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Immunology

IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E_2

(IgE regulation/lymphokines/IgE-binding factors/CD23)

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ABSTRACT **The effect of human recombinant interleukin 4 (IL-4) on antibody production by normal peripheral blood mononuclear cells enriched for B cells was investigated. IL-4 preferentially induced IgE synthesis *in vitro*. In addition, a low induction of IgG production was observed, whereas IL-4 had no effect on IgA and IgM synthesis. The IL-4-induced IgE production by B cells required T cells and monocytes but was specifically inhibited by an anti-IL-4 antiserum indicating that, although IL-4 acts indirectly, it is responsible for the induction of IgE synthesis. IL-4-induced IgE production was blocked in a dose-dependent way by interferon γ (IFN-γ), interferon α (IFN-α), and prostaglandin E_2. IFN-γ also inhibited IL-4-induced IgG production. These inhibitory effects of IFN-γ and IFN-α on IgE production cannot be attributed to toxic effects since IFN-α induced IgM production in the presence of IL-4, whereas IFN-γ was ineffective in inhibiting IgG production induced by IL-2. IFN-γ, IFN-α, and prostaglandin E_2 also inhibited IL-4-induced expression of the low-affinity receptor for the Fc portion of IgE (CD23) on B cells, indicating that there is an association between CD23 expression and IL-4-induced IgE production. This theory was supported by the finding that IL-4-induced IgE production was inhibited by $F(ab')_2$ fragments of an anti-CD23 monoclonal antibody.**

Recently, it has been demonstrated that recombinant human interleukin-4 (IL-4) has pleiotropic effects. IL-4 induces proliferation of activated B cells, T cells, thymocytes, and natural killer clones (1–3). In addition, IL-4 has been shown to induce class II major histocompatibility complex antigens and low-affinity receptors for the Fc portion of IgE, which represent the B-cell activation marker CD23 on B cells (4–7). The biological function of CD23 is unclear, but both murine and human studies have indicated that B cells bearing the low-affinity receptors for the Fc portion of IgE release factors that modulate IgE production (8, 9).

Most studies on human IgE synthesis *in vitro* have been carried out with B lymphocytes from severely atopic individuals, whose cells are activated *in vivo* and produce IgE spontaneously *in vitro* (10). Normal B cells generally failed to produce IgE under experimental conditions that resulted in the differentiation of B cells.

However, various studies have shown that cocultivation of alloreactive and autoreactive T-cell clones with peripheral blood B cells resulted not only in IgE production but also in IgG and IgM production (11–13). IgE production could also be induced by T-cell clones activated by phytohemagglutinin or anti-CD3 monoclonal antibodies (mAbs) in the apparent absence of specific interaction with highly purified tonsil B cells (14). Moreover, supernatants of a T-cell line specifically activated by parasite antigens induced IgE production by normal unactivated B cells (15). These results suggested that upon activation normal T-cell clones produce factors capable of inducing B cells to produce IgE.

It has been shown in animal models that production of IgE is regulated by both antigen-specific and isotype-specific regulation mechanisms, which are mediated by lymphocytes secreting IgE-binding factors that may enhance or suppress IgE synthesis (8, 16). Comparable mechanisms have been identified in the regulation of human IgE production (9, 17–19). Recently, Coffman *et al.* (20) have demonstrated that murine IL-4 induced IgE production by murine spleen B cells activated by lipopolysaccharide. These observations and the finding that IL-4 induces CD23 on B cells, which is implicated in the regulation of IgE production, prompted us to investigate if recombinant human IL-4 could stimulate IgE production by human B cells.

In the present paper, it is demonstrated that human recombinant IL-4 induces the production of IgE by peripheral blood lymphocytes (PBL) and that IL-4-induced IgE production is blocked by interferon-γ (IFN-γ), interferon-α (IFN-α), prostaglandin E_2 (PGE_2), and a mAb against CD23.

