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# IgE secretion by Epstein–Barr virus-infected purified human B lymphocytes is stimulated by interleukin 4 and suppressed by interferon γ

**(allergy/polyclonal B-cell activation/immunoglobulin isotypes/class switching)**

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**ABSTRACT** **The cytokine interleukin 4 (IL-4) has been shown to induce lipopolysaccharide-activated murine B cells to differentiate into IgE-secreting cells and to stimulate IgE secretion by cultured human peripheral blood lymphoid cells. It is unclear, however, whether this effect of IL-4 on human peripheral blood lymphoid cells is a direct effect on the B cell because IL-4 can stimulate T cells and monocytes as well as B cells and does not induce purified human B cells to secrete immunoglobulin. To investigate this issue we studied the ability of IL-4 to induce IgE secretion by purified human B cells (93–96% CD20⁺, <1% CD3⁺) that were cultured with Epstein–Barr virus (EBV). Although B cells cultured with IL-4 alone did not secrete Ig and B cells cultured with EBV alone secreted IgM, IgG, and IgA but <150 pg of IgE per ml, the combination of EBV and IL-4 induced an IgE response that ranged from 11.4 to 40.3 ng/ml of culture supernatant after 26 days of culture. While IL-4 also enhanced IgM, IgG, and IgA secretion, as well as proliferation by EBV-infected B cells, these effects were less pronounced, occurred earlier during culture, and required a lower concentration of IL-4 than did the stimulation of IgE secretion. Furthermore, interferon γ at 10 units per ml was found to inhibit IL-4/EBV-induced IgE secretion without inhibiting the other stimulatory effects of IL-4. We conclude that (*i*) IL-4 and interferon γ can act directly on polyclonally activated human B cells to respectively stimulate and suppress IgE secretion and (*ii*) IL-4, in addition to its specific effect on IgE secretion, has a general stimulatory effect on the growth and differentiation of EBV-infected human B cells.**

Because IgE plays a critical role in the stimulation of mast cell degranulation and the generation of allergic symptoms that result from this process (1–3), recent evidence that two cytokines regulate IgE secretion may have clinical importance. Studies in the mouse have demonstrated that (*i*) interleukin 4 (IL-4) can induce purified, lipopolysaccharide-activated B lymphocytes to secrete IgE in the absence of other T cell stimuli (4); (*ii*) IL-4 is required for T-cell-dependent induction of IgE secretion both *in vitro* and *in vivo* (5–7); (*iii*) IL-4 induces transcription of a germ-line ε chain transcript (8); and (*iv*) interferon γ (IFN-γ) specifically inhibits the differentiation of B lymphocytes into IgE-secreting cells (9–11). *In vitro* studies with human lymphocytes support the possibility that these two cytokines also regulate human IgE secretion: (*i*) Culture of human peripheral blood cells with IL-4 induces an IgE response, which can be inhibited by the addition of IFN-γ to the culture system (12, 13); (*ii*) culture of purified human B cells with cloned helper T cells induces an IgE response that is directly proportional to the quantity of IL-4 produced by the T cells and inversely proportional to the quantity of IFN-γ produced by these cells (14). However, because all these studies with human cells required the presence of T lymphocytes and monocytes to induce an IgE response and because both IL-4 and IFN-γ can affect the growth of T as well as B lymphocytes (15–18), these studies do not distinguish whether IL-4 and IFN-γ affect IgE secretion directly at the B-cell level or indirectly, by inducing T cells to produce additional cytokines that can stimulate or suppress IgE secretion. Studies in which anti-Ig antibodies, protein A-bearing *Staphylococcus aureus*, or bacterial endotoxin were used to stimulate human B lymphocytes showed no clear direct stimulatory role for IL-4 in inducing an IgE response (19); indeed, interleukin 2 (IL-2), in the absence of IL-4, has been reported to induce purified B cells cultured with *S. aureus* to secrete IgE, and IL-4 has been found to inhibit *S. aureus* plus IL-2-induced secretion of all Ig isotypes, including IgE (20, 21).

