

Interleukin-4 and low-affinity receptor for IgE on B cells in peripheral blood of patients with atopic bronchial asthma

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Background: A greater frequency of type 2 helper cells producing IL-4 without interferon- γ is thought to be responsible for the elevated IgE in serum of atopic subjects. However, the proportion of B cells responding to IL-4 by an increased synthesis of IgE is also higher in atopic subjects than in nonatopic subjects.

Objective: Important questions are whether the elevated IgE in atopic subjects is due to overproduction of IL-4 by T cells, the enhanced sensitivity of B cells to IL-4, or both and whether functional alterations of T and B cells are related to the development of allergic diseases.

Methods: Spontaneous and IL-4-induced CD23 expression on B cells was examined to evaluate the response of B cells to IL-4, and production of IL-4 by concanavalin-A-stimulated peripheral blood mononuclear cells (PBMCs) was measured to evaluate the T-cell function in nonatopic normal subjects, atopic normal subjects, and patients with symptomatic bronchial asthma.

Results: IL-4-induced expression of CD23 on B cells was greater in normal atopic subjects and atopic patients with bronchial asthma than in normal nonatopic subjects. IL-4 generated by concanavalin A-stimulated PBMCs was also higher in normal atopic subjects and atopic patients with bronchial asthma than in normal non-atopic subjects. The expression of CD23 on B cells and IL-4 generation by concanavalin-A-stimulated PBMCs were not different between normal atopic subjects and atopic patients with bronchial asthma.

Conclusions: Both B-cell and T-cell functions are enhanced in atopic subjects. However, neither enhanced B-cell nor T-cell function is a hallmark in development of allergic diseases. (*J Allergy Clin Immunol* 1996;97:1121-8.)

Key words: Atopy, bronchial asthma, IgE, IL-4, CD23

Atopy is characterized by the predisposition to respond to environmental allergens with hyperproduction of IgE antibodies. Atopy is suggested to be one of the important inducing factors in allergic diseases. Although diagnostic value is limited, total serum IgE concentration tends to be higher in

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Abbreviations used

Con-A:	Concanavalin-A
Fc ϵ RII:	Low-affinity receptor for IgE
FVC:	Forced vital capacity
PBMCs:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
PEFR:	Peak expiratory flow rate
TCM:	Tissue culture medium

allergic adults and children compared with nonatopic individuals.¹ Serum IgE level shows a two- to fourfold increase in sensitive patients during pollen seasons.^{2,3}

Two essential signals are necessary for IgE production by B cells. One is a B-cell activation signal, provided by a costimulatory factor on T-cell mem-

branes, and the other is an IgE isotype-specific signal provided by activated T cell–derived IL-4.⁴ IgE binding factor is a cleaved portion of the low-affinity receptor for IgE (FcεRII), also known as CD23 antigen. IL-4 acts indirectly on the production of IgE by induction of CD23.⁵ IL-4 increases the expression of CD23 on B cells in vitro, and the highest levels of expression are obtained by having both IgE and IL-4 in the culture.⁶ Fc receptor for IgE on B cells increases in patients with seasonal atopic rhinitis, especially when they are exposed to specific allergens.⁷

B cells from atopic subjects spontaneously synthesize IgE, whereas there is no production in nonatopic subjects.⁸⁻¹⁰ This suggests that atopic subjects' B lymphocytes are activated in vivo. Relative deficiency of T suppressor cells was thought to be responsible for enhanced production of IgE in atopic subjects.^{9,10} However, selected helper T-cell clones are more responsible for IgE production by B cells in patients with allergy.¹¹ Helper function for IgE is detectable only among the IL-4–producing clones.^{12,13} T-lymphocyte clones in atopic donors have a greater frequency of type 2 helper cell (T_{H2}) producing IL-4 without interferon-γ than those in nonatopic donors.¹² A marked difference was found in the expression of CD28 on T_{H2} clones from atopic donors compared with those of type 1 helper cell (T_{H1}) clones from nonatopic subjects.¹² These findings suggest that the T cells of atopic donors may produce a greater amount of IL-4, which might be detected in peripheral blood circulation. The spontaneous expression of FcεRIIb on B cells of atopic individuals¹⁴ suggests a recent encounter of peripheral blood B cells with IL-4, which is derived from activated T cells. In addition, the proportion of peripheral blood mononuclear cells (PBMCs) responding to IL-4 with an increased synthesis of IgE is higher in atopic donors than in nonatopic donors.¹⁵ This suggests that atopic subjects differ from nonatopic subjects with respect to the generation of IL-4 by T cells and/or the sensitivity of B cells to the biologic effects of IL-4.

