presence of atopic disease. PBMCs and plasma were isolated and cryopreserved.

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Abbreviations used

ARI: Acute respiratory illness
HDM: House dust mite
IL-4R: IL-4 receptor
OR: Odds ratio
SLRI: Severe lower respiratory tract illness

Parents kept a daily symptom diary and recorded the presence of symptoms of acute respiratory illness (ARI), such as runny/blocked nose, cough, and wheeze, as well as the presence of fever (temperature $>38^{\circ}\text{C}$). If their child had any of these symptoms, the study center was contacted within 24 hours, triggering a visit from the study doctor, who assessed the child; follow-up telephone calls were made to the family every 2 weeks until the ARI was resolved. The study doctor used information collected from the telephone contacts and the diary cards to classify ARI episodes (see the **Methods** section in this article's Online Repository at www.jacionline.org), and data relating to severe episodes before age 2 years are used below to develop early predictors of susceptibility to persistent wheeze.

Antibody assays

Antibodies were measured using ImmunoCAP (Phadia AB, Uppsala, Sweden), comprising total IgE and specific IgE and IgG4 levels to house dust mite (HDM) and a broad panel of additional allergens (see the **Methods** section in this article's Online Repository).

In vitro culture of PBMCs and cytokine measurement

Standard methodology was used, as detailed previously (see the **Methods** section in this article's Online Repository).^{11,12}

Statistical analyses

Univariate analyses of group differences in antibody and cytokine mRNA expression used the Mann-Whitney *U* test. Spearman correlation was used to examine the relationship between HDM-IgE titers at 5 years and HDM-stimulated cytokine responses. After adjustment of measurements less than assay detection limits (see the **Methods** section in this article's Online Repository), continuous variables were $\log_{10}$ transformed for use in regression analyses. Cases missing data for variables were excluded from relevant regression analyses. This was never more than a few subjects, and therefore no techniques for replacing missing data, such as imputation, were used. The cutoff level of significance used for regression analyses was a *P* value of less than .05. Prediction and cross-sectional analysis of HDM-IgE titers were performed by using stepwise linear regression, whereas wheezing phenotype at 5 years was assessed with forward-step logistic regression. All analyses were performed with SPSS software for the Mac OS system (SPSS, Inc, Chicago, Ill).

RESULTS**Postnatal development of IgE responses in atopic and nonatopic children**

Fig 1 shows the age-related increase in the frequency of sensitization to inhalant allergens in the study cohort. Forty-six percent of the cohort were sensitized to 1 or more aeroallergens by age 5 years, and 26% were sensitized to food allergens (overall population figures for this age range are 40% and 22%, respectively). The dominant inhalant allergen affecting this population is HDM, and the frequency of sensitization increased progressively over the observation period, reaching 37% at outcome age.

Fig E1 (available in this article's Online Repository at www.jacionline.org) tracks total IgE titers over the first 5 years of life

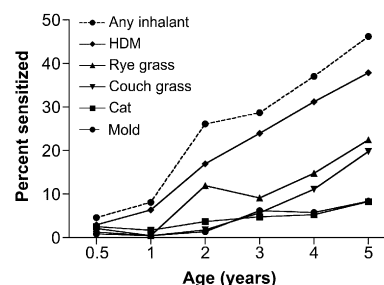


FIG 1. Rates of sensitization to inhalant allergens. Data shown are percentage of the cohort at each age who were sensitized, as defined by relevant allergen-specific IgE titers of 0.35 kU/L or greater.

in individual children. Total IgE levels increased progressively after birth and did so more rapidly in children sensitized by age 5 years relative to nonsensitized children (see Fig E1, A), and the trajectory of this increase in total IgE levels in individual subjects was generally regular within each group (see Fig E1, B). This contrasts with the pattern seen for specific IgE responses to the inhalant allergens, as exemplified by HDM. Among the group who were sensitized to HDM at age 5 years (Fig 2, right), beyond the first birthday, specific IgE titers in sera of individual children almost invariably maintained their upward trajectories, suggesting the progressive consolidation of underlying immune responses that were primed during infancy.¹¹ In contrast, HDM-specific IgE titers among children who remained below the sensitization threshold (Fig 2, left) fluctuated cyclically within the low range throughout the observation period. A similar cyclical pattern was seen in IgE titers against the other inhalant allergens and also those to food allergens, including peanut (data not shown).

Prediction of sensitization at age 5 years based on specific IgE levels at earlier ages

The cyclical nature of early IgE responses to inhalant allergens questions the usefulness of such measures as prognostic indices in relation to assessment of risk for persistent sensitization in preschool children. We next addressed this issue in more detail, focusing on responses to the dominant aeroallergen HDM. Fig 3 shows the proportion of children who were sensitized to HDM at age 5 years in relation to their individual peak HDM-specific IgE titers attained by age 2 years. Thus 93.3% of children who reached a titer of 0.35 kU/L or greater remained at or above this sensitization threshold at age 5 years; if this analysis was extended to include their IgE titers over the first 3 years, then this figure for percentage persistence remained high at 86.4% (not shown). If the cutoff was decreased to 0.20 kU/L up to age 2 years (Fig 3), the figure became 86.1%.

