Unique profile of IL-4 and IFN-γ production by peripheral blood mononuclear cells in infants with atopic dermatitis

Mitsuaki Kimura, MD, PhD, Satoru Tsuruta, MD, PhD, and Takami Yoshida, MD, PhD
Shizuoka, Japan

Although the level of house dust mite (HDM)–specific lymphocyte proliferation is high in infants with atopic dermatitis (AD), the level of HDM-specific IgE antibody (HDM-IgE-RAST) is usually very low or negative. To elucidate the cause of the deficient HDM-specific IgE antibody formation in infants with AD, we examined the profile of IL-4 and IFN-γ production by HDM-stimulated PBMCs. The amount of IL-4 production was higher in infants with AD and in children with AD (3 to 15 years) than in the nonatopic control subjects. Although the amount of IFN-γ production in children with AD was lower than that found in nonatopic children, it was higher in infants with AD than in nonatopic infants. This result suggests that HDM-specific helper T lymphocytes in infants with AD have not yet differentiated into T_{H2} but rather stayed at the stage of T_{H0}. The level of IgE-RAST for egg white (EW) is already elevated in infants with AD. The amount of IL-4 produced by EW-stimulated PBMCs was comparable to that produced by HDM-stimulated PBMCs in infants with AD. However, the amount of IFN-γ produced by EW-stimulated PBMCs was distinctly lower than that produced by HDM-stimulated PBMCs in infants with AD. Although there was no correlation between the amount of IL-4 production by HDM-stimulated PBMCs and the level of HDM-IgE-RAST in infants with AD, the amount of IL-4 production by EW-stimulated PBMCs was closely correlated with the level of EW-IgE-RAST. These results suggest that it is not the lack of IL-4 but rather a relative increase in IFN-γ production by HDM-specific helper T lymphocytes that causes the deficiency of HDM-specific IgE-antibody synthesis in infants with AD. (J Allergy Clin Immunol 1998;102:238-44.)

Key words: *Atopic dermatitis, infants, IL-4, IFN-γ, lymphocyte proliferation, house dust mite, egg white, IgE*

Abbreviations used
AD: Atopic dermatitis
EW: Egg white
HDM: House dust mite
IgE-RAST: RAST for IgE antibody
PBMC: Peripheral blood mononuclear cell
SIF: Stimulation index measured by flow cytometry

House dust mite (HDM) allergen is now widely accepted as a major cause of atopic dermatitis (AD) in adults.[1,2] In patients with AD, the levels of HDM-specific IgE antibody (IgE-RAST) and HDM-specific lymphocyte proliferation are usually elevated with a positive correlation between them.[3] In contrast to adults, the level of HDM-IgE-RAST is usually negative in infants with AD despite an increase in the level of IgE-RAST for food allergens such as egg white (EW).[4,5] Therefore food allergens, but not HDM, are assumed to play an important role in the development of infantile AD.[6]

Recently, some investigators reported that the avoidance of HDM in infancy suppressed the development of HDM-related airway allergic disorders in childhood,[7,8] suggesting that sensitization to HDM occurs during infancy. We demonstrated that the level of HDM-specific lymphocyte proliferation expressed as stimulation index measured by flow cytometry (SIF) was distinctly higher in infants with AD than in nonatopic infants.[9] Moreover, the level of HDM-SIF is closely correlated with the severity of infantile AD, the absolute number of peripheral blood eosinophils, and the amount of IL-5 production by HDM-stimulated peripheral blood mononuclear cells (PBMCs) in infants with AD.[9] Thus HDM appears to play an important role in the development of infantile AD by inducing IL-5 secretion by HDM-specific helper T lymphocytes.

