Interleukin 13 Is a B Cell Stimulating Factor

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Summary

The recently cloned human interleukin 13 (IL-13) is a novel cytokine expressed in activated T cells that has been shown to inhibit inflammatory cytokine production by lipopolysaccharide-activated monocytes. The protein encoded by the IL-13 cDNA is the human homologue of a mouse Th2-product called P600. Here, we show that IL-13 acts at different stages of the B cell maturation pathway: (*a*) it enhances the expression of CD23/FcεRII and class II MHC antigens on resting B cells; (*b*) it stimulates B cell proliferation in combination with anti-Ig and anti-CD40 antibodies; and (*c*) it induces IgE synthesis. Thus, the spectrum of the biological activities of IL-13 on B cells largely overlaps that previously ascribed to IL-4. The present observations suggest that IL-13 may be an important factor, in addition to IL-4, in the development of allergic diseases.

Th1 and Th2 T cell clones were first distinguished on the basis of their pattern of cytokine secretion (1, 2). Th1 cells produce IL-2, IFNγ, and lymphotoxin, whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-10 (3). It is now clear that these two Th cell subsets also display distinct functional capabilities. Th1 cells play an important role in cell-mediated immunity and are good mediators of delayed-type hypersensitivity and cytotoxic T cell responses (3, 4). Conversely, Th2 cells are particularly effective in providing help for humoral responses (5).

Human IL-13 has been recently cloned from a cDNA library established from activated PBMC (6). IL-13 cDNA encodes a polypeptide of 12.4 kD with four potential *N*-glycosylation sites presenting 60% amino acid homology with a previously cloned mouse Th2 T cell product known as P600 (7, 8). No biological activity has been reported so far for P600, but human IL-13 shares with two other Th2-derived cytokines (IL-4 and IL-10) the capacity to inhibit cytokine synthesis by activated monocytes (6, 9, 10). Due to the specific expression of P600 by Th2 cells, the human counterpart of this molecule could be considered as a potential modulator of B cell responses. Here, we report on the effects of recombinant human IL-13 on the activation, proliferation, and differentiation of human B cells.

Materials and Methods

Reagents and Cytokines. Rabbit anti-human Igs (heavy and light chains) coupled to polyacrylamide beads were purchased from Bio-Rad Laboratories (Richmond, CA) and were used at a final dilution of 1/600, which provided the optimal costimulatory effect in proliferation assays. Formalinized particles of *Staphylococcus aureus* strain Cowan I (SAC)[1] were purchased as Pansorbin from Calbiochem-Behring Corporation (La Jolla, CA) and were used at a final concentration of 0.005% (vol/vol). Recombinant IL-2 (3×10^6 U/mg) and purified recombinant IL-4 (2×10^7 U/mg) were purchased from Amgen Biologicals (Thousand Oaks, CA). Purified recombinant IL-10 was purchased from Immugenex (Los Angeles, CA) and was used at a final concentration of 100 ng/ml throughout the study. Recombinant IL-13 was purified from culture supernatants of stably transfected CHO cell lines, as described elsewhere (6). The anti-CD40 mAb G28-5 (11) was kindly provided by Dr. E. A. Clark (University of Washington, Seattle, WA) and was used at a final concentration of 100 ng/ml. PE-conjugated anti-CD23 mAb and FITC-conjugated monoclonals directed against HLA-DR, DP, DQ antigens were purchased from Becton Dickinson & Co. (Mountain View, CA). Culture supernatants of the OKT3 (CD3 mAb) and OKT11 (CD2 mAb) hybridoma cell lines were used for depletion of T cells from tonsils. The blocking rabbit anti-human IL-4 polyclonal antibody was purchased from Genzyme (Cambridge, MA) and was used at the final concentration of 10 μg/ml. The corresponding negative control was a preimmune rabbit serum. The mouse Ltk$^-$ cell line D1B1 (12), stably expressing the murine IgG Fc receptor (FcγRII) was kindly provided by Dr. C. Sautès (INSERM U. 255, Institut Curie, Paris, France).

