

Development of the Capacity of Peripheral Blood Mononuclear Cells to Produce IL-4, IL-5 and IFN- γ upon Stimulation with House Dust Mite in Children with Atopic Dermatitis

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Key Words

IL-4 · IL-5 · IFN- γ · House dust mite · Atopic dermatitis, children

Abstract

Background: Imbalances of IL-4 and IFN- γ production are widely known to increase IgE synthesis in allergic individuals, and IL-5 is known to play a critical role in the pathogenesis of various allergic diseases. However, little is known about how Th cells specific to house dust mite (HDM) develop the capacity to produce these cytokines in children with atopic dermatitis (AD). **Objective:** This study aims to clarify when HDM-specific Th cells develop the capacity to produce IL-4, IL-5 and IFN- γ in children with AD. **Methods:** The production of IL-4, IL-5 and IFN- γ by peripheral blood mononuclear cells (PBMCs) upon stimulation with HDM was measured in 91 children with AD and 37 control subjects. The changes in the cytokine production with age were analyzed transectionally and longitudinally. **Results:** IL-4 production by HDM-stimulated PBMCs in children with AD was already increased before age 1. Thereafter, it continuously rose until reaching a near-maximum level at age 2. Growth-related changes in the production of IL-5 resembled those in the production of IL-4 and peaked at age 1. The production of

these cytokines was very low in control subjects up to age 2 and then gradually increased, albeit never above the levels measured in AD children. The production of IFN- γ in children with AD reached a near-maximum level during the first year of life and diminished after age 3. Although IFN- γ production in controls was lower than that in AD children during infancy, it continuously rose even after age 3 and surpassed the levels measured in AD children. The level of serum HDM-specific IgE antibody began to increase after age 1 following the rise of IL-4 production. Essentially the same relationship among IL-4, IFN- γ and IgE synthesis was seen in a longitudinal study of 6 AD infants, in whom HDM-specific IgE was initially negative but later turned positive. **Conclusions:** These findings suggest that the capacity of HDM-specific Th cells to produce IL-4, IL-5 and IFN- γ develops and effectively matures during the first 3 years of life in children with AD.

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Introduction

IgE antibody is thought to play a critical role in the pathogenesis of allergic disorders by triggering the type I allergic reaction [1]. IgE synthesis has been shown to be

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Table 1. Cytokine production in children with AD

Age	IL-4*** fg/ml	IL-5* pg/ml	IFN- γ (NS) pg/ml	HDM-IgE**** U _A /ml
0–6 months	<65 (<65–240) (9)	7.0 (<1.0–21.0) (9)	2.3 (<1.0–13.5) (9)	0.4 (0.3–0.9) (9)
7–11 months	140 (<65–398) (11)	18.8 (<1.0–169) (10)	5.5 (<1.0–16.5) (11)	0.2 (0.1–54.4) (11)
1 year	348 (<65–975) (14)	37.3 (<1.0–293) (16)	5.8 (<1.0–31.5) (16)	1.1 (0.1–132.7) (16)
2 years	410 (<65–1,750) (15)	33.0 (<1.0–370) (16)	6.2 (<1.0–135) (16)	89.1 (0.2–153.5) (16)
3–7 years	388 (<65–1,860) (22)	35.0 (<1.0–209) (24)	2.7 (<1.0–37.5) (24)	110.0 (0.2–171.9) (24)
8–15 years	460 (175–1,525) (13)	30.9 (10.4–402) (14)	3.2 (<1.0–29.0) (15)	102.3 (8.0–133.3) (15)

IL-4, IL-5 and IFN- γ production by PBMCs upon stimulation with HDM in children with AD is shown as the median and range along with the serum level of HDM-IgE. The number of subjects is indicated in parentheses. The significance of the alteration with growth is indicated as follows: * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$. NS = Not significant.

enhanced by IL-4 secreted by Th2 cells, and suppressed by IFN- γ produced by Th1 cells [2–4]. Consistent with this, the IL-4 production is increased in allergic patients, whereas IFN- γ production is diminished or unchanged [5–10]. However, the profile of cytokine production is considerably altered with age. We demonstrated increases in both IL-4 and IFN- γ production by peripheral blood mononuclear cells (PBMCs) upon stimulation with house dust mite (HDM), a major indoor allergen [11, 12], in infants with atopic dermatitis (AD), suggesting that HDM-specific Th cells had not yet fully differentiated into Th2 cells in those patients [13]. This is consistent with the absence of HDM-specific IgE antibody (HDM-IgE) in most infants with AD [13, 14].