Although there is a significant positive correlation between the levels of HDM-IgE-RAST and HDM-SIF in children with AD, no correlation is observed between these levels in infants with AD because of the deficiency of HDM-specific IgE antibody synthesis.[9] The production of IgE antibody is regulated reciprocally by a pair of T lymphocyte–derived cytokines, IL-4 and IFN-γ; the former enhances and the latter suppresses IgE synthesis.[10,11] Many investigators have reported an increase in IL-4 production, a decrease in IFN-γ secretion, or both by T lymphocytes on stimulation with polyclonal mitogen or HDM in patients with AD.[12-15] Although the

From the Department of Allergy and Clinical Immunology, Shizuoka Children's Hospital, Shizuoka, Japan.
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Reprint requests: Mitsuaki Kimura, MD, PhD, Department of Allergy and Clinical Immunology, Shizuoka Children's Hospital, Urushiyama 860, Shizuoka City 420-8660, Japan.

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TABLE I. Profile of subjects

Group	*n*	Age	HDM-IgE-RAST (U_A/mL)	HDM-SIF
Infants (0 yrs)				
AD	13	8 mos (3-11 mos)	0.28 (0.15-132.6)	229.2 ± 79.0*
CTRL	6	6 mos (2-11 mos)	0.23 (0.19-0.39)	120.8 ± 21.3
Children (3-15 yrs)				
AD	22	7 yrs (3-15 yrs)	119.4 (15.2-171.8)	166.6 ± 52.5†
CTRL	8	10 yrs (4-15 yrs)	0.23 (0.13-0.31)	123.0 ± 16.4

Age and HDM-IgE-RAST are presented as median and range. HDM-SIF is presented as mean ± 1 SD.
CTRL, Nonatopic control subjects.
*Significantly higher than that in CTRL infants ($P < .01$) and in children with AD ($P < .01$).
†Significantly higher than that in CTRL children ($P < .05$).

TABLE II. Amount of IL-4 and IFN-γ production by HDM-stimulated PBMCs

	AD	CTRL
0 yrs		
IL-4 (fg/mL)	143* (<65.0-290) (*n* = 13)	<65.0 (<65.0) (*n* = 6)
IFN-γ (pg/mL)	5.2† (<1.0-165) (*n* = 13)	<1.0 (<1.0-3.7) (*n* = 6)
3-15 yrs		
IL-4 (fg/mL)	424 (165-1860) (*n* = 19)	395 (<65.0-690) (*n* = 7)
IFN-γ (pg/mL)	12.7‡ (<1.0-75.0) (*n* = 18)	35.0 (2.9-92) (*n* = 7)

The amount of cytokines in the supernatants is presented as median and range.
CTRL, Nonatopic control subjects.
*Significantly higher than that in CTRL subjects ($P < .01$).
†Significantly higher than that in CTRL subjects ($P < .05$).
‡Significantly lower than that in CTRL subjects ($P < .05$).

amount of IL-4 production was reported to be smaller in neonates than in children or adults,[16,17] T lymphocytes in infants appear to be able to induce IgE antibody formation because food allergen–specific IgE antibody can be produced even in infants.[4,5] To elucidate the reason for the lack of HDM-specific IgE antibody, we examined the pattern of IL-4 and IFN-γ production by PBMCs on stimulation with HDM in infants with AD.

METHODS

Subjects

Heparinized peripheral blood was obtained at the time of routine blood examination from 35 patients with AD who visited us from October 1996 to July 1997 after informed consent was obtained (Table I). As controls, heparinized blood was collected similarly from 14 nonatopic children who did not have any hematologic, immunologic, or infectious diseases. AD was treated only by topical use of steroid ointment without any systemic administration of steroids. This study was approved by the Ethics Review Board of the Shizuoka Children's Hospital.

Cell culture

PBMCs were collected by gradient centrifugation over Ficoll-Paque (Pharmacia) and resuspended at a concentration of 1×10^6 cells/mL in RPMI-1640 medium (Gibco) supplemented with 10% heat-inactivated FCS (Xavier Investments, Wacol, Australia), 2 mmol/L glutamine, and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). A portion of the cell suspension (0.5 mL) was added in each well in duplicate on a 48-well culture plate (Falcon 3078, Becton Dickinson) and cultured for 4 days for IL-4 measurement or for 6 days for measurement of IFN-γ and lymphocyte proliferation in a humidified atmosphere of 5% CO_2 at 37° C with or without 50 μg/mL HDM (Der f)[18-20] or 500 μg/mL EW extract (Torii Pharmaceutical Co., Tokyo, Japan). The concentration of allergen and the duration of the culture were determined by preliminary experiments. The endotoxin level in the RPMI-1640 medium containing 50 μg/mL HDM is less than 1 ng/mL (measured with an endotoxin-specific test kit, Endospecy; Seikagaku Kogyo Co. Ltd., Tokyo, Japan). After incubation, the supernatant was collected in an Eppendorf tube (Iwaki Glass Co. Ltd., Chiba, Japan) and stored at –80° C for measurement of cytokines. Cells were transferred into a 5-mL glass tube (Iwaki) for analysis of lymphocyte proliferation.

