

In Vitro IgE, Interleukin 4, and Interferon Gamma Production in Children with Differing Serum IgE Concentrations

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ABSTRACT

Interleukin 4 (IL-4) and interferon-gamma (IFN- γ) have been shown to play a role in the regulation of *in vitro* IgE production by cells from adults. Little information is available regarding the role of these cytokines in influencing IgE production by cells from children. Children ages 3–17 were classified into two groups based upon serum IgE concentrations (>600 U/ml vs. <200 U/ml). Lymphocytes were evaluated for phenotype and IgE production. *In vitro* IL-4 and IFN- γ production was measured as was the effect of these cytokines on *in vitro* IgE production by cells obtained from subjects in the two study groups. Children with elevated serum IgE concentrations produced greater amounts of IL-4 than those with lower serum concentrations of IgE. At low dosages of IL-4 (10U) cells from the patients with lower serum IgE concentrations were more sensitive to the effects of IFN- γ , while at higher concentrations of IL-4 (300U) cells from those with higher serum concentrations of IgE were more sensitive to the effects of IFN- γ . The group with higher serum IgE levels had a significantly higher proportion of CD4+ T cells and a greater proportion of cells positive for CD4 and CD29. There are differences in lymphocyte phenotype and IL-4 production in patients with high and normal concentrations of serum IgE. These differences may be important in understanding the basis for different serum IgE concentrations in children.

INTRODUCTION

THE ROLE OF CYTOKINES in the regulation of IgE synthesis has been extensively studied over the past few years.^(1–6) Such studies have clearly demonstrated that IL-4 and IFN- γ play a role in regulating IgE synthesis *in vitro*. One of the consequences of these studies has been an attempt to use IFN- γ therapeutically in a limited number of patients with elevated IgE and severe atopic disease.^(7–10)

Studies evaluating the production and the regulatory role of IL-4 and IFN- γ in IgE synthesis have largely been performed using cells derived from adults. In this study we evaluated the *in vitro* cytokine production of cells from children with different serum concentrations of IgE. We also studied the *in vitro* synthesis of

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IgE and the effect of IL-4 and INF- γ had on IgE synthesis. Our findings indicate that children with higher serum values of IgE produce greater amounts of IL-4 *in vitro*. Such insights may provide new approaches for therapeutic interventions in the future.

MATERIALS AND METHODS

Lymphocyte phenotype studies

Lymphocyte phenotype was determined using a Coulter EPICS flow cytometer and direct conjugate two-color-labeled monoclonal antibodies. Direct dye conjugates of matching isotope Ig were used as controls. The cell population was selected for analysis using forward angle and side light scatter bitmap gating.

Cytokine production and assay

Peripheral blood mononuclear cells (PBMC) were resuspended in RPMI-1640 (BioWhittaker) supplemented with 10% fetal bovine serum at 1×10^6 cells/ml. Cultures were performed in 12×75 culture tubes and incubated for 72 h at 37°C and 5% CO₂ in media alone and in media with phytohemagglutinin (PHA) (Sigma) and phorbol myristate acetate (PMA) (Sigma). Supernatants were harvested and kept frozen until tested. The kits used were IFN- γ (Genzyme) and IL-2, IL-4 (R&D systems).

IgE production

IgE synthesis was determined using a modification of a technique previously reported.⁽¹¹⁾ The PBMC were isolated from fresh heparinized venous blood by centrifugation over a Ficoll-Hypaque density gradient. Cells (5×10^5 cells/ml) were cultured in Iscoves' Modified Dulbecco's Medium (Sigma) with and without IL-4 (Genzyme). Supernatants were harvested at day 10 and frozen until assayed for IgE.

IgE concentrations in culture supernatants were determined by ELISA. To determine preformed IgE vs. spontaneously synthesized IgE, 5×10^5 cells/ml were incubated in the presence of the protein inhibitor cyclohexamide. Spontaneous IgE synthesis was determined by the following calculation:

$$\text{Spontaneous IgE synthesis} = \text{IgE without IL-4} - \text{IgE without IL-4 but with cyclohexamide}$$

The relative sensitivity of cells to IL-4 and IFN- γ as measured by IgE production was determined by culturing cells in the presence of varying concentrations of these cytokines. For each study, cells from a normal subject were run in parallel.

Serum IgE determinations

IgE was determined at LeBonheur Children's Medical Center using a microparticle enzyme immunoassay (EIA).

Study populations

A total of 45 children ranging in age from 3 to 17 years were studied. Informed consent was obtained from the parents of these children and the study was approved by the University of Tennessee Institutional Review Board. Subjects were placed into one of two groups: those with serum IgE concentrations below 200 U/ml (<5 –189 U/ml, mean 64.1 ± 60.1) and those with concentrations greater than 600 U/ml (601–2312 U/ml, mean 987.2 ± 525.0). Subjects were selected by review of the IgE determinations performed in the laboratory at LeBonheur Children's Hospital. The mean age was 6.5 ± 2.8 years in the low-IgE group and 8.2 ± 3.1 years in the group with the higher IgE concentrations. The patients with the higher IgE concentrations were more likely to have been diagnosed as having underlying conditions such as atopic dermatitis, asthma, and allergic rhinitis (14 of 14) than those with the lower IgE levels (10 of 31; $p = 0.034$).

