Journal of Cellular Biochemistry 39:253–264 (1989)
Molecular and Cellular Mechanisms of Human
Hypersensitivity and Autoimmunity 37–48

Modulation of IL-4-Induced Human IgE Production In Vitro by IFN-γ and IL-5: The Role of Soluble CD23 (s-CD23)

J. Pène, I. Chrétien, F. Rousset, F. Brière, J.Y. Bonnefoy, and J.E. de Vries

UNICET, Laboratory for Immunology, BP 11, 69572, Dardilly, France

IL-4 specifically induced IgE production by peripheral blood lymphocytes or by tonsil or spleen cells from healthy donors. IL-4-induced IgE synthesis was dependent on $CD4^+$ T cells and monocytes and was blocked by IFN-γ, IFN-α, and prostaglandin E-2 (PGE-2). These substances also inhibited IL-4-induced CD23 expression and subsequent release of soluble CD23 (s-CD23). In addition, IgE production was blocked by F(ab')2 fragments of an mAb against CD23. In contrast, IL-5 enhanced IL-4-induced IgE production, provided IL-4 was added at nonsaturating concentrations. This increase in IgE production correlated quantitatively with an enhanced release of s-CD23. Collectively, these results indicate that there is a correlation between s-CD23 release and IgE production. However, s-CD23 fractionated from supernatants of the lymphoblastoid cell line RPMI-8866 was ineffective in inducing IgE production in the absence of IL-4, but acted synergistically with suboptimal concentrations of IL-4. In addition, it is demonstrated that alloreactive T-cell clones produced varying concentrations of IL-4, IL-2, or IFN-γ upon stimulation. Only supernatants of 2/4 of these T-cell clones induced a low degree of IgE synthesis, but in the presence of anti-IFN-γ antibodies, all four supernatants induced a strong induction of IgE production. This IgE synthesis was blocked specifically by anti-IL-4 antibodies, indicating that IL-4 is the sole inducer of IgE synthesis. Our findings demonstrate that IL-4-induced IgE production involves complex interactions of T cells, B cells, and monocytes and is positively modulated by IL-5 and s-CD23 but down-regulated by IFN-γ, IFN-α, and PGE-2, respectively.

Key words: IFN-α, regulation IgE response

IL-4 is produced by T cells and has pleiotropic effects. Recombinant IL-4 has been shown to act as a growth factor for activated B cells [1], activated T cells [2], and lymphokine-activated killer cells [3]. Furthermore, IL-4 induces the expression of class II MHC antigens and low-affinity Fc receptors for IgE (CD23) on normal and malignant B cells [4–6]. Soluble truncated forms of CD23 can be released by B cells and enhance spontaneous IgE production by B cells from atopic individuals

Received May 13, 1988; accepted August 23, 1988.

[7,8]. Recently, it has been reported that murine IL-4 induces IgE production by activated murine spleen cells [9,10]. In addition, other studies have shown that supernatants of T lymphocytes from patients with the hyper-IgE syndrome, from highly atopic individuals, or from parasite specific T-cell lines can induce significant IgE production by normal B cells [11–14], indicating that T cells produce factors that induce IgE synthesis. These results prompted us to investigate whether recombinant IL-4 or IL-4 produced by activated T-cell clones induced IgE production by normal human B cells.

Here, we show that human IL-4 specifically induces IgE production by normal human B cells. This IgE-inducing effect of IL-4 required $CD4^+$ T cells and monocytes and was blocked by IFN-γ, IFN-α, and PGE-2 and enhanced by IL-5. In addition, alloreactive human T-cell clones and their supernatants were found to induce low levels of IgE synthesis. This IgE-inducing effect was mediated by naturally produced IL-4 and blocked by IFN-γ that was produced simultaneously by these T-cell clones upon activation by alloantigen.

MATERIALS AND METHODS

Reagents

IL-2 and IL-5 (5 μg/ml) were provided by Drs. R. Kastelein and J. Wideman (DNAX Research Institute, Palo Alto, CA). Recombinant IL-4 (specific activity 10^7 U/mg) and IFN-γ (specific activity 10^7 IU/mg) were a gift from Drs. P. Trotta and S. Nagabhushan (Schering Corporation, Bloomfield, NJ). The mAb SPV-T3b (anti-CD3) was described previously [15]. The mAbs NKH-1, T3 (anti-CD3), T11 (anti-CD2), T4 (anti-CD4), and T8 (anti-CD8) were purchased from Coulter (Hialeah, FL). The mAb Leu M3 (anti-CD14) that detects monocytes and the mAbs B1 (anti-CD20) and B2 (anti-CD21) were obtained from Becton and Dickinson (Mountain View, CA) and Coulter, respectively. The mAb 25, directed against the low-affinity receptor for the Fc part or IgE (FcϵRII, CD23), was described elsewere [16]. The polyclonal anti-IFN-γ antiserum was raised in a rabbit. The immunoglobulin fraction was purified by affinity chromatography on protein-A columns according to the instructions of the manufacturer (Bio-Rad, Richmond, CA). This antiserum blocks the induction of class I and class II MHC antigens on human melanoma cells by recombinant IFN-γ and neutralizes the inhibitory effects of IFN-γ on the cytopathic effects of encephalomyocardites virus. The anti-IFN-γ antiserum is specific for IFN-γ and does not react with recombinant IL-1 α and β, IL-2, IL-3, IL-4, IL-5, GM-CSF, and IFN-α as determined in ELISAs. The rabbit anti-IL-4 antibody was purified as described above. This antiserum is specific for IL-4. It binds with IL-4 in solution and blocks IL-4-induced T- and B-cell proliferation and IL-4-induced expression of CD23 on the Burkitt lymphoma cell line Jijoye. Fluorescein-conjugated F(ab')2 goat anti-mouse Ig was purchased from Grub (Vienna, Austria). Cycloheximide was obtained from Sigma (St Louis, MO).

