

Prenatal versus postnatal sensitization to environmental allergens in a high-risk birth cohort

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Background: The timing of allergen sensitization is controversial, with conflicting evidence suggesting transplacental priming versus exclusively postnatal priming. Resolution of this question is important in relation to rational design of allergy prevention strategies, particularly the issue of allergen avoidance during pregnancy.

Objective: To elucidate the kinetics of sensitization in high-risk children during their first 2 years of life.

Methods: We prospectively studied house dust mite (HDM)-specific IgE and IgG₄ antibody production and associated T-cell immunity in a cohort of 200 high-risk infants. Parallel antibody studies tracked responses against a broader panel of inhalant and dietary allergens including peanut.

Results: HDM-induced T_H2 responses in PBMC from 6 months onward, particularly IL-4 and IL-5, correlated increasingly strongly with sensitization outcomes at 2 years, and a contrasting negative relationship was observed with IFN- γ response capacity. HDM-induced T-cell responses in cord blood, although common, were unrelated to subsequent sensitization. Transient HDM-IgE (and IgG₄) production frequently peaked at 6 or 12 months before returning to baseline, which suggests the onset of protective tolerance. This finding contrasted with progressively increasing HDM-IgE titers in children sensitized by 2 years of age. Comparably contrasting patterns were observed in peanut-specific responses in sensitized versus nonsensitized children.

Conclusion: Priming of T_H2 responses associated with persistent HDM-IgE production occurs entirely postnatally, as HDM reactivity in cord blood seems nonspecific and is unrelated to subsequent development of allergen-specific T_H2 memory or IgE.

Clinical implications: These findings question the scientific basis for existing recommendations for allergen avoidance by high-risk women during pregnancy. (J Allergy Clin Immunol 2007;119:1-11.)

Key words: Atopy, cytokines, IgE, IgG₄, T-cell memory, T_H2

Interest has increased in the potential control of diseases such as atopic asthma via prevention of sensitization to environmental allergens during early life. Accordingly, it is important to determine in the relevant high-risk (HR) groups, the time frame over which priming against such allergens most frequently occurs. In this regard, a variety of data links risk for sensitization to seasonal allergens to birth in spring,¹⁻⁴ and this has been widely interpreted as evidence for active priming of the immune system in early postnatal life. However, it has also been shown that priming to some perennial and/or food allergens displays a similar season-dependent pattern,⁵ which suggests that other more complex mechanisms may underlie this association.

It has additionally been suggested on the basis of allergen-induced T-cell reactivity in cord blood⁶⁻¹⁰ that sensitization can also occur transplacentally. Supportive data for such a mechanism include the cloning of allergen-reactive T-cells from cord blood and their subsequent genotyping to confirm fetal origin.¹⁰ The mechanism(s) underlying putative prenatal sensitization are unclear, but it could involve transplacental transport of immunogenic allergen fragments or IgG/allergen complexes.¹¹⁻¹³ Several precedents exist for such fetal priming,^{14,15} including the demonstration that maternal vaccination can trigger fetal antibody class switching.¹⁶ These and additional epidemiologic findings have prompted recommendations from the United Kingdom Committee on Toxicity of Chemicals in Food (COT)^{17,18} and the New

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Abbreviations used

CBMC: Cord blood mononuclear cell
 HDM: House dust mite
 HR: High risk
 OD: Odds ratio
 RTE: Recent thymic emigrant
 SPT: Skin prick test

Zealand Ministry of Health¹⁹ for avoidance of peanut products by pregnant atopic women and a related (albeit milder) caution from the American Academy of Pediatrics.²⁰ Moreover, they have stimulated a range of intervention studies aimed at prevention of food allergy via maternal avoidance of dietary allergens,²¹⁻²⁴ and similar approaches are being followed with respect to inhalant allergy.^{25,26}

However, the concept of fetal sensitization has been challenged, notably on the grounds of a lack of a quantitative relationship between maternal allergen exposure and T-cell responses in their newborn offspring.^{27,28} Moreover, a recent expert review²⁹ has questioned the clinical evidence for a protective effect of maternal allergen avoidance. Despite this question, the COT recommendations have not changed, and it is unclear how these guidelines and the associated debate have impacted on the community at large.²² A need for definitive evidence exists to help in finally resolving this controversial issue.

The current study has addressed a series of immunologic questions relevant to this debate, focusing in particular on the relationship between priming of the specific T_H cells responsible for driving immunoglobulin class switching and the ensuing production of specific IgE antibody. Our approach involves sampling a cohort of subjects at HR of atopy at regular intervals from birth to age 2 years, tracking the development of T-cell immunity and associated antibody production against the archetypal inhalant allergen house dust mite (HDM), with parallel analyses on IgE/IgG₄ responses to a panel of additional inhalant and food allergens including peanut.