In this study we compared the spontaneous and IL-4–induced CD23 expression on B cells in peripheral blood of atopic patients with bronchial asthma, atopic normal control subjects, and nonatopic normal control subjects to evaluate differences in the response of B cells to IL-4. We also compared the concentration of IL-4 in peripheral blood and culture supernatants to evaluate the IL-4–generating ability of T cells among the groups.

METHODS

This study was approved by the Soonchunhyang University Human Subjects Committee. All subjects gave informed consent before participation.

Study subjects

The study subjects included 18 patients with symptomatic atopic bronchial asthma (6 had allergic rhinitis), 17 atopic normal subjects, and 42 nonatopic normal subjects. The presence of atopy was determined by positive immediate responses to one or more of 48 aeroallergen extracts, commonly found in Korea, on skin prick test. All patients with atopic bronchial asthma had positive specific IgE to one or more of three allergens: house dust, *Dermatophagoides farinae*, and *D. pteronyssinus*. The onset of symptoms in the patients with atopic bronchial asthma varied from 1 to 12 years ago. Each group was age- and sex-matched. Forced vital capacity (FVC), FEV₁, and peak expiratory flow rate (PEFR) were measured. PC₂₀ of histamine was measured by Cockcroft's method¹⁶ with concentrations from 0.025 mg/ml to 25 mg/ml.

The profiles of study subjects are summarized in Table I. FVC, FEV₁, and PEFR were significantly lower in the patients with symptomatic atopic bronchial asthma than in atopic and nonatopic normal subjects. PC₂₀ ranged from 0.025 mg/ml to 3.8 mg/ml with geometric means of 1.23 mg/ml in the patients with atopic bronchial asthma, which was significantly lower than those in normal atopic and nonatopic control subjects. IgE concentrations were higher in sera of both atopic patients with bronchial asthma and atopic normal subjects than in sera of nonatopic normal subjects.

Separation of PBMCs

Venous blood was drawn into preservative-free heparin, and mononuclear cells (PBMCs) were obtained by centrifugation over Ficoll-Hypaque (specific gravity of 1.077; Pharmacia, Uppsala, Sweden), washed twice in phosphate-buffered saline (PBS), and resuspended in RPMI-1640 medium (Gibco, Grand Island, N.Y.) supplemented with 1% nonessential amino acid, 20 mmol/L glutamine, 50 mmol/L 2-mercaptoethanol, and 50 mg/ml gentamicin. The latter solution was referred to tissue culture medium (TCM). PBMCs (5×10^6 /ml) were incubated in the presence of 100 U/ml of IL-4 (Genzyme, Cambridge, Mass.) for 48 hours to measure the responsiveness of B cells to IL-4. PBMCs were stimulated with 10 μg/ml concanavalin-A (Con-A) for 24 hours, then supernatants were collected to measure IL-4.

Measurement of CD23 expression on B cells in peripheral blood

PBMCs were suspended in PBS containing 0.1% bovine serum albumin and 0.2% sodium azide. Specific binding of monoclonal antibodies was analyzed by dual immunofluorescence stain according to the standard

TABLE I. Profiles of study subjects

	Nonatopic normal subjects	Atopic normal subjects	Atopic bronchial asthma
No.	42	17	18
Age (yr)	28.5 ± 1.4	30.4 ± 2.3	29.5 ± 2.0
Sex (M:F)	26:16	10:7	10:8
IgE (U/ml)	104 ± 19	323 ± 63*	357 ± 79A*
FVC (%)	97.5 ± 0.8	96.4 ± 0.7	84.3 ± 3.1†
FEV ₁ (%)	99.5 ± 0.9	97.3 ± 1.2	76.2 ± 2.5†
PEFR (%)	98.0 ± 0.9	98.7 ± 1.4	68.4 ± 3.9†
PC ₂₀ (mg/ml)	24.3 ± 0.5	22.5 ± 1.0	1.23 ± 0.23†

FVC, FEV₁, and PEFR are expressed as percent predicted.

All data are expressed as means ± SEM.