MATERIALS AND METHODS

Cytokines and Reagents. IL-4 was used as a supernatant from COS-7 cells transfected with the pcD or pEBT vector containing the human IL-4 cDNA clone (1). One unit of IL-4 is defined as the concentration of IL-4 resulting in half-maximal proliferation of phytohemagglutinin-activated T lymphoblasts. Mock-transfected preparations consisting of culture supernatants of COS-7 cells transfected with an unrelated cDNA clone were used as controls. In certain experiments, purified recombinant IL-4 was used. Purified recombinant IL-4 (specific activity of 10^7 units/mg), IFN-α, and recombinant IFN-γ (specific activity of 10^7 units/mg) were kindly provided by T. Nagabhushan and P. Trotta (Schering Research). The rabbit anti-IL-4 antiserum, raised in our laboratory, is specific for IL-4 and does not react with IL-1α and -1β, IL-2, IL-3, IL-5, low molecular weight B-cell growth factor, IFN-γ, IFN-α, or granulocyte/macrophage colony-stimulating factor. This anti-IL-4 antiserum blocks the biological activity of IL-4 as judged by its capacity to

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Abbreviations: IL-1, IL-2, IL-3, IL-4, IL-5, interleukin 1, 2, 3, 4, and 5, respectively; PBL, peripheral blood lymphocytes; IFN-γ, interferon γ; IFN-α, interferon-α; PGE_2, prostaglandin E_2; mAb, monoclonal antibody; SRBC, sheep erythrocytes.

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inhibit IL-4-induced expression of CD23 on B cells and IL-4-induced proliferation of activated B and T cells (I.C., J.B., A. van Kimmenade, and Y. Abrams, unpublished observations). Recombinant human IL-2 was kindly provided by R. Kastelein (DNAX Research Institute, Palo Alto, CA). mAb 25 (IgG1) is directed against CD23, and $F(ab')_2$ fragments were prepared as described (5). mAb SPV-L3 is specific for HLA-DQ (21). mAbs Leu-4 (anti-CD3), Leu-M3 (anti-CD14), and Leu-16 (anti-CD20) were obtained from Becton Dickinson, and mAb B1 was from Coulter. mAb AMF-7 (IgG1) is specific for a melanoma-associated cellular adhesion molecule and was used as a control mAb. mAb HNK-1, specific for natural killer cells, was kindly provided by T. Hercend (Institute Gustave Roussy, Villejuif, France). PGE_2 and cycloheximide were obtained from Sigma.

Cells and Cell Cultures. PBL were isolated from heparinized blood from healthy donors by centrifugation over Ficoll/Hypaque. To remove adherent cells, 20–50 × 10^6 PBL were incubated in 20 ml of culture medium consisting of RPMI-1640 supplemented with 10% heat-inactivated (30 min, 56°C) fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) (Flow Laboratories) in 75-cm^2 tissue culture flasks (Falcon) for 2–4 hr. Nonadherent cells were recovered, and the B cells were enriched by one round of depletion of sheep erythrocyte (SRBC) rosette-forming cells by centrifugation over Ficoll/Hypaque. These PBL enriched for B cells were collected from the interface, washed three times with medium, and used for antibody production. T cells were recovered by lysis of erythrocytes with isotonic NH_4Cl/Tris treatment for 10 min at 4°C, and these T cells (>98% $CD3^+$) were washed, resuspended in the culture medium, and used for the reconstitution experiments. The adherent monocytes used for reconstitution experiments were collected by scraping them carefully from the bottom of the culture flask with a rubber policeman. These cells were >99% viable and consisted of 95% $CD14^+$ cells. B cells were further enriched from the non-SRBC rosette-forming cell population by standard panning procedures in which the remaining contaminating T cells and monocytes were removed by binding to Leu-4 (anti-CD3) and Leu-M3 (anti-CD14) mAbs coated to plastic. For antibody production, 5 × 10^5 non-SRBC rosette-forming cells or 2.5 × 10^5 purified B cells were incubated for 9–12 days in 1 ml of culture medium in 12- × 75-mm sterile plastic tubes (Falcon). Supernatants were collected after centrifugation (5 min, 450 × *g*) and were measured immediately or after storage at −20°C.