METHODS

Subjects were part of an ongoing prospective birth cohort of HR children as previously described.³⁰ All aspects of the study were approved by our institutional Human Ethics Committee. At birth and at 6, 12, and 24 months of age, peripheral blood was collected. In addition, the children were assessed for skin prick test (SPT) reactivity to a standard panel of allergens comprising HDM (*Dermatophagoides pteronyssinus*), perennial rye, cat pelt, *Alternaria tenuis*, *Aspergillus fumigatus*, cow's milk, and hen's egg (Hollister-Stier, Spokane, Wash) at 6 and 24 months as performed previously.³¹

Antibody assays

Antibody titers were determined via the ImmunoCAP 100 assay (Phadia AB, Uppsala, Sweden) as described for IgE^{32,33} and IgG₄³⁴ (detection limits 0.35 kU/L and 0.15 mg/L, respectively). In light of earlier suggestions of the potential usefulness of data on IgE levels

in young children below the standard clinical cut-off of 0.35 kU/L,^{35,36} a zero control was included in the IgE ImmunoCAP assay, which extended the limits of detection to 0.1 kU/L.^{37,38} Additionally, cumulative titers of IgE and IgG₄ against a panel of allergens (hens egg, cows milk, peanut, shrimp, *D pteronyssinus*, cat, dog, birch, timothy, ragweed, and wall pellitory [*Parietaria judaica*]), designated in text below as "polyclonal," were determined via the Phadiatop Infant versions of ImmunoCAP.³⁹

Cell preparation and culture

Cord (CBMCs) and peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved for later batch analysis.⁴⁰ CBMCs or PBMCs were cultured for 48 hours alone or with HDM or phytohemagglutinin (PHA) as performed previously.⁴⁰ The HDM preparation was devoid of detectable lypopolysaccharide-like activity as per earlier studies.⁴⁰ Cell pellets were resuspended into RNeasy lysis buffer (Ambion, Austin, Tex) and stored at -20°C until extraction (RNeasy lysis buffer, Ambion).

Cytokine detection

Cytokine protein levels above background were determined in supernatants by time-resolved fluorescence⁴⁰ (TRF; limits of detection 10 pg/mL); cytokine-specific mRNA was determined by quantitative polymerase chain reaction (PCR) and normalized against the housekeeping gene *UBE2D2*.⁴¹

Statistical analysis

Group differences were assessed via the Mann-Whitney *U* test and differences between individuals over time by the Wilcoxon matched pairs signed-rank test. Correlations between HDM SPT wheal size/IgE titers and immunologic outcomes were determined using the Kendal τ rank correlation. For multiple stepwise linear regression, and stepwise logistic regression, cytokine and antibody responses were log transformed, with values below the limits of detection (including zero values) being designated as half the limits of detection.

RESULTS

Development of T-cell immunity to HDM allergen

Fig 1 illustrates PBMC responses to HDM among HR infants stratified by HDM-SPT reactivity at age 2 years, measured via PCR. CBMCs from the majority (overall 72%) of subjects displayed 1 or more positive cytokine response to HDM, and respective IL-9, IL-13 and IFN- γ responses were often higher in CBMCs than in later infant samples. Parallel analyses of cytokine protein were also performed employing TRF; these data were highly correlated with respective mRNA (IL-5 Kendal τ rank .426, $P = .000$; IL-13: .448, $P = .000$; IFN- γ : .436, $P = .000$), but the PCR methodology was overall more sensitive and consistent in discriminating atopic and nonatopic groups, and hence, ensuing analyses (except Table I) use the latter.

The most clear-cut distinction between cytokine responses in subjects grouped on the basis of HDM SPT status at 2 years were observed with respect to HDM-specific IL-4 and IL-5 outcome measures. Significant group differences are evident by 6 months for IL-4 and by 12 months for IL-5, and these differences follow predicted patterns (namely, relative T_H2-polarity in the atopic group) and remain stable. Note also the progressive