Measurement of allergen-specific lymphocyte proliferation

The antigen-specific lymphocyte proliferation was measured by using flow cytometry as described elsewhere.[9] Briefly, for fixation, cells were transferred into a 5-mL glass tube (Iwaki) after completion of a 6-day culture period, washed twice with PBS, resuspended in 0.6 mL of –20° C 70% ethanol, and kept at 4° C for longer than 4 hours. To remove RNA, after washing twice with PBS, the fixed cells were incubated in 500 μL PBS containing 100 μg/mL RNase (Sigma Chemical Co.) at 37° C for 1 hour. Cellular DNA was stained by adding propidium iodide (Sigma) at a final concentration of 40 μg/mL. The amount of DNA per cell was analyzed by flow cytometry (EPICS Elite, Coulter Corporation), and the percentage of the S-phase cells distributed in the flat area between the peaks of G0/G1 - and G2/M-phase cells was measured. The percentage of the S-phase cells was converted to stimulation index and expressed as SIF (stimulation index measured by flow cytometry).

Measurement of IL-4, IFN-γ, and IgE-RAST

IL-4 and IFN-γ in the culture supernatant were measured with ELISA kits (Cytoscreen US human IL-4 and Cytoscreen human IFN-γ; BioSource International, Camarillo, Calif). The sensitivity was 65 fg/mL for IL-4 and 1.0 pg/mL for IFN-γ. IgE-RAST for

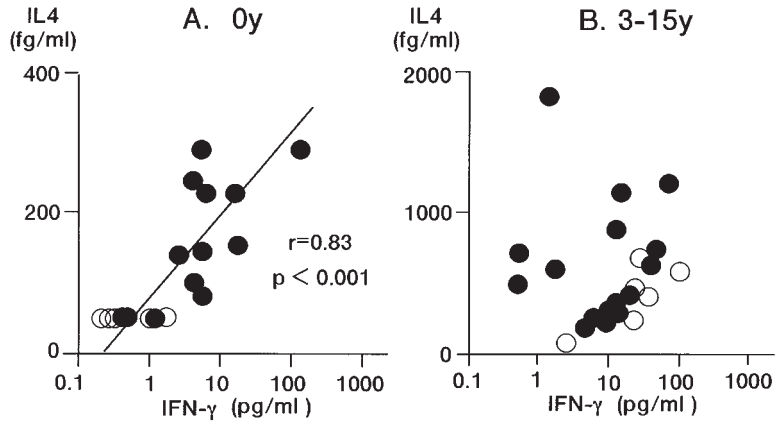

FIG. 1. Correlation between amounts of IL-4 and IFN-γ production by HDM-stimulated PBMCs in infants (**A**) and in children (**B**). *Open* and *closed circles* represent nonatopic control subjects and subjects with AD, respectively.