Recently, there has been much interest in the prevention of HDM-IgE synthesis in order to hamper the development of allergic disorders [15–17]. Some investigators demonstrated that countermeasures to reduce HDM and other indoor allergens from early childhood are to some extent effective in hindering the production of HDM-IgE in children as they grow older [16, 17]. In order to develop the most effective intervention against HDM-IgE synthesis, it is very important to clarify when HDM-specific Th cells differentiate into Th2.

IL-5, another representative Th2 cytokine [18], is also thought to play an important role in the development of various allergic disorders including AD [12, 19, 20]. HDM-specific Th2 cells have been demonstrated to be increased in the peripheral blood of patients with AD [21]. Recently, we demonstrated that IL-5 production by PBMCs upon stimulation with HDM is significantly higher in children with AD than in nonallergic controls [22]. Interestingly, IL-5 production by HDM-stimulated

PBMCs is also increased in infants with AD in whom HDM-IgE is negative, suggesting that IL-5 also contributes to the development of infantile AD [23]. However, IL-5 production by HDM-stimulated PBMCs in infants with AD is still lower than that in older children with AD [23]. It remains unknown when HDM-specific Th2 cells acquire a mature capacity to produce IL-5 in younger children with AD.

In this study, we closely examined the age-dependent changes in IL-4, IL-5 and IFN- γ production by PBMCs upon stimulation with HDM in children with AD. Our results suggest that HDM-specific Th cells practically complete the differentiation into Th2 cells during the first 3 years of life.

Materials and Methods

Subjects

After having obtained informed consent, we drew peripheral blood from 91 children with AD who were referred to our institute between October 1996 and March 2000. In view of the difference in cytokine production between patients with AD and those with bronchial asthma [22], subjects with bronchial asthma were excluded. No subjects had ever received systemic corticosteroids, and the examination was not performed during the acutely aggravated phase. Although the level of serum HDM-IgE was very low in infants with AD (0–11 months) (table 1), all of them were positive (>0.7 U_A/ml) for serum IgE antibody against egg white. As controls, blood samples were collected in a similar fashion from 37 age-matched nonallergic children who were apparently healthy and free from any allergic, hematologic, immunologic or infectious diseases. This study was approved by the Ethics Review Board of the Shizuoka Children's Hospital.

Table 2. Cytokine production in nonallergic controls

Age	IL-4*** fg/ml	IL-5* pg/ml	IFN-γ** pg/ml
0–6 months	<65 (5)	<1.0 (<1.0–1.9) (5)	<1.0 (<1.0–4.5) (5)
7–11 months	<65 (<65–120) (8)	2.9 (<1.0–10.5) (8)	2.6 (<1.0–12.8) (8)
1 year	<65 (<65–105) (7)	4.1 (<1.0–12.0) (6)	5.0 (<1.0–30.8) (7)
2 years	<65 (<65–113) (5)	2.0 (<1.0–9.0) (5)	5.2 (<1.0–25.9) (5)
3–7 years	153 (<65–747) (5)	28.0 (<1.0–54.0) (5)	8.3 (1.0–28.7) (5)
8–15 years	423 (220–690) (6)	19.5 (<1.0–64.8) (7)	37.5 (17.1–205) (7)

The production of IL-4, IL-5 and IFN-γ by PBMCs upon stimulation with HDM in nonallergic control subjects, in whom serum HDM-IgE was negative (<0.35 U_A/ml), is presented as the median and range. The number of subjects is indicated in parentheses. The significance of the alteration with growth is indicated as follows: * p < 0.05; ** p < 0.01; *** p < 0.001.