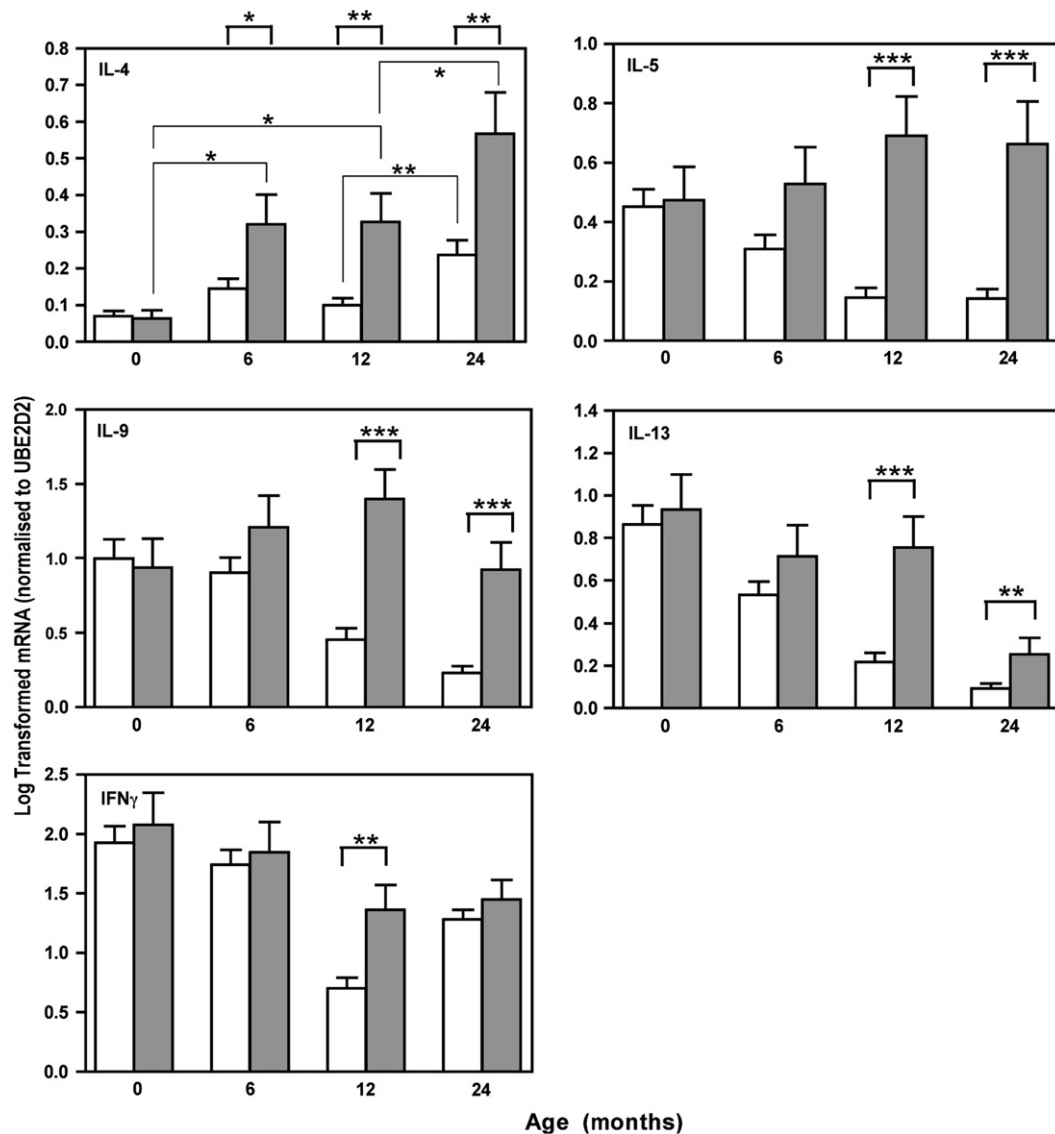


FIG 1. HDM-stimulated cytokine production. Data are shown as means + SEs for those who are HDM SPT-negative (open bars; $n = 113, 117, 101$, and 151 at birth, 6, 12, and 24 months) versus those who are HDM SPT-positive (gray bars; $n = 33, 32, 34$, and 43) at 24 months. Samples with 0 assay values are plotted here and below as $0.5 \times$ detection limit. Differences between groups: * $P < .05$; ** $P < .01$; *** $P < .001$.

time-dependent increase in IL-4 responses in the SPT⁺ group. No CBMC cytokine responses displayed significant intergroup differences. Moreover, no allergen-specific HDM response in CBMC (protein or mRNA) correlated with later atopic status as measured by HDM-induced SPT wheal size at outcome age (Table I) or HDM-specific IgE titers (not shown). This result contrasted with a consistent pattern of positive correlations for the 6-month and particularly the 12-month samples.

Postnatal development of HDM-specific IgE and IgG₄

The HDM-specific response profiles for the overall study population are illustrated in Fig 2, A, stratified as in Fig 1 by SPT outcome. At the population level,

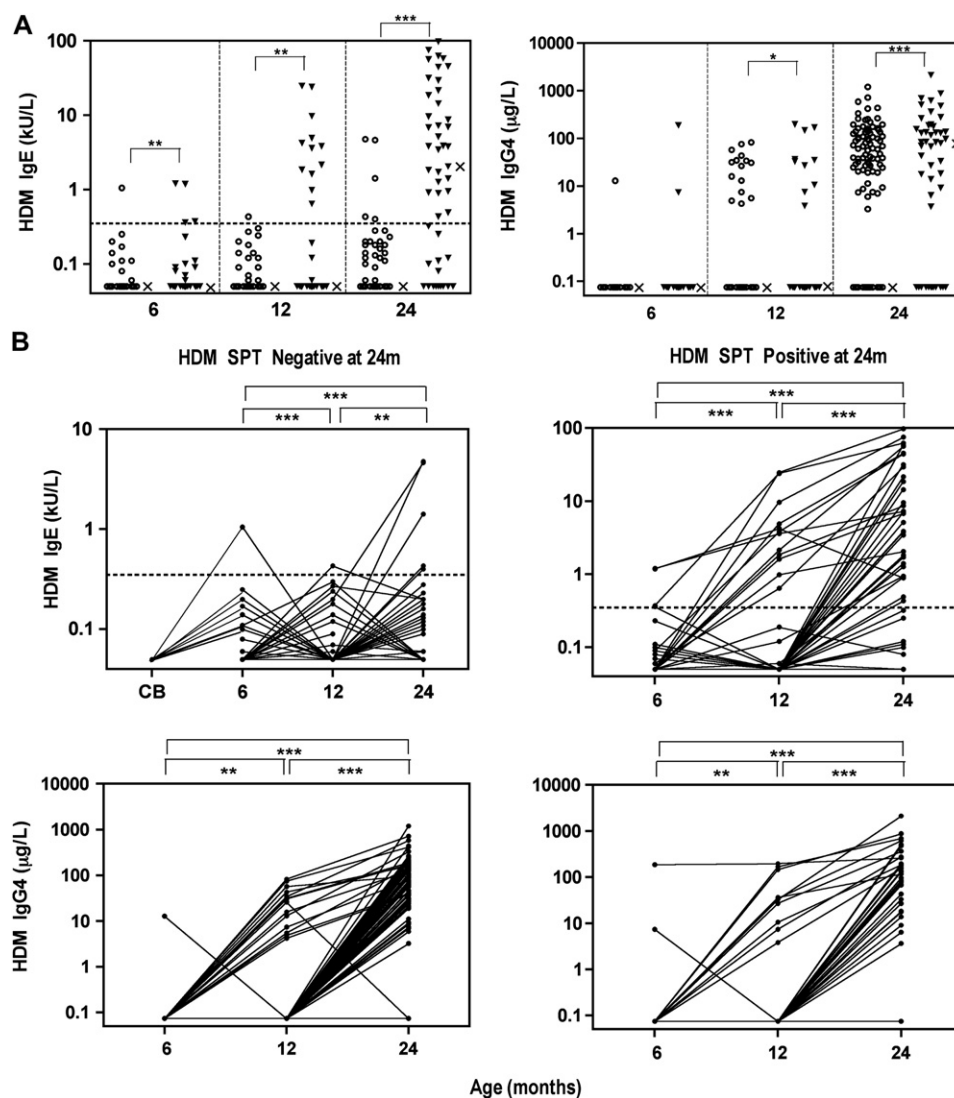
HDM-specific IgE titers were significantly increased in the SPT⁺ group from 6 months onward and consolidated thereafter. The situation with respect to IgG₄ was qualitatively similar. In particular, at 2 years, 44% of the HDM-SPT⁻ children displayed measurable HDM-specific IgG₄ titers, in contrast to 74% of the HDM-SPT⁺ group. Note also the high frequency of putative positive IgE readings, which are below the 0.35 kU/L clinical cut-off but above the 0.1 kU/L detection limits (particularly at 6 and 12 months) among the group who remains HDM-SPT⁻, the significance of which is discussed in more detail below.