To investigate whether IL-4 and IFN-γ can act directly on human B lymphocytes to regulate IgE secretion we have studied a system in which the infection of highly purified normal human peripheral blood B lymphocytes with Epstein–Barr virus (EBV) induces these cells to proliferate and to secrete IgM, IgG, and IgA, but not detectable IgE. Although addition of IL-4 to the culture system enhances cell growth and production of all Ig isotypes, we find that IgE production is enhanced much more than that of the other isotypes. Addition of IFN-γ along with IL-4 to the EBV-stimulated cultures inhibits IgE production without affecting production of the other Ig isotypes.

## MATERIALS AND METHODS

**B-Cell Purification.** Peripheral blood mononuclear cells were obtained by leukopheresis of five healthy normal donors and depleted of polymorphonuclear leukocytes and erythrocytes by centrifugation over a layer of Ficoll/Hypaque. Lymphocytes were partially depleted of macrophages by fractionation in Sepracell-MN according to the manufacturer's instructions (Sepratech, Oklahoma City, OK), then incubated for 45 min with a mixture of mouse monoclonal antibodies 35.1, 64.1, G17.2.8, G10-1, G3-7, FC2.2, and 60.1 (gift of J. A. Ledbetter, Oncogene, Seattle, WA), which are specific for surface markers CD2, CD3, CD4, CD8 (present on T cells), CD7, CD16, and CD11 (present on macrophages

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Abbreviations: IL, interleukin; EBV, Epstein–Barr virus; IFN-γ, interferon γ; cIg, intracytoplasmic immunoglobulin; FITC, fluorescein isothiocyanate.
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and/or natural killer cells), respectively (22–25). The cells were then washed and incubated for 1 hr at 4°C with iron-containing beads conjugated with goat anti-mouse IgG antibody (Dynal, Great Neck, NY) at a ratio of four beads per cell. Cells bound to beads and free beads were removed magnetically from the cell suspension, after which the remaining cells were washed twice before being placed in culture. Immunofluorescence staining, coupled with fluorescence-activated cell sorter analysis, demonstrated that 93–96% of the resulting cell population bore the B-cell-specific surface marker B1 (Coulter), whereas cells that were stained by fluorescein-labeled antibodies specific for T-cell, macrophage, or natural killer-specific markers (Leu 4, Leu M3, Leu 7, and Leu 11; Becton Dickinson) were not detectable (<1%). The absence of cells stained by these fluorescein-labeled antibodies cannot be accounted for by blocking of antigenic determinants with unlabeled monoclonal antibodies because no cells were stained detectably by a fluorescein-labeled anti-mouse Ig antibody.

**Analysis of Ig Secretion.** Ig levels in supernatants of cultured cells were analyzed by ELISA in 96-well flat-bottomed polystyrene microtiter plates (Dynatech). To assay IgE levels, wells were coated overnight with goat anti-human IgE antibody at 10 $\mu$g/ml ($\epsilon$ chain-specific, Cappel Laboratories) in 0.1 M $NaHCO_3$ buffer, pH 9.6. Wells were washed, blocked with phosphate-buffered saline/0.1% Tween 20/1% bovine serum albumin, and filled with 100 $\mu$l of serial 4-fold dilutions of culture supernatants. After a 2-hr incubation step wells were sequentially filled with 100-$\mu$l aliquots of a 1:500 dilution of a mouse monoclonal antibody to human IgE (Hybritech), a 1:20,000 dilution of a biotin conjugate of goat anti-mouse IgG antibody (Jackson ImmunoResearch), a 1:2000 dilution of a peroxidase-streptavidin conjugate (BRL), and substrate (3,3', 5,5'-tetramethylbenzidine plus $H_2O_2$; Kirkegaard and Perry, Gaithersburg, MD) according to the manufacturer's instructions. After 10 min the reaction was stopped by addition of 1 M $H_3PO_4$, and wells were analyzed for absorbance at 450 nm in an ELISA reader (Dynatech). An IgE myeloma protein standard at 150 pg/ml (gift of R. Wistar, Naval Medical Research Institute) generated an $A_{450}$ of twice the background level, whereas IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgM myeloma proteins each at up to 50 $\mu$g/ml (also gifts of R. Wistar) failed to generate a detectable reaction in this assay. To quantitate IgM, IgG, or IgA in culture supernatants, wells were first coated with goat anti-human Ig antibody at 1 $\mu$g/ml and then incubated sequentially with culture supernatant dilutions, peroxidase conjugates of goat antibody to IgM (diluted 1:2000), IgG (1:5000), or IgA (1:2000), respectively (Cappel Laboratories), followed by substrate. Purified human IgA, IgG, and IgM standards were purchased from Cappel Laboratories.