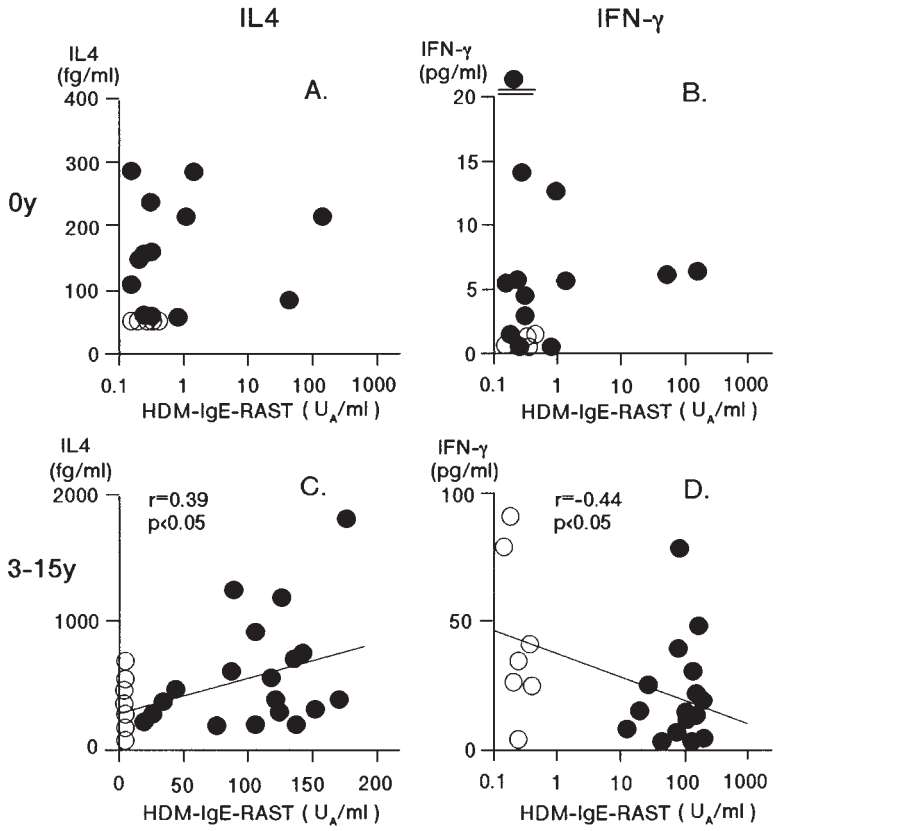

FIG. 2. Correlation of level of HDM-IgE-RAST with amount of IL-4 (**A** and **C**) or IFN-γ (**B** and **D**) production by HDM-stimulated PBMCs in infants (**A** and **B**) and in children (**C** and **D**). *Open* and *closed circles* represent nonatopic control subjects and subjects with AD, respectively.

HDM (Der f) or EW was measured by using a CAP-RAST radioimmunoassay kit (Pharmacia).

Statistics

SIF is expressed as the mean ± 1 SD, and IgE-RAST, IL-4, and IFN-γ are expressed as the median and the range. The significance of the difference was examined by Student's *t* test for SIF, and the Wilcoxon rank-sum test for IL-4 and IFN-γ. Correlation between the levels of IL-4 and IFN-γ in infants is expressed by the Spearman rank correlation coefficient.

RESULTS

Increase of IL-4 and IFN-γ production by HDM-stimulated PBMCs in infants with AD

In children (age range, 3 to 15 yrs), the amount of IL-4 produced by HDM-stimulated PBMCs was higher in patients with AD than in nonatopic control subjects, although the difference was not significant (Table II). In contrast, the amount of IFN-γ produced by