Fig 2, B tracks HDM-specific antibody in individuals over time. These assays were initially restricted to samples collected at and beyond 6 months, and 2 distinct response patterns were evident. Notably, HDM-specific IgE

TABLE I. Correlation between HDM SPT wheal size at 24 months and HDM-stimulated cytokine responses at birth, and at 6 and 12 months within the whole population

Sample time	Statistic	Protein				mRNA				
		IL-5	IL-10	IL-13	IFN- γ	IL-4	IL-5	IL-9	IL-13	IFN- γ
CB	Kendal τ rank correlation	0.028	0.065	0.124	0.097	-0.107	0.066	0.071	0.078	0.117
	<i>P</i> value	.694	.334	.065	.167	.192	.385	.331	.287	.095
6 mo	Kendal τ rank correlation	0.179	-0.015	0.183	0.054	0.228	0.136	0.198	0.176	0.090
	<i>P</i> value	.021	.834	.010	.459	.004	.074	.005	.017	.191
12 mo	Kendal τ rank correlation	0.369	0.029	0.268	0.194	0.200	0.379	0.366	0.279	0.244
	<i>P</i> value	.000	.682	.000	.009	.014	.014	.000	.000	.001

CB, Cord blood.

**FIG 2.** HDM-specific IgE and IgG4 titers. **A**, Antibody titers in subjects HDM SPT-negative at 24 months (circles; $n = 171$) or HDM SPT-positive (triangles; $n = 47$). Each X designates a median. **B**, Tracking antibody titers in individuals who are HDM SPT-negative versus HDM SPT-positive at outcome age [differences between group (A) or individual titers (B) designated by asterisks as per Fig 1].

responses in children who were SPT⁺ at age 2 years typically trend progressively upward beyond 6 months, in marked contrast to those who were SPT⁻, the majority of whom tracked along the y-axis (below detection limits).

However, a subpopulation of the subjects who were SPT⁻ at outcome displayed transient low-level HDM-specific IgE responses that seemed to peak at either 6 or 12 months, trending down thereafter. Follow-up analyses on cord