Using the 2-year data, a cutoff HDM-IgE titer of 0.35 kU/L to designate high risk for sensitization at 5 years had a positive predictive value of 93.3% and a negative predictive value of 75.2%; at the lower cutoff of 0.20 kU/L, these values were 86.1% and 74.1%, respectively.

HDM-specific T_H memory responses

We next focused on T-cell responses to HDM. We have previously reported on HDM-specific cytokine responses in these children during infancy and demonstrated clear increases in HDM-induced IL-4, IL-5, IL-9, IL-13, and also IFN- γ production in

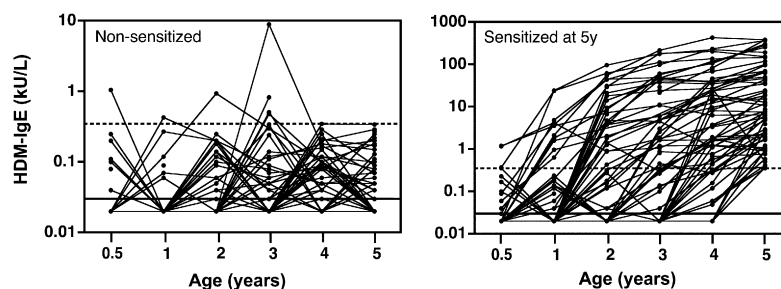


FIG 2. Age-related changes in IgE titers. Fluctuations in HDM-specific IgE titers in individual children who were not (*left*) or were (*right*) sensitized at age 5 years. The dotted line indicates the 0.35 kU/L sensitization threshold.

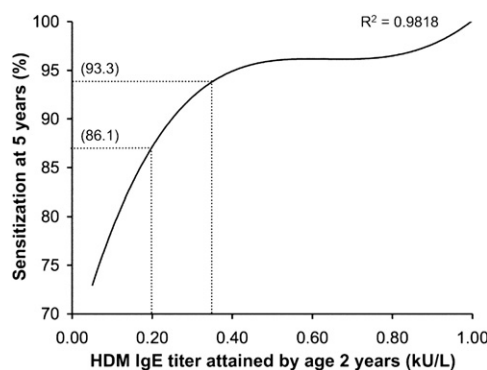


FIG 3. Relationship between HDM-IgE titers in individual children at 2 and 5 years. Data shown represent the percentage sensitized to HDM at 5 years among subjects exceeding set IgE cutoffs at 2 years computed at 0.5 kU/L intervals (line of best fit and associated R^2 value computed in Excel). In the examples shown, 86.1% of children whose HDM-IgE levels were 0.20 kU/L or greater at 2 years were sensitized at 5 years versus 93.3% of those whose 2-year levels were 0.35 kU/L or greater.

association with specific IgE, particularly from age 12 months.¹¹ We have now followed these HDM-specific cytokine responses to age 5 years (Fig 4); data from the birth to 2-year time points reported earlier¹¹ are included here for reference and restratified as above. We focused on mRNA as opposed to protein data based on our earlier findings¹¹ that the more sensitive quantitative RT-PCR methodology is better equipped to handle the low-level T_H memory responses characteristic of these early age groups and resulting group data are less spread. Protein data for IL-5, IL-9, IL-13, and IFN- γ collected in parallel demonstrate similar differences between the sensitized/nonsensitized groups (not shown).

We additionally expanded these analyses to include assessment of IL-4 receptor (IL-4R) mRNA production induced by HDM in light of other studies demonstrating the importance of this gene in T_H2 memory responses.¹² The hallmark T_H2 effector cytokines IL-4, IL-5, IL-9, and IL-13 become prominent in the HDM-specific differential expression signature of the atopic subjects from 1 year onward, and from 3 years, this is joined by IL-4R and also a T_H1 (IFN- γ) component (Fig 4). Note also that patterns of HDM-specific cytokine expression in individual children, as exemplified by IL-4 (see Fig E2 in this article's Online Repository at www.jacionline.org), fluctuate cyclically over time comparable with that seen above for specific IgE.

Table I shows a Spearman correlation matrix showing the strength and consistency of associations between individual HDM-specific cytokine responses at each age and HDM sensitization at 5 years. As reported previously, at age 2 years,¹¹ increasingly strong associations are seen for the T_H2 cytokines from 6 months onward and to a lesser extent for IFN- γ , and this pattern remains consistent out to age 5 years. In contrast, IL-4R expression does not show this association until age 3 years.

Cytokine responses and sensitization status: Multivariate analyses

Initial linear regression analysis was undertaken to identify determinants of HDM-specific IgE titers at age 5 years, integrating measures of humoral and cellular immunity from samples collected at that age. Predictable positive associations were seen with T_H2 cytokines (notably HDM-specific IL-4 and IL-13), and negative associations were seen with the T_H1 cytokine IFN- γ (see Table E1 in this article's Online Repository at www.jacionline.org). Table II (left) uses cytokine and antibody data from the age 2 years sample collection to predict HDM-IgE titers at 5 years. The significant variables in this model were current HDM-specific IgE titer at the times of sampling together with HDM-induced IL-9.