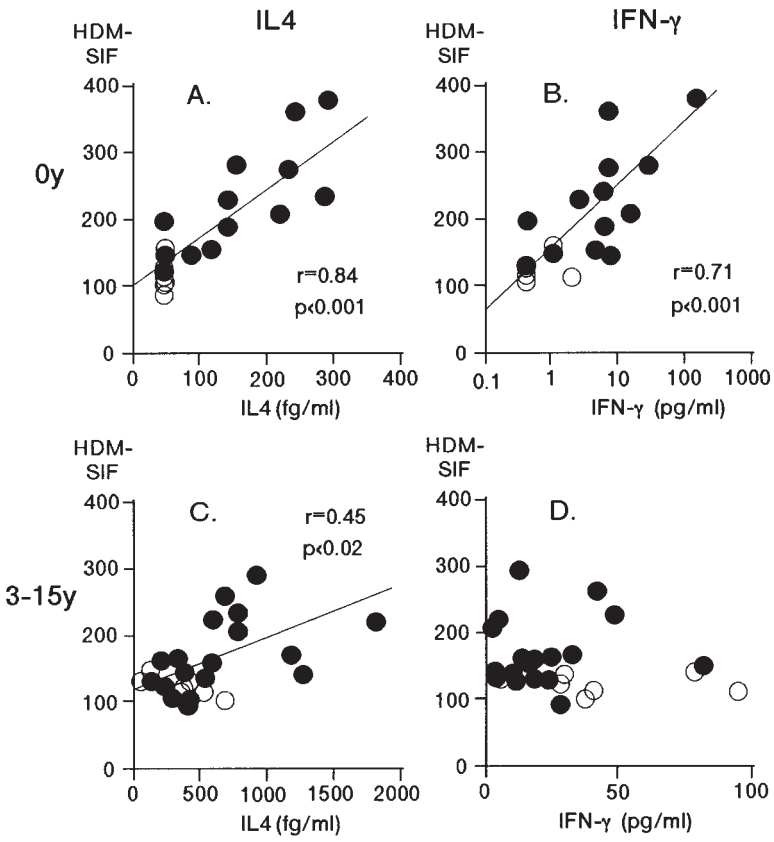

FIG. 3. Correlation of the level of HDM-SIF with the amount of IL-4 (**A** and **C**) or IFN-γ (**B** and **D**) production by HDM-stimulated PBMCs in infants (**A** and **B**) and in children (**C** and **D**). *Open* and *closed circles* represent nonatopic control subjects and subjects with AD, respectively.

HDM-stimulated PBMCs was significantly lower in children with AD than in nonatopic control subjects (Table II). The amounts of IL-4, as well as IFN-γ, produced by HDM-stimulated PBMCs were lower in infants with AD than in children with AD (Table II). However, both were significantly higher in infants with AD than in nonatopic control infants (Table II). There was a close positive correlation between the amounts of IL-4 and IFN-γ produced by HDM-stimulated PBMCs in infants with AD, whereas no apparent correlation was observed between them in children with AD (Fig. 1).

In children the amount of IL-4 produced by HDM-stimulated PBMCs showed a significant positive correlation with the level of HDM-IgE-RAST (Fig. 2, *C*), whereas the amount of IFN-γ production was inversely correlated with the level of HDM-IgE-RAST (Fig. 2, *D*). In contrast to children, the amount of either IL-4 or IFN-γ produced by HDM-stimulated PBMCs did not correlate with the level of HDM-IgE-RAST in infants (Fig. 2, *A* and *B*).

Correlation between cytokine production and lymphocyte proliferation of HDM-stimulated PBMCs in infants with AD

In children there was a significant positive correlation between the level of HDM-specific lymphocyte proliferation (HDM-SIF) and the amount of IL-4 produced by HDM-stimulated PBMCs (Fig. 3, *C*), whereas no correlation was observed between the level of HDM-SIF and the amount of IFN-γ produced by HDM-stimulated PBMCs (Fig. 3, *D*). In infants, not only the amount of IL-4 but also the amount of IFN-γ produced by HDM-stimulated PBMCs was intimately correlated with the level of HDM-SIF (Fig. 3, *A* and *B*).

TABLE III. Profile of infants enrolled in the study of EW-specific cytokine production by PBMCs

Group	*n*	Age (mos)	EW-IgE-RAST (U_A/mL)	EW-SIF
AD	9	7 (3-11)	16.3 (0.32-116.4)	161.8 ± 58.6
CTRL	5	6 (2-10)	0.23 (0.15-1.16)	105.4 ± 22.7

Age and EW-IgE-RAST are presented as median and range. EW-SIF is presented as mean ± 1 SD.
CTRL, Nonatopic control subjects.