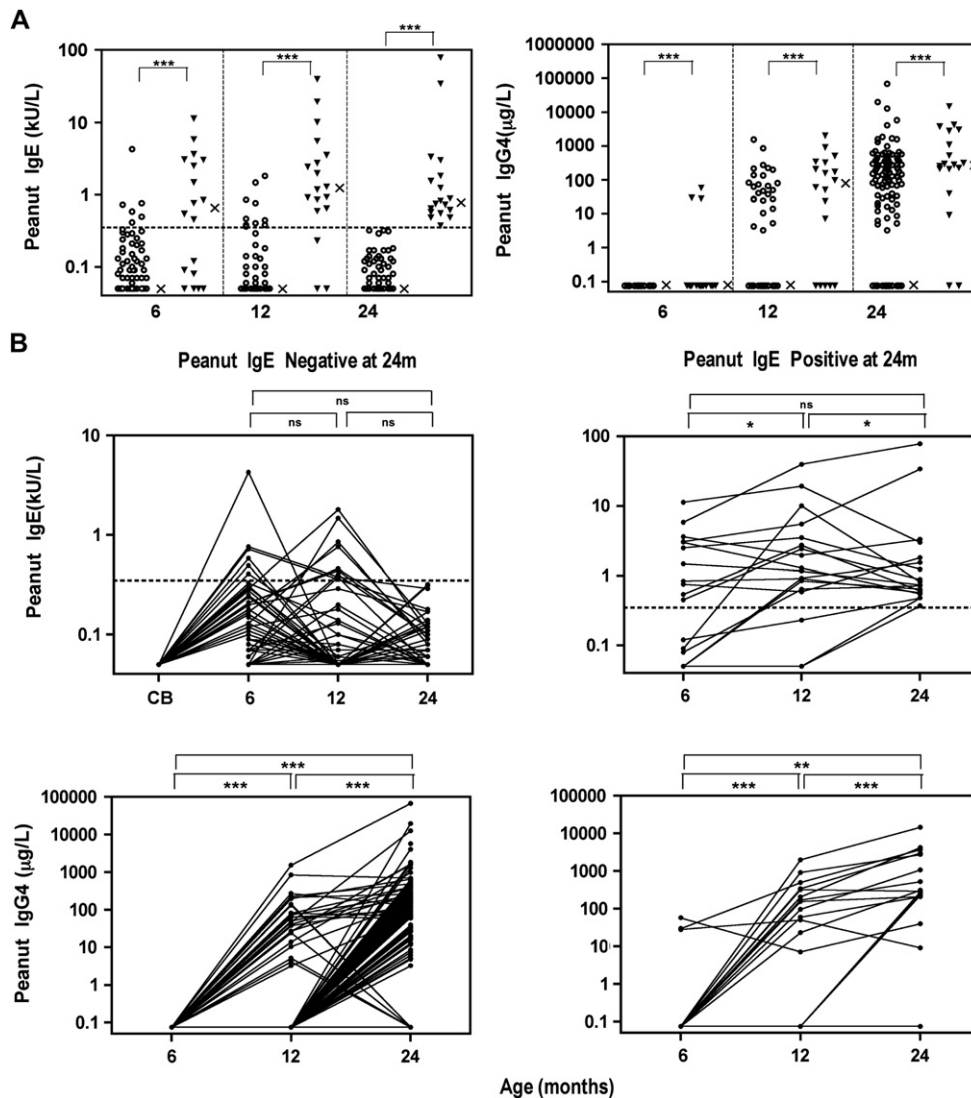


FIG 3. Peanut-specific IgE and IgG₄ titers. **A**, Antibody titers in subjects sensitized (peanut-specific IgE ≥ 0.35 kU/L; triangles; $n = 18$) versus nonsensitized (circles; $n = 199$) at 24 months. **B**, Tracking of antibody titers in individuals. Data expressed as per Fig 2.

blood samples from the subset of subjects displaying 6-month titers greater than or equal to 0.1 kU/L (Fig 2, B) demonstrated that their HDM-specific IgE titers at birth were below this detection limit. This pattern is not evident for corresponding IgG₄ responses. IgE and IgG₄ responses at 6 months were not significantly related, but these responses were weakly correlated at 12 (Kendal τ rank correlation 0.146; $P = .009$) and 24 months (0.148; $P = .004$). Fig 3 illustrates corresponding patterns of IgE and IgG₄ antibody titers against peanut, which mirror those for HDM, of particular note being the high frequency of children showing early transient “peak” peanut-specific IgE responses at 6 months; 90% of the latter remained low or continued to trend downward to 24 months (not shown). Comparable patterns were also observed for cat and grass (not shown). It is additionally pertinent to note that these fluctuating serum IgE responses were also accompanied

by transient SPT responses; notably, 14 infants who displayed SPT reactivity to 1 or more allergens at the 6-month time point were completely SPT negative at 24 months (not shown).

Fig 4 illustrates polyclonal antibody responses within the study population after stratification on the basis of overall atopic status at outcome age 2 years. Phadiatop Infant scores for both IgE and IgG₄ increased progressively in the atopics over the observation period, which indicates generalized upregulation of T_H2-dependent responses in this group. The relevant IgE scores were statistically increased at all time points in the atopics relative to the nonatopics (Fig 4, A), but this discrimination was not evident for IgG₄.

Tracking mixed allergen-specific IgE profiles in individual subjects by Phadiatop Infant (Fig 4, B) demonstrated biphasic patterns that were comparable with