**Cytokines.** Recombinant human IL-4 was synthesized by transfected C127 mouse mammary tumor cells as a 17.5-kDa glycoprotein (A. R. Seetharam, T. G. Warren, and A.D.L., unpublished work). The protein was purified from serum-free conditioned medium by sequential chromatography on a Pharmacia Fast S column and a Vidac $C_4$ column. Homogeneity of the purified lymphokine was demonstrated by amino-terminal sequence analysis, PAGE, and elution as a sharp symmetrical peak on a $C_4$-microbore reverse-phase column (Applied Biosystems). Purified IL-4 at 250 pg/ml had one unit of activity, as defined as that concentration required to stimulate half-maximal expression of membrane CD23 by the human Burkitt lymphoma Jiyoye cell line. Recombinant interleukin 1 was a gift of Hoffman–La Roche; IL-2 was a gift of Cetus; and interleukin 3, IFN-$\gamma$, and interleukin 6 were gifts of the Genetics Institute (Cambridge, MA).

**Culture System.** Purified B lymphocytes ($10^5$) were cultured in 48-well culture plates (Costar) in 1 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum (GIBCO), 2 mM L-glutamine, gentamicin at 0.5 mg/ml, and $2 \times 10^{-5}$ M 2-mercaptoethanol plus varying doses of recombinant cytokines at 37°C in an atmosphere that contained 5% $CO_2$. Every 4–5 days 50% of the culture medium was replaced with an equal volume that contained the same cytokine concentration. Culture supernatants of the EBV-producing B95.8 marmoset cell line, which contained $3 \times 10^5$ infectious units of EBV, were added to cells at the start of culture.

**Identification of Cells with Intracytoplasmic Ig (cIg).** To identify cells that were synthesizing large quantities of Ig, cultured cells were first centrifuged over a layer of Ficoll/Hypaque to remove dead cells, then mixed with formalin-fixed chicken erythrocytes at a 100:1 ratio and centrifuged onto a glass microscope slide with a cytocentrifuge (Shandon Southern Instruments, Sewickley, PA). Cells were air-dried, fixed for 1 hr in methanol at 4°C, dried, and stained for 30 min with fluorescein-labeled mouse monoclonal antibodies to IgM or IgE (Becton Dickinson), washed, mounted in glycerol, and examined with a Leitz Ortholux phase/fluorescence microscope. The percentage of $cIgM^+$ cells was determined by counting lymphoid cells under both phase and UV light; the percentage of $cIgE^+$ cells was determined by counting under UV light the number of lymphoid cells that displayed green cytoplasmic fluorescence in fields that contained a total of 100 chicken erythrocytes (and, therefore, 10,000 lymphocytes) (7). Chicken erythrocytes were identified by their oval shape and orange nuclear fluorescence.