**p* < 0.01 versus nonatopic normal subjects.

†*p* < 0.01 versus atopic and nonatopic normal subjects.

method recommended by Becton-Dickinson Microbiology Systems (Cockeysville, Md.). Aliquots containing 1×10^6 cells were incubated for 30 minutes at 4° C in the presence of saturating concentrations of fluorescein isothiocyanate- and phycoerythrin-conjugated monoclonal antibodies. Monoclonal antibodies for CD19 (Leu 12) were obtained in conjugated form with fluorescein isothiocyanate and CD23 (low-affinity receptor for IgE) in conjugated form with phycoerythrin from Becton-Dickinson and Company (Franklin Lakes, N.J.). The cells were washed in PBS and fixed in 1 ml of 1% paraformaldehyde in PBS.

Cytofluorometric analysis of the cells for the surface immunofluorescence was performed with FACSCAN (Becton-Dickinson). The cells were excited with the 488 nm argon laser. The filter set used to separate the two signals was recommended by the manufacturer for fluorescein isothiocyanate and phycoerythrin. For each sample, 10,000 cells were analyzed. The data were collected and analyzed on the Consort 40 program (Becton-Dickinson).

Measurement of IL-4

The concentrations of IL-4 in sera and supernatants were measured by enzyme immunoassay with an Inter-test 4 kit (Genzyme). Briefly, various dilutions of standards (45 to 3000 pg/ml) and samples were incubated in anti-IL-4 monoclonal antibody-coated, flat-bottomed, 96-well microtiter plates. After washing, rabbit anti-IL-4 polyclonal antibody and then biotin-labeled goat anti-rabbit Ig were applied. A horseradish peroxidase-streptavidin reagent was added, then reaction with 0-phenylenediamine was halted at the appropriate point by addition of H₂SO₄. The optical density of the wells was determined at 492 nm. The absorbance of the test wells was then compared with the standard curve and converted to a numerical value. The detection limit was approximately 45 pg/ml. The intraassay coefficient of variation was 8.8%, and the interassay coefficient of variation was 10.6%. The value below the detection limit was regarded as 0 pg/ml.

Statistics

The differences between groups were compared by using the nonparametric Kruskal-Wallis H test for continuous data, and if results were significant, the Mann-Whitney test with Bonferroni's correction was applied to compare any two groups. Wilcoxon signed-rank test was used for paired samples. The relationships between two parameters were studied by using Spearman's rank correlations. The difference was considered significant when the *p* value was less than 0.05. The results are expressed as mean ± SEM, unless otherwise stated.

RESULTS

Spontaneous and IL-4-induced expression of CD23 on B cells

In peripheral blood, percentages of B cells among lymphocytes were the same in nonatopic normal subjects (10.4% ± 0.7%, *n* = 42), atopic normal subjects (12.0% ± 1.7%, *n* = 17), and patients with bronchial asthma (9.7% ± 1.3%, *n* = 18). The expression rates of CD23 on B cells were higher in atopic normal subjects and patients with bronchial asthma than in nonatopic normal subjects (27.7% ± 3.8% vs 16.0% ± 1.7%, *p* < 0.01 and 32.5% ± 4.5% vs 16.0% ± 1.7%, *p* < 0.01, respectively). There was no significant difference in the expression rates of CD23 on B cells between atopic normal subjects and patients with bronchial asthma (Fig. 1).

In the presence of IL-4 (100 U/ml), the expression rates of CD23 on B cells significantly increased in all study subjects, except one nonatopic normal subject, when compared with those in the presence of TCM only. IL-4-induced expression of CD23 on B cells was higher in atopic normal control subjects (41.4% ± 5.5%, *n* = 15) and atopic patients with bronchial asthma (35.6% ±