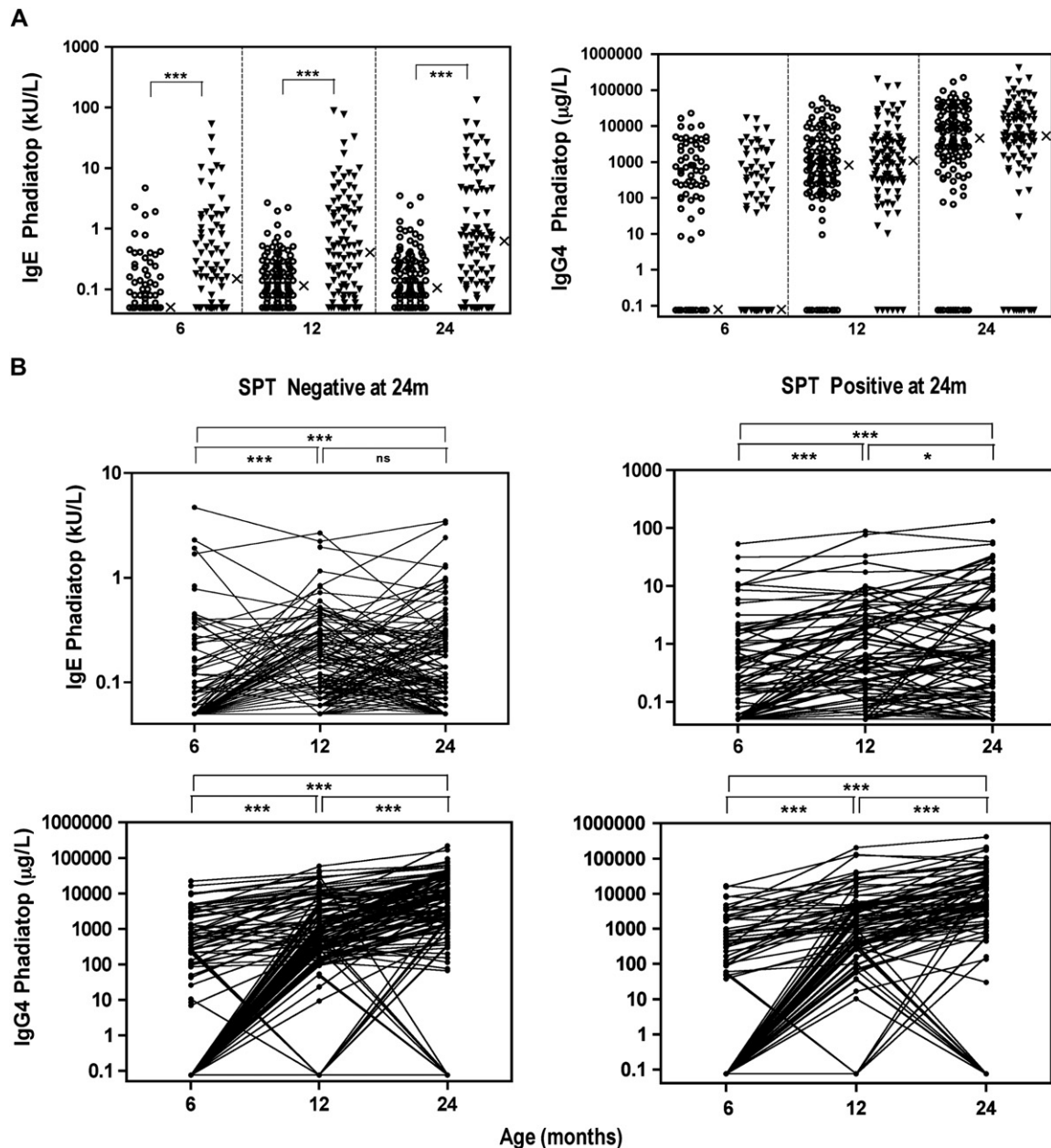


FIG 4. Phadiatop IgE and IgG₄ titers. **A**, Phadiatop IgE and IgG₄ titers in individuals stratified by atopic status at 24 months. SPT-positive response to any allergen in the test panel (*triangles*; n = 94); SPT negatives (*circles*; n = 125). **B**, Tracking of Phadiatop titers in individuals. Data expressed as per Fig 2.

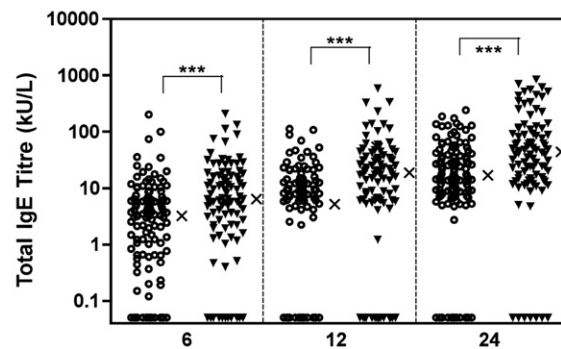
those observed in Fig 2, A for HDM-specific responses, but they were predictably more frequent as Phadiatop measures multiple specificities. At the population level, overall polyclonal IgE responses at 12 months are significantly increased relative to 6 months in both atopics and nonatopics, but they continued to rise thereafter only in the atopics (Fig 4, B). A biphasic response pattern can again be observed in a subset of subjects, for both IgE and IgG₄. Total IgE increased progressively in both atopics and nonatopics, with the rate of climb being highest in the atopics (Fig 5). A subset of infants (n = 61) again

displays an apparently biphasic pattern of overall IgE production, which shows titers that decline after 6 or 12 months of age.

Relationships between cellular and humoral immune responses to allergens

In parallel with the antibody studies, a series of analyses were carried out on corresponding cellular immune responses, which focus principally on HDM. Initial univariate analyses examined relationships between HDM-specific cytokine responses and corresponding IgE titers

A



B

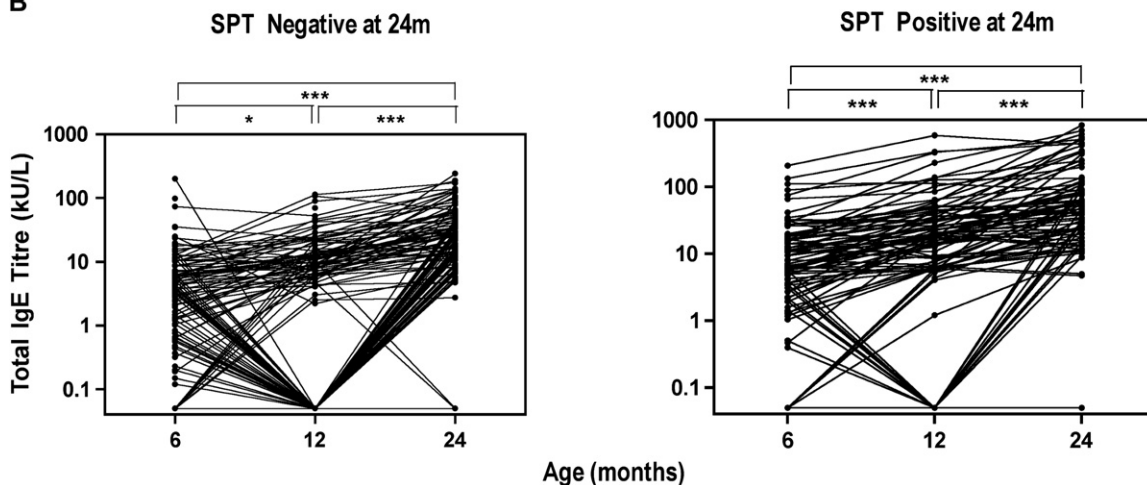


FIG 5. Total IgE titers. **A**, IgE titers in the same subjects shown in Fig 4. **B**, tracking of total IgE titers in individuals. Data expressed as per Fig 2.