B- and T-Cell Purification

Peripheral blood mononuclear cells (PMNC) and tonsil cells were purified as described elsewhere [17,18]. Spleens were removed from healthy individuals because of trauma. Spleen B cells were purified by removing the adherent cells by adherence to plastic culture flasks (75 cm^2, Falcon, Oxnard, CA). The adherent cells (> 90%

monocytes as judged by their staining with the anti-CD14 mAb) were collected and used for reconstitution experiments. The nonadherent cells were collected, and the B cells were obtained by two rounds of depletion of sheep erythrocyte (Esh) rosette-forming cells. B cells were further enriched by depletion of contaminating T cells and NK cells by one round of reversed rosetting using anti-CD3, anti-CD2, and NKH-1 mAbs and magnetic beads (Dynabeads M450, Dynal, Skøyen, Norway) coated with goat anti-mouse IgG. The rosette-forming cells were depleted with a magnet. The purified B cells, containing 90% $CD20^+$ cells and < 1% $CD3^+$ cells, were collected, washed three times with culture medium, and used for antibody production. The rosette-forming T-cells were collected, and after the Esh were lysed, the T cells were washed three times in culture medium. B cells, B cells plus $CD4^+$ T cells, or B cells plus $CD8^+$ T cells were depleted by one round of reversed rosetting with anti-CD4, anti-CD8 (T4 or T8) mAbs, and anti-B cell mAbs (B1 and B2) and human erythrocytes conjugated with anti-murine Ig (Tago, Burlingame, CA), followed by one round of reversed rosetting with the anti-CD4, anti-CD8, and anti-B cells mAbs and magnetic beads coated with goat anti-mouse IgG. The remaining T cells, $CD4^+$ (depleted of $CD8^+$ cells) and $CD8^+$ (depleted of $CD4^+$ cells) T-cell subpopulations, were collected, washed three times with culture medium, and used for the reconstitution experiments. $CD4^+$ and $CD8^+$ T-cell populations were more than 95% $CD3^+$ as judged by staining with anti-CD3 mAb. The $CD4^+$ subset contained 7% $CD8^+$ cells, whereas the $CD8^+$ subset contained 8% $CD4^+$ cells.

T-Cell Clones and T-Cell Clone Supernatants

PMNC from healthy donor FA and hyper-IgE patient BG with serum IgE levels of 30,000 IU/ml were isolated by centrifugation over Ficoll/Hypaque and stimulated in a mixed leukocyte culture (MLC) with irradiated (5,000 rad) cells of the EBV-transformed B-cell lines JY or UD53. T-cell clones were obtained as described in detail elsewhere [19,20]. All cultures were carried out in RPMI-1640 supplemented with 10% fetal calf serum (FCS), penicillin (100 IU/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM), all obtained from Flow Laboratories (Irvine, Scotland). Four alloreactive T-cell clones were selected. Two clones derived from donor FA (FA-10 and FA-28) proliferated specifically in response to JY, whereas the clones from donor BG (BG-24 and BG-39) proliferated in response to UD53. The T-cell clones (10^6) were stimulated with 10^5 irradiated JY or UD53 cells in 1 ml culture medium in 24-well tissue culture plates (Linbro, Flow Laboratories, McLean, VA). Supernatants were collected after 4 days of incubation at 37°C and 5% CO_2.

IgE Production

Induction of IgE production by recombinant IL-4 was measured in cultures in which 5×10^5 peripheral blood lymphocytes (PBL), tonsil cells, or spleen cells partially depleted of adherent cells or highly purified spleen B cells were incubated with various concentrations of recombinant IL-4 or T-cell clone supernatants (20% v/v). The cultures were carried out in 1 ml culture medium in 2-ml tissue culture wells (Linbro) for 9 days at 37°C and 5% CO_2. After this culture period, supernatants were collected and tested for the presence of IgE (and IgG, IgA, and IgM, see below). Where indicated, recombinant IL-4 or supernatants of the T-cell clones were tested in the presence of the polyclonal anti-IFN-γ antiserum or of the polyclonal anti-IL-4 antiserum. Preimmune rabbit sera were used as control sera.

ELISAs for IgE, IgG, IgA, and IgM

IgE production was measured in a sandwich ELISA as described elsewhere [17]. The limit of the sensitivity of the assay was 150 pg IgE/ml. The production of IgG, IgA, and IgM were measured in standard ELISAs as described previously [17]. The sensitivities of the assays were 1 ng/ml, 4 ng/ml, and 2 ng/ml for IgG, IgA, and IgM, respectively.