ELISAs for IgE, IgG, IgA, and IgM. Flat-bottomed microtiter plates (Nunc) were coated with rabbit anti-IgE immunoglobulins (Dako, Glostrup, Denmark) diluted 1:2000 in a bicarbonate buffer (pH 9.6). After incubation for 18 hr at 4°C, the plates were washed four times with phosphate-buffered saline (PBS)/0.05% Tween (Merck) and were incubated with RPMI-1640/10% fetal calf serum to saturate protein binding sites for 1 hr at 20°C. Next, the culture supernatants were diluted to the appropriate concentration in PBS/0.05% Tween, added to the plates, and incubated for 6 hr at 20°C. The plates were washed four times with PBS/0.05% Tween, and a murine anti-IgE mAb (Hybritech, San Diego, CA) was added at a final dilution of 1:5000 and was incubated for 18 hr at 20°C. After four washes with PBS/0.05% Tween, the plates were finally incubated for 4 hr at 20°C with goat anti-mouse immunoglobulins coupled to alkaline phosphatase (Sigma; dilution of 1:1000) that were preadsorbed on normal rabbit serum. After washing, IgE was measured by incubation with *p*-nitrophenyl phosphate (Sigma) in 1 M diethanolamine/HCl buffer, pH 9.8, and the plates were read with a micro ELISA autoreader (Dynatech, MR 580) at 405 nm. A standard IgE serum (Behring, Marburg, F.R.G.) was used as a reference. The limit of the sensitivity of the assay was 150 pg of IgE per ml.

The production of IgG, IgM, and IgA was measured in standard ELISAs in which rabbit anti-IgG (dilution of 1:2000) or rabbit anti-IgM (1:1000) (Behringwerke) or goat anti-IgA (1:10,000) (Pasteur Institute, Paris) were used as coating antibodies. Next the plates were incubated with the appropriate dilutions of the culture supernatants and finally with goat anti-IgG (1:250) or goat anti-IgM (1:250) (Behring) or rabbit anti-IgA (1:10,000) (Pasteur Institute) antibodies coupled to alkaline phosphatase. IgG, IgM, and IgA standards (Behring) were used as references. The sensitivities of the assays were 1 ng/ml, 4 ng/ml, and 2 ng/ml for IgG, IgM, and IgA, respectively.

Immunofluorescence. Phenotyping of the cell cultures was carried out by immunofluorescence assays as described previously (5). The percentages of T cells, B cells, and monocytes were determined by using the mAbs Leu-4, Leu-16 and Leu-M3, respectively, and fluorescein isothiocyanate-labeled $F(ab')_2$ fragments of goat anti-mouse immunoglobulin (Grub, Vienna). Expression of CD23 was detected by binding of mAb 25 $F(ab')_2$ fragments and fluorescein isothiocyanate-labeled $F(ab')_2$ fragments of goat anti-mouse immunoglobulin (Grub). Fluorescence was measured with a fluorescence-activated cell sorter model 440. For double-fluorescence analysis, cells were incubated simultaneously with B1- or Leu-4 fluorescein isothiocyanate-conjugated mAbs (Coulter and Becton Dickinson, respectively) and biotinylated mAb 25. The binding of biotinylated mAb 25 was assessed by using phycoerythrin-conjugated streptavidin (Becton Dickinson). Green fluorescence (B1 or Leu-4) is plotted along the *y* axis; red fluorescence (mAb 25) is plotted along the *x* axis in Fig. 1.

RESULTS

IL-4 Induces IgE Synthesis by Normal PBL. PBL enriched for B cells (non-SRBC rosette-forming cells) cultured in the presence of IL-4 produced relatively large quantities of IgE, whereas supernatants from mock-transfected COS-1 cells were ineffective (Table 1). Further purification of these enriched B cells by standard panning procedures resulted in B-cell populations contaminated with ≈10% $CD3^+$ T cells and <1% monocytes. Surprisingly, these purified B cells failed to produce IgE upon incubation with IL-4. IgE production was restored in a dose-dependent fashion by reconstitution with autologous T cells. Although only two concentrations of T cells were tested in this experiment, a dose-dependent effect was observed. Optimal IgE production was obtained by the addition of autologous T cells at a T-cell to B-cell ratio of 2:1 and 10% autologous monocytes. These results indicate that both T cells and monocytes are required for IgE production by normal B cells. Cycloheximide added at a concentration of 50 μg/ml completely prevented IL-4-induced IgE synthesis, indicating that IL-4 indeed induces *de novo* IgE production.