Positive correlation between IL-4 production by EW-stimulated PBMCs and the level of EW-IgE-RAST

Although HDM-IgE-RAST is usually negative in infants with AD, the level of EW-IgE-RAST is almost always elevated. To see whether such a difference could be explained by the difference in the profile of cytokine production, we measured the amounts of IL-4 and IFN-γ

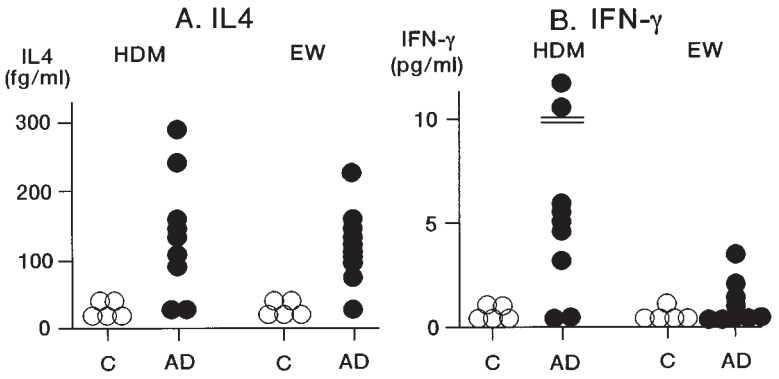

FIG. 4. Amounts of IL-4 (**A**) and IFN-γ (**B**) produced by HDM-stimulated PBMCs are compared with those produced by EW-stimulated PBMCs in infants. A pair of HDM- and EW-stimulated samples was measured at the same time. *Open* and *closed circles* represent nonatopic control subjects and subjects with AD, respectively. *C,* Nonatopic control subjects.

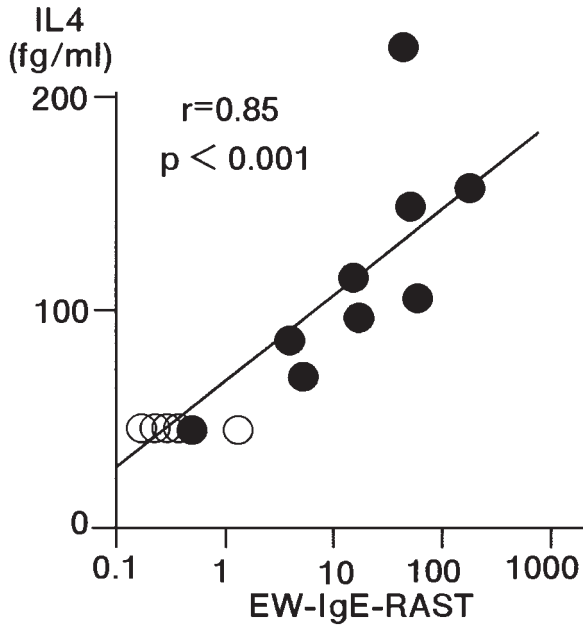

FIG. 5. Correlation of level of EW-IgE-RAST with amount of IL-4 production by EW-stimulated PBMCs in infants. *Open* and *closed circles* represent nonatopic control subjects and subjects with AD, respectively.

produced by EW-stimulated PBMCs simultaneously with those produced by HDM-stimulated PBMCs in 9 infants with AD and 5 nonatopic control subjects (Table III).

PBMCs of infants with AD could produce a detectable amount of IL-4 on stimulation with EW (median [range], 100 fg/mL [<65.0 to 223 fg/mL]), which was significantly higher than that produced by EW-stimulated PBMCs in nonatopic infants (all cases, <65.0 fg/mL; $P < .01$) (Fig. 4, *A*). The amount of IL-4 produced by EW-stimulated PBMCs in infants with AD was comparable to that produced by HDM-stimulated PBMCs (140 fg/mL [<65.0 to 290 fg/mL]) (Fig. 4, *A*). In contrast, the amount of IFN-γ produced by EW-stimulated PBMCs was distinctly lower than that produced by HDM-stimulated PBMCs (< l.0 pg/mL [<l.0 to 3.1 pg/mL] vs 5.2 pg/mL [<l.0 to 165 pg/mL]; $P < .05$) (Fig. 4, *B*). There was a close positive correlation between the amount of IL-4 production by EW-stimulated PBMCs and the level of EW-IgE-RAST (Fig. 5).