B Cell Preparations. B cells were isolated from tonsils as previ-

[1] *Abbreviations used in this paper:* B-CLL, chronic lymphocytic leukemia B cells; GC, germinal center; MFI, mean fluorescence intensity; PBA, polyclonal B cell activator; SAC, *Staphylococcus aureus* strain Cowan I.

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ously described (13). Briefly, after a rosetting step with sheep red blood cells, nonrosetting cells were further incubated with anti-CD2 and anti-CD3 mAbs before negative selection performed with magnetic beads coated with anti-mouse IgG (Dynabeads; Dynal, Oslo, Norway). The purity of the B cell populations obtained by this procedure was routinely superior to 98% as estimated by flow cytometry analysis using anti-CD19, -CD20, -CD2, -CD3, and -CD14 mAbs. sIgD$^+$ and sIgD$^-$ B cells were isolated as described previously (13) using a preparative magnetic cell separation system (MACS®; Becton Dickinson & Co.). The purity of both B cell populations was routinely superior to 95%.

Leukemic B cells were obtained from a patient with hematologically diagnosed chronic lymphocytic leukemia. B cells were isolated from peripheral blood, according to the procedure described above, and contained more than 95% leukemic cells as estimated by the expression of the CD5 antigen, which is constitutively expressed on chronic lymphocytic leukemia B cells (B-CLL).

Cultures. All cultures were performed in RPMI 1640 supplemented with 10% selected heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2% Hepes (all from Gibco Laboratories, Grand Island, NY). For CD23 and class II MHC antigen induction experiments, B cells (10^6/ml) were cultured for 48 h in the absence of stimulating agent, or in the presence of IL-4 or IL-13 used at 100 ng/ml. At the end of the culture period, cells were stained with PE-conjugated anti-CD23 or FITC-conjugated anti-HLA-DR, DP, or DQ monoclonals and analyzed on a FACScan® using the Lysis software. (Becton Dickinson & Co.) Propidium iodide (2 μg/ml) was added to each sample (except when staining was performed with PE-conjugated antibodies) before flow cytometry analysis to gate out nonviable cells. For proliferation assays, B cells were seeded at 1.5 × 10^5 cells/well in 96-well microtiter trays in a final volume of 0.1 ml. Cytokines and polyclonal B cell activators (anti-Ig or anti-CD40 antibodies) were added at the onset of the culture. Each culture point was performed in triplicate. At the times specified in the text, DNA synthesis was determined by pulsing the cells with [^{3}H]thymidine ([^{3}H]TdR) for the last 16 h of the culture period. For differentiation assays performed with polyclonal B cell activators (SAC and anti-CD40 mAb), B cells were seeded at 3 × 10^5 cells per well in 96-well microtiter trays, in a final culture volume of 0.25 ml. When SAC was used as the stimulating agent, the addition of cytokines was made either at the onset of the culture (costimulation assay) or was delayed for 2 d (restimulation assay). For assays aimed at determining the influence of IL-13 on IgE synthesis, two distinct activation systems were used. In the first one, B cells were co-cultured with mitomycin-treated (75 μg/ml, 45 min, 37°C) T cells activated by phytohemagglutinin-P (PHA; Difco Laboratories, Inc., Detroit, MI). In these experiments, 3 × 10^6 T cells, purified from peripheral blood by filtration on nylon wool columns, were added together with cytokines and PHA (1 μg/ml) to 5 × 10^5 B cells, in 24-well plates, in a final volume of 1 ml. In the second one, purified B cells were activated by the anti-CD40 mAb G28-5 presented by the D1B1 cell line, stably expressing the murine FcγRII. In these experiments, 5 × 10^5 B cells were co-cultured in 24-well plates with 2 × 10^5 irradiated (7,000 rad) D1B1 cells and the G28-5 mAb (100 ng/ml) in the presence of IL4 (200 ng/ml) or IL-13 (100 ng/ml), with or without addition of anti-IL4 or control antisera (10 μg/ml). Each culture point was performed in six replicates (T/B cell co-cultures) or in quadruplicates (co-cultures with D1B1 cells). For determination of immunoglobulin production, culture supernatants were harvested at day 10 and IgM, IgG, IgA, and IgE levels were measured in standard ELISAs (14, 15).