at the sampling times. No positive associations were observed before 2 years, presumably because of the low frequency of sensitized subjects producing specific IgE before this age (Fig 2, A). However, at 2 years, over 40 children were sensitized, and strong positive associations were evident within the overall cohort between their HDM-specific IgE titers and their HDM-induced T_H2 cytokine responses, notably IL-4 (Kendal τ rank correlation 0.203; $P = .001$), IL-5 (0.260; $P = .000$), IL-9 (0.224; $P = .000$), and IL-13 (0.176; $P = .004$). Table II illustrates the results of logistic regression modeling of relationships between atopic reactivity to HDM and immune response variables at 6, 12, and 24 months. At the earliest sampling time (6 months), IL-4 responses to HDM were detectable in a subset of the study population, and positive responses at this time correlated strongly with having HDM-specific IgE antibody titers at 2 years above the 0.35 kU/L clinical cut-off for sensitization [odds ratio (OR) 11.8] and with a positive SPT reaction (OR 3.0). The frequency and magnitude of positive allergen-specific T-cell responses increased with age. By 12 months, IL-5 responses correlated positively with subsequent atopic outcomes at age 2 years, and IgE reactivity at 12 months showed a similar relationship. At outcome age itself, SPT reactivity correlated strongly with IgE titer, whereas

having HDM-specific IgE titers above the clinical cut-off correlated strongly with the IL-9 component of the HDM-specific T-cell response. Of note, the T_H1 response capacity at ages 1 and 2 years as measured by polyclonal (PHA) IFN- γ production correlated inversely with IgE responsiveness.

These relationships are explored in more detail in Table III, which illustrates the results of multiple linear regression modeling of HDM-specific IgE and SPT responses at outcome age. Within the overall population, the IL-4, IL-5, and IL-9 components of the HDM-specific response were positively associated with IgE titers and the negative association observed with the T_H1 cytokine IFN- γ in Table II was reiterated in this analysis. Among the atopic subset selected on the basis of IgE titers above the clinical cut-off, the strongest positive signals were observed for IL-5. The strongest determinant of HDM-specific SPT wheal size was the corresponding IgE titer, with an additional (weaker) signal from IL-5.

With respect to HDM-specific IgG₄, linear regression modeling demonstrated a significant relationship between serum titers at 24 months and *in vitro* HDM-specific IL-9 responses ($t = 2.877$; $P = .004$) and a negative relationship between 12 month titers and the IFN- γ component

TABLE II. Logistic regression modeling of relationship between atopic reactivity to HDM at 24 months and immune response variables during sequential blood sampling

Sample time	Outcome measure*	
6 mo	Positive SPT response to HDM at 24 mo IL-4/HDM (OR 3.093 [95% CI 1.091–8.764], $P = .034$)	IgE titer to HDM at 24 mo ≥ 0.35 kU/L IL-4/HDM (OR 11.847 [95% CI 3.568–39.332], $P = .000$)
12 mo†	IL-5/HDM (OR 5.510 [95% CI 2.283–13.299], $P = .000$) IgE/HDM (OR 3.582 [95% CI 1.537–8.351], $P = .003$)	IFN- γ /PHA (OR 0.470 [95% CI 0.238–0.926], $P = .029$) IL-5/HDM (OR 9.603 [95% CI 3.496–26.375], $P = .000$)
24 mo	IgE/HDM (OR 6.893 [95% CI 3.868–12.282], $P = .000$)	IFN- γ /PHA (OR 0.326 [95% CI 0.127–0.833], $P = .019$) IL-9/HDM (OR 3.644 [95% CI 2.279–5.828], $P = .000$)

*Log HDM-specific mRNA and log PHA-stimulated protein cytokine levels are included in both models. HDM-specific log IgE and IgG₄ are included in both models to predict SPT responsiveness. Confounding factors of gender, dual parental atopic history, and day care attendance in the first 2 years of life are included in each model.

†Male gender (OR 7.705 [1.699–34.947], $P = .008$) and parental history (OR 5.137 [1.387–19.023], $P = .014$) were independently associated with IgE titer at 24 mo at this time point.

TABLE III. Determinants of HDM wheal size and HDM-specific IgE titer in serum at 24 months by multiple stepwise linear regression modelling*

Outcome parameter	Study group	Immune response index	Coefficient (SE)	t	P value
Log HDM-IgE titer	Whole population	Log IL-5/HDM	0.493 (0.176)	2.802	.006
		Log IL-9/HDM	0.342 (0.129)	2.661	.008
		Log IFN- γ /PHA	−0.311 (0.125)	−2.489	.014
		Log IL-4/HDM	0.236 (0.118)	1.997	.047
Log HDM-IgE titer	IMMUNOCAP positive only	Log IL-5/HDM	0.611 (0.065)	9.460	.000
		Log IL-5/PHA	0.415 (0.149)	2.780	.010
HDM wheal size	Whole population	Log IgE/HDM	1.757 (0.127)	13.830	.000
		Log IL-5/HDM	0.469 (0.216)	2.170	.031
HDM wheal size	IMMUNOCAP positive only	Log IgE/HDM	2.639 (0.796)	3.316	.003

*Log HDM-specific mRNA and log PHA-stimulated protein cytokine levels included in both models from the 24-month sample point. HDM-specific log IgE and IgG₄ included in both models to predict HDM wheal size.

of the *in vitro* HDM response at the same time point ($t = -2.142$; $P = .034$).