## RESULTS

**IL-4 Increases Ig Secretion by EBV-Infected B Lymphocytes.** Supernatants of purified B lymphocytes from four or five normal individuals were cultured with EBV for 26 days in the absence of exogenous cytokines; these cultures contained easily detectable quantities of IgM, IgA, and IgG but no detectable IgE (<150 pg/ml) (Fig. 1). Addition of $2 \times 10^3$ units of IL-4 to cultures increased supernatant concentrations of IgM, IgG, and IgA by an average of 11-, 10-, and

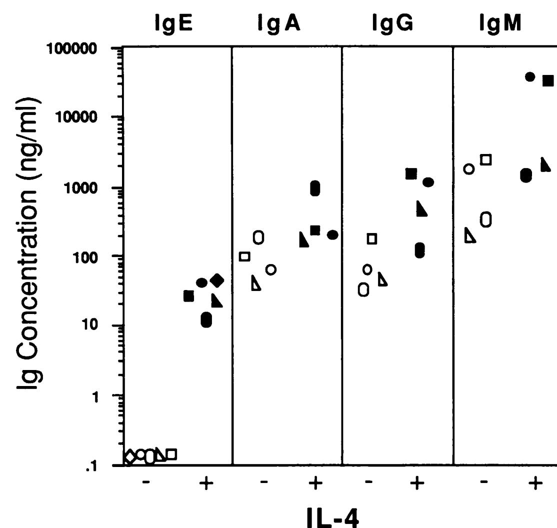


FIG. 1. Effect of IL-4 on EBV-induced Ig secretion. Purified human peripheral blood B cells were cultured for 26 days at $10^5$ cells in 1 ml of culture medium with $3 \times 10^7$ infectious units of EBV in the presence or absence of $2 \times 10^3$ units of purified recombinant IL-4. Half of the culture medium was replaced every 4–5 days with fresh medium that contained an equal concentration of IL-4. Day-26 culture supernatants were tested for Ig concentrations by isotype-specific ELISAs. Each symbol represents an individual experiment, and each experiment was performed with cells from a different donor. Open symbols indicate results of cultures that lacked IL-4; closed symbols indicate results of cultures that contained IL-4.

4-fold, respectively. The effect of this cytokine on IgE secretion was much more marked; in the presence of IL-4 culture supernatants had average IgE concentrations of 11.4–40.3 ng/ml, an increase, on average, of >180-fold over baseline (Fig. 1). Thus, while IL-4 increases production of all Ig isotypes by EBV-infected B lymphocytes, the increase in IgE production is at least 10-fold greater than that of other Ig isotypes. In contrast, the addition of recombinant IL-1, IL-2, IL-3, or IL-6 to purified B cells cultured with EBV failed to induce detectable IgE secretion, and B cells cultured with 2–2000 units of IL-4 in the absence of EBV also failed to secrete detectable IgE (data not shown).

**IL-4 Increases the Percentage of EBV-Infected B Cells That Synthesize IgE.** To determine whether IL-4 increased the percentage of cells that synthesize large quantities of IgE, in addition to the total quantity of IgE secreted, purified B cells cultured for 26 days with EBV in the presence or absence of IL-4 at $2 \times 10^3$ units/ml were fixed on glass slides, stained with fluorescein isothiocyanate (FITC)-labeled anti-IgM or anti-IgE antibodies, and examined by fluorescence microscopy for the percentage of cells with bright intracytoplasmic fluorescence. IL-4 increased the percentage of $cIgM^+$ cells from 17 to 41 and increased the percentage of $cIgE^+$ cells from <0.010 to 0.267. Thus, the large increase in IgE secretion is accompanied by a sizable increase in the percentage of cells that synthesize large quantities of IgE and most likely secrete this isotype.