Results

IL-13 Enhances Expression of CD23 and Class II MHC Antigens on B Cells. To examine the capacity of IL-13 to exert an influence on the early B cell activation steps, both tonsillar B cells and leukemic B cells from a B-CLL patient were assessed for CD23 expression after a 48-h culture period in the presence of 100 ng/ml purified recombinant IL-13 or IL-4. The results from one representative experiment are illustrated by Fig. 1. As described earlier (16, 17), CD23 was constitutively expressed on the leukemic clone (Fig. 1 *E*), whereas it was only distributed on a subset of normal B cells (Fig. 1 *A*). In agreement with previously published reports (17), normal and leukemic B cells (Fig. 1, *B* and *F*, respectively) almost totally lost the expression of CD23, in the absence of stimulant. Both IL-13 (Fig. 1, *C* and *G*) and IL-4 (Fig. 1, *D* and *H*) were able to induce reexpression of CD23 on leukemic and normal B cells. However, the proportion of

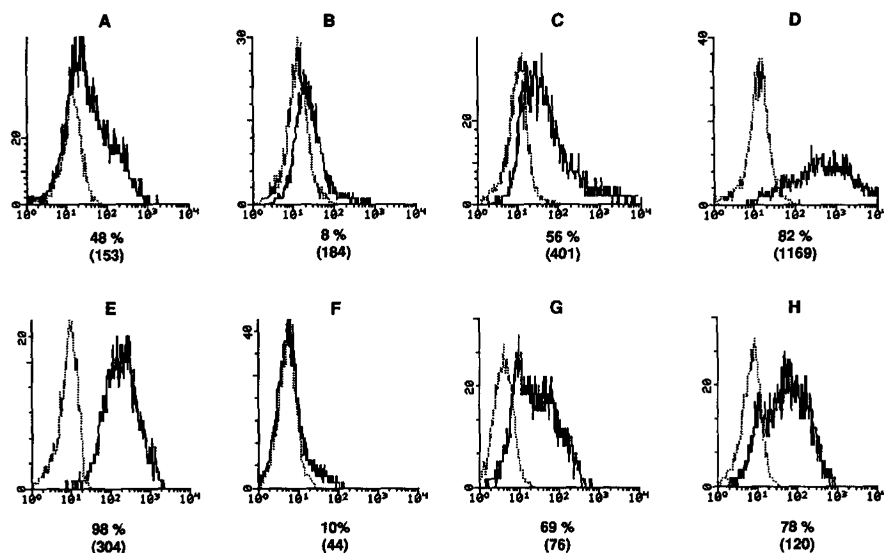

Figure 1. Expression of CD23 on normal and leukemic B cells cultured with IL-13 or IL-4. Purified tonsillar B cells (*A–D*) or B-CLL cells (*E–H*) were cultured for 48 h with purified recombinant IL-13 (100 ng/ml; *C* and *G*) or with purified recombinant IL-4 (100 ng/ml; *D* and *H*). A control, in which B cells were cultured in the absence of stimulant (*B* and *F*) was also included in the experiment. The constitutive expression of CD23 on freshly isolated tonsillar B cells and B-CLL cells is shown in *A* and *E*, respectively. The fluorescence histograms obtained after staining with the PE-conjugated anti-CD23 mAb (*plain lines*) were superimposed with the negative control performed with an isotype-matched unrelated mAb (*dashed lines*). The proportion of CD23-expressing cells as well as the mean fluorescence intensity of the fluorescence histogram (*in parentheses*) are indicated below the FACS® profiles. Fluorescence is expressed on a logarithmic scale. The figure is representative of three separate experiments.