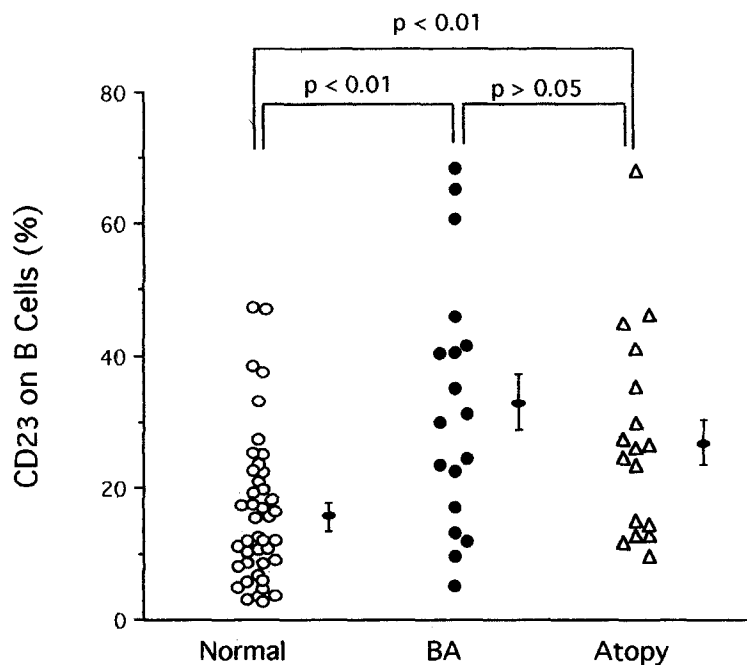


FIG. 1. Spontaneous expression of CD23 on peripheral blood B cells. The expression rates of CD23 on B cells were higher in atopic normal subjects (*Atopy*) and patients with bronchial asthma (*BA*) when compared with those of nonatopic normal subjects (*Normal*).

5.7%, $n = 12$) than in nonatopic normal control subjects ($21.8\% \pm 2.6\%$, $n = 24$, $p < 0.05$, respectively) (Fig. 2).

The IL-4-induced increment of CD23 expression on B cells was calculated as percentages of CD23 on B cells in the presence of IL-4 minus those in the presence of culture medium only. The IL-4-induced increment of CD23 on B cells was higher in atopic normal control subjects ($31.0\% \pm 5.16\%$, $n = 15$) than in nonatopic normal control subjects ($15.6\% \pm 2.6\%$, $n = 24$, $p < 0.05$). However, there was no difference in IL-4-induced increment of CD23 on B cells between atopic patients with bronchial asthma ($21.6\% \pm 4.7\%$, $n = 12$) and nonatopic normal or atopic normal control subjects.

Concentration of IL-4 in serum and generation of IL-4 by PBMCs

Detectable levels of IL-4 were present in sera from one of 42 normal nonatopic subjects, 10 of 16 atopic normal subjects, and nine of 18 atopic patients with bronchial asthma. The detection rates and mean concentrations of IL-4 in sera were significantly higher in atopic normal subjects and patients with bronchial asthma than those in normal subjects ($p < 0.01$, respectively). The mean concentrations of IL-4 were the same in sera of

atopic patients with bronchial asthma and atopic normal subjects (44 ± 14 pg/ml vs 118 ± 54 pg/ml) (Fig. 3).

IL-4 was not spontaneously generated by cultured PBMCs of all study subjects except one nonatopic normal control subject and two patients with bronchial asthma. Twenty-four hours after stimulation with Con-A, IL-4 was detected in supernatants of all study subjects. The generation of IL-4 was significantly higher in atopic normal subjects (210 ± 51 pg/ml, $n = 11$) and patients with bronchial asthma (290 ± 46 pg/ml, $n = 9$) when compared with that of normal control subjects (110 ± 11 pg/ml, $n = 13$, $p < 0.01$ and $p < 0.05$, respectively) (Fig. 4).

CD23 expression on B cells and serum IL-4 concentration according to serum IgE concentration

The normal upper limit of serum IgE was 350 U/ml, which was calculated as mean ± 2 SD of serum IgE in nonatopic normal subjects. Atopic normal control subjects and patients with asthma were divided into two groups: high IgE groups and normal IgE groups. The percentages of CD23 on B cells were significantly higher in the high IgE group of patients with bronchial asthma ($53.5\% \pm 5.2\%$, $n = 6$) when compared with those in the high IgE