**Effects of IL-4 Concentration on Increases in Cell Number and Ig Secretion.** Although IL-4 caused increases in cell number and in the concentration of all Ig isotypes tested in culture supernatants, these effects were dissociated to some extent by differences in the dose–response curves (Fig. 2). IL-4 at 2 units per ml stimulated a maximal increase in cell number as well as substantial increases in IgG and IgM concentrations; however, this dose failed to induce detectable IgE secretion. IgE secretion first became detectable when IL-4 was added to cultures at a dose of 6.6 units per ml. Increasing the IL-4 concentration to 20 units per ml stimulated a considerable further increase in IgE secretion, but increases in IL-4 concentration beyond this level had little effect on secretion of IgE. No decreases in secretion of any of the measured Ig isotypes were seen at IL-4 doses as high as 2000 units per ml, although the number of live cells recovered from cultures declined when IL-4 concentrations of 66 units per ml or higher were used. These observations suggest that the mechanisms through which IL-4 induces increases in cell number, the secretion of IgE, and the secretion of other Ig isotypes may differ; these observations further indicate that increased IgE secretion cannot simply be a consequence of an IL-4-induced increase in lymphocyte proliferation or survival.

**IgE Is Produced Late by B Cells Cultured with EBV Plus IL-4.** By the 10th day of culture of B cells with EBV plus IL-4, the number of cells per culture increased by a factor of 4–5, and considerable quantities of IgM and IgG accumulated in the culture supernatant (Fig. 3). In contrast, IgE was either not detectable (<150 pg/ml) or just barely measurable in culture supernatants at this time (280 pg/ml in the experiment shown in Fig. 3). In addition, the percentage increase in culture supernatant IgE concentration between days 10 and 14 was considerably greater than the percentage increases in the culture supernatant concentrations of IgM or IgG during this time period. Thus, induction of IgE secretion is regulated differently from induction of IgG and IgM secretion and may

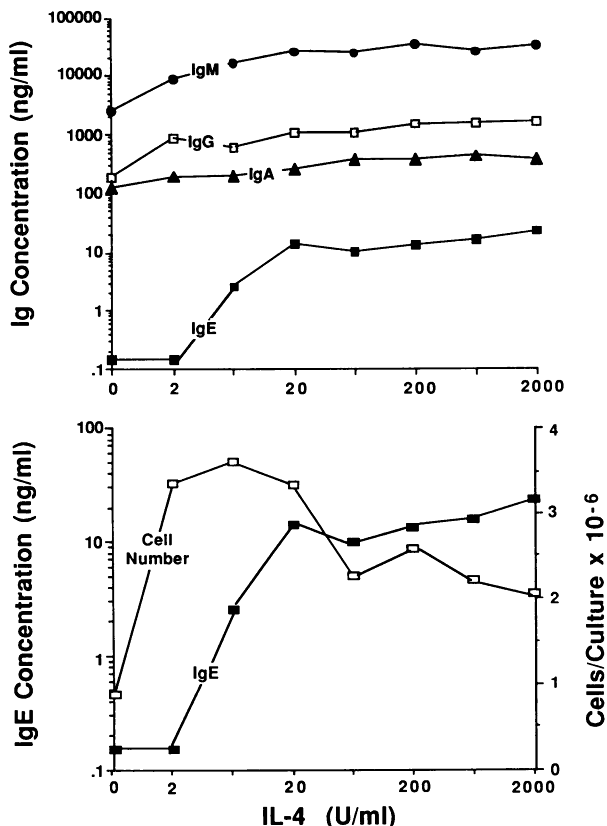


FIG. 2. Effect of IL-4 concentration on proliferation and Ig secretion by EBV-infected human B cells. Purified human peripheral blood B cells were cultured as described in the legend to Fig. 1 with EBV plus various concentrations of IL-4. Half of the culture medium was replaced every 4–5 days with fresh medium that contained an equal concentration of IL-4. Day-26 culture supernatant Ig concentrations were determined by ELISA; live cell counts were determined by trypan blue exclusion. Upper and lower panels show data from the same experiment. U, units.

