

IL-4 production by PBMCs on stimulation with mite allergen is correlated with the level of serum IgE antibody against mite in children with bronchial asthma

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Background: Although IL-4, IL-13, and IFN- γ are known to affect IgE synthesis, it remains unclear which one plays the most important role in *in vivo* IgE synthesis in atopic patients. **Objective:** The aim of this study is to clarify the difference in importance among these cytokines in up-regulation of IgE synthesis in atopic patients.

Methods: We measured IL-4, IL-13, and IFN- γ production by PBMCs on stimulation with house dust mite (HDM) in 23 children, 3 to 15 years old, with bronchial asthma (BA) and analyzed the correlation with HDM-specific IgE antibody levels expressed as HDM IgE radioallergosorbent test (RAST) results.

Results: The production of IL-4 and IL-13 by PBMCs on stimulation with HDM was significantly higher in children with BA than in nonatopic control subjects (IL-4, 752.9 ± 365.9 vs 312.3 ± 230.0 fg/mL, $P < .001$; IL-13, 21.9 pg/ml [<12.0 - 77.6] vs <12.0 , $P < .01$). IL-4 production showed a close positive correlation with HDM IgE RAST ($r = 0.71$, $P < .001$), which was distinctly stronger than that between IL-13 production and HDM IgE RAST ($r = 0.46$, $P < .05$). IFN- γ production was neither different between children with BA and nonatopic control subjects (7.24 [1.54 - 33.90] pg/mL vs 11.2 [1.66 - 75.9] pg/mL) nor correlated with HDM IgE RAST levels. Essentially the same result was obtained by stimulation of PBMCs with a purified HDM major allergen Der f 1.

Conclusion: IL-4 is likely to be the most important cytokine in up-regulation of *in vivo* IgE synthesis against HDM in children with BA. (J Allergy Clin Immunol 2000;105:327-32.)

Key words: Bronchial asthma, house dust mite, *Candida albicans*, IL-4, IFN- γ , IL-13, IgE synthesis

IgE antibody mediates immediate hypersensitivity reactions and is thought to play an important role in the pathogenesis of various atopic disorders, including bronchial asthma (BA).¹ IgE synthesis was demonstrated to be regulated by 2 cytokines, IL-4 and IFN- γ (the former

Abbreviations used

BA:	Bronchial asthma
CA:	<i>Candida albicans</i>
HDM:	House dust mite
IgE-RAST:	Radioallergosorbent test for IgE antibody
T _H :	Helper T

enhances whereas the latter suppresses it).^{2,3} Correspondingly, many investigators have reported increased IL-4 production or decreased IFN- γ synthesis by helper T (T_H) cells in atopic patients.⁴⁻¹¹ Recently another cytokine, IL-13, was found to have an enhancing effect on IgE synthesis by B cells.¹²⁻¹⁴ An increase in IL-13 production in atopic patients was also demonstrated.¹⁵⁻¹⁷

Although these 3 cytokines were shown to have the potential to regulate IgE synthesis, their actual importance in *in vivo* IgE synthesis remains unclear. Some investigators have studied cytokine production by PBMCs on stimulation with polyclonal activators. However, the profiles of cytokine production by T_H cells were reported to be distinctly different from allergen to allergen.^{4,5,18} Thus the significance of each cytokine in the regulation of IgE synthesis against a given allergen should be analyzed in an allergen-specific manner. In this study we measured the production of IL-4, IL-13, and IFN- γ by PBMCs in primary culture on stimulation with house dust mite (HDM) in children with BA. It was found that IL-4 production had the strongest correlation with the level of serum HDM-specific IgE antibody, suggesting that IL-4 plays the most crucial role in the up-regulation of IgE synthesis against HDM in children with BA.