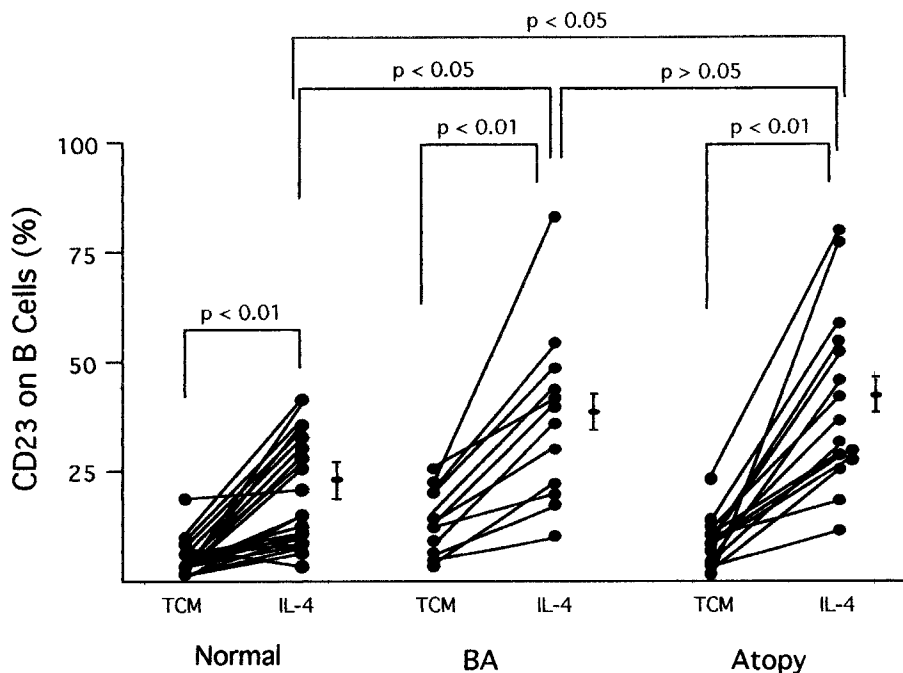


FIG. 2. IL-4-induced expression of CD23 on peripheral blood B cells. In the presence of IL-4 (100 U/ml), the expression rates of CD23 on B cells significantly increased when compared with those in the presence of culture media only (TCM). IL-4-induced expression of CD23 on B cells was higher in atopic normal control subjects and atopic patients with bronchial asthma than in nonatopic normal control subjects.

group of atopic normal control subjects ($31.2\% \pm 7.8\%$, $n = 6$) ($p < 0.05$). Among patients with bronchial asthma, the high IgE group had significantly higher CD23 expression on B cells than the normal IgE group ($53.5\% \pm 5.2\%$ vs $21.9\% \pm 3.1\%$, $p < 0.01$). IL-4 concentrations in sera were not different between high and normal IgE groups of atopic normal control subjects and patients with bronchial asthma (Table II).

Correlations between serum IgE, IL-4, and CD23 on B cells

Serum IgE concentration correlated with percentages of CD23 on B cells when observed in the entire study population ($r = 0.58$, $n = 77$, $p < 0.001$), as well as in atopic patients with bronchial asthma ($r = 0.66$, $n = 18$, $p < 0.01$). Serum IL-4 concentration correlated with percentages of CD23 on B cells ($r = 0.24$, $n = 76$, $p < 0.05$) and serum IgE concentrations ($r = 0.26$, $n = 76$, $p < 0.05$) when observed in the entire study population; however, concentrations were not correlated when observed in atopic normal subjects or patients with bronchial asthma (Table III).

DISCUSSION

In this study we demonstrated that atopic subjects had an enhanced response of B cells to IL-4 and hyperfunctioning of T cells as compared with nonatopic normal control subjects. Enhanced response of B cells was demonstrated by the findings that the spontaneous and IL-4-induced expression of CD23 on B cells was higher in the atopic subjects than in the nonatopic subjects. Spontaneous expression of FcεR on B cells was elevated in atopic patients in other studies.^{7, 17, 18} Patients with severe atopic dermatitis have greater percentages of FcεR-positive lymphocytes than mildly atopic and nonatopic control subjects.¹⁷ In addition, proportions of PBMCs responding to IL-4 with an increased synthesis of IgE are significantly higher in atopic donors than in nonatopic donors.¹⁵ These findings indicate that atopic subjects are different from nonatopic normal subjects in terms of responsiveness of B cells to the biologic effect of IL-4.