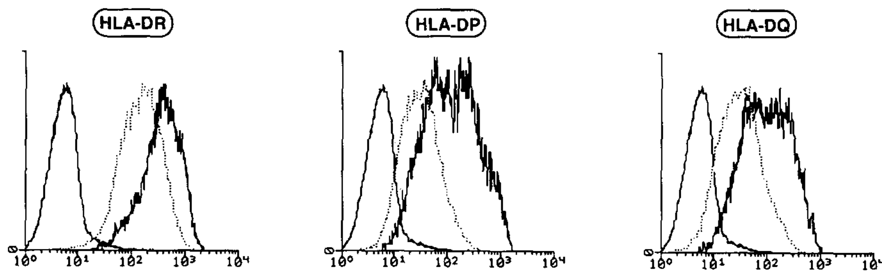

Figure 2. IL-13–mediated enhancement of class II MHC antigen expression on leukemic B cells. B-CLL cells were cultured for 48 h in the presence of IL-13 at 100 ng/ml (*plain lines*) or in the absence of any stimulant (*dashed lines*), and analyzed by flow cytometry after staining with FITC-conjugated anti-HLA-DR, DP, and DQ monoclonals. The negative control performed with an isotype-matched unrelated mAb is also shown (*first plain line histogram on the left of each panel*). Data are shown on a logarithmic scale. The MFI values of the fluorescence histograms for DR, DP, and DQ were 560, 272, and 171, respectively, for cultures stimulated with IL-13, vs. 181, 44, and 38 for untreated cultures. The figure is representative of three experiments.

CD23-positive cells as well as the density of CD23 antigens per cell, estimated by the mean fluorescence intensity (MFI) of the fluorescence histogram, were consistently lower in cultures stimulated with IL-13 than in cultures stimulated with IL-4. The half-maximal effect of IL-13 on CD23 induction, estimated on normal tonsillar B cells, was reached with concentrations of IL-13 on the order of 3 ng/ml (data not shown). In contrast with IL-4, IL-13 totally failed to enhance CD23 expression on the Burkitt lymphoma cell line Jijoye (data not shown). As illustrated by Fig. 2, IL-13 was also able to significantly increase expression of the HLA-DR, DP, and DQ antigens on B-CLL cells. At optimal concentrations of both cytokines, the signal provided by IL-13 for enhancement of class II MHC antigens was of similar amplitude to that provided by IL-4 (data not shown), and up to sixfold increases in MFI were observed for HLA-DP antigens in response to IL-13.

IL-13 Stimulates Growth of Activated B Cells. Purified recombinant IL-13 and IL-4 were next compared for their capacity to stimulate DNA synthesis from human tonsillar B cells activated either through cross-linking of surface Igs using immobilized anti-Ig antibodies or through ligation of CD40 antigens using the anti-CD40 mAb G28-5. As shown in Fig. 3, IL-13 enhanced [^{3}H]TdR incorporation of activated human B lymphocytes, in a dose-dependent fashion, in both culture systems, a plateau being reached at 50 ng/ml. As observed for CD23 induction, the half-maximal effect of IL-13 on B cell proliferation was obtained for a concentration of ~3 ng/ml. On average, at optimal concentration of both cytokines, the levels of [^{3}H]TdR incorporation induced by IL-13 were approximately two times lower than those induced by IL-4, regardless of the activating stimulus applied. No stimulation of B cell growth was observed when IL-13 was tested in the absence of costimulating agent (data not