Detection of IL-4, IL-2, and IFN-γ

IL-4 was measured in an ELISA as described previously [21]. The polyclonal anti-IL-4 antiserum was used as a catcher antibody. An anti-IL-4 mAb (11 B4, IgG 2A) was obtained after immunizing rats with recombinant IL-4 purified from supernatants of Cos-7 cells transfected with the cDNA clone encoding human IL-4. A peroxidase conjugated goat anti-rat Ig (Tago) was used for labeling, and ABTS (2,2′ Azinobis-3-ethyl-benzthiazoline sulfonic acid, Sigma) was used as substrate. Optical densities were measured with a Dynatech MR 580 Autoreader. The sensitivity of the assay was 0.1 ng IL-4/ml.

IFN-γ was measured in a sandwich ELISA in which two monoclonal anti-IFN-γ antibodies detecting different epitopes on the molecule were used as catcher and tracer antibodies, respectively, as described previously [21]. The sensitivity of this assay was 0.2 ng IFN-γ/ml. IL-2 activity was measured by the proliferation-inducing activity of the supernatants on the murine CTLL cell line as described previously [21].

Detection of s-CD23

Soluble CD23 was determined in a radioimmunoassay, as described previously [22]. In this assay, the capacity of s-CD23 to block the binding of ^{121}I-labeled mAb 25 to the EBV-transformed B-cell line RPMI-8866 was determined.

Fractionation of s-CD23

Fractionation of s-CD23 from 50-fold concentrated culture supernatants of RPMI-8866 was carried out by means of Sephadex G100 columns as described elsewhere [22].

RESULTS

IL-4 added to PBL, tonsil cell, or spleen cell populations obtained from different healthy donors induced IgE synthesis in a dose-dependent fashion (Fig. 1). Significant IgE production was already observed at IL-4 concentrations of 10–20 U/ml, whereas maximal IgE production plateaued at IL-4 concentrations of 200–300 U/ml. IL-4-induced IgE synthesis was specific for IL-4 since IgE production by IL-4 was completely blocked by a polyclonal anti-IL-4 antiserum. A control preimmune rabbit serum was ineffective (not shown). In the presence of cycloheximide (50μg/ml), no IgE production was observed, indicating that IL-4 induced de novo IgE synthesis. IL-4 added to highly purified spleen B cells containing $< 1\%$ $CD3^+$ T cells and $< 1\%$ monocytes failed to induce IgE production (Table I). Reconstitution with different concentrations of autologous T cells resulted in the induction of IgE synthesis in a dose-dependent manner. IgE synthesis was further increased when also 10% autolo-

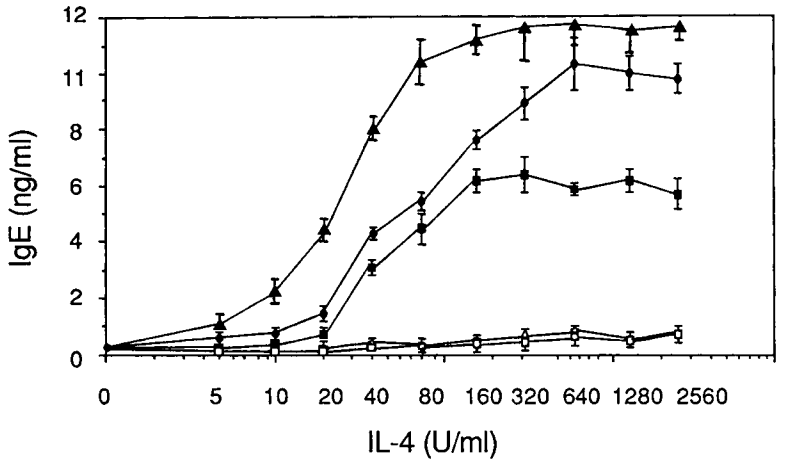

Fig. 1. Dose response and specificity of IL-4-induced IgE production by PMNC and tonsil and spleen cells. IL-4 was purified from Cos-7 cell transfection supernatants. PMNC contained 9% $CD20^+$, 83% $CD3^+$, and 4% $CD14^+$ cells. Tonsil cells comprised 63% $CD20^+$, 30% $CD3^+$, and 3% $CD14^+$ cells. Spleen cells contained 62% $CD20^+$, 34% $CD3^+$, and 3% $CD14^+$ cells. The anti-IL-4 antiserum was added at a final concentration of 1:500. PMNC (●); PMNC + anti-IL-4 (○); tonsil cells (■); tonsil cells + anti-IL-4 (□); spleen cells (▲).

TABLE I. Induction of IgE Synthesis by IL-4 Is Indirect and Requires T Cells and Monocytes

	IgE production (ng/ml)
Experiment 1	
Spleen cells	
(65% $CD20^+$, 35% $CD3^+$, 4% $CD14^+$)	25.2
B cells	
(90% $CD20^+$, < 1% $CD3^+$, < 1% $CD14^+$)	0.5
B cells + T cells (10:1)	9.7
+ T cells (3:1)	18.0
+ T cells (1:1)	22.6
+ $CD4^+$ T cells (1:1)	30.1
+ $CD8^+$ T cells (1:1)	0.4
+ T cells (1:1) + MON (10%)	36.2
Experiment 2	
Spleen cells	
(50% $CD20^+$, 35% $CD3^+$, 2% $CD14^+$)	7.5
B cells	
(98% $CD20^+$, < 1% $CD3^+$, < 1% $CD14^+$)	< 0.3
B cells + $CD4^+$ T cells (3:1)	3.2
+ $CD4^+$ T cells (1:1)	4.6
+ $CD4^+$ T cells (0.5:1)	6.8
+ $CD8^+$ T cells (3:1)	< 0.3
+ $CD8^+$ T cells (1:1)	< 0.3
+ $CD8^+$ T cells (0.5:1)	< 0.3

IL-4 was added at a concentration of 200 U/ml. T cells contained 91% $CD3^+$, 66% $CD4^+$, and 31 % $CD8^+$ cells. Monocytes (MON) consisted of 93% $CD14^+$ cells.

gous monocytes were present. These results indicate that although IL-4 specifically induces IgE production by normal B cells, the effects of IL-4 are insufficient and require T cells and monocytes.