Because the expression of CD23 on B cells is elevated in seasons when atopic subjects are exposed to specific allergens,⁷ we selected atopic subjects, who were sensitive to only house dust

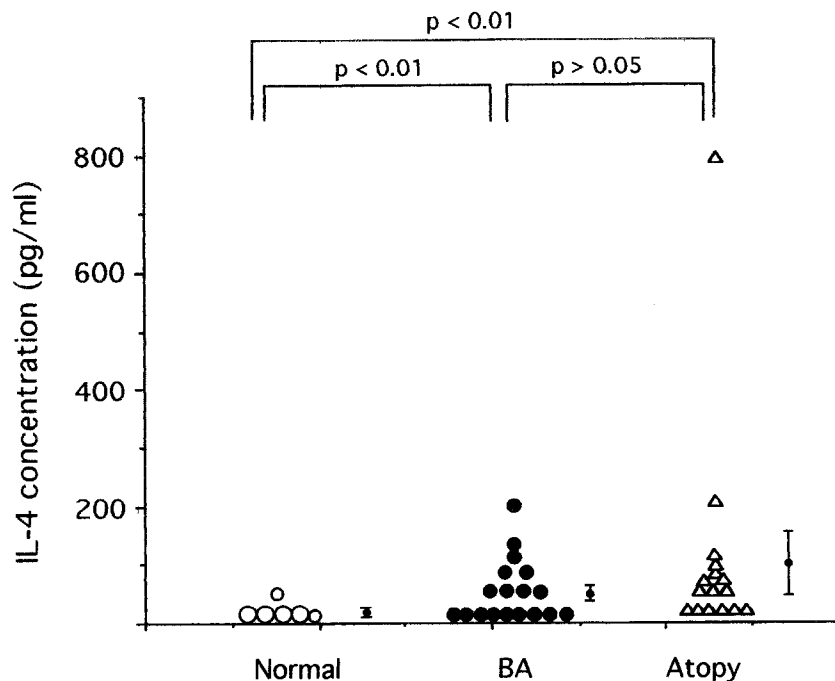


FIG. 3. Concentration of IL-4 in sera of the study population. Detectable levels of IL-4 were present in sera from one of 42 normal nonatopic subjects (*Normal*), 10 of 16 atopic normal subjects (*Atopy*), and nine of 18 atopic patients with bronchial asthma (*BA*). The detection rates and mean concentrations of IL-4 in serum were significantly higher in atopic normal subjects and patients with bronchial asthma than in normal subjects. A larger open circle represents 10 subjects.

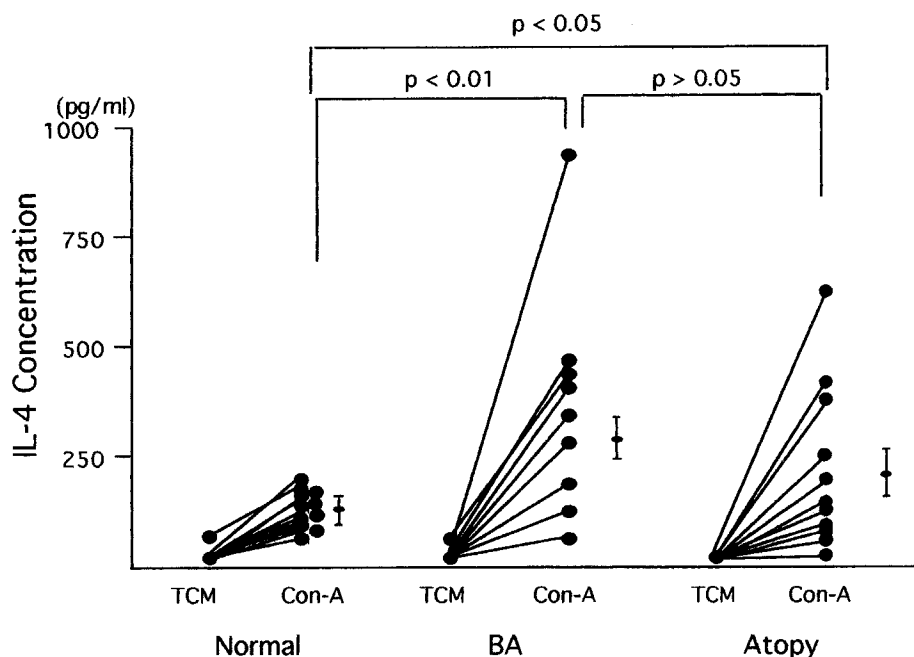


FIG. 4. Con-A-stimulated generation of IL-4 by PBMCs. IL-4 was not spontaneously generated by cultured PBMCs (TCM) of all study subjects except one normal control subject (*Normal*) and two patients with bronchial asthma (*BA*). Twenty-four hours after stimulation with Con-A, generation of IL-4 was significantly higher in atopic normal subjects (*Atopy*) and patients with bronchial asthma when compared with that in normal control subjects.