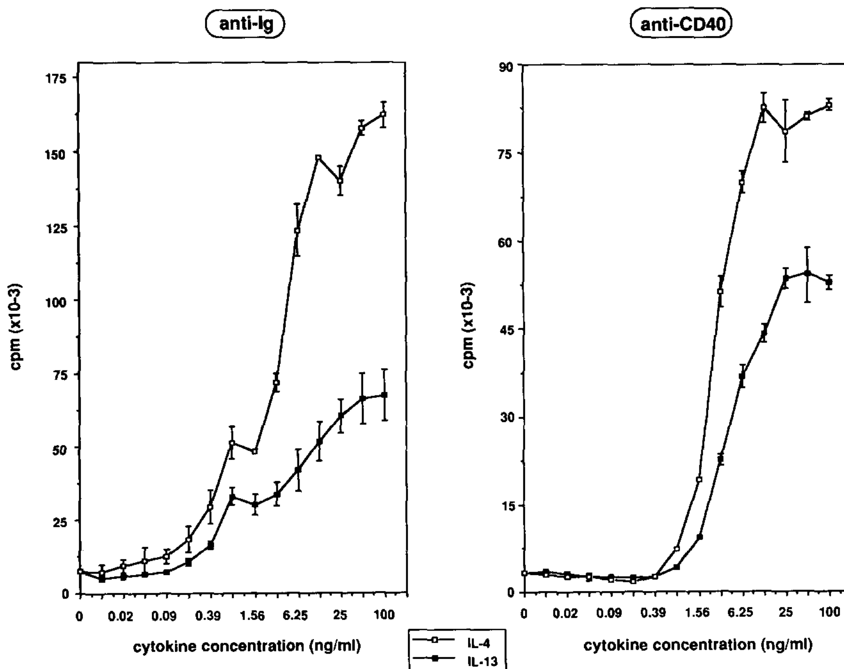

Figure 3. Titration of the growth-promoting activity of IL-13 on B cells activated through sIgs or CD40. 1.5 × 10^5 B cells were cultured with serial dilutions of purified recombinant IL-13 or purified recombinant IL-4, in combination with immobilized anti-Ig antibodies (1/600 dilution) or with the anti-CD40 mAb G28-5 (100 ng/ml). [^{3}H]TdR incorporation levels were assessed at day 3, after a 16-h pulse. The mean ± SD rates of incorporation from triplicate wells of a typical experiment are shown. The figure is representative of three separate experiments.

shown). To examine whether IL-13 could modulate the growth-promoting effect of other B cell stimulatory cytokines, anti-Ig or anti-CD40–activated B cells were cultured with optimal concentrations of IL-2 (20 U/ml), IL-4 (200 ng/ml), or IL-10 (100 ng/ml) in the presence or absence of an optimal concentration of IL-13 (100 ng/ml). As indicated by the data summarized in Table 1, IL-2 and IL-10, but not IL-4, were able to cooperate with IL-13 to induce proliferation of activated B cells. As expected from previous results (18), IL-2 failed to stimulate DNA synthesis from anti-CD40–activated B cells, whereas IL-10 was virtually inactive on anti-Ig–activated B cells. Accordingly, enhancement by IL-13 of the growth-promoting effects of IL-2 and IL-10, was observed in the anti-Ig and anti-CD40 activation systems, respectively. As demonstrated by kinetics studies (Fig. 4), cooperation between IL-13 and IL-2 or between IL-13 and IL-10, for B cell growth, was observed throughout the culture period, with the exception of the earlier time point. At the peak of the proliferative response (day 2 for anti-Ig–activated B cell, day 3 for anti-CD40–activated B cells), the levels of stimulation reached with IL-13 + IL-2 or IL-13 + IL-10, were approximately equivalent to the sum of the responses elicited by each factor alone.

Tonsil B cells can be separated into two major B cell populations, on the basis of surface IgD expression (13). $sIgD^+$ B cells are representative of virgin B cells of the follicular mantle, whereas $sIgD^-$ B cells include germinal center (GC) B cells as well as extra-follicular B cells. Thus, $sIgD^+$ and $sIgD^-$ B cells were compared for their ability to respond by DNA synthesis to IL-13 used in combination with immobilized anti-Ig antibodies or with the anti-CD40 mAb G28-5.

