

IFN- γ Plays a Dominant Role in Upregulation of *Candida*-Specific IgE Synthesis in Patients with Atopic Dermatitis

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Key Words

Candida albicans · IgE · Atopic dermatitis · IL-4 · IFN- γ

Abstract

Background: Although *Candida albicans* (CA) is known to induce Th1 clones that suppress IgE synthesis, serum IgE antibody against CA is often increased in atopic patients. This study aims to elucidate the mechanism of IgE synthesis against CA in atopic patients. **Methods:** We measured the production of IL-4 and IFN- γ by peripheral blood mononuclear cells (PBMCs) from atopic patients upon stimulation with CA and examined the correlation with the level of serum IgE antibody against CA. **Results:** The level of serum CA-specific IgE antibody (CA-IgE) was significantly higher in patients with atopic dermatitis (AD) than in patients with bronchial asthma (BA) (geometric mean = 3.6 vs. 0.27 U_A/ml, $p < 0.02$) (U_A = unit allergen), while there was no difference in the level of house dust mite-specific IgE antibody between them (67.6 vs. 87.1 U_A/ml). Although IL-4 production by PBMCs upon stimulation with CA in patients with AD was not significantly different from that in patients with BA (mean = 359.1 vs. 515.3 fg/ml), IFN- γ production was significantly lower in the former than in the latter group (8.1 vs. 56.2 pg/ml, $p < 0.001$).

Consequently, the ratio of IL-4/IFN- γ production was apparently higher in patients with AD than in those with BA, which corresponds to the difference between them in the level of serum CA-IgE. A significant negative correlation was seen in patients with AD between IFN- γ production by CA-stimulated PBMCs and the level of serum CA-IgE ($p < 0.05$). **Conclusions:** IgE synthesis against CA in atopic patients may be precipitated not by enhancing IL-4 production, but by reducing IFN- γ secretion.

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Introduction

House dust mite (HDM) is a major allergen causing various atopic disorders, including bronchial asthma (BA) and atopic dermatitis (AD) [1, 2]. It induces allergic reactions by cross-linking HDM-specific IgE antibody (HDM-IgE) bound to high-affinity IgE receptors expressed on the surface of mast cells [3]. In atopic patients, the serum HDM-IgE level is usually high [1, 4]. This may be ascribed to the peculiar capacity of HDM to preferentially activate Th2 cells [5–8], which exclusively secrete IL-4 to enhance IgE synthesis [9–11]. In contrast, *Candida albicans* (CA) has been reported to induce mainly IFN- γ pro-

Table 1. Profile of subjects

	Nonatopic	AD	BA
Number	10	21	20
Age, years	9 (3–15)	7 (3–15)	8 (3–14)
Sex, m/f	7/3	6/15	15/5
IgE, IU/ml	38.0 (9.1–159)	2,041 (741–5,623)	708 (229–2,188)
HDM-IgE, U _A /ml	0.21 (0.15–0.30)	67.7 (27.5–166)	87.1 (51.3–148)
CA-IgE, U _A /ml	0.15 (0.12–0.19)	3.6 (0.63–20.9)	0.27 (0.12–0.58)

Age is presented as the median with range in brackets. Serum IgE, HDM-IgE and CA-IgE levels are shown as the geometric means with a range of 1 SD in brackets.

duction by Th1 cells, which suppresses IgE synthesis [5]. However, it has been demonstrated that not only HDM-IgE but also CA-specific IgE antibody (CA-IgE) production is often enhanced in atopic patients [12–14]. So far, little is known about how CA-IgE synthesis is upregulated in atopic patients.

In this study, we measured IL-4 and IFN- γ production by peripheral blood mononuclear cells (PBMCs) from atopic patients upon stimulation with CA and analyzed the correlation with serum CA-IgE level. It was found that although the serum CA-IgE level was not correlated with IL-4 production, it showed a significant negative correlation with IFN- γ production by CA-stimulated PBMCs in atopic patients, suggesting that CA-IgE synthesis is enhanced not by increasing IL-4 secretion but by reducing IFN- γ production.

Materials and Methods

Subjects

After obtaining informed consent, peripheral blood was drawn from 21 patients with AD and 20 patients with BA who were brought to our institute between October 1996 and March 1999 (see table 1). The subjects were limited to those who had experienced either AD or BA. Because cytokine production by PBMCs is shown to be lower in younger children [15–17], the subjects were limited to those aged 3–15 years. Fourteen and 17 patients with AD and BA, respectively, had been admitted because of acute exacerbation of symptoms. No patients with AD had been administered systemic corticosteroids, and blood samples were collected avoiding the acutely aggravated phase. For patients with BA, the examination was done during the asymptomatic period. Although short-acting corticosteroids were often administered systemically during acute asthma attacks, no subject had received them within a week before examination. As controls, blood samples were collected in a similar fashion from 10 age-matched nonatopic children who were apparently healthy and did not have any allergic, hematologic, immunologic, or infectious diseases.