The study was performed in two phases. The first phase consisted of determining the lymphocyte phenotype of cells from both study groups as well as culturing cells from these subjects for purposes of study-

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ing cytokine production. The second phase of the study was to compare *in vitro* IgE production by subjects from the two study groups and to evaluate the effect of different concentrations of IL-4 and IFN- γ on this *in vitro* response. A single patient in the high-IgE group was excluded from analysis because his *in vitro* IgE production was over four times the value of any other patient.

Data analysis

Comparisons between the two groups for each measurement were done using a Mann-Whitney analysis. Statistical significance was considered to be a $p < 0.05$ after the Bonferroni/Dunn adjustment.

RESULTS

Lymphocyte phenotypes and cytokine production

Table 1 shows the differences when lymphocyte phenotypes were compared in the two groups. No significant differences were seen in the proportion of cells positive for CD19 (B cells), CD3 (T cells), CD8 (Tc), and membrane-associated CD23 (IgE receptor). There was no difference in the proportion of T cells expressing DR antigen (activated T cells) between the two groups.

The proportion of T cells that were CD4+ positive was significantly higher in the group with the higher IgE levels. A greater proportion of the CD4+ cells in this latter group also were positive for the CD29 marker.

Table 1 also shows the results from studies designed to measure *in vitro* IL-2, IL-4, and IFN- γ production in the two groups of patients. There was no difference in unstimulated cytokine production. Following stimulation with PHA and PMA, there were similar levels of production of IL-2 and IFN- γ in the two groups. IL-4 production was significantly greater in the group of patients with the higher IgE concentrations.

In vitro IgE synthesis

Patients in the group with the higher serum concentration of IgE had more preformed and spontaneous IgE production, but these differences did not achieve statistical significance (Table 2). When cells were cultured in the presence of 10U of IL-4 there was no significant increase in IgE production over spontaneous IgE synthesis in either group. However, in the presence of 300 U IL-4 there was an increase in IgE production in both groups. These data suggest that cells of patients with higher concentrations of IgE are not more sensitive to the effects of IL-4 than are cells from patients with lower concentrations of serum IgE.

We next designed an experiment to examine whether cells from the patients with higher IgE serum levels were more resistant to the downregulating effects of IFN- γ (Fig. 1). These studies show that at low doses

TABLE 1. LYMPHOCYTE PHENOTYPES

Marker ^a	Low IgE (<200 U/ml)	High IgE (>600 U/ml)	p
CD3	66.7 $\pm$ 5.5	68.9 $\pm$ 4.2	NS
CD4	39.5 $\pm$ 6.2	46.5 $\pm$ 3.8	<0.002
CD8	28.6 $\pm$ 5.9	25.7 $\pm$ 3.8	NS
CD29+ CD4+	13.4 $\pm$ 7.0	19.3 $\pm$ 6.3	0.02
CD23	6.8 $\pm$ 4.1	6.3 $\pm$ 2.4	NS
CD19	16.3 $\pm$ 5.5	14.6 $\pm$ 4.5	NS
<i>In vitro cytokine production^b</i>			
IFN- γ	295 $\pm$ 681	240 $\pm$ 424	NS
IL-2	1161 $\pm$ 1977	728 $\pm$ 722	NS
IL-4	32.7 $\pm$ 44.5	66.9 $\pm$ 54.6	0.02

^aValues are given as percentage positive (mean $\pm$ SD).

^bValues are given in pg/ml (mean $\pm$ SD).

TABLE 2. *IN VITRO* IgE SYNTHESIS

	Low IgE (<200 U/ml)	High IgE (>600 U/ml)	<i>p</i>
Spontaneous IgE	307 ± 489	943 ± 986	0.15
Preformed IgE	132 ± 162	295 ± 226	0.36
Stimulated IgE (300 UIL-4)	9687 ± 6350	9435 ± 5586	0.94

of IL-4, IgE production by cells from the group of children with higher IgE concentrations were less likely to decrease in the presence of either 10 or 100U IFN- γ . By contrast, in the presence of a higher concentration of IL-4, cells from patients with higher serum IgE were much more sensitive to the effects of IFN- γ (Fig. 2).