IL-4-Induced IgE Production Is Blocked by Anti-IL-4 Antibodies, IFN-γ, IFN-α, and PGE_2. The induction of IgE synthesis by IL-4 was inhibited by the polyclonal anti-IL-4 antiserum in a dose-dependent way, whereas the control preimmune rabbit serum was ineffective. A typical experiment is shown in Table 2, experiment 1. Complete inhibition by the anti-IL-4 antiserum was observed at a dilution of 1: 250, whereas, at a dilution of 1:10,000, IgE production was still more than 80% inhibited. IL-4 did not induce significant levels of IgA and IgM but, in most experiments, a low production of IgG was obtained, which was also blocked by the anti-IL-4 antiserum. IFN-γ or IL-2 failed to induce IgE production. These results indicate that induction of IgE synthesis is specific for IL-4.

Recently, we demonstrated that IL-4 induced the expression of CD23 on normal B cells (6). This IL-4-induced CD23 expression was completely blocked by IFN-γ, indicating that

Table 1. Effect of IL-4 on IgE production by normal B cells

Cell culture*	Addition(s)†	IL-4, 100 units/ml	IgE production, pg/ml
Non-SRBC rosette-forming cells			
(5 × 10^5 per ml)	—	−	<150
		−‡	<150
		+	11,300 ± 208
B cells (2.5 × 10^5 per ml)	—	−	<150
		+	<150
B cells (2.5 × 10^5 per ml)	CD3$^+$ cells (2.5 × 10^5 per ml)	+	5,800 ± 351
B cells (2.5 × 10^5 per ml)	CD3$^+$ cells (5 × 10^5 per ml)	+	9,100 ± 184
B cells (2.5 × 10^5 per ml)	CD3$^+$ cells (5 × 10^5 per ml) + 10% CD14$^+$ cells	+	21,300 ± 178
Non-SRBC rosette-forming cells + cycloheximide (50 μg/ml)	—	+	<150

*Non-SRBC rosette-forming cells contained 36% CD20$^+$, 43% CD3$^+$, 7% CD14$^+$, and 5% HNK$^+$ cells. B-cell populations contained 85% CD20$^+$, 10% CD3$^+$, and 1% CD14$^+$ cells.
†CD3$^+$ T cells and CD14$^+$ monocytes used for reconstitution experiments were obtained as described in *Materials and Methods*.
‡Supernatant from mock-transfected COS-7 cells was added.

IFN-γ can act as an antagonist of IL-4 (6). Therefore, the effect of IFN-γ on IL-4-induced IgE production was investigated. The presence of IFN-γ added at a concentration of 100 units/ml during a 9-day culture period resulted in a complete inhibition of IL-4-induced IgE production, whereas inhibitions of 80% were obtained at a concentration of 50 units/ml. The low induction of IgG induced by IL-4 was also inhibited by IFN-γ (Table 2, experiment 1). In this system, IFN-γ did not inhibit IL-2-induced IgG production, indicating that IFN-γ only antagonized the activity of IL-4.

The inhibition of IL-4-induced IgE production was not specific for IFN-γ, since IFN-α and to a lesser extent PGE_2, which inhibit IL-4-induced expression of CD23 on B cells, both blocked IL-4-induced IgE production in a dose-dependent way (Table 2, experiment 2). In contrast to its inhibitory effects on IL-4-induced IgE production, IFN-α was found to induce the production of IgM in the presence of IL-4. IFN-α tested alone was ineffective. IFN-α and PGE_2 are known to be produced by monocytes (21, 22), which therefore may account for the suppression of IgE production observed in the presence of relatively high monocyte concentrations (data not shown). These data indicate that factors that inhibit CD23 induction by IL-4 on B cells also blocked IL-4-induced IgE production, which suggests an association between CD23 expressed on B cells and IgE production.