In order to determine whether autologous $CD4^+$ or $CD8^+$ T cells were necessary for IL-4-induced IgE production, reconstitution experiments were carried out with $CD4^+$ or $CD8^+$ T cells. In Table I, it is shown that in the presence of $CD4^+$ T cells, IL-4-induced IgE production. In contrast, in the presence of $CD8^+$ T cells, no IgE synthesis was obtained. These results indicate that the IL-4-induced IgE production requires $CD4^+$ T cells. Whether the $CD8^+$ T cells suppress IL-4 induced IgE production mediated by $CD4^+$ T cells is presently under investigation.

We demonstrated previously that IL-4 induces the expression of CD23 on normal and malignant B cells and that this effect was antagonized by IFN-γ, indicating that IFN-γ can act as an antagonist of IL-4 [5,6]. In Figure 2, it is shown that IFN-γ blocked IL-4-induced IgE production in a dose-dependent way and that complete inhibition of IgE synthesis was obtained at IFN-γ concentrations of 100 U/ml. Inhibition of IL-4-induced IgE production was not specific for IFN-γ, since also IFN-α and, to a lesser extent, PGE-2 blocked IL-4-induced IgE synthesis. Collectively, these results indicate that IFN-γ, IFN-α, and PGE-2 that have been shown to block IL-4-induced CD23 expression also block IL-4-induced IgE production, suggesting that there is a correlation between CD23 expression and IgE production.

IL-4 induces not only expression of CD23 on the membrane of normal B cells, but it also induces the subsequent release of soluble CD23 [22]. The notion that either cell-bound CD23 or s-CD23 is associated with IL-4-induced IgE production was supported by blocking studies with the anti-CD23 mAb 25 (IgG1). F(ab)2 fragments of mAb 25 blocked IL-4-induced IgE production in a dose-dependent way (Table II). At concentrations of 25 μg/ml, complete inhibition of IgE synthesis was obtained, whereas the isotype control mAb AMF-7 (IgG1) that is directed against a human melanoma-associated antigen and purified mouse IgG and mouse IgM were ineffec-

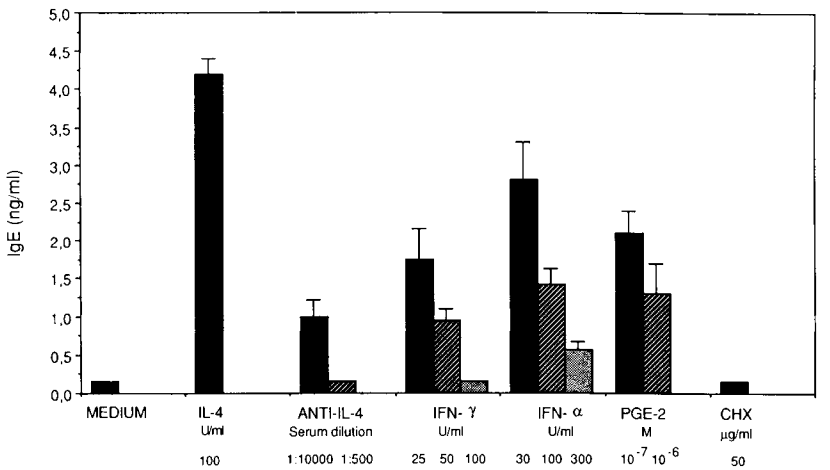

Fig. 2. Induction of IgE production by normal PMNC by IL-4 and its inhibition by anti-IL-4 antibodies, IFN-γ, IFN-α, and PGE-2. PMNC enriched for B cells contained 38% $CD20^+$, 50% $CD3^+$, and 4% $CD14^+$ cells. Black, striped and checked bars represent IgE production in the presence of different dilutions of the factors or molecules indicated.

TABLE II. Effects of mAb 25 on IL-4-Induced IgE Production

		IgE production (ng/ml)	
	Antibody added	Exp. 1	Exp. 2
Medium	—	⩽ 0.15	⩽ 0.15
IL-4	—	21.4 ± 0.5	14.3 ± 2.3
	+ mAb 25 (25 μg/ml)	⩽ 0.15	⩽ 0.15
	+ mAb 25 (10 μg/ml)	6.4 ± 1.9	2.8 ± 1
	+ mAb 25 (1 μg/ml)	15.3 ± 2.3	10.7 ± 3.1
	+ AMF-7	21.7 ± 1.2	15.9 ± 1.8
	+ SPV-L-3	28.4 ± 0.7	NT
	+ IgM	21.2 ± 0.9	15.6 ± 1.5
	+ IgG	20.3 ± 1	14.9 ± 2

IL-4 was added at a concentration of 100 U/ml. $F(ab')_2$ fragments of mAb 25 were used. AMF-7, SPV-L-3 (anti-HLA-DQ), and purified murine IgM and IgG were used at concentrations of 25 μg/ml.