DISCUSSION

The scientific basis for recommending maternal allergen avoidance for prevention of infant sensitization rests primarily on the demonstration in cord blood of T cells that respond *in vitro* to allergens,^{7,9,10} inferring previous *in utero* priming. However, recent studies from our group have cast doubt on the phenotype of these cells. Notably, it is now evident that most putative T_H-memory responses in neonates can be accounted for via the activity of recent thymic emigrant (RTE) CD4⁺ T cells expressing the naive CD45RA⁺ phenotype.⁴² These RTE cells express altered antigen receptors that lack the fine specificity of conventional T cells, and as a consequence, they can interact at low affinity with a broad range of antigens/allergens not previously encountered. T cells with comparably “promiscuous” antigen recognition properties have also been demonstrated in neonatal mice.⁴³ An additional characteristic of the human neonatal RTE is that, after an initial round of allergen-triggered proliferation and cytokine production, they typically apoptose, unless “rescued” via high levels of cytokines such as IL-2.⁴² It is pertinent to

note that a feature of the methodology in our earlier studies to generate allergen-specific T-cell clones from cord blood¹⁰ involved the use of high levels of IL-2, which accounts for their survival in the cloning cultures.

If cord blood allergen-responsive T cells are indeed short-lived RTE as opposed to long-lived T_H memory cells, it follows that specific allergen responsiveness in cord blood may be unrelated to subsequent allergen responder phenotype. Consistent with this conclusion: (1) HDM-induced IL-9, IL-13, and IFN- γ responses in cord blood display clear evidence of transience during infancy (Fig 1); and (2) the predicted patterns of positive associations between T_H2 cytokine responses and subsequent sensitization outcomes, and parallel negative associations with T_H1 responses, do not develop until at or beyond 6 months (Tables I–III). Moreover, the broad concordance from 6 months and beyond between the kinetics of expansion of HDM-specific T_H2 immunity, in particular relating to production in the atopics of IL-4, which drives IgE-B-cell switching (Fig 1 and Table II) and corresponding production of the T_H2-dependent antibodies IgE and IgG₄, provides a plausible and internally consistent argument that relevant T-cell and B-cell priming are both essentially postnatal processes.

Additionally, postnatal tracking of IgE production in individual children who do versus who do not develop

sensitization provides an additional level of information relating to the immunologic basis for natural resistance to allergy in nonatopic children. As illustrated in Figs 2 and 3, a significant proportion of children who remained nonatopic produced detectable levels of allergen-specific IgE that fluctuates within the low range, peaking in individual children at 6 or 12 months of age before returning to baseline, and this pattern is reiterated in total IgE levels (Fig 5) and, as noted, in patterns of SPT reactivity. These findings are consistent with earlier prospective studies,⁴⁴ including those that have tracked nonatopic children beyond infancy to school age^{35,45} and are consistent with animal model studies that have tracked the waxing and waning of specific IgE responses over prolonged periods of exposure to aeroallergens.⁴⁶ The biphasic nature of IgE responses in these latter models is now recognized as the signature of an underlying process involving initial T_H2-cell activation followed by development of protective immunologic tolerance,⁴⁷ and a similar set of mechanisms is believed to operate in nonatopic humans during initial exposure to allergens, to prevent sensitization.⁴⁸ The results presented here identify infancy/early childhood as the age range during which this protective mechanism commonly operates, and by extension as the logical life phase for implementation of intervention therapies aimed at enhancing the efficiency of this process, as suggested earlier.⁴⁸ The latter approach is currently under trial in a double-blind placebo-controlled multicenter study in Australia and the United States focusing on HR infants and children.⁴⁹

The principal implications of this study relate to the validity of current recommendations for dietary allergen avoidance by atopic women during pregnancy. Notably, our data refute the principal immunologic evidence underpinning these guidelines. The current study represents the most systematic analysis to date of the timing of allergen-specific immunologic priming, which focuses on HR infants who as a group are the most likely to manifest *in utero* sensitization, if such a process occurs. We have approached this issue by monitoring age-dependent changes in IgE/IgG₄ and corresponding T_H-cell cytokine responses, focusing in particular on HDM, with subsidiary analyses of additional IgE/IgG₄ responses, including peanut and other specificities as pools measured via the Phadiatop Infant test. The broad consistency achieved between measures of T_H2-polarized immunity and IgE/IgG₄ responses, and the parallel negative association observed with T_H1 reactivity, attest to the robustness of the methodology employed. On that basis, we argue that the finding of lack of association between cord blood T-cell reactivity to allergen and subsequent IgE or SPT responses, and the development of an increasingly strong association between these parameters beyond 6 months of age, suggests that the key events associated with development of the T_H2-polarized allergen-specific memory that underpins stable atopic sensitization occurs postnatally as opposed to *in utero*. As such, and taken together with other recent data on the role of RTE in neonatal T-cell responses,⁴² we suggest that previous findings of putative allergen-responsive T cells in cord blood do not provide credible support

for the notion of intrauterine sensitization, and as such add further weight to the doubts raised as to the validity of existing recommendations on maternal allergen avoidance.²² We stress that these findings pertain specifically to HR children and that applicability to the overall population remains to be determined.

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