TABLE II. CD23 expression on B cells and serum IL-4 concentration according to serum IgE concentration

	No.	Serum IgE (U/ml)	CD23(+) B cells (%)	IL-4 (U/ml)
Nonatopic normal control subjects	42	104.0 ± 19.1	16.0 ± 1.7	0 ± 0
Atopic normal control subjects				
High IgE group	6	588.7 ± 87.0	31.2 ± 7.8*	0.18 ± 0.13*
Normal IgE group	11	164.2 ± 33.1	25.7 ± 4.2	0.08 ± 0.05*
Patients with atopic asthma				
High IgE group	6	710.0 ± 150.6	53.5 ± 5.2*†‡	0.07 ± 0.02*
Normal IgE group	12	181.1 ± 30.5	21.9 ± 3.1	0.03 ± 0.02*

All data are expressed as means ± SEM.

**p* < 0.05 versus nonatopic normal control subjects.

†*p* < 0.05 versus high IgE group of atopic normal control subjects.

‡*p* < 0.01 versus normal IgE group of patients with atopic asthma.

TABLE III. Spearman's correlation coefficient of serum IgE, IL-4, and CD23 expression on B lymphocytes

	Total	Nonatopic normal subjects	Atopic normal subjects	Atopic patients with bronchial asthma
No.	76	42	17	18
IgE-IL4	0.26*	0.54†	0.03	0.14
IgE-CD23(+) B cells	0.58†	0.41	0.25	0.66‡
IL-4-CD23(+) B cells	0.24*	0.15	0.11	0.40

**p* < 0.05.

†*p* < 0.001.

‡*p* < 0.01.

mites, to avoid the seasonal variations in exposure to related allergens. House dust mites are persistent allergens in Korea through all seasons.¹⁹

IL-4 is a major cytokine that upregulates CD23 expression on B cells. In our study IL-4 upregulated CD23 expression on B cells in nonatopic subjects, as well as atopic subjects. The expression of CD23 is controlled at two levels: the cleavage of CD23 and cellular expression of CD23. Serum IgE upregulates CD23 on B cells as a result of stabilization by prevention of cleavage.²⁰ IL-4 directly increases FcεRIIb on B cells and monocytes. FcεRIIb is expressed on peripheral blood lymphocytes in atopic individuals, but it is not detectable in nonatopic normal subjects.¹⁴

The presence of IL-4 in serum and a greater production of IL-4 by Con-A-stimulated PBMCs of the atopic subjects indicate that atopic subjects have an enhanced activity of T cells compared with normal control subjects. The serum concentration of IL-4 is elevated in other allergic diseases such as atopic eczema and food anaphylaxis and is significantly correlated with the serum levels of IgE.²¹ Increased production of IL-4 by mitogen-stimu-

lated PBMCs in vitro is higher in atopic dermatitis and the ratio of in vitro IL-4 to interferon-γ production correlates positively with serum IgE.²² These findings support the presence of different T-cell clones in PBMCs of the atopic subjects.

It is unclear whether the number of T-cell clones reactive to Con-A increases or the capability of IL-4 generation by T cells increases, and it is unclear whether the sites of IL-4 synthesis are in target organs or in peripheral blood. The lack of spontaneous generation of IL-4 by PBMCs suggests that IL-4 in serum may originate from target organs in our study. There is evidence of local IL-4 synthesis in atopic patients with bronchial asthma. The concentrations of IL-4 are higher in bronchoalveolar lavage fluid from patients with atopic asthma than in those from nonatopic normal subjects, and lymphocytes in bronchoalveolar lavage fluid release greater amounts of IL-4 in culture supernatants.²³ This finding indicates that atopic subjects have predominant T_{H2}-type clones in peripheral blood¹² and bronchial trees.²⁴

FcεR-positive cells are significantly elevated up to 4.7% from 1.2%, during the grass pollen sea-

sons, when patients have symptoms. FcεR-positive peripheral blood lymphocytes increase in patients with allergy during natural exposure to allergens in the absence of measurable increases of total and specific IgE. The enumeration of FcεR-positive lymphocytes is a more sensitive indicator of host responses than total IgE and specific IgE changes.⁷ However, enhanced function of either B cells or T cells is not a hallmark in the development of allergic diseases, because the expression of CD23 on B cells and IL-4 generation by T cells were not different between atopic normal control subjects and atopic patients with bronchial asthma in our study.

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