Current wheeze

The outcome of principal interest in this study was wheeze, and at age 5 years, the prevalence of current wheeze in this cohort was 28% (compared with 21.5% in the overall population). Data collected at age 5 years were initially used to identify risk variables associated with wheeze at this age by means of logistic regression, and the HDM-specific IgE titer was positively associated with this outcome (see Table E2 in this article's Online Repository at www.jacionline.org). Data from year 2 were also used to identify variables predictive of risk for wheeze at age 5 years. By using univariate logistic regression (see Fig E3 in this article's Online Repository at www.jacionline.org), the risk for wheeze at age 5 years increased linearly with increasing IgE titers at age 2 years, in particular anti-inhalant IgE. When using a broader matrix of 2-year variables to predict wheeze at 5 years by means of multivariate logistic regression, the significant risk variables identified were HDM-IgE and a history of severe lower respiratory tract illness (SLRI) in the first 2 years of life (Table II).

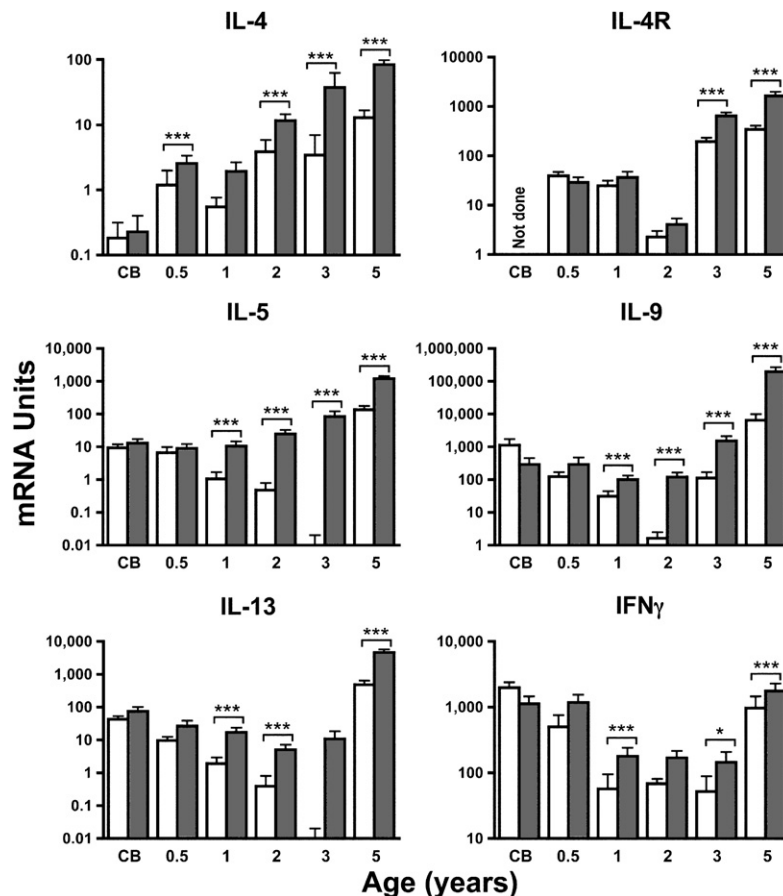


FIG 4. Postnatal development of HDM-specific T_H memory responses. Data shown are HDM-specific cytokine responses as group means $\pm$ SEs, with stratification by sensitization outcome at age 5 years. Dark bars designate sensitized at 5 years, and light bars indicate not sensitized. * $P < .05$, and *** $P < .001$, Mann-Whitney U test. CB, Cord blood.

TABLE I. Correlation between HDM-IgE level at 5 years and HDM-stimulated cytokine responses at birth and 0.5, 1, 2, 3, and 5 years

Age (y)	HDM-stimulated mRNA					
	IL-4	IL-4R	IL-5	IL-9	IL-13	IFN- γ
0	0.081 (.388)	ND	0.065 (.486)	-0.018 (.846)	0.033 (.727)	-0.043 (.647)
0.5	0.338 (.000)	-0.069 (.463)	0.229 (.013)	0.155 (.097)	0.201 (.030)	0.110 (.240)
1	0.194 (.051)	0.032 (.747)	0.362 (.000)	0.366 (.000)	0.298 (.002)	0.261 (.008)
2	0.229 (.005)	0.082 (.320)	0.423 (.000)	0.411 (.000)	0.284 (.000)	0.119 (.148)
3	0.215 (.013)	0.332 (.000)	0.297 (.001)	0.413 (.000)	0.191 (.028)	0.193 (.027)
5	0.496 (.000)	0.261 (.004)	0.588 (.000)	0.481 (.000)	0.487 (.000)	0.301 (.001)

Data shown are Spearman Rho (P value).

ND, Not done.