TABLE III. Production of IL-4, IL-2, and IFN-γ by T-Cell Clones After Alloantigen Activation

T-cell clones	IL-4 (pg/ml)	IL-2 (IU/ml)	IFN-γ (pg/ml)
FA-10	3,440	18	640
FA-28	1,925	10	4,700
BG-24	840	14	< 200
BG-39	1,990	6	2,500

Clones FA-10, FA-28, and BG-39 are $CD4^+$, whereas BG-24 is $CD8^+$. Supernatants of the T-cell clones were collected 5 days after activation by the allospecific stimulator cells.

tive. Also, the mAb SPV-L3 that reacts with HLA-DQ antigens expressed on B cells did not block IgE production. These results indicate that membrane CD23 or s-CD23 is associated with IL-4-induced IgE production.

Having established that recombinant IL-4 induces IgE production by normal human B cells, we investigated whether supernatants of alloreactive human T-cell clones containing IL-4 could induce IgE synthesis by normal B cells. Four clones, FA-10, FA-28, BG-24, and BG-39, were selected. These clones produced variable concentrations of IL-4 after activation by their specific alloantigens (Table III). In addition, variable concentrations of IFN-γ and IL-2 were produced simultaneously. FA-28 and BG-39 were relatively strong IFN-γ producers (4,700 and 2,500 pg/ml, respectively), whereas FA-10 synthesized 640 pg/ml. BG-24 produced levels of IFN-γ that were below the sensitivity of our ELISA (< 200 pg/ml).

Supernatants of FA-10 and BG-24 induced a low degree of IgE production by PBL of normal donors, whereas supernatants of FA-28 and BG-39 were ineffective. However, as demonstrated in the experiment shown in Table IV, in the presence of a polyclonal anti-IFN-γ antiserum, IgE production induced by supernatants of FA-10 and BG-24 was considerably enhanced, whereas the supernatants of FA-28 and BG-39 also induced significant levels of IgE. IgE production induced by the supernatant of BG-24 in the presence of anti-IFN-γ antibodies was completely blocked by the anti-IL-4 antiserum (Fig. 3). In contrast, the IgG, IgM, and IgA production induced by the supernatant of BG-24 was not affected by the anti-IL-4 antiserum (Fig. 3).

These results indicate that naturally produced IL-4 is responsible for the induction of IgE synthesis by normal B cells, but that final IgE production is the net result of the inducing effects of IL-4 and the suppressive effects of IFN-γ that is produced simultaneously upon alloactivation.

TABLE IV. Production of IgE by Enriched B Cells Induced by Supernatants of Alloantigen-Activated T-Cell Clones in the Presence of Anti-IFN-γ Antibodies

T-cell clone supernatant (20% v/v)	IgE production (ng/ml)	
		+ anti-IFN-γ
—	⩽ 0.15	⩽ 0.15
FA-10	0.42 ± 0.02*	6.44 ± 0.09**
FA-28	⩽ 0.15	1.84 ± 0.11**
BG-24	2.63 ± 0.4**	7.8 ± 0.12**
BG-39	⩽ 0.15	1.38 ± 0.03**

T-cell clone supernatants were collected 5 days after activation with the irradiated allospecific stimulator cells. Enriched B cells contained 35% $CD20^+$, 52% $CD3^+$, and 6% $CD14^+$ cells. Anti-IFN-γ antiserum was added at a dilution of 1:150. The preimmune rabbit serum used as control (1:150 diluted) was ineffective (not shown).

*$P \leqslant .05$ as determined by Student's t-test.

**$P \leqslant .01$ as determined by Student's t-test.

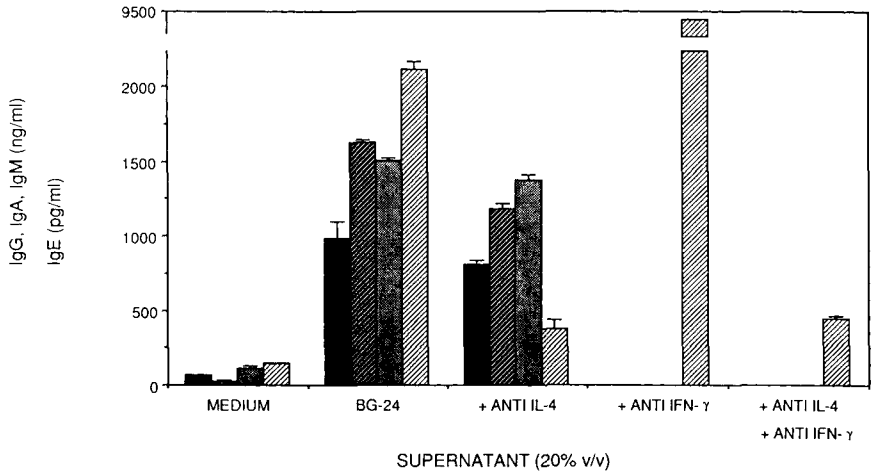

Fig. 3. Induction of IgE production by supernatants of BG-24 in the presence or absence of anti-IFN-γ antibodies is blocked by anti-IL-4 antibodies. Supernatant of BG-24 was added at a concentration of 15% (v/v). The anti-IL-4 antiserum was added at a dilution of 1:500, and the anti-IFN-γ antiserum, at a dilution of 1:150. Fifteen percent supernatant of BG-24 contained no detectable IgE, 8 ng/ml IgG, ⩽4 ng/ml IgM, and 4 ng/ml IgA. IgG (■); IgM (▨); IgA (▩); IgE (▨).