IL-4-Induced IgE Production Is Also Blocked by mAb 25 (Anti-CD23). To further analyze the relation between CD23 and IgE production, the effect of mAb 25 (IgG1) on IL-4-induced IgE production was investigated. In Table 3, it is shown that $F(ab')_2$ fragments of mAb 25 added at different concentrations inhibited IL-4-induced IgE production in a dose-dependent way. At a concentration of 25 μg/ml, mAb

Table 2. Induction of IgE production by normal PBL by IL-4 and its inhibition by anti-IL-4 antibodies, IFN-γ, IFN-α, and PGE_2

Lymphokine(s) added	IgG, ng/ml	IgM, ng/ml	IgA, ng/ml	IgE, pg/ml
		Experiment 1		
None	36 ± 3	<10	68 ± 4	<150
IL-4 (100 units/ml)	88 ± 5	<10	103 ± 10	4235 ± 130
IL-4 + anti-IL-4 (1:250)	38 ± 3	<10	85 ± 5	<150
IL-4 + anti-IL-4 (1:10,000)	47 ± 4	<10	79 ± 6	811 ± 28
IL-4 + normal rabbit serum (1:250)*	ND	ND	ND	4680 ± 80
IL-4 + IFN-γ (100 units/ml)	38 ± 5	<10	76 ± 6	<150
IL-4 + IFN-γ (50 units/ml)	40 ± 3	<10	88 ± 5	960 ± 22
IFN-γ (100 units/ml)	41 ± 2	<10	59 ± 4	<150
IL-2 (40 units/ml)	703 ± 37	<10	60 ± 25	<150
IL-2 (40 units/ml) + IFN-γ (100 units/ml)	648 ± 30	<10	62 ± 5	<150
		Experiment 2		
None	10 ± 2	21 ± 3	56 ± 8	<150
IL-4 (100 units/ml)	27 ± 9	43 ± 0	37 ± 2	1942 ± 67
IL-4 + IFN-α (300 units/ml)	13 ± 2	543 ± 8	38 ± 4	219 ± 9
IL-4 + IFN-α (100 units/ml)	14 ± 3	520 ± 25	38 ± 5	599 ± 5
IL-4 + IFN-α (30 units/ml)	17 ± 3	329 ± 10	37 ± 2	1173 ± 11
IFN-α (100 units/ml)	15 ± 3	40 ± 4	60 ± 5	<150
IL-4 + PGE_2 (10^{-6} M)	15 ± 0	36 ± 5	67 ± 1	642 ± 54
IL-4 + PGE_2 (10^{-7} M)	12 ± 2	50 ± 1	63 ± 1	979 ± 28
IL-4 + cycloheximide (50 μg/ml)	5 ± 0	<10	21 ± 3	176 ± 4

In experiment 1, the PBL enriched for B cells contained 32% CD20$^+$, 50% CD3$^+$, 5% CD14$^+$, and 4% HNK-1$^+$ cells. In experiment 2, the PBL enriched for B cells contained 39% CD20$^+$, 48% CD3$^+$, and 6% CD14$^+$. ND, not determined.
*Preimmune rabbit serum was used as control.

Table 3. Inhibition of IL-4-induced IgE production by mAb 25 directed against CD23

Addition(s)	IgG, ng/ml	IgM, ng/ml	IgA, ng/ml	IgE, pg/ml
Medium	17 ± 1	25 ± 0	41 ± 4	195 ± 39
IL-4 (100 units/ml)	25 ± 0	40 ± 0	40 ± 2	3411 ± 138
IL-4 + mAb 25* (25 μg/ml)	30 ± 3	37 ± 2	48 ± 3	<150
IL-4 + mAb 25* (10 μg/ml)	12 ± 0	21 ± 1	36 ± 0	880 ± 25
IL-4 + mAb 25* (2 μg/ml)	24 ± 3	33 ± 3	44 ± 4	2120 ± 50
IL-4 + mAb AMF-7† (25 μg/ml)	27 ± 3	37 ± 3	42 ± 3	3620 ± 62
IL-4 + mAb SPV-L3‡ (1:100)	ND	ND	ND	4980 ± 85

The B-cell population contained 38% $CD20^+$, 50% $CD3^+$, and 4% $CD14^+$ cells. ND, not determined.
*mAb 25 was added in the form of $F(ab')_2$ fragments.
†mAb AMF-7 was purified from ascites.
‡mAb SPV-L3 was added at 1:100 dilution of ascites.