We finally focused on the significant predictors from year 2 data in Table II, restricting the selection to those based on information that would be available in a routine clinical setting (ie, HDM-IgE status and infection history). Logistic regression modeling was used to generate a probability matrix (Table III) describing risk for wheeze at age 5 years as a function of titer of HDM-IgE attained by the second birthday (cohort range, 0.015-97.5 kU/L IgE) and cumulative number of episodes of SLRI experienced during the first 2 years (range, 0-10 episodes). For example, at a log IgE titer of -0.456 (0.35 kU/L), the risk for subsequent persistent wheeze is 48.9% with 3 SLRI episodes, increasing to 75.2% with 6 episodes. Comparable matrices were

generated by using compendium data derived by means of summation of individual titers of specific IgEs or data from the Phadiatop test, which covers multiple IgE specificities (see Table E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

The available evidence indicates that resistance to sensitization to ubiquitous environmental allergens is an active process involving the development of various forms of immunologic tolerance. In the gastrointestinal tract this involves generation of "oral tolerance" to dietary allergens, and in the respiratory tract

TABLE II. Prediction of 5-year outcomes using regression analysis

Log of 2-y variables	HDM-IgE titer: Linear regression				Current wheeze: Logistic regression		
	B	SE	t	P value	OR	95% CI	P value
HDM-IgE	0.852	0.089	9.565	.000	1.781	1.236-2.567	.002
HDM IL-9 mRNA	0.188	0.059	3.174	.002			NS
No. of SLRIs in first 2 y	—	—	—	—	1.471	1.175-1.840	.001

The linear regression model for prediction of HDM-IgE titer at 5 years comprised the corresponding 2-year total and allergen-specific IgE levels (HDM, cat, peanut, rye, and mold), PHA-induced cytokines (IL-5, IL-10, IL-13, and IFN- γ), HDM-induced cytokine mRNA (IL-4, IL-4R, IL-5, IL-9, IL-13, and IFN- γ), and sex. The logistic regression model for prediction of wheeze at 5 years comprised corresponding 2-year current wheeze, total and allergen-specific IgE levels, PHA-induced cytokine and HDM-induced cytokine mRNA, number of SLRIs in the first 2 years of life, and sex.

OR, Odds ratio.

TABLE III. Predicted probabilities of wheeze at 5 years

Log 2-y HDM-IgE	Number of SLRIs up to 2 y										
	0	1	2	3	4	5	6	7	8	9	10
−1.8	.113	.158	.216	.288	.373	.467	.562	.654	.735	.803	.857
−1.6	.127	.176	.238	.315	.403	.499	.594	.682	.759	.823	.872
−1.4	.141	.195	.263	.343	.435	.530	.624	.709	.782	.841	.886
−1.2	.158	.216	.288	.373	.466	.562	.654	.735	.803	.857	.898
−1.0	.176	.238	.315	.403	.498	.593	.682	.759	.822	.872	.909
−0.8	.195	.262	.343	.434	.530	.624	.709	.782	.840	.886	.919
−0.699*	.205	.275	.358	.450	.546	.639	.722	.793	.849	.892	.924
−0.6	.216	.288	.373	.466	.562	.653	.735	.803	.857	.898	.928
−0.456*	.232	.307	.394	.489	.584	.674	.752	.817	.868	.906	.934
−0.4	.238	.315	.403	.498	.593	.682	.759	.822	.872	.909	.936
−0.2	.262	.343	.434	.530	.624	.709	.782	.840	.885	.919	.943
0.0	.288	.372	.466	.562	.653	.735	.803	.857	.898	.928	.950
0.2	.315	.403	.498	.593	.682	.759	.822	.872	.909	.936	.956
0.4	.343	.434	.530	.623	.709	.781	.840	.885	.919	.943	.961
0.6	.372	.466	.561	.653	.734	.803	.857	.898	.928	.950	.965
0.8	.403	.498	.593	.681	.759	.822	.872	.909	.936	.956	.969
1.0	.434	.529	.623	.708	.781	.840	.885	.919	.943	.961	.973
1.2	.465	.561	.653	.734	.802	.856	.898	.928	.950	.965	.976
1.4	.497	.592	.681	.758	.822	.871	.909	.936	.956	.969	.979
1.6	.529	.623	.708	.781	.840	.885	.919	.943	.961	.973	.981
1.8	.561	.652	.734	.802	.856	.898	.928	.950	.965	.976	.984
2.0	.592	.681	.758	.822	.871	.909	.936	.956	.969	.979	.986

SLRIs were defined as episodes of lower respiratory tract infection accompanied by wheeze or temperature of greater than 38°C.

*−0.699 = log-transformed 0.20 kU/L; −0.456 = log-transformed 0.35 kU/L.

the corresponding process by which responsiveness to aeroallergens is controlled is known as “inhalation tolerance.”¹³ Data from a number of cohort studies have established that the life phase in which these processes most commonly occurs is infancy, when the immunologically naive immune system first makes direct contact with the outside environment.^{11,14-17} Tolerance induction is mediated in part through regulatory T cells and also involves cross-regulation between populations of allergen-specific T_H1 and T_H2 cells, and evidence of these competing signals was demonstrated within early HDM-specific T_H memory cell responses from this high-risk cohort during infancy and out to age 2 years.¹¹