Cell Culture

PBMCs were collected from heparinized blood by gradient centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden) and resuspended at a concentration of 1×10^6 cells/ml in RPMI 1640 medium (Gibco, Grand Island, N.Y., USA) supplemented with 10% heat-inactivated fetal calf serum (Xavier Investments, Wacol, Australia), 2 mM glutamine and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). The cell suspension was dispensed in duplicate into a 48-well culture plate (0.5 ml/well) (Falcon 3078, Becton Dickinson, Lincoln Park, N.J., USA) and cultured for 4 days for IL-4 measurement or 6 days for IL-5 and IFN-γ measurement in a humidified atmosphere of 5% CO₂/95% air at 37°C with 50 µg/ml HDM (Der f) crude extract [23–26] (Torii Pharmaceutical Co., Tokyo, Japan). The level of endotoxin in RPMI 1640 medium containing 50 µg/ml HDM was lower than 1 ng/ml. The supernatant was collected in an Eppendorf tube and stored at –80°C.

Measurement of IL-4, IL-5, IFN-γ and IgE Antibody

IL-4, IL-5 and IFN-γ in the culture supernatant were measured with enzyme-linked immunosorbent assay (ELISA) kits (Cytoscreen-US human IL-4, Cytoscreen human IL-5 and IFN-γ, BioSource International, Camarillo, Calif., USA). The sensitivity was 65 fg/ml for IL-4 measurement and 1 pg/ml for IL-5 and IFN-γ. HDM-IgE in the serum was examined by the CAP system (Pharmacia).

Statistics

Data are presented as the median and range. The significance of the difference in cytokine production between AD and control subjects and the correlation between IL-4 and IFN-γ production in AD children were estimated by the Mann-Whitney test and Spearman's rank correlation coefficient, respectively. The significance of age-dependent changes in cytokine production was examined by the Kruskal-Wallis test. The alteration of data with growth in the longitudinal study was analyzed by the Wilcoxon signed-rank test. Interaction between age and the disease in the production of each cytokine was estimated by two-factor ANOVA. The result was judged to be significant if the p value was less than 0.05.

Results

IL-4, IL-5 and IFN-γ Production in Nonallergic Subjects

IL-4, IL-5 and IFN-γ production by HDM-stimulated PBMCs was undetectable in nonallergic infants before the age of 6 months (table 2). Thereafter, the production of these cytokines increased gradually until reaching a maximum level after the age of 8 years (fig. 1).

IL-4 and IL-5 Production in Children with AD

IL-4 production by HDM-stimulated PBMCs was already detectable during the first year of life in most infants with AD (table 1). Thereafter, the level rapidly increased until reaching a near-maximum level at age 2. The level of serum HDM-IgE began to rise after age 1 in parallel with the rise of IL-4 production (table 1). The changes in the production of IL-5 by PBMCs from children with AD resembled those of IL-4 (table 1) and peaked at age 1 (fig. 1b).

IFN-γ Production in Children with AD

The production of IFN-γ by HDM-stimulated PBMCs from children with AD reached a near-peak level before age 1 (table 1). Thereafter, it remained unchanged until the children reached their third year, when it began to decrease somewhat. Unlike the pattern seen in the production of the other cytokines and IFN-γ in nonallergic controls, the overall alteration of IFN-γ production with age in children with AD was not significant (tables 1, 2).

Although INF-γ production by HDM-stimulated PBMCs was higher in children with AD than in their nonallergic counterparts up to the age of 2, PBMCs from non-

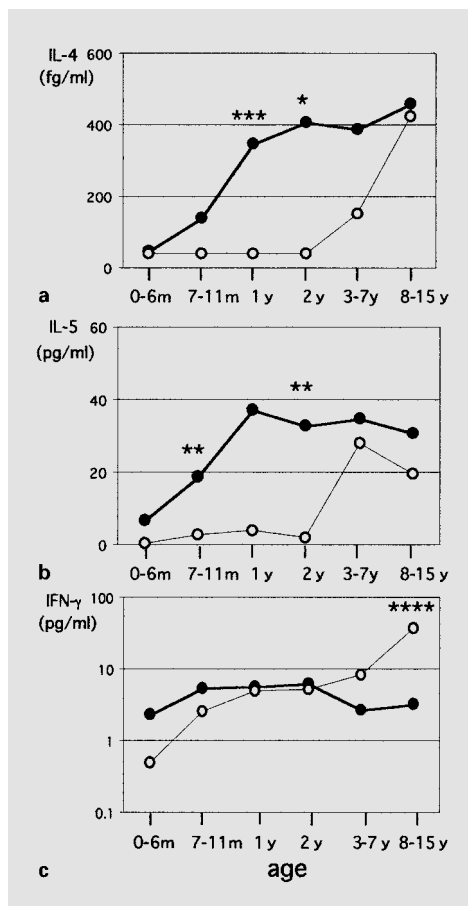


Fig. 1. Comparison of age-dependent changes in IL-4, IL-5 and IFN- γ production between children with AD and nonallergic controls. The alteration of IL-4 (a), IL-5 (b) and IFN- γ production (c) in nonallergic control subjects (○) and children with AD (●) is shown. The significance of the difference between children with AD and nonallergic controls is indicated as follows: * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$. m = Months; y = year(s).