IL-5 added to PBL was ineffective in inducing IgE production, but in the presence of IL-4, IL-5 enhanced IgE synthesis in a dose-dependent fashion. One out several experiments is shown in Figure 4A. Augmentation of IgE production was observed only when IL-4 was present at nonsaturating concentrations. At saturating concentrations of IL-4, IL-5 was ineffective. In addition, it is shown that in these assays, IL-5 strongly enhanced the release of s-CD23 (IgE-BF) in the presence of suboptimal concentrations of IL-4, whereas no increase in s-CD23 release was observed at saturating concentrations of IL-4 (Fig. 4B). IL-5 tested alone did not induce s-CD23 release. IgE production and s-CD23 release in the presence of IL-4 or combinations of IL-4 and IL-5 were blocked by IFN-γ. Taken together, these data indicate that IL-5 enhances IL-4-induced IgE synthesis and there is a quantitative

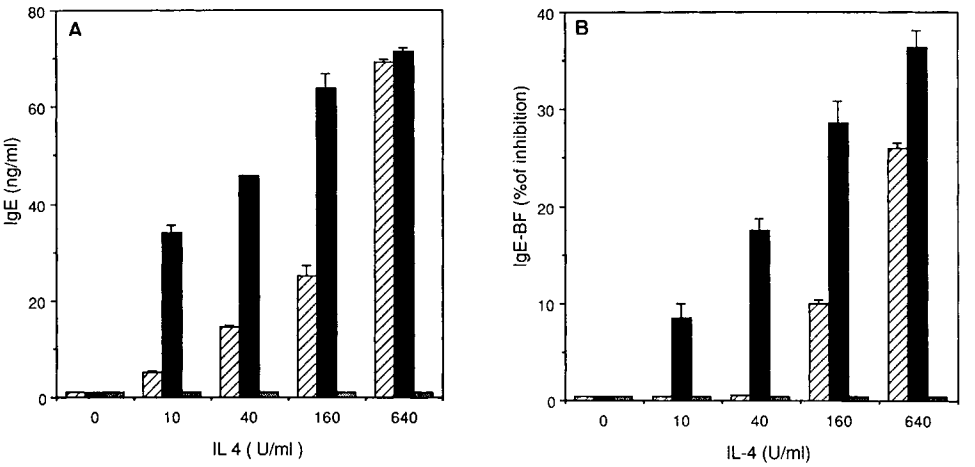

Fig. 4. IL-5 enhances IL-4-induced IgE production and s-CD23 release. PMNC contained 7% $CD20^+$, 85% $CD3^+$, and 4% $CD14^+$ cells. IL-5 (total protein concentration was estimated at 5 μg/ml) was added at a final concentration of 0.25%. **A,** IgE synthesis; **B,** s-CD23 was measured in the supernatant of the culture after a tenfold concentration. IL-4 (▨); Il-4 + Il-5 (■); IL-4 + Il-5 + IFN-γ (100 U/ml).

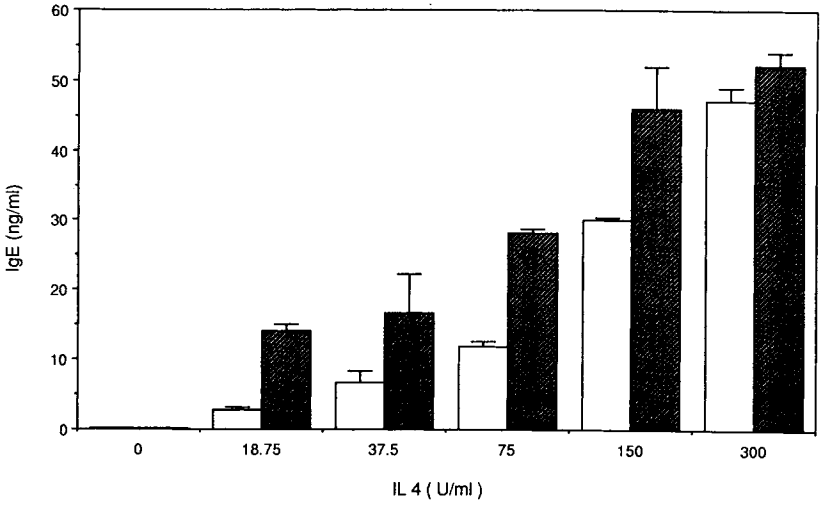

Fig. 5. The effect of s-CD23 on Il-4-induced IgE synthesis. PMNC contained 13% $CD20^+$, 78% $CD3^+$, and 5% $CD14^+$ cells. IL-4 (□); IL-4 + s-CD23 added at concentration of 0.5% (v/v)(■).

correlation between the release of s-CD23 and IgE production in the presence of IL-4 or combinations of IL-4 and IL-5.