A notable feature of the immune response profiles in this cohort during infancy was the cycling of IgE antibody production against both inhalant and dietary allergens within a range of less than the 0.35 kU/L sensitization threshold in the group who remained nonsensitized by age 2 years.¹¹ The present study tracked cellular and humoral immune responses in the cohort out to age 5 years, and as shown in Fig 2, this cyclical pattern of low-level IgE production in nonatopic subjects is maintained throughout, reinforcing the view that the maintenance of stable tolerance is an ongoing dynamic process. It is likely that the underlying mechanism

involves the interplay between countermanding T_H1 and T_H2 signals during this period. In particular, the data from Table E1 demonstrate a clear reciprocal relationship between the influence of the T_H2 (IL-4 and IL-13) and T_H1 (IFN- γ) components of HDM-specific memory responses and HDM-specific IgE titers at age 5 years, continuing the same strong trend observed in the responses of these high-risk subjects during infancy.¹¹

This suggests that the principles of the murine T_H1/T_H2 paradigm in relation to regulation of IgE responses by immune deviation mechanisms¹⁸ are broadly applicable in this preschool age group. However, it should be stressed that we and others have also demonstrated that beyond the preschool years, this concept becomes progressively less applicable as T_H1 cytokines become increasingly more prominent within the T_H memory responses of atopic subjects.¹⁹⁻²¹ Moreover, T_H1 cytokines, such as IFN- γ , become part of the cytokine response profiles associated with asthma-associated clinical phenotypes, in particular airway hyperresponsiveness.^{19,22,23} Similar observations have been made in murine systems, indicating progressive loss of susceptibility to T_H1/T_H2 cross-regulation as T_H memory clones mature over time,²⁴ possibly as a result of changes in cytokine receptor expression.

We have also observed significant qualitative changes in HDM-specific T_H memory responses over time in the cohort. As shown in Fig 4, from as early as 6 months, the T_H2 polarity of the HDM-specific cytokine response becomes evident with respect to IL-4 in the HDM-induced expression signature of subjects who progress to sensitization at age 5 years, and the other T_H2 cytokines become prominent thereafter. However, the key receptor for the T_H2 cell growth factor IL-4 does not become part of this expression signature until the 3-year sampling point (Fig 4). As shown in Fig 2, beyond this time point, “cycling” of T_H2-dependent IgE production in this group effectively ceased, and IgE titers in the vast majority locked into a common upwardly trending pattern. An important role of IL-4R expression is to stabilize T_H2 memory through promotion of IL-4–induced survival and expansion of IL-4–secreting cells,^{24,25} which in turn drive growth and differentiation of IgE-secreting B cells.²⁶ The notable absence of an IL-4R component from the overall T_H2 expression signature at ages preceding 3 years (Fig 4) might account for the relative instability of IgE responses before that time point.

We and others^{27–29} have also previously shown that T-cell responses during early infancy are dominated by immunologically naive recent thymic emigrants that have functionally altered antigen receptors, enabling low-affinity interactions with environmental antigens in the absence of genuine T_H memory priming. These cells respond to external antigens in a cross-reacting fashion, triggering transient proliferation and cytokine production, which is terminated by apoptosis.²⁹ However, the cytokines produced represent a potential source of short-lived bystander help in a range of immune responses, and this might account, in part, for the transient nature of T cell–dependent immune responses during infancy.^{29–31} Replacement of these recent thymic emigrants with functionally competent naive T cells occurs progressively over the first few years of life, and this might contribute toward eventual age-dependent stabilization of immunologic memory responses, including against allergens.

As noted above, there is increasing interest in the general concept of early intervention in children at high risk of sensitization to inhalant allergens, in particular because of the strong connection with risk for ensuing development of persistent wheeze.^{2–5} Although the underlying mechanism or mechanisms are incompletely understood, it is evident that the effects of atopy in this context involve interactions with those resulting from respiratory tract infections^{1,7} and, furthermore, that maximization of risk for development of persistent wheeze ensues when these events occur contemporaneously during the first years of life.⁹ These findings are reflected in the regression analyses in Table III using data from the cohort at 2 years to predict their risk for wheeze at age 5 years, the results of which identify IgE and infection-related variables as significant predictors.

An important question arising from these findings is whether they can potentially be applied in a clinical setting to assist in the early identification of at-risk children. It should be stressed that the children in this cohort are already at higher risk than the population average because they all come from families with 1 or more atopic parent, and therefore for this study, the question is restricted to the atopic family history–positive subgroup. With respect to the level of their risk for sensitization by age 5 years, the analyses reported here suggest that exceeding a threshold level of HDM-IgE equivalent to 0.20 kU/L by their second birthday was strongly predictive of sensitization by age 5 years (positive predictive value, 86.1%), with this figure increasing to 93.3% if

their HDM-IgE level 0.35 kU/L during the same period. In both cases the negative predictive value was on the order of 75%, indicating that the principal value of quantitative atopic assessment as a prognostic index lies in its targeted use in subjects exemplified by the members of this atopic family history–positive cohort who are already known to be at increased risk of atopy and in whom confirmatory evidence of disease initiation is needed to justify commencement of some form of active treatment. This would be of particular interest in relation to the selection of subjects for early immunologic interventions aimed specifically at prevention of persistent sensitization, if these findings can be validated in other cohorts.