DISCUSSION

Both IL-4 and IFN- γ have been demonstrated to play an important role in regulating IgE synthesis.^(1-6,12) Most studies evaluating the regulatory roles of these agents have been performed in adult subjects. In the study described here, children with various levels of serum IgE were evaluated to determine the potential importance of these cytokines in the regulation of *in vitro* IgE synthesis. Our hypothesis was that patients with higher serum concentrations of IgE would be likely to produce either more IL-4 or less IFN- γ than those with lower serum IgE levels. Such findings appear to be present in many patients with the hyper IgE syndrome.⁽¹³⁻¹⁵⁾ We also hypothesized that patients with high and low serum IgE concentration might demonstrate a differential in relative sensitivity to either IL-4 or IFN- γ . In addition, we were interested in examining cells from these patients for possible lymphocyte phenotype differences.

Based on the observations made in this study, it appears that cells from children with higher IgE levels produce more IL-4 *in vitro* than cells from children with lower serum IgE levels. These findings are consistent with a recent report that compared children with atopic dermatitis and high serum IgE with a control group.⁽¹⁶⁾

A second hypothesis that we attempted to test was that cells from children with increased serum IgE would have a different sensitivity to IL-4 and/or IFN- γ than those with lower serum IgE. We evaluated this by examining *in vitro* IgE synthesis in the presence of varying concentrations of these cytokines. Both groups responded similarly to IL-4. In the presence of low-dose IL-4 (10 U) and low-dose IFN- γ (10 U), IgE production by patients with lower serum IgE concentrations fell by 67% while IgE production in the other group did not decline at all.

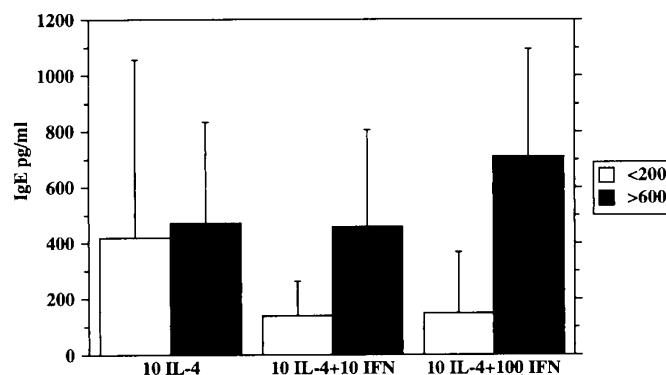


FIG. 1. At low concentrations of IL-4, the effect of IFN- γ was more apparent in the patients with lower IgE serum concentrations.

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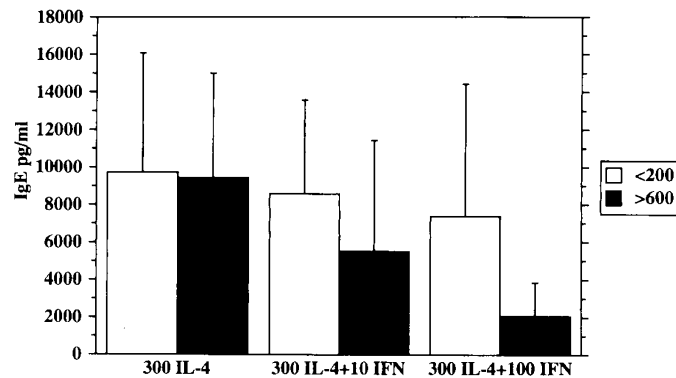


FIG. 2. At high concentrations of IL-4 (300U), the downregulating effect of IFN- γ was more evident in cells from patients with higher serum IgE concentrations.

In the presence of the high dose of IL-4 (300 U) and low dose IFN- γ (10 U), *in vitro* IgE from cells of patients with high levels of serum IgE fell 42% while that of patients with lower serum levels fell only 12%. One explanation for this paradoxical observation is that cells from patients with higher concentrations of IgE may be more sensitive to a variety of regulatory cytokines. Other factors (e.g., increased production of IL-4, IL-13) may ultimately override greater sensitivity of IFN- γ or may influence the degree of responsiveness to other cytokines.

The role of different T-cell subsets in regulating IgE synthesis is unclear. Phenotypic analysis of lymphocytes from these children revealed a significant difference in the proportion of cells bearing both the CD4+ and CD4+ CD29+ phenotypes. This latter subpopulation of cells is said to represent a more immunologically mature cell population.⁽¹⁷⁾ One study in children with atopic dermatitis revealed that these children had a decreased proportion of CD4+ CD29+ cells compared to a control group and a group with asthma.⁽¹⁷⁾ Another study has demonstrated that CD4+ CD45R0+ cells that are likely to be similar to CD4+/CD29+ cells are efficient helper cells for IgE production in the presence of IL-2 or IL-2 plus IL-4.⁽¹⁸⁾ The findings presented regarding a difference in CD4+ phenotype and increased IL-4 production here are compatible with the idea that even in children there may be a shift toward a TH₂ dominant phenotype in patients with higher IgE synthesis.

Recognition of the subtleties of IgE regulation and potential differences between children and adults may provide insight that could result in improved therapy for these children.

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