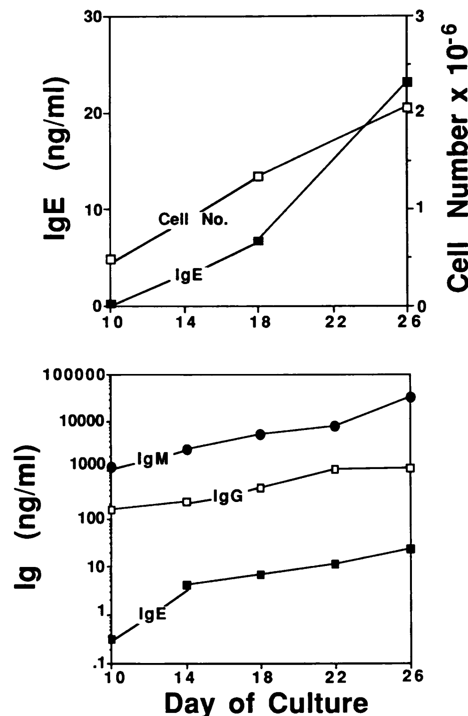


FIG. 3. Kinetics of proliferation and Ig secretion by EBV/IL-4-stimulated B cells. Purified human peripheral blood B cells were cultured with EBV plus IL-4 at $2 \times 10^3$ units per ml as described in the legend to Fig. 1. Ig concentrations in culture supernatants were determined by ELISA; cell counts were performed in the presence of trypan blue to determine numbers of live cells. Upper and lower panels show data from the same experiment.

require more prolonged stimulation with EBV plus IL-4 than does induction of B-cell growth or IgM or IgG secretion.

**T Cells Do Not Increase in Number During the Culture of Purified B Cells with EBV and IL-4.** Because T cells have been required for the generation of an IgE response by cultured human lymphoid cells in other systems and because the generation of an IgE response by purified B cells cultured with EBV plus IL-4 takes >10 days, it was necessary to determine whether T lymphocytes, although undetectable at the initiation of culture, increase in number during this time period. To investigate this possibility we stained cells after 8 and 16 days of culture with FITC-labeled antibodies specific for T cells (anti-CD3), B cells (anti-CD20), or with a FITC-labeled control antibody and then analyzed these cells for fluorescence intensity with a fluorescein-activated cell sorter. Less than 1% of cells from either group were stained significantly by anti-CD3, whereas a large percentage of unfractionated peripheral blood mononuclear cells that had been cultured for 8 days with IL-4 plus EBV were stained brightly by this reagent (Fig. 4). Furthermore, virtually all cells cultured with EBV and IL-4 continued to bear the B-cell marker CD20 after 8 or 16 days of culture. Thus, induction of IgE secretion by the combination of EBV and IL-4 cannot be explained by T-lymphocyte outgrowth during culture.

**IFN-γ Specifically Inhibits IgE Production by B Cells Cultured with EBV Plus IL-4.** Because IFN-γ inhibits IL-4 effects in many murine and human systems (9, 10, 12, 26), we studied the effect of this cytokine on the induction of proliferative and Ig-secretory responses by B cells cultured with EBV plus IL-4. At doses >100 units per ml, IFN-γ blocked all B-cell proliferative and Ig-secretory responses to EBV plus IL-4 or to EBV alone (data not shown), presumably because the antiviral effects of IFN-γ interfered with EBV infection of B lymphocytes. However, at 10 units per ml, IFN-γ inhibited IgE secretion by 95% without affecting secretion of IgG or IgM (Fig. 5) or B-cell proliferation (data not shown). Thus, induction of an IgE response is more sensitive to the inhibitory effects of IFN-γ in this system than are some other events stimulated by IL-4. Although IFN-γ probably inhibits IgE production in this system by specifically blocking IL-4 induction of an IgE response, we cannot rule out the possibility that IFN-γ selectively prevents EBV infection of those B cells that can act as precursors of IgE-secreting cells.