Cell Culture

PBMCs were obtained from heparinized blood samples 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 μ g/ml streptomycin). Then, 0.5 ml of the cell suspension was delivered in duplicate to each well of a 48-well culture plate (Falcon 3078, Becton Dickinson, Lincoln Park, N.J., USA) and cultured for 4 days for IL-4 measurement and 6 days for IFN- γ measurement in a humidified atmosphere of 5% CO₂ at 37 °C with or without 100 μ g/ml CA or 50 μ g/ml HDM (Der f) crude extract (Torii Pharmaceutical Co., Tokyo, Japan) [18–21]. The endotoxin level in RPMI-1640 medium containing 100 μ g/ml CA or 50 μ g/ml HDM was lower than 1 ng/ml. Maximum IFN- γ production was obtained under these conditions. Although maximum IL-4 production was seen by stimulation of PBMCs with 20 μ g/ml CA or 10 μ g/ml HDM, a comparable amount of IL-4 was produced under the above-mentioned conditions as well. After incubation, the supernatant was collected in an Eppendorf tube (Iwaki Glass Co., Chiba, Japan) and stored at –80 °C until the measurement of cytokines.

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

IL4 and IFN- γ in the culture supernatant were measured with the use of enzyme-linked immunosorbent assay (ELISA) kits (Cytoscreen-US human IL4 and Cytoscreen human IFN- γ , respectively; BioSource International, Camarillo, Calif., USA), whose sensitivities were 65 fg/ml and 1 pg/ml, respectively. Serum IgE antibody and allergen-specific IgE antibody levels were examined by a radioimmunoassay kit (Ab beads IgE kit Eiken, Eiken Chemical Co., Tokyo, Japan) and the CAP system (Pharmacia), respectively.

Statistics

Data for IL-4 are expressed as means $\pm$ 1 SD, while those for serum IgE, IgE-RAST and IFN- γ are shown as geometric means and a range of 1 SD. For the purpose of analysis, if the amount of cytokine was below the lower limit of measurement, values of 50 fg/ml and 0.5 pg/ml were arbitrarily assigned to IL-4 and IFN- γ , respectively. The difference and correlation were regarded as significant if the p value was lower than 0.05.

Table 2. IL-4 and IFN- γ production by CA-stimulated PBMCs in atopic patients

	IL-4, fg/ml	IFN- γ , pg/ml	IL-4/IFN- γ
Nonatopic ¹	312.8 $\pm$ 264.2	51.3 (8.1–323.6)	6.1
AD ²	359.1 $\pm$ 274.5	8.1 (1.7–38.9)	44.3
BA ³	515.3 $\pm$ 166.5	56.2 (17.8–177.8)	9.2

The production of IL-4 and IFN- γ are presented as the mean $\pm$ 1 SD and the geometric mean and a range of 1 SD, respectively.

¹ n = 10 for IL-4 and IFN- γ .

² n = 14 for IL-4 and n = 21 for IFN- γ .

³ n = 14 for IL-4 and n = 19 for IFN- γ .

Results

Differences in Serum CA-IgE Level between Patients with AD and BA

There were no significant differences between patients with AD and BA in terms of serum HDM-IgE level (table 1). However, the serum CA-IgE level was significantly higher in the former than in the latter group [3.6 (0.63–20.9) vs. 0.27 (0.12–0.58) U_A/ml, $p < 0.02$ (U_A = unit allergen)]. CA-IgE was positive (>0.7 U_A/ml) in 76.1% (16/21) of the patients with AD and 15.0% (3/20) of the patients with BA.

Differences in Cytokine Production by CA-Stimulated PBMCs between Patients with AD and BA

IL-4 production by CA-stimulated PBMCs in patients with AD was lower than that in patients with BA (359.1 $\pm$ 274.5 vs. 515.3 $\pm$ 166.5 fg/ml) (fig. 1), although the difference was not significant. On the other hand, PBMCs from patients with AD produced a decidedly smaller amount of IFN- γ upon stimulation with CA than those from patients with BA [8.1 (1.7–38.9) vs. 56.2 (17.8–177.8) pg/ml, $p < 0.001$] (fig. 1). Consequently, the ratio of IL-4 to IFN- γ production was higher in patients with AD than in those with BA (44.3 vs. 9.2) (table 2). The ratio of IL-4/IFN- γ production by CA-stimulated PBMCs in patients with BA was comparable to that in nonatopic control subjects (6.1).