As shown in Table III, it might be possible to further extend this general principle to aid in early identification of children at risk of persistent wheeze by coupling early sensitization data with information on early infection history. Thus the matrix in Table III predicts that in children who attain an HDM-IgE level equivalent to 0.20 kU/L (log–0.699) by age 2 years, the risk for development of persistent wheeze varies from 20.5% to 92.4%, depending on the number of episodes of SLRI they experience before their second birthday; in children who cross the 0.35 kU/L (log–0.456) threshold, the risk ranges from 23.2% to 93.4% depending on infection history. Other (in particular cumulative) measures of sensitization to allergens can also be used in a similar fashion (see Table E3).

In conclusion, the results of this study add further weight to the growing evidence that the risk for development of persistent wheeze is underpinned by susceptibility to early atopic sensitization and early respiratory tract infections. Furthermore, they suggest that integration of quantitative measures relating to the operation of these pathways might provide highly effective tools for asthma risk assessment, in particular by aiding in discrimination between atopic children with a positive family history requiring preventive as opposed to symptomatic treatment. In this regard we acknowledge the 2 major limitations of this study, notably modest sample size and restriction to atopic family history–positive subjects. As such, our present results should be viewed as proof-of-concept only, but they nevertheless justify larger follow-up studies, in particular in unselected populations that include subjects at low disease risk and in geographic areas where aeroallergens other than HDM predominate.

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Clinical implications: Improved ability to identify children at high risk of persistent allergic sensitization or of persistent asthma will allow appropriate selection for clinical trials or treatment with primary prevention strategies.

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METHODS

In vitro culture of PBMCs

Cryobanked cord blood mononuclear cells or PBMCs were cultured for 48 hours in AIM-V medium with 4×10^{-5} mol/L 2-mercaptoethanol alone or with 10 μ g/mL HDM extract (prepared in house) or 1 μ g/mL PHA (Murex Diagnostics Ltd, Dartford, United Kingdom). Cells were cultured in round-bottomed 96-well plates (Nunc, Roskilde, Denmark) at a concentration of 1.0×10^6 cells/mL for all time points and stimuli except for HDM stimulation of cells isolated from cord blood or at 0.5 and 1 year, which were cultured at 2×10^6 cells/mL.

Allergen test panel

Allergen specificities tested with the Phadia ImmunoCAP were as follows: HDM (*Dermatophagoides pteronyssinus*), rye grass pollen (*Lolium perenne*), cat, couch grass (*Cynodon dactylon*), mold mix (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata*, and *Helminthosporium halodes*), peanut, and food mix (egg white, milk, fish, wheat, peanut, and soybean). In studies using the Infant Phadiatop, the specificities tested were HDM (*D. pteronyssinus*), cat, dog, egg white, milk, peanut, shrimp, timothy grass, birch, ragweed, and *Parietaria judaica*; the results of this test represent a summation of titers against these specificities.

Cytokine mRNA measurements

mRNA levels for IL-4, IL-4R, IL-5, IL-9, IL-13, and IFN- γ and the reference gene *UBE2D2* were measured from culture supernatants by means of quantitative RT-PCR on the ABI Prism 7900HT (Applied Biosystems, Austin, Tex) using QuantiTect SYBR Green (Qiagen, Doncaster, Victoria, Australia). Cell pellets were stored at -20°C in RNeasy lysis buffer (Qiagen, Austin, Tex) before extraction with the RNeasy lysis buffer (Qiagen, Austin, Tex) before extraction with the RNeasy lysis buffer (Qiagen, Hilden, Germany) with oligo(dT) (Promega, Madison, Wis) and RNasin (Promega), and cDNA was diluted 1:5 before PCR. cDNA-specific PCR assays were developed in house, and relative cDNA copy numbers were measured from serial dilution plasmid standard curves, with copy numbers for all targets

normalized against those for *UBE2D2* for each cDNA sample. Amplification of unique products was verified by means of dissociation curve analysis.

Log transformation of cytokine data

Cytokine mRNA Δ values of 0 or less were converted to 0.000001 to allow $\log_{10}$ transformation; antibody values below the limits of detection were ascribed a value equivalent to half the limit of detection for each assay.

Classification of ARI

The study doctor used information collected from the follow-up telephone contacts, as well as the diary cards, to classify the episodes of ARI. Complete diary card data were available for 85% of the children for the 2-year period used in these analyses; none of the missing data pertained to ARI. The episodes of ARI were classified as follows.

Upper respiratory tract illness. Any episode of runny/blocked nose or cough in the absence of other respiratory symptoms (no tachypnea, difficulty breathing, wheeze, or rattly chest) was classified as an upper tract respiratory illness.

Lower respiratory tract illness. Episodes that were associated with wheeze or rattly chest, evidence of respiratory distress, or both were considered to be LRIs, and in earlier studies in the cohort we have detected pathogens in the majority of PNAs collected at the time of assessment.^{E1} Rattle/rattly chest was defined as moist, wet noisy breath sounds from the child's chest. Wheeze was defined as a high-pitched whistling sound heard coming from the chest on expiration. Episodes of lower respiratory tract illness were defined as severe (SLRIs) if a temperature of greater than 38°C or wheeze was present.