allergic counterparts produced more IFN- γ than those from AD children past the age of 3 (fig. 1c).

As for the production of IFN- γ , but not for the production of IL-4 or IL-5, two-factor ANOVA showed a significant interaction between growth (age) and the disease state (allergic or nonallergic) ($p < 0.0005$).

Correlation between IL-4 and IFN- γ Production

There was a close positive correlation between IL-4 and IFN- γ production in infants with AD (fig. 2b,c). Thereafter the positive correlation weakened with age, and no correlation was found in children aged 2 (fig. 2d). Later, in the age group of 3–7 years, a significant inverse correlation was seen (fig. 2e). Still later, in the age group of

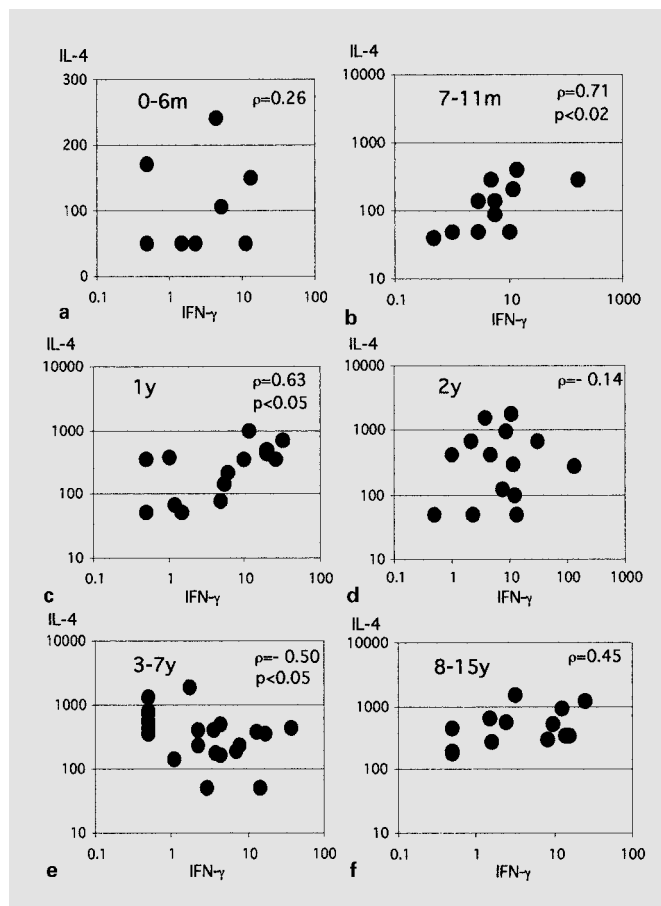


Fig. 2. Correlation between IL-4 and IFN- γ production. Growth-related changes in correlation between IL-4 (fg/ml) and IFN- γ (pg/ml) production by HDM-stimulated PBMCs in children with AD are shown. a 0–6 months. b 7–11 months. c 1 year. d 2 years. e 3–7 years. f 8–15 years.

8–15 years, the correlation between IL-4 and IFN- γ production weakened again (fig. 2f).

Longitudinal Study of Cytokine Production

The change in cytokine production before and after the initiation of HDM-IgE synthesis was monitored in 6 infants with AD up to a maximum of 3 years (fig. 3). Although the level of serum HDM-IgE in these subjects was negative (<0.35 U_A/ml) at the first examination before age 1, it rose to 33.5 U_A/ml (0.99–71.0) at the final examination.