Negative Correlation between IFN- γ Production by CA-stimulated PBMCs and Serum CA-IgE Level

There was a significant positive correlation in patients with AD between serum HDM-IgE level and the production of IL-4 (fig. 2), but not of IFN- γ (fig. 2B), by PBMCs upon stimulation with HDM. In contrast, although the

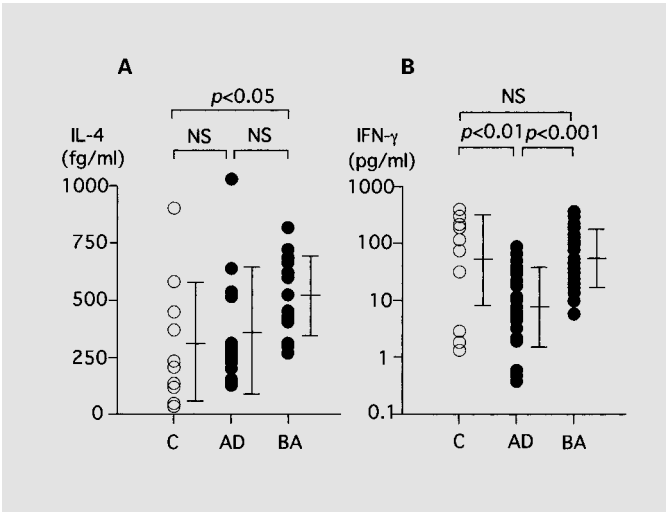


Fig. 1. IL-4 (A) and IFN- γ (B) production by PBMCs upon stimulation with CA in patients with AD and BA. C = Nonatopic controls; NS = not significant.

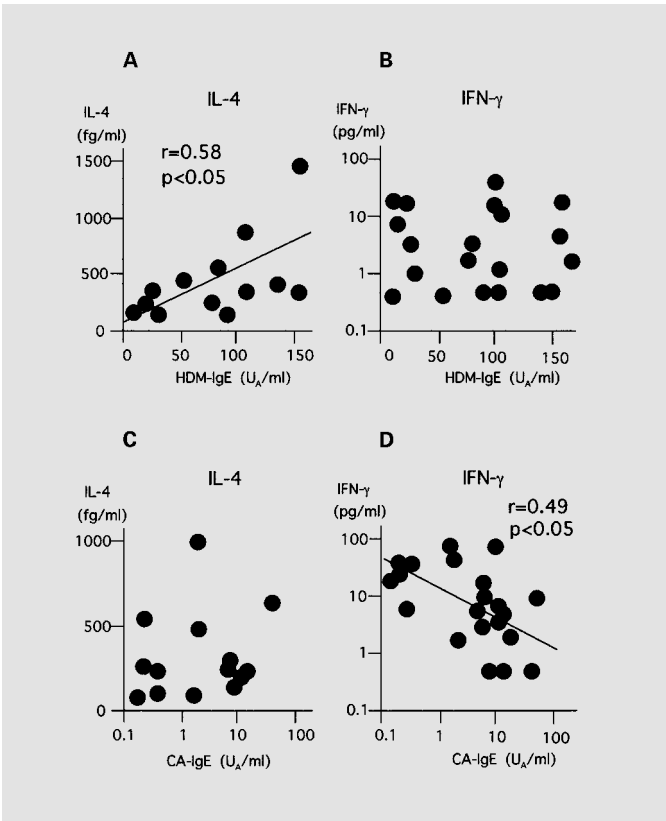


Fig. 2. Correlation of the serum HDM-IgE (A, B) and CA-IgE level (C, D) with IL-4 (A, C) and IFN- γ (B, D) production by PBMCs upon stimulation with HDM (A, B) or CA (C, D) in patients with AD.

serum CA-IgE level was not correlated with IL-4 production by PBMCs upon stimulation with CA (fig. 2C), it showed a significant negative correlation with IFN- γ production by CA-stimulated PBMCs (fig. 2D).

Discussion

HDM is well known as activating Th2 cells [5–8], which corresponds to an increase in IgE antibody formation against HDM in atopic patients [1, 4]. On the other hand, CA has been demonstrated to stimulate Th1 cells to produce IFN- γ , which suppresses IgE synthesis, even in atopic patients [5]. This suggests that CA-IgE cannot be produced in nonatopic individuals, nor atopic patients. However, the serum CA-IgE level is often increased in atopic patients [12–14]. Interestingly, it has been shown that the incidence of positive CA-IgE is higher in patients with AD than in those with BA, although there were no apparent differences between them in the incidence of positive HDM-IgE [22]. We confirmed this in the present study (table 1). Comparing the profile of cytokine production upon stimulation with CA between patients with AD and BA may be a promising way to obtain clues to the mechanism that enables IgE synthesis against CA in atopic patients.