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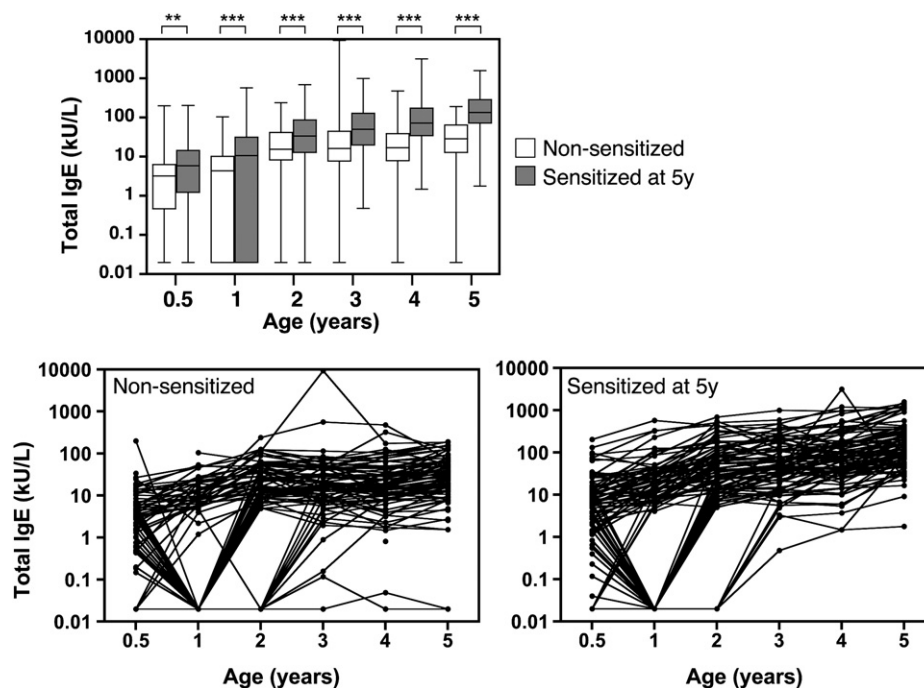


FIG E1. Age-dependent changes in total IgE levels in high-risk children during the first 5 years of life. **A**, Group data shown as median, interquartile range, and minimum/maximum values. ** $P < .01$ and *** $P < .001$. **B**, Age-dependent changes in individual children; population stratified by sensitization status at age 5 years.

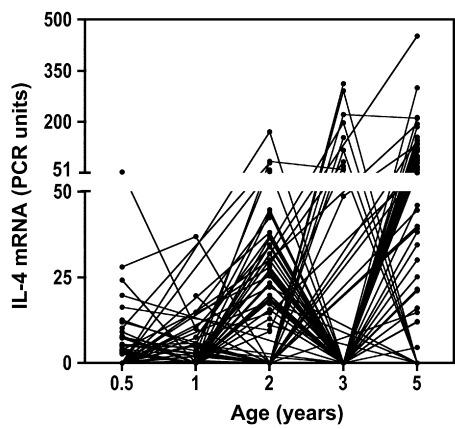


FIG E2. T_H2 memory responses to HDM allergen in individual children. HDM-induced IL-4R mRNA production in the overall study population at different time points is shown. Each line represents an individual subject.

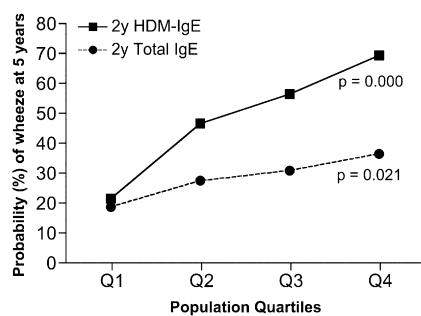


FIG E3. Probability of wheeze at age 5 years in relation to IgE titers at age 2 years. The population was quartiled by IgE titers, and probability of wheeze at 5 years as a function of IgE titer at 2 years was computed by using univariate logistic regression.

TABLE E1. Determinants of HDM-IgE titer at 5 years: Linear regression analysis

Log of 5-y variables	B	SE	t	P value
HDM IL-4 mRNA	0.339	0.065	5.244	.000
HDM IL-13 mRNA	0.254	0.064	3.967	.000
Male sex	0.547	0.213	2.565	.012
PHA IFN- γ	-0.369	0.166	-2.226	.028
HDM IFN- γ mRNA	-0.128	0.059	-2.157	.033

The regression model comprises 5-year PHA-induced cytokines (IL-5, IL-10, IL-13, and IFN- γ), HDM-induced cytokine mRNA (IL-4, IL-4R, IL-5, IL-9, IL-13, and IFN- γ), and sex.

TABLE E2. Cross-sectional multivariate logistic regression analysis of wheeze at 5 years

Log of 5-y variables	OR	95% CI	P value
HDM-IgE	1.808	1.342-2.438	.000

The regression model comprises 5-year total and allergen-specific IgE (HDM, cat, peanut, rye, and mold), PHA-induced cytokines (IL-5, IL-10, IL-13, and IFN- γ), HDM-induced cytokine mRNA (IL-4, IL-4R, IL-5, IL-9, IL-13, and IFN- γ), and sex.