Table 1. *Interplay of IL-13 and Other Cytokines on B Cell Growth*

Cytokines	IL-13	[³H]Thymidine incorporation: Anti-Ig	[³H]Thymidine incorporation: Anti-CD40
		cpm × 10⁻³	
–	–	4.7	12.2
–	+	33.9	21.9
IL-2	–	33.3	14.0
IL-2	+	65.3	25.2
IL-4	–	84.3	45.6
IL-4	+	84.6	42.7
IL-10	–	4.8	33.4
IL-10	+	25.8	48.6

Purified B cells were seeded at 1.5×10^5 cells/well, in microtiter trays, and stimulated with immobilized anti-Ig antibodies or with the anti-CD40 mAb G28-5 in the absence or presence of IL-2 (10 U/ml) or IL-10 (100 ng/ml), used either alone or in combination with IL-13 (100 ng/ml). Cultures were terminated at 72 h after a 1-μCi [^{3}H]TdR pulse for the final 16 h. Results are expressed as mean of triplicate determinations. SD never exceeded 10% of the mean values. The table is representative of three separate experiments.

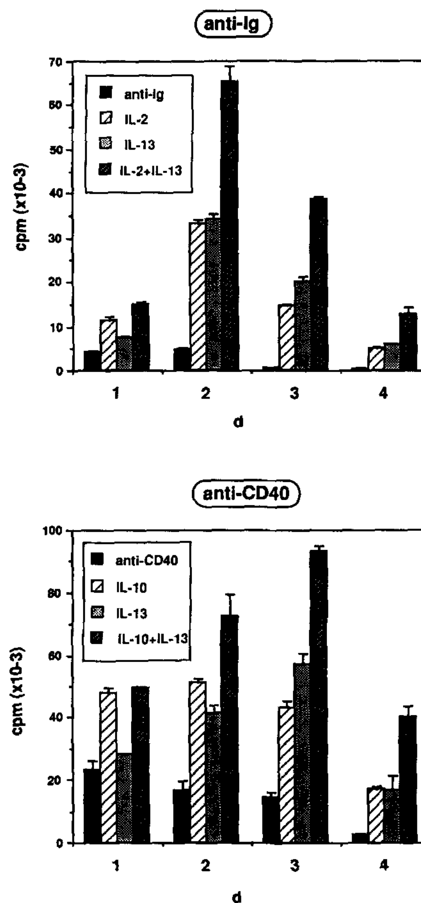

Figure 4. Effect of combinations of IL-13 and IL-2 or IL-13 and IL-10 on the proliferative response of activated B cells. 1.5×10^5 B cells were cultured with IL-13 (100 ng/ml), IL-2 (10 U/ml), IL-13 + IL-2, in the presence of immobilized anti-Ig antibodies (1/600 dilution); or with IL-13, IL-10 (100 ng/ml), IL-13 + IL-10, in the presence of the anti-CD40 mAb G28-5 (100 ng/ml). Cells were pulsed with [^{3}H]TdR at the indicated times, cultures being terminated 16 h later. Results correspond to the mean ± SD values of three separate experiments.

As a control, the influence of IL-2 and anti-Ig antibodies on the growth response of both populations was also examined. As shown in Fig. 5, $sIgD^+$ B cells displayed an enhanced responsiveness to the growth stimulatory effect of IL-13, when compared with $sIgD^-$ B cells, in both the anti-Ig and anti-CD40 activation systems. The levels of DNA synthesis induced by IL-13 in the former population were, on average, two to five times superior to those induced in the latter. In contrast, the proliferative response of $sIgD^-$ B cells to the combination of IL-2 and immobilized anti-Ig antibodies was markedly higher than that of $sIgD^+$ B cells, at all time points. This observation therefore rules out the possibility of an impaired responsiveness of $sIgD^-$ B cells to mitogenic stimuli. Taken together, these findings indicate that IL-13 is a B cell growth-factor whose preferential cellular target is virgin $sIgD^+$ B cells.