25 completely inhibited IL-4-induced IgE synthesis. mAb 25 specifically blocked IL-4-induced IgE production, since the very low IgG, IgM, and IgA production was not significantly affected. The theory that mAb 25 specifically inhibits IgE synthesis was supported by the finding that mAb 25 does not affect pokeweed mitogen-induced IgG, IgM, and IgA production (data not shown). Control mAb AMF-7 (IgG1), which is directed against a melanoma-associated surface antigen, and control mAb SPV-L3, which is specific for HLA-DQ expressed on the B cells in the cultures (23), had no blocking effect. These data strongly support the theory that CD23 is associated with IgE production.

Expression of CD23 on B Cells Cultured in the Presence of IL-4 or Combinations of IL-4 and IFN-γ or IFN-α. The association between CD23 expression on B cells and IgE production was confirmed by immunofluorescence studies (Fig. 1). PBL enriched for B cells cultured in the presence of IL-4 for 7 days expressed CD23 as judged by their staining with mAb 25 (Fig. 1*A*). Addition of IFN-γ (100 units/ml) or IFN-α (300 units/ml) to the cells cultured with IL-4 completely inhibited IL-4-induced expression of CD23 on the cells (Fig. 1 *B* and *C*). Also PGE_2 at concentrations of 10^{-7} M strongly blocked CD23 expression (data not shown). Moreover, immunofluorescence studies using double-labeling methods showed that CD23 could be detected on B cells only (Fig. 1*D*). Collectively, these data indicate that IFN-γ, IFN-α, and PGE_2 have antagonistic effects on both IL-4-induced CD23 expression on B cells and on IL-4-induced IgE production, demonstrating that there is a strong association between CD23 expression on the B cells and the production of IgE.

DISCUSSION

In the present study, we demonstrate that IL-4 can induce IgE production by PBL without preactivation *in vitro*. However, purified blood B cells cannot be induced to produce IgE. Reconstitution experiments show that IL-4-induced IgE production by B cells is indirect and requires T cells and monocytes. Although the effects of IL-4 are indirect, induction of IgE production by normal human B cells is specific for IL-4 since a polyclonal anti-IL-4 antiserum completely blocks IL-4-induced IgE synthesis. IL-4 predominantly induces IgE production but, in the majority of the experiments, a low but consistent production of IgG is observed that is also blocked by anti-IL-4 antibodies.

In addition, we recently demonstrated that alloreactive T-cell clones produced IL-4 upon activation by the relevant alloantigen and that these supernatants, in addition to inducing IgG, IgM, and IgA production, could induce IgE production by normal B cells, provided that anti-IFN-γ antibodies were present. This IgE synthesis induced by natural IL-4 could be abrogated by anti-IL-4 antibodies (24).

IL-4-induced IgE production is blocked by IFN-γ, IFN-α, and PGE_2 in a dose-dependent way. In addition, IFN-γ also blocks the induction of IgG production by IL-4. These results indicate that IFN-γ, IFN-α, and PGE_2 have antagonistic effects on IL-4-induced IgE production. Interestingly, these factors are also shown to block IL-4-induced expression of CD23 on B cells, indicating a correlation between the expression of CD23 and IgE production. Recently, it has been demonstrated in murine systems that B cells could release factors that bind IgE and modulate IgE production (8). Also human CD23-bearing B cells release a factor that binds to IgE, reacts with anti-CD23 mAbs, and enhances ongoing IgE synthesis by B cells from atopic individuals (9, 19). This IgE-binding factor appeared to be a proteolytic cleavage product of CD23 (4, 25, 26). We found that IL-4 enhanced the release of a 25-kDa molecule that binds to IgE and that reacts with mAb 25 (27).