METHODS

Subjects

Heparinized peripheral blood was obtained during routine blood examinations from 23 patients with BA without atopic dermatitis at our institute between October 1996 and March 1999 after informed consent was obtained (Table I). The diagnostic criterion for BA is recurrent episodes of acute onset of dyspnea with wheezing that is improved by treatment with a bronchodilator. Patients who had no IgE antibody against HDM were excluded. The examination was done during the asymptomatic period. Although 20 patients had

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TABLE I. Profile of subjects

	BA	Controls
No.	23	15
Age (y)	7 (3-14)	6 (3-15)
Sex (male/female ratio)	16:7	9:6
Serum IgE (IU/mL)	831.8 (281.3-2454.7)	38.9 (12.0-125.9)
IgE-RAST (U _A /mL)		
HDM	85.1 (49.0-147.9)	<0.35
po (%)	100	0
CA	0.27 (0.12-0.58)	<0.35
po (%)	13.0	0

IgE-RAST, Radioallergosorbent test for IgE antibody; po (%), percentage of subjects with IgE-RAST level higher than 0.7 U_A/mL; CA, *Candida albicans*.

undergone temporary administration of systemic corticosteroids during acute exacerbations, none had received them within 1 week before testing. Because cytokine production by PBMCs is lower in younger children,¹⁹⁻²¹ the study subjects were limited to 3- to 15-year-old subjects. As controls, heparinized blood was collected similarly from 15 age-matched nonatopic children who were apparently healthy and did not have any allergic, hematologic, immunologic, or infectious diseases. 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, Uppsala, Sweden) and resuspended at a concentration of 1×10^6 cells/mL in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated FCS (Xavier Investments, Wacoal, Australia), 2 mmol/L glutamine, and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). Then, a 0.5-ml cell suspension was delivered to each well in duplicate on a 48-well culture plate (Falcon 3078, Becton Dickinson, Lincoln Park, NJ) and incubated in a humidified atmosphere of 5% carbon dioxide at 37°C with or without 50 µg/mL HDM (Der f) crude extract (Torii Pharmaceutical, Tokyo, Japan).²²⁻²⁵ In some experiments 2 µg/mL Der f 1 (Asahi Brewery, Tokyo, Japan), a purified major HDM allergen,^{24,26} or 100 µg/mL *Candida albicans* (CA) crude extract (Torii) was used as a stimulant. On the basis of preliminary experiments, culture time was set for 4 days for IL-4 measurement or for 6 days for IL-13 and IFN-γ measurement. The level of endotoxin in the RPMI 1640 medium containing 50 µg/mL HDM or 100 µg/mL CA was less than 1 ng/mL (measured by an endotoxin-specific quantification kit Endospecy, Seikagaku Kogyo, Tokyo, Japan). After incubation, the supernatant was collected in an Eppendorf tube (Iwaki Glass, Chiba, Japan) and stored at -80°C until use.

Measurement of IL-4, IL-13, IFN-γ, and IgE antibody

IL-4, IL-13, and IFN-γ in the culture supernatant were measured with the use of ELISA kits (Cytoscreen US human IL-4, Cytoscreen human IL-13 and IFN-γ, BioSource International, Camarillo, Calif) whose sensitivities were 65 fg/mL, 12 pg/mL, and 1 pg/mL, respectively. The level of serum IgE antibody against HDM (Der f) and CA was estimated by the radioallergosorbent test for IgE antibody (IgE-RAST) with use of a CAP-RAST RIA kit (Pharmacia). IgE-RAST levels higher than 0.7 U_A/mL were counted as positive.

TABLE II. Cytokine production by PBMCs on stimulation with HDM in children with BA and in nonatopic control subjects

	BA	Control	Statistical significance
IL-4 (fg/mL)	752.9 ± 365.9 (n = 19)	312.3 ± 230.0 (n = 14)	$P < .001$
IL-13 (pg/mL)	21.9 (<12-77.6) (n = 22)	<12 (n = 7)	$P < .01$
IFN-γ (pg/mL)	7.24 (1.54-33.90) (n = 22)	11.2 (1.66-75.9) (n = 14)	NS

NS, Not significant.