IL-4 production during this period significantly increased from 95.0 fg/ml (<65 –398) to 450 fg/ml (210–975; $p < 0.05$). In 5 of 6 infants, IL-4 production rose lin-

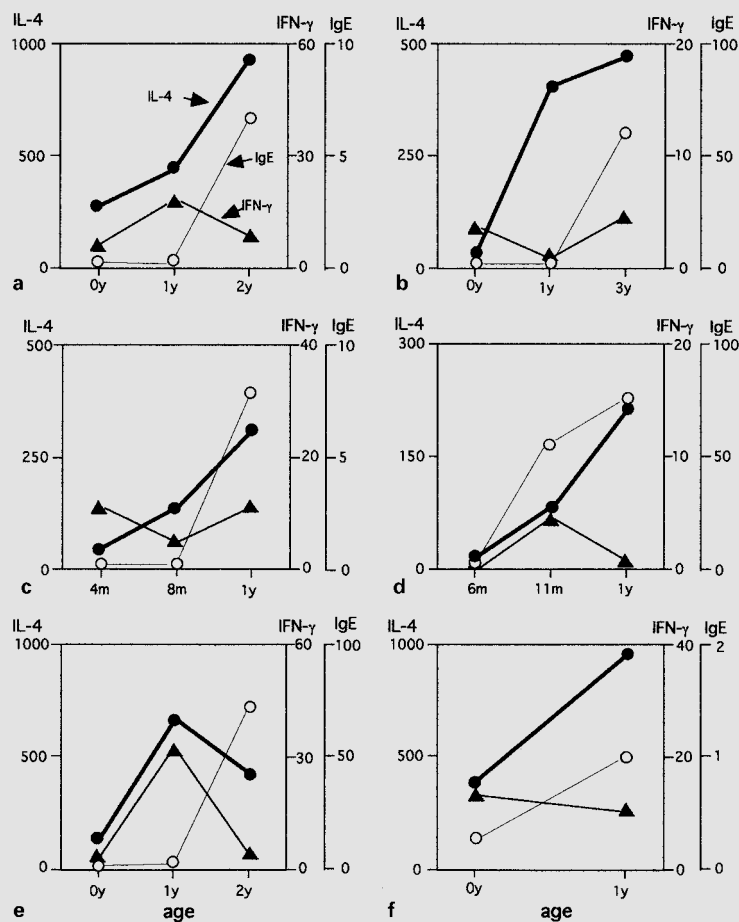


Fig. 3. Longitudinal study of cytokine production in children with AD. Alteration with age of IL-4 (fg/ml; ●) and IFN- γ (pg/ml; ▲) production in 6 infants with AD is shown along with the change in the level of serum HDM-IgE (U_A/ml; ○) that was initially negative and later turned positive. m = Months; y = year(s).

early (fig. 3a–d, f). Although the sixth infant (fig. 3e) exhibited lower IL-4 production at the third examination at age 2 than at the second examination at age 1 (410 vs. 675 pg/ml), the level at age 2 was still higher than that of the nonallergic counterparts (table 2). Moreover, the ratio of IL-4 to IFN- γ production at age 2 (410/3.6) was higher than that at age 1 (675/31.5).

By contrast, IFN- γ production was not significantly altered during this period [initially 4.4 pg/ml (<1.0–14.3) and finally 6.8 pg/ml (1.5–11.5)]. Although IFN- γ production was transiently increased in 3 subjects (fig. 3a, b, d), it had fallen again by age 3.

Discussion

IgE synthesis is thought to be enhanced by IL-4 and suppressed by IFN- γ [3, 4]. It has been reported that HDM-specific Th cells in allergic subjects have a tendency to differentiate into Th2 cells, which exclusively secrete IL-4 [2, 18, 21, 27]. Indeed, the profile of cytokine production by HDM-stimulated PBMCs corresponds to that of Th2 cells in older children with AD or bronchial asthma (3–15 years) [10, 13]. On the other hand, while even infants with AD exhibit an increased production of cytokines by HDM-stimulated PBMCs [13, 23], the profile of cytokine production is consistent not with that of Th2 but rather that of Th0 cells (or Th1/Th2). In other words, PBMCs from infants with AD produce higher levels of both IL-4 and IFN- γ compared to nonallergic controls [13]. It remains unknown when HDM-specific Th2 cells

mature in humans. In this study, we clarified the time course of the development of the imbalance of IL-4 and IFN- γ production by HDM-specific Th cells in children with AD.