In order to test the role of s-CD23 more precisely, s-CD23 fractionated from supernatants of RPMI-8866 was tested on its capacity to induce IgE synthesis. s-CD23 was ineffective in inducing IgE production when tested alone (Fig. 5), indicating that s-CD23 cannot replace IL-4. However, s-CD23 enhanced IgE production in the presence of IL-4, provided IL-4 was present at suboptimal concentrations. At saturating concentrations of IL-4, s-CD23 was ineffective.

DISCUSSION

In the present study, we demonstrate that both recombinant and naturally produced IL-4 present in supernatants of activated T-cell clones can induce IgE production by B cells from healthy donors. IgE induction was specific for IL-4, because it was completely blocked by an anti-IL-4 antiserum. Although the anti-IL-4 antiserum blocked IL-4-induced IgE production, the IgE inducing effect of IL-4 was indirect, since it required both T cells and monocytes. Interestingly, reconstitution experiments indicated that IgE synthesis occurred only in the presence of autologous $CD4^+$ T cells, whereas IL-4 was unable to induce IgE production in the presence of $CD8^+$ T cells. The finding that $CD4^+$ T cells are required for the induction of IgE production, whereas $CD8^+$ T cells are not, is consistent with recent data reported by Ohta et al., who showed that $CD4^+$ T cells enhanced pokeweed mitogen (PWM) induced IgE synthesis by PBL of atopic individuals, whereas $CD8^+$ T cells were suppressive [23]. The actual role of the $CD4^+$ and $CD8^+$ T cells is currently investigated, but since we demonstrated that IL-4 activates T cells [2] and monocytes [24], it seems not unlikely that $CD4^+$ or $CD8^+$ T cells upon activation by IL-4 could produce factors that either enhance or suppress IgE synthesis similarly as has been described for murine models [25].

IL-4-induced IgE production was completely blocked by IFN-γ and IFN-α, and, to a lesser extent, by PGE-2. These factors were also found to inhibit IL-4-induced induction of CD23 on normal and malignant B cells [4–6], indicating that they act as antagonists of IL-4. In contrast, IL-5 that was ineffective in inducing IgE production when tested alone enhanced IL-4-induced s-CD23 release and IgE synthesis particularly at suboptimal IL-4 concentrations. These data show that there is a correlation among induction of CD23 expression on B cells, the subsequent release of s-CD23, and IgE production by IL-4 or combinations of IL-4 and IL-5.

Further analysis of the role of s-CD23 showed that s-CD23 fractionated from supernatants of RPMI-8866 did not induce IgE production, but s-CD23 enhanced IgE production induced by suboptimal concentrations of IL-4. These data indicate that s-CD23 modulates IL-4-induced IgE synthesis, but cannot replace IL-4. Furthermore, it is important to note that despite the fact that IL-4 induces s-CD23 release from highly purified B cells [22], this does not result in IgE production. It is demonstrated here that in order to obtain optimal IgE synthesis, autologous $CD4^+$ T cells and monocytes are required, indicating that the effect of s-CD23 is indirect. The notion that s-CD23 is associated with IgE production is also supported by the finding that the anti-CD23 mAb 25 (which reacts with s-CD23) blocked IL-4-induced IgE synthesis in dose-dependent fashion.

Supernatants of the $CD4^+$ alloreactive T-cell clones FA-10, FA-28, and BG-39 and $CD8^+$ T-cell clone BG-24 were found to contain variable concentrations of IL-4 upon activation by the relevant alloantigenic stimulus. In addition, all four T-cell clones produced IFN-γ simultaneously, although BG-24 was a low producer. Despite the presence of IL-4, supernatants of FA-10 and BG-24 induced a low degree of IgE synthesis, whereas supernatants of FA-28 and BG-39 were completely negative. In addition, supernatants of all four T-cell clones induced variable amounts of IgG, IgM, and IgA, indicating that they contained B-cell growth and differentiation factors inducing the synthesis of these isotypes. The failure to induce IgE synthesis was due to the presence of IFN-γ, since considerable IgE production was induced by all four

supernatants in presence of anti-IFN-γ antibodies. Finally, IgE production induced by supernatant of BG-24 in the presence of anti-IFN-γ antibodies was completely blocked by the anti-IL-4 antiserum, whereas the induction of IgG, IgM, and IgA synthesis induced by this supernatant was not significantly affected. These results indicate that IL-4 is the sole inducer of IgE synthesis.

All four T-cell clones tested here produced, in addition to IL-4 and IFN-γ, also IL-2 simultaneously. This is in contrast with recent data obtained with murine T-cell clones indicating the existence of two types of $CD4^+$ helper T-cell clones, which can be distinguished on the basis of the type of lymphokines they produce upon activation [26]. Type 1 helper T cells were found to produce IL-2, IFN-γ, and lymphotoxin simultaneously, whereas type 2 helper T cells produce exclusively IL-4 and IL-5. It is clear that the four allospecific T-cell clones tested here do not fall in these categories. Furthermore, our results indicate also that the $CD8^+$ T-cell clone BG-24 can produce IL-4. These results are in line with another study of our laboratory that indicated that 21/22 $CD4^+$ and 6/23 $CD8^+$ T-cell clones produced IL-2, IL-4, and IFN-γ simultaneously [21]. Whether these differences are species related or are due to different cloning, culture, or immunization conditions is not clear yet.