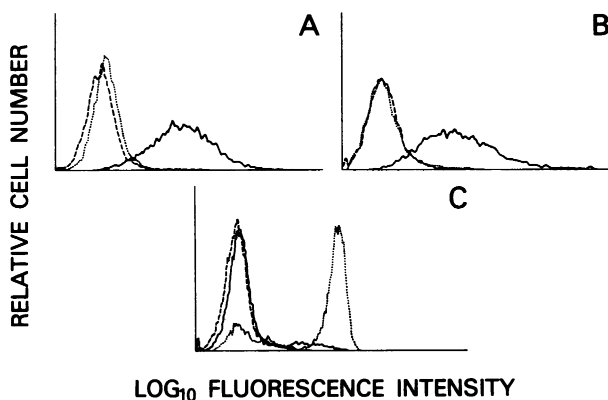


FIG. 4. No increase in T-cell number occurs during culture of purified human peripheral blood B cells with EBV plus IL-4. Purified human peripheral blood B cells were cultured as described in the legend to Fig. 1 for 8 days (*A*) or 16 days (*B*) stained with FITC-B1 (—), FITC-Leu-4 (anti-CD3) (···), or as a negative control, FITC-normal mouse IgG (–––), and analyzed for fluorescence intensity by flow microfluorimetry. The flow microfluorogram of similarly stained and analyzed unfractionated peripheral blood lymphocytes that were cultured for 8 days with EBV plus IL-4 is shown in *C*.

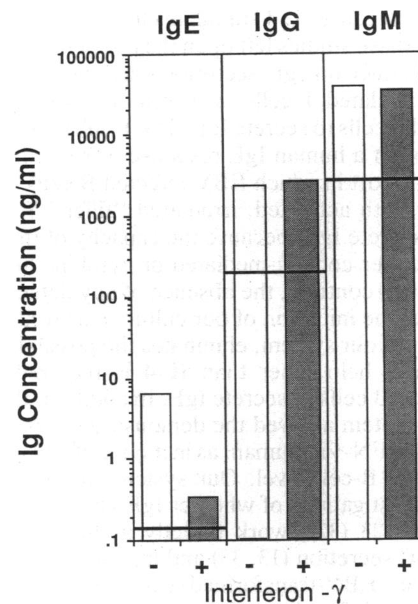


FIG. 5. IFN-γ specifically suppresses IgE secretion by EBV/IL-4-stimulated human peripheral blood B cells. Purified human peripheral blood B cells were cultured for 26 days as described in the legend to Fig. 1 with EBV alone, EBV plus IL-4 at $2 \times 10^3$ units per ml or EBV plus IL-4 at $2 \times 10^3$ units per ml and IFN-γ at 10 units per ml. Half of the culture medium was replaced every 4–5 days with fresh medium that contained an equal concentration of cytokines. Culture supernatant Ig concentrations were determined by ELISA. Horizontal lines indicate Ig concentrations of supernatants of cells cultured with EBV alone, open bars indicate Ig concentrations of supernatants of cells cultured with EBV plus IL-4, and shaded bars indicate Ig concentrations of supernatants of cells cultured with EBV plus IL-4 and IFN-γ.

## DISCUSSION

Our studies show that highly purified human peripheral blood B lymphocytes can be stimulated by EBV plus the cytokine IL-4 to secrete IgE. EBV, in the absence of IL-4, induced the secretion of IgM, IgG, and IgA, but not IgE, whereas IL-4, in the absence of EBV, failed to stimulate any Ig secretion. These observations are analogous to the results of previous studies in the mouse, which demonstrated that (*i*) the combination of IL-4 and lipopolysaccharide induces purified B cells to secrete IgE as well as IgM and IgG; (*ii*) lipopolysaccharide in the absence of IL-4 stimulates secretion of IgM and IgG, but not IgE; and (*iii*) IL-4, by itself, does not stimulate Ig secretion (4, 27). We do not yet know whether IL-4 modifies the distribution of IgG subclasses secreted by human EBV-infected B cells, as it does for murine lipopolysaccharide-activated B cells (27–29); nor do we have an explanation for the long time required for the combination of EBV and IL-4 to induce a detectable IgE response by human peripheral blood B cells. This delay in the secretion of IgE may explain an earlier report that human B cells do not secrete IgE in response to EBV plus IL-4 (30) because the culture period in that study was only 10 days and we have frequently failed to detect IgE secretion before 14 days of culture. Another cause for failure to detect IgE secretion by B cells cultured with EBV plus IL-4 is that impure IL-4 preparations may contain an inhibitor of IgE secretion; partially purified preparations of recombinant IL-4 used in our initial studies required much higher concentrations of IL-4 to induce IgE secretion than did the highly purified recombinant IL-4 preparations used for the studies described here.