The production of IL-4 by PBMCs upon stimulation with CA was higher in patients with BA than in those with AD, although the difference was not significant. This forms a sharp contrast to a very low serum CA-IgE level in patients with BA. Thus, while we demonstrated that IL-4 plays a critical role in the upregulation of HDM-IgE synthesis [8], this is not the case in CA-IgE synthesis. On the contrary, IFN- γ production by CA-stimulated PBMCs was distinctly lower in patients with AD than in those with BA. As a result, the ratio of IL-4/IFN- γ production by PBMCs upon stimulation with CA was markedly higher in patients with AD than in those with BA. Thus, the reduction in IFN- γ production appears to be related to enhanced CA-IgE production in patients with AD.

In addition to the analysis of the ratio of IL-4/IFN- γ production, it may be useful to examine the correlation between cytokine production and the serum level of allergen-specific IgE antibody for determining the cytokine that plays the dominant role in upregulation of IgE synthesis against a given allergen. In the case of HDM, IL-4 production by PBMCs upon stimulation with HDM is strongly enhanced in children with BA, while IFN- γ production is not increased [8]. Moreover, it was demonstrated that there is a close positive correlation between

the production of IL-4, but not IFN- γ , by PBMCs upon stimulation with HDM and serum HDM-IgE level in patients with BA. These findings concordantly indicate that IL-4 plays a dominant role in the upregulation of HDM-IgE formation [8].

In contrast, IFN- γ production by PBMCs upon stimulation with HDM is lower in patients with AD than in nonatopic control subjects, while IL-4 production is to some degree higher in the former than in the latter [17]. Although AD patients complicated with BA were excluded from this study, essentially the same finding was seen between patients with AD and nonatopic control subjects [IL-4 481.2 ± 440.3 (n = 13) vs. 334.5 ± 202.7 fg/ml (n = 10); IFN- γ 2.8 (<1.0–12.0) (n = 20) vs. 20.4 (4.1–102.4) pg/ml (n = 10), $p < 0.01$). Based on these findings, both cytokines appear to contribute equally to the upregulation of HDM-IgE synthesis in patients with AD. However, the serum HDM-IgE level in patients with AD is significantly correlated with the production of IL-4, but not IFN- γ , by HDM-stimulated PBMCs (fig. 2), suggesting that IL-4 plays a primary role in the upregulation of HDM-IgE synthesis, even in patients with AD.

While IL-4 production by PBMCs upon stimulation with CA is not elevated in patients with AD, IFN- γ production is significantly diminished (fig. 1). Because IFN- γ suppresses IgE synthesis [10], it could theoretically upregulate IgE synthesis by a reduction in its secretion. Indeed, some investigators reported a decrease in IFN- γ production by PBMCs in atopic patients [23, 24], especially in those with AD [25–28], suggesting that the reduction of IFN- γ works in some cases as a mechanism of upregulation of *in vivo* IgE synthesis. However, so far, no allergen has been demonstrated to upregulate IgE synthesis using such a mechanism. In this study, in addition to a reduction in IFN- γ production, it was found that there was a significant negative correlation between the serum CA-IgE level and IFN- γ production by CA-stimulated PBMCs (fig. 2D). In contrast, the serum CA-IgE level in patients with AD did not show any correlation with IL-4 production by PBMCs upon stimulation with CA (fig. 2C). Taken together, these results suggest that in the case of CA IFN- γ is likely to play a primary role in the upregulation of IgE synthesis through diminution of its production.

Wierenga et al. [5] reported that Th1 clones alone were established from PBMCs from a patient with AD upon stimulation with CA, which does not correspond to our results. However, it is worth noting that CA-IgE was negative in the patient enlisted in the former study. We found in the present study that in patients with BA whose serum CA-IgE level was negative or low, IFN- γ production by

PBMCs upon stimulation with CA was high and comparable to that in nonatopic control subjects (fig. 1). Moreover, in patients with AD, the production of IFN- γ by CA-stimulated PBMCs was higher in those with a low CA-IgE

level than in those with a high CA-IgE level (fig. 2D). Thus, the results of Wierenga et al. [5] may be valid only in patients with AD whose serum CA-IgE level is negative.

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