Statistics

Data on IgE-RAST, IL-13, and IFN-γ are presented as the geometric mean and range of 1 SD. The production of IL-4 is shown as the mean ± 1 SD. For the purpose of analysis, if cytokine production was below the detection limit of the kit, 50 fg/mL, 5 pg/mL, and 0.5 pg/mL were arbitrarily allocated to IL-4, IL-13, and IFN-γ production, respectively. The significance of the differences in data on IL-4 and IFN-γ production was examined by the Student *t* test. The Spearman's rank sum test was used for estimating the significance of the difference in data on IL-13 production.

RESULTS

Difference in cytokine production between children with BA and nonatopic control subjects

IL-4 and IL-13 production by PBMCs from children with BA on stimulation with HDM were significantly higher than that in nonatopic control subjects (Table II). On the other hand, the amount of IFN-γ production did not differ between them.

Difference in the profile of cytokine production between HDM and CA

To confirm the allergen-specific regulation of cytokine production, we compared the profile of IL-4 and IFN-γ production between HDM and CA. IL-4 production by PBMCs from children with BA on stimulation with CA was significantly lower than that on stimulation with HDM (515.3 ± 166.5 fg/mL [$n = 14$], $P < .05$), whereas IFN-γ production on stimulation with CA was significantly higher than that on stimulation with HDM ($56.2 [17.8-177.8]$ pg/mL [$n = 20$], $P < .001$) (Fig 1).

Correlation between cytokine production by PBMCs and the level of serum HDM-specific IgE antibody

A significant correlation was observed between IL-4 production by HDM-stimulated PBMCs and the level of serum HDM-specific IgE antibody expressed as HDM-IgE-RAST ($r = 0.71$, $P < .001$) (Fig 2, A). Although the amount of IL-13 produced by HDM-stimulated PBMCs was also significantly correlated with the level of HDM-

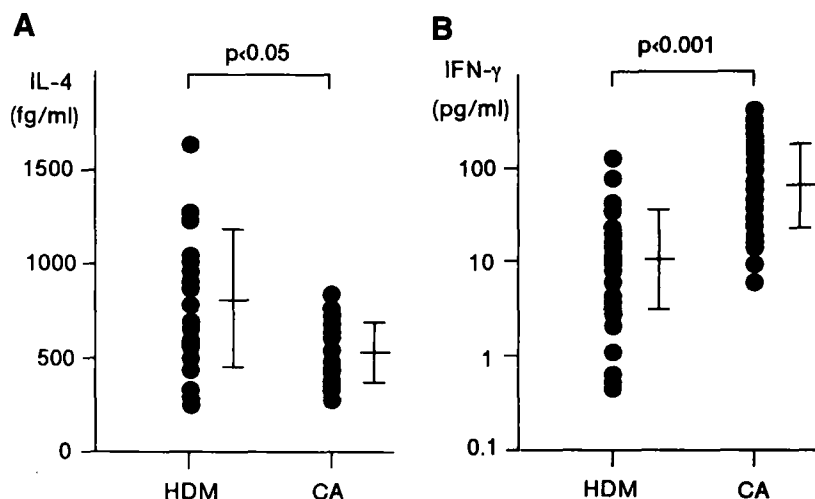


FIG 1. Difference in profile of cytokine production between HDM- and CA-stimulated PBMCs. Production of IL-4 (A) and IFN- γ (B) by PBMCs from children with BA on stimulation with HDM and CA are shown.