DISCUSSION

Although HDM-IgE-RAST is usually negative in infants with AD, we previously showed that lymphocyte proliferation and IL-5 production by PBMCs on stimulation with HDM were already elevated in infants with AD.[9] Because there was a close positive correlation among the HDM-SIF level, the severity of AD, the degree of eosinophilia, and the amount of IL-5 production by HDM-stimulated PBMCs, HDM-specific helper T cells do not seem to be bystanders but to be involved in the development of infantile AD, probably by increasing and activating eosinophils by means of IL-5 production. In this study we further demonstrated that HDM also induced the production of IL-4 and IFN-γ by PBMCs in infants with AD. These findings strongly suggest that the sensitization of T lymphocytes to HDM occurs in infancy.

This study demonstrated some features in IL-4 and IFN-γ production in infants with AD. First, the amount of cytokine production per million PBMCs was smaller in infants than in older children (3 to 15 yrs). This may agree with reports indicating that the amount of IL-4, as well as IFN-γ, production by PBMCs is smaller in neonates than in adults.[16,17] However, because the absolute number of helper T lymphocytes in infants is more than twice that found in older children,[21] the difference in the amount of in vivo cytokine production between infants with AD and children with AD might be smaller than that shown here.

Second, the amount of IFN-γ production by HDM-stimulated PBMCs was significantly higher in infants with AD than in nonatopic control infants. Moreover, there is a significant positive correlation between the amounts of IL-4 and IFN-γ production by HDM-stimulated PBMCs in infants with AD. Helper T lymphocytes are believed to be composed of two major subtypes, T_{H1}

and T_{H2}.[22-24] The former produces a large amount of INF-γ but no or only a small amount of IL-4, whereas the latter acts inversely. T_{H2} cells are assumed to play a critical role in the development of atopic disorders and have been reported to be a dominant T_H subtype in the affected tissue or PBMCs in atopic patients.[12-15] Our result for the pattern of cytokine production by HDM-stimulated PBMCs in older children with AD agrees with previous reports because the amount of IFN-γ production is significantly smaller in children with AD than in nonatopic control subjects. However, because the amounts of not only IL-4 but also IFN-γ production are elevated in infants with AD, their dominant HDM-specific helper T lymphocytes seem to belong not to T_{H2} but to T_{H0} cells, which are defined as less differentiated helper T lymphocytes with the ability to produce both IL-4 and IFN-γ.[25,26]

In contrast to HDM-IgE-RAST, the level of EW-IgE-RAST is usually high in infants with AD. To determine which cytokine is responsible for the lack of HDM-specific IgE antibody, we compared the pattern of cytokines produced by HDM-stimulated PBMCs with that produced by EW-stimulated PBMCs in infants with AD. The amount of IL-4 produced by EW-stimulated PBMCs is comparable to that produced by HDM-stimulated PBMCs, whereas the amount of IFN-γ produced by EW-stimulated PBMCs is definitely lower than that produced by HDM-stimulated PBMCs in infants with AD. There was a close positive correlation between the amount of IL-4 production and the level of EW-IgE-RAST in infants. The result indicates that the amount of IL-4 produced by EW-stimulated PBMCs, which is equal to that produced by HDM-stimulated PBMCs, is enough to enhance IgE antibody formation if the amount of IFN-γ production is low. Therefore a relatively high amount of IFN-γ production by HDM-specific T lymphocytes appears to be responsible for the deficiency in HDM-specific IgE antibody in infants with AD.

Finally, there was a close positive correlation between the amount of cytokine production and the level of HDM-SIF in infants with AD. In children the correlation between the proliferation and the production of cytokines is not as clear as in infants. For example, the amount of IFN-γ production by HDM-stimulated PBMCs in children with AD did not correlate with the level of HDM-SIF. Although a significant positive correlation was observed between the amount of IL-4 production by HDM-stimulated PBMCs and the level of HDM-SIF in both children with AD and infants with AD, the degree in the former is lower than that in the latter. The correlation between the amount of IL-5 production by HDM-stimulated PBMCs and the level of HDM-SIF is also closer in infants with AD than in children with AD.[9] Although the significance and the mechanism of these findings remain to be elucidated, there seems to be a tighter linkage between the proliferation and the production of cytokines in T_{H0} cells than in T_{H2} cells. HDM-SIF could be used as a good indicator of the cytokine-producing function of HDM-specific helper T lymphocytes in infants with AD.

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