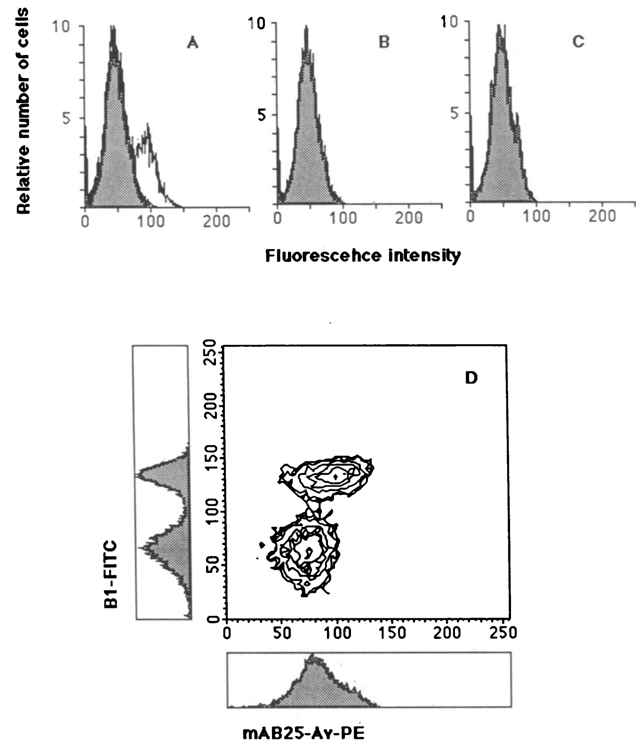

FIG. 1. CD23 expression on B cells after 7 days of culture in the presence of IL-4 or combination of IL-4 and IFN-γ or IFN-α. IL-4 was added to PBL enriched for B cells by depletion of adherent cells and SRBC rosette-forming cells (40% $CD20^+$, 42% $CD3^+$, 5% $CD14^+$, 3% HNK-1^+). CD23 expression on non-SRBC rosette-forming cells cultured for 7 days with IL-4 (100 units/ml) (*A*), IL-4 and IFN-γ (100 units/ml) (*B*), or IL-4 and IFN-α (300 units/ml) (*C*) was determined. Fluorescence intensity is expressed on a logarithmic scale. (*D*) Double labeling of cells with B1 and mAb 25 antibodies was performed. B1-FITC, B1 fluorescein isothiocyanate-conjugated mAb; mAb 25-Av-PE, biotinylated mAb 25 plus phycoerythrin-conjugated streptavidin.

By taking into account that induction of membrane CD23 expression on B cells results in the subsequent release of this truncated soluble form of CD23 (soluble CD23) and that soluble CD23 is associated with IgE production, it is tempting to speculate that the inhibition of IL-4-induced expression of CD23 by IFNs and PGE_2 causes the inhibition of IgE production. This implies that the blocking effects observed with the anti-CD23 mAb 25, which are specific for IgE, can be explained by its capacity to prevent the release of soluble CD23 or to neutralize its biological activity. On the other hand, it cannot be excluded that membrane-bound CD23 is also associated with IgE production. In this case, it may be possible that binding of mAb 25 to surface CD23 confers a negative signal to the B cells, which finally results in the prevention of IgE production. In addition, it has been reported recently that IFN-γ blocked the transcription of IL-4-induced CD23 mRNA in normal B cells, supporting the notion that the absence of membrane-bound CD23 and/or secreted IgE-binding factor accounts for the lack of IgE production (28). Direct evidence that soluble CD23 is associated with IgE production comes from preliminary experiments in which soluble CD23 partially purified from supernatants of the $CD23^+$ Epstein–Barr virus-transformed B-cell line RPMI 8866 strongly enhanced IgE production by normal B cells induced by suboptimal concentrations of IL-4 (29). Soluble CD23 was unable to induce IgE production when tested in the absence of IL-4.

Collectively, these data indicate that IL-4 is the sole inducer of IgE production. The regulatory effects of soluble CD23 are indirect, since highly purified non-preactivated B cells can be induced by IL-4 to release CD23 (26), but T cells and monocytes are required to synthesize IgE. Since we recently demonstrated that IL-4 induced the activation of T cells (3) and monocytes (30), it is possible that factors produced by these cells upon activation by IL-4 contribute to IgE synthesis by normal B cells.

Our data are in line with those of Coffman *et al.* (20) who showed that murine IL-4 induced IgE and IgG1 production by lipopolysaccharide-preactivated murine B lymphoblasts in the virtual absence of T cells and macrophages, suggesting that preactivation of the B cells probably circumvents the requirement for T cells and monocytes. Also, in these murine systems, IL-4-induced IgE production could be blocked by IFN-γ (31).

Finally, our study shows that IL-4-induced IgE production by normal B cells provides an excellent model to dissect the multicellular and multifactorial events that are associated with the regulation of IgE production in humans.

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