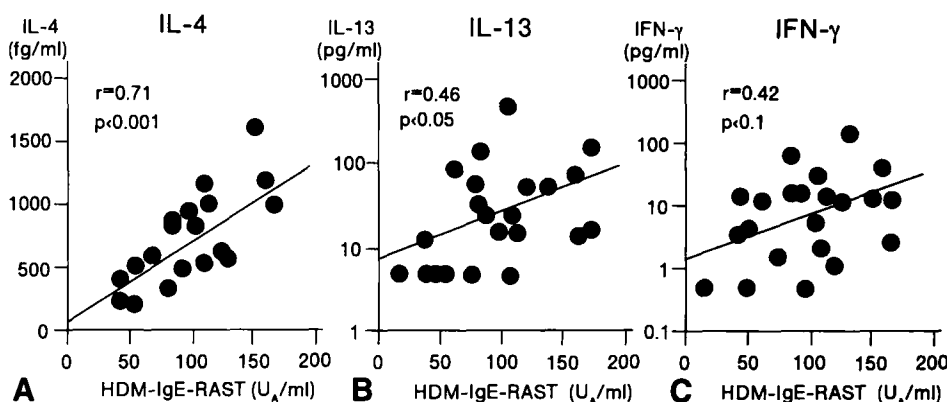


FIG 2. Correlation of cytokine production by HDM-stimulated PBMCs and level of HDM-IgE-RAST in children with BA. Correlations in children with BA of level of serum HDM-IgE-RAST with amount of IL-4 (A), IL-13 (B), and IFN- γ (C) produced by PBMCs on stimulation with HDM are shown.

IgE-RAST ($P < .05$) (Fig 2, B), it was markedly weaker than that between IL-4 production and the HDM-IgE-RAST level. There was no significant correlation between HDM-specific IFN- γ production and the level of HDM-IgE-RAST (Fig 2, C).

IL-4 and IFN- γ production by PBMCs on stimulation with purified HDM allergen Der f 1

A strong correlation was observed between the level of HDM-IgE-RAST and IL-4 production by PBMCs stimulated with a purified major HDM allergen Der f 1 ($r = 0.83$, $P < .01$) (Fig 3, A). On the contrary, there was no correlation between IFN- γ production by PBMCs on stimulation with Der f 1 and the level of HDM-IgE-RAST (Fig 3, B).

Correlations among IL-4, IL-13, and IFN- γ production

There was no significant correlation between IL-4 and IFN- γ production by PBMCs on stimulation with HDM

(Fig 4, A) or purified Der f 1 (Fig 5). In contrast, a significant correlation was seen between IL-13 and IFN- γ production ($r = 0.65$, $P < .01$) (Fig 4, B). IL-13 production was also significantly correlated with IL-4 production ($r = 0.56$, $P < .02$) (Fig 4, C).

DISCUSSION

IgE antibody against HDM is usually increased in atopic patients and is thought to play a crucial role in the pathogenesis of atopic disorders.¹ IgE synthesis is now thought to be mainly regulated by IL-4 and IFN- γ ; the former is produced by T_H2 cells to up-regulate IgE synthesis, whereas the latter is produced by T_H1 cells to down-regulate IgE synthesis.^{2,3,27,28} However, it has not yet been confirmed whether both cytokines contribute equally to the up-regulation of IgE synthesis in atopic patients. The analysis of the correlation between cytokine production by PBMCs on stimulation with HDM and the level of serum HDM-specific IgE antibody per-