The production of IL-4 by PBMCs upon stimulation with HDM could not be detected before age 2 in nonallergic subjects (table 2). Thereafter, it increased very slowly until reaching a maximum level after age 8, as previously reported by Tang and Kemp [28]. In contrast, the elevation of IL-4 production by HDM-stimulated PBMCs was already remarkable before age 1 in children with AD, suggesting that the sensitization of Th cells to HDM begins during the first year of life. The discrepancies in both IL-4 and IL-5 production between allergic and nonallergic subjects were quite distinct in younger age groups (0–2 years) (fig. 1a, b). Thus, the measurement of these cytokines produced by HDM-stimulated PBMCs appears to have great value as an indicator of the sensitization of Th cells to HDM in younger allergic children who do not yet produce detectable levels of HDM-IgE.

The pattern of an age-dependent alteration of IFN- γ production by HDM-stimulated PBMCs in children with AD is apparently different from the patterns of IL-4 and IL-5 production (table 1): namely it nearly peaked during the first year of life. This may explain why HDM-IgE is not produced in AD infants in spite of the ability of their HDM-specific Th cells to secrete IL-4. A relatively high IFN- γ production probably suppresses the action of IL-4 during this period. It is also suggested that a sharp increase in IL-4 production without any significant increase in IFN- γ production after age 1 leads to the development of HDM-IgE synthesis.

The change with growth in IFN- γ production by HDM-stimulated PBMCs was also distinctly different between allergic and nonallergic subjects. Although IFN- γ production was higher in AD children than in nonallergic controls up to age 2, PBMCs from the AD children older than 3 years produced less IFN- γ than those from the nonallergic counterparts. This inversion of cytokine production between allergic and nonallergic individuals appears to be a phenomenon most clearly reflecting the maturation of Th2 cells in children with AD.

We previously demonstrated that IL-4 production is positively correlated with IFN- γ production in infants with AD, indicating that Th cells in those patients still retain the feature of Th0 cells [13]. In the present study, the correlation between IL-4 and IFN- γ production in children with AD was found to fluctuate markedly as the children grew (fig. 2). Although a significant positive correlation was seen in infants, a significantly negative one

was seen in the children aged 3–7. This result suggests that HDM-specific Th cells predominantly have the characteristics of Th2 cells in children aged 3–7, i.e. in the age range when the serum level of HDM-IgE reaches its peak level (table 1). Since IL-4 production in the age group of 3–7 years did not differ from that in the age group of 2 years, the inverse correlation between IL-4 and IFN- γ production may be ascribed to the diminution of IFN- γ production (table 1). While it remains unknown why the inverse correlation between IL-4 and IFN- γ production disappeared in children aged 8–15, it might be responsible for a slight decrease of the serum HDM-IgE level (table 1).

For further clarification of the relationship between the change in cytokine production and the development of IgE synthesis in early childhood, the alteration of cytokine production by HDM-stimulated PBMCs was prospectively studied in 6 infants with AD, in whom HDM-IgE was initially negative but confirmed to become positive within a few years. The result of this prospective study was essentially the same as that obtained by the transectional analysis. Thus, it seems valid that an increase in IL-4 production with growth without any remarkable alteration of IFN- γ secretion has a significant relevance to the initiation of HDM-IgE synthesis.

Some investigators have attempted to prevent HDM-IgE synthesis by reducing HDM in the environment during early childhood, when HDM-IgE is not yet produced [15–17]. Although this approach was demonstrated to be effective to some extent, HDM-IgE synthesis could not be completely inhibited [16, 17]. One factor limiting the success of this approach is the difficulty in eradicating HDM from the environment. Another possible factor is the early sensitization of Th cells to HDM, which occurs much earlier in life than previously believed. If we consider the proliferative response of lymphocytes to HDM [23] and an increase in cytokine production by HDM-stimulated PBMCs in infants with AD (table 1), the sensitization of Th cells to HDM appears to occur within 6 months after birth. For this reason, it is not so easy to start environmental control before the sensitization of Th cells to HDM. However, this study suggests that, in spite of their sensitivity to HDM, HDM-specific Th cells in allergic infants have not yet fully differentiated into Th2 cells. Thus, if a safe modulator of the maturation of Th cells is developed [29, 30], it may be of special value in preventing the production of HDM-IgE in these subjects by blocking the differentiation into Th2 cells.

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