Collectively our data indicate that the relative activities of IL-4 and IFN-γ regulate the amplitude of the IgE response in vitro. Whether these lymphokines are also operational in vivo remains to be determined. However, recently it has been shown that murine IL-4 and IFN-γ, analogous to their effects in vitro, also regulate IgE synthesis in murine models in vivo [27,28]. Elevated IgE levels in mice infected with *Nippostrongylus brasiliensis* or injected with anti-IgD antibodies were specifically inhibited by administration of an anti-IL-4 mAb [27] or by IFN-γ [28]. Therefore, it is tempting to speculate that the enhanced IgE levels observed in atopic individuals or patients suffering of helminthic infections are related to an increased IL-4 and/or a decreased IFN-γ production.

ACKNOWLEDGMENTS

We like to thank Drs. K. Arai, N. Arai, T. Yokota, and H. Spits for their support, Miss C. Peronne for technical assistance, and Mrs. N. Courbière for secretarial help.

REFERENCES

1. Defrance T, Vanbervliet B, Aubry J P, Takebe Y, Arai N, Miyajima A, Yokota T, Lee F, Arai K, de Vries JE, Banchereau J: J Immunol 139:1135, 1987.
2. Spits H, Yssel H, Takebe Y, Arai N, Yokota T, Lee F, Arai K, Banchereau J, de Vries JE: J Immunol 139:1142, 1987.
3. Spits H, Yssel H, Paliard X, Kastelein R, Figdor GC, de Vries JE: J Immunol 141:29, 1988.
4. Kikutani H, Inui S, Sato R, Barsumian EL, Owaki H, Yamasaki K, Kalaho, Uchibayashi N, Hardy RR, Hirano T, Taimasawa S, Sakiyama F, Suemura M, Kishimoto T: Cell 47:657, 1986.
5. Defrance T, Aubry JP, Rousset F, Vanbervliet B, Bonnefoy JY, Arai N, Takebe Y, Lee F, Arai K, de Vries JE, Banchereau J: J Exp Med 165:1459, 1987.
6. Rousset F, de Waal Malefijt R, Slierendregt B, Aubry JP, Defrance T, Banchereau J, de Vries JE: J Immunol 140:2625, 1988.
7. Sarfati M, Rector E, Wong M, Rubio-Trujillo M, Sehon AH, Delespesse G: Immunology 53:197, 1984.
8. Sarfati M, Rector E, Wong M, Rubio-Trujillo M, Sehon AH, Delespesse G: Immunology 53:207, 1984.

9. Coffman RL, Ohara J, Bond MW, Carty J, Zlotnick A, Paul WE: J Immunol 136:4538, 1986.
10. Snapper CM, Finkelman FD, Paul WE: J Exp Med 167:183, 1988.
11. Saryan JA, Leung DYM, Geha RS: J Immunol 130:242, 1983.
12. Romagnani S, Maggi E, Delprete GF, Ricci M: Clin Exp Immunol 52:85, 1983.
13. Leung DYM, Young MC, Wood N, Geha RS: J Exp Med 163:713, 1986.
14. Nutman TD, Volkman J, Hussain R, Fauci AS, Ottesen E: J Immunol 134:1175, 1985.
15. Spits H, Keizer G, Borst J, Terhorst C, Hekman A, de Vries JE: Hybridoma 2: 423, 1983.
16. Bonnefoy JY, Aubry JP, Peronne C, Wijdenes J, Banchereau J: J Immunol 138:2970, 1987.
17. Pène J, Rousset F, Brière F, Chrétien I, Bonnefoy JY, Spits H, Yokota T, Arai N, Arai K, Banchereau J, de Vries JE: Proc Natl Acad Sci USA 85:6880, 1988.
18. Pène J, Rousset F, Brière F, Chrétien I, Bonnefoy JY, de Vries JE: Eur J Immunol 18:929, 1988.
19. Spits H, Yssel H, Terhorst C, de Vries JE: J Immunol 128:95, 1982.
20. Spits H, Yssel H,Thompson A, de Vries JE: J Immunol 131:131, 1983.
21. Paliard X, de Waal Malefijt R, Yssel H, Blanchard D, Chrétien I, Abrams J, de Vries JE, Spits H: J Immunol 141:849, 1988.
22. Bonnefoy JY, Defrance T, Peronne C, Ménétrier C, Rousset F, Pène J, de Vries JE, Banchereau J: Eur J Immunol 18:117, 1988.
23. Ohta K, Rozzo SJ, Hayward A, Harbeck RJ, Kirkpatrick CH: J Allergy Clin Immunol 81:401, 1988.
24. Te Velde AA, Klomp J, Yard BA, de Vries JE, Figdor CG: J Immunol 140:1548, 1988.
25. Ishizaka K: Annu Rev Immunol 2:159, 1984.
26. Mosmann TR, Cherwinski H, Bond BW, Giedlin M, Coffman RL: J Immunol 136:2348, 1986.
27. Finkelman FD, Katona IM, Urban J, Snapper CM, Ohara J, Paul WE: Proc Natl Acad Sci USA 83:9675, 1986.
28. Finkelman FD, Katona IM, Mosmann T, Coffman RL: J Immunol 140:1022, 1988.