OR, Odds ratio.

TABLE E3. Predicted probabilities of wheeze at 5 years: Number of SLRIs up to 2 years and log total specific IgE levels at 2 years

	No. of SLRIs up to 2 y										
Log 2-y sum-specific IgEs	0	1	2	3	4	5	6	7	8	9	10
−1.8	.054	.077	.109	.152	.208	.277	.359	.450	.545	.637	.719
−1.6	.064	.090	.127	.175	.237	.312	.399	.492	.586	.675	.752
−1.4	.074	.105	.147	.201	.269	.349	.440	.534	.627	.710	.782
−1.2	.087	.122	.169	.229	.303	.389	.482	.576	.665	.744	.809
−1.0	.101	.141	.194	.260	.340	.429	.524	.617	.702	.775	.834
−0.8	.117	.163	.222	.294	.378	.471	.566	.656	.736	.803	.856
−0.699*	.127	.175	.237	.312	.399	.492	.586	.675	.752	.816	.866
−0.6	.136	.187	.252	.330	.419	.513	.606	.693	.767	.828	.876
−0.456*	.151	.206	.276	.357	.449	.543	.635	.718	.788	.845	.888
−0.4	.157	.214	.285	.368	.460	.555	.646	.727	.796	.851	.893
−0.2	.181	.244	.321	.408	.502	.596	.683	.759	.822	.871	.908
0.0	.207	.276	.358	.450	.544	.636	.719	.789	.845	.889	.921
0.2	.236	.311	.398	.492	.586	.674	.751	.816	.866	.904	.933
0.4	.268	.349	.439	.534	.626	.710	.782	.840	.884	.918	.942
0.6	.302	.388	.481	.575	.665	.743	.809	.861	.901	.930	.951
0.8	.339	.429	.523	.616	.701	.774	.834	.880	.915	.940	.958
1.0	.378	.470	.565	.655	.735	.802	.856	.897	.927	.949	.964
1.2	.418	.512	.606	.692	.767	.828	.875	.911	.938	.956	.970
1.4	.460	.554	.645	.727	.795	.850	.893	.924	.947	.963	.974
1.6	.502	.596	.683	.759	.822	.871	.908	.935	.955	.969	.978
1.8	.544	.635	.718	.788	.845	.888	.921	.945	.961	.973	.982
2.0	.585	.673	.751	.815	.866	.904	.932	.953	.967	.977	.984

	No. of SLRIs up to 2 y										
Log 2-y Phadiatop-IgE	0	1	2	3	4	5	6	7	8	9	10
−1.8	.081	.113	.158	.215	.285	.368	.460	.554	.645	.726	.795
−1.6	.090	.127	.175	.237	.312	.398	.491	.585	.673	.751	.815
−1.4	.101	.141	.194	.260	.339	.429	.523	.616	.700	.774	.833
−1.2	.113	.157	.214	.285	.368	.460	.554	.645	.726	.795	.850
−1.0	.127	.175	.236	.312	.398	.491	.585	.673	.751	.815	.865
−0.8	.141	.194	.260	.339	.429	.523	.615	.700	.774	.833	.879
−0.699*	.149	.204	.272	.354	.444	.539	.630	.714	.784	.842	.886
−0.6	.157	.214	.285	.368	.460	.554	.645	.726	.795	.850	.892
−0.456*	.170	.230	.304	.389	.482	.576	.665	.744	.809	.861	.901
−0.4	.175	.236	.311	.398	.491	.585	.673	.751	.815	.865	.904
−0.2	.194	.260	.339	.428	.523	.615	.700	.773	.833	.879	.914
0.0	.214	.285	.368	.460	.554	.645	.726	.795	.850	.892	.924
0.2	.236	.311	.398	.491	.585	.673	.750	.815	.865	.904	.932
0.4	.260	.339	.428	.523	.615	.700	.773	.833	.879	.914	.940
0.6	.285	.368	.459	.554	.645	.726	.795	.850	.892	.923	.946
0.8	.311	.398	.491	.585	.673	.750	.815	.865	.904	.932	.952
1.0	.339	.428	.522	.615	.700	.773	.833	.879	.914	.940	.958
1.2	.368	.459	.554	.645	.726	.795	.850	.892	.923	.946	.963
1.4	.398	.491	.585	.673	.750	.814	.865	.904	.932	.952	.967
1.6	.428	.522	.615	.700	.773	.833	.879	.914	.939	.958	.971
1.8	.459	.554	.644	.726	.795	.850	.892	.923	.946	.963	.974
2.0	.491	.585	.673	.750	.814	.865	.904	.932	.952	.967	.977

In the top panel, results from individual subjects' sera from individual ImunoCaps specific for HDM, cat, rye, couch, mold, and peanut were summated. In the bottom panel total specific IgE levels were determined by using the ImmunoCap Infant Phadiatop test.

*−0.699 = log-transformed 0.20 kU/L; −0.456 = log-transformed 0.35 kU/L.