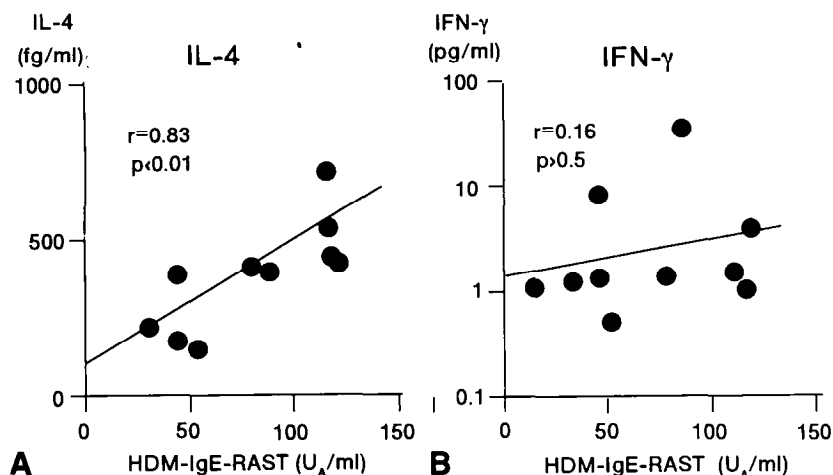


FIG 3. Correlation of cytokine production by Der f 1-stimulated PBMCs and level of HDM-IgE-RAST in children with BA. Correlations in children with BA of level of serum HDM-IgE-RAST with amount of IL-4 (A) and IFN- γ (B) produced by PBMCs on stimulation with purified major HDM allergen Der f 1 are shown.

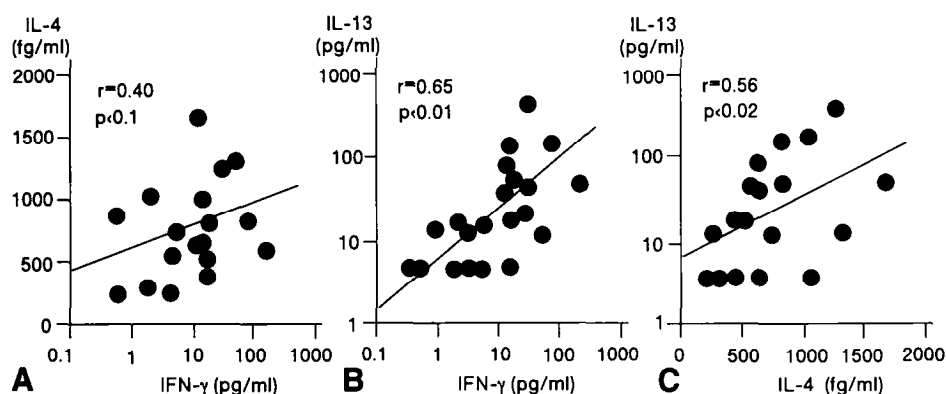


FIG 4. Correlations among cytokine production by HDM-stimulated PBMCs in children with BA. Correlations in children with BA between production of IL-4 and IFN- γ (A), IL-13 and IFN- γ (B), and IL-13 and IL-4 (C) are shown.

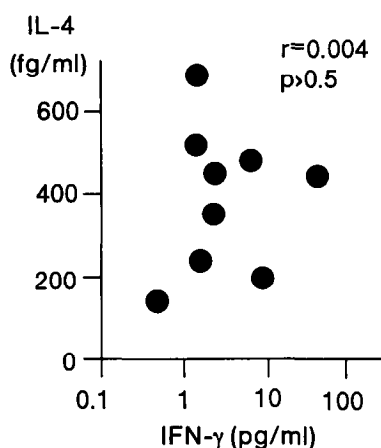


FIG 5. Correlation between IL-4 and IFN- γ production by PBMCs on stimulation with Der f 1. Correlation in children with BA between IL-4 and IFN- γ production by PBMCs on stimulation with Der f 1 is shown.

formed in this study suggests that there is a clear difference in their importance in up-regulating HDM-specific IgE antibody secretion in children with BA.

Because IgE synthesis is influenced by various factors, including the physicochemical nature of allergens themselves,^{4,5,18} the role of each cytokine in IgE synthesis against a given allergen should be studied in an allergen-specific manner. An imbalance of cytokine production in atopic patients has often been demonstrated by establishing allergen-specific T-cell clones.^{4,5} Although this method is effective in demonstrating the imbalance of cytokine production, the process of establishing cell lines may considerably alter the original nature of T_H cells through repetitive stimulation under the presence of artificially supplemented cytokines.