The results of our study complement previous reports that human peripheral blood cells are stimulated by IL-4 to secrete IgE, provided that T cells are present, and that human T-cell clones that secrete IL-4 can induce purified B cells to secrete

IgE (12–15). Because IL-4 stimulates human T cells as well as B cells (15), these studies left open the possibility that the IL-4 stimulatory effect on IgE secretion was entirely indirect, in that IL-4 stimulated T cells to secrete a second lymphokine that induced B cells to secrete IgE. The T-cell requirements for the induction of a human IgE response were also unresolved by a recent report in which EBV-infected B cells, cultured at low density with activated, irradiated "filler" T cells, were induced to secrete IgE, because the capacity of the filler cells to provide either contact-mediated or cytokine help was not defined (31). In contrast, the absence of any detectable T-cell population at the initiation of our cultures, as well as at 8 and 16 days later in our system, eliminates the possibility that any form of T-cell help other than IL-4 is required to induce EBV-infected B cells to secrete IgE. In addition, the lack of T cells in our system allowed the demonstration that the inhibitory effect of IFN-$\gamma$ in human, as in mouse (9, 10), can operate directly at the B-cell level. Our system should similarly be useful for investigations of whether IgE binding factors, such as soluble CD23 (32), work directly at the B-cell level to stimulate IgE secretion (13, 33) and may allow the cloning of IgE-secreting, EBV-transformed cells (34–36). Such cells would be useful for determining whether IL-4 and IFN-$\gamma$ continue to regulate IgE secretion by a cell at this stage of differentiation as well as for investigating whether induction of IgE secretion by EBV-infected cells is accompanied by deletion of upstream Ig heavy chain constant region genes. Investigation of the status of Ig heavy chain constant region genes upstream to the gene for the constant region of the $\varepsilon$ chain in B cells induced by EBV plus IL-4 to secrete IgE has become particularly important in view of the recent report that no deletion of these genes occurs in B cells that secrete IgE when infected with EBV and cultured with irradiated, activated T cells (31).

Stimulation of IgE secretion was not the only effect of IL-4 on the growth and differentiation of EBV-infected B cells in our study; this cytokine also substantially increased B-cell proliferation and the secretion of IgM, IgG, and IgA. These additional effects, however, may operate through a different mechanism than does the stimulation of IgE secretion because (*i*) these effects occur earlier than IgE secretion, (*ii*) as in the mouse these effects can be induced by lower IL-4 concentrations than are required to induce IgE secretion, and (*iii*) these effects are not inhibited by a concentration of IFN-$\gamma$ that effectively blocks IgE secretion. These considerations are relevant to the use of agents that inhibit IL-4 production or the effects of IL-4 on the B cell as possible therapies for IgE-mediated disorders because they show that it is sometimes possible to block IL-4-mediated IgE secretion without inhibiting other effects of IL-4. Also of interest in this regard is the observation in murine *in vivo* studies that an anti-IL-4 antibody can block IgE production by >99% without affecting production of other Ig isotypes (37). This observation cannot be explained simply by dose–response considerations and is consistent with the possibility that IL-4, while critical for the stimulation of IgE secretion, may have an auxiliary or redundant role of relatively little importance *in vivo* in generating other humoral immune responses.

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