In this study we measured cytokine production by PBMCs in primary culture in an allergen-specific manner, which seems to better reflect the state of in vivo cytokine production than established T_H clones do. With

use of this method, the difference in the profile of cytokine production between HDM- and CA-stimulated PBMCs was demonstrated (Fig 1). The production of IL-4 and IFN- γ by PBMCs on stimulation with CA were lower and higher, respectively, than those on stimulation with HDM. This is correspondent to the difference between the level of serum CA- and HDM-IgE-RAST in children with BA: the former is markedly lower than the latter (Table I). Thus the method used in this study appears to be useful to analyze cytokine production in an allergen-specific manner.

Although IL-5 and IFN- γ production by PBMCs on stimulation with HDM has already been examined in patients with BA,¹¹ little is known about IL-4 production by HDM-stimulated PBMCs in primary culture. This study demonstrated that IL-4 production by HDM-stimulated PBMCs in children with BA was significantly higher than that in nonatopic controls, whereas there was no apparent difference in IFN- γ production between them. Moreover, there was a strong correlation between the production of IL-4, but not IFN- γ , and the level of HDM-IgE-RAST. To our knowledge, such a clear correlation between them is first demonstrated here. Thus IL-4 seems to play a more crucial role than IFN- γ does in the up-regulation of *in vivo* IgE synthesis against HDM in children with BA.

IL-13 has also been demonstrated to facilitate the IgE class switch of B cells and to enhance IgE synthesis.¹²⁻¹⁴ It was shown by us (Table II) and other investigators¹⁵⁻¹⁷ that the production of IL-13 was significantly higher in patients with BA than in nonatopic control subjects. Given these findings, the importance of IL-4 could not be distinguished from that of IL-13 in the up-regulation of HDM-specific IgE synthesis in patients with BA. However, a distinct difference was found between them by analyzing the correlation between cytokine production and the level of HDM-IgE-RAST: the latter was more closely correlated with IL-4 production than with IL-13 production. These findings suggest that the action of IL-4 is more potent than IL-13 on *in vivo* IgE antibody production against HDM in children with BA.

To further clarify the characteristics of cytokine production by HDM-stimulated PBMCs in patients with BA, we then analyzed the correlations among the production of each cytokine. There was no significant correlation between IL-4 and IFN- γ production (Figs 4, A, and 5), corresponding to their different roles in IgE synthesis^{2,3} and lymphocytic cell sources.^{27,28} The production of IL-13 was significantly correlated with that of IL-4 (Fig 4, C). Surprisingly, there was a close correlation between IL-13 and IFN- γ production (Fig 4, B), which was stronger than that between IL-13 and IL-4 production. Taken together, it is suggested that, although the secretion of IL-13 partly mimics that of IL-4, the regulation of the former is more complicated than the latter. Although the reason for the difference in correlation with IFN- γ production between IL-4 and IL-13 is so far unknown, it might be related to the difference in lymphocytic cell source, namely, although IL-4 is produced

only by T_H2 cells, IL-13 is produced by both T_H1 and T_H2 cells.²⁹ Recently IL-13 was shown to cause inflammation in the bronchial tissue, similar to that in patients with BA, independently of IgE antibody or eosinophils.³⁰ Considering that the effect of IL-13 on IgE synthesis is weaker than that of IL-4,^{14,31} the main biologic role of IL-13 may not be the up-regulation of IgE synthesis but rather to cause inflammation in the bronchial tissue through its unique action.

In conclusion, although IL-4, IL-13, and IFN- γ were shown to have the ability to regulate IgE synthesis, IL-4 is likely to be the dominant cytokine in the up-regulation of HDM-specific IgE synthesis in children with BA. The contribution of IL-13 to the enhancement of IgE synthesis against HDM may be smaller than that of IL-4. It remains to be confirmed whether the result presented here is valid in adult patients with BA.

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