IL-13 Induces IgE Synthesis. We next examined the activity of IL-13 on B cell differentiation. IL-13 was first compared

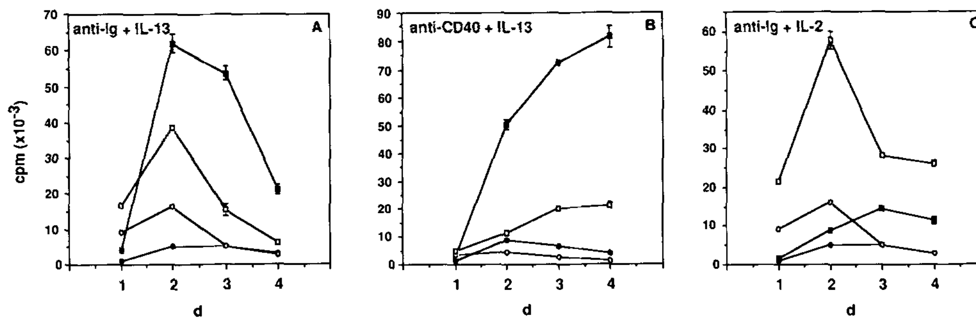

Figure 5. Response of sIgD+ and sIgD− B cells to the growth-stimulatory effect of IL-13. Purified sIgD+ (*closed symbols*) and sIgD− B cells (*open symbols*) isolated from tonsils were costimulated with: (*a*) IL-13 (100 ng/ml) and immobilized anti-Ig antibodies (1/600 dilution, *A*); (*b*) IL-13 and the anti-CD40 mAb G28-5 (100 ng/ml; *B*); and (*c*) IL-2 (10 U/ml) and immobilized anti-Ig antibodies (*C*). The levels of stimulation induced by the activating agent alone (PBA) in both B cell populations are indicated for each culture condition. B cells were cultured at the cell density of 1.5×10^5 cells per well in 96 well-microtiter trays. Cells were pulsed with [^{3}H]TdR at the indicated times, cultures being terminated 16 h later. Results correspond to the mean ± SD values of triplicate determinations. The figure is representative of three separate experiments. —●—, sIgD+/PBA; —■—, sIgD+/PBA + cytokine; —○—, sIgD−/PBA; —□—, sIgD−/PBA + cytokine.

with IL-2 for its capacity to induce polyclonal Ig secretion from activated B cells, in four distinct assay systems: (*a*) co-culture with SAC; (*b*) co-culture with the anti-CD40 mAb G28-5; (*c*) co-culture with SAC and anti-CD40; (*d*) restimulation of B cells preactivated with SAC for 2 d. The latter culture condition was included in our study in order to avoid underestimation of the Ig-inducing activity of IL-13 due to its presence during the initial activation with SAC. Indeed, the development of the stimulatory effect of IL-4 on B cell differentiation had been shown to be prevented when SAC and IL-4 were added simultaneously on B cells (19). As shown in Table 2, IL-13 failed to stimulate IgM, IgG, and IgA synthesis, whatever the mode of B cell activation used. However, a low but reproducible IgE response was observed when B cells were co-cultured with IL-13 and soluble anti-CD40 antibodies. In spite of its capacity to enhance the proliferative response induced by IL-2 and IL-10, IL-13 did not affect the IgM, IgG, and IgA synthesis elicited by these two cytokines in the SAC restimulation assay (Fig. 6) or in the three other culture systems described above (data not shown). IL-13 also

Table 2. *Effect of IL-13 on Ig Secretion by Human B Cells Activated by SAC or Soluble Anti-CD40 Antibodies*

		Immunoglobulin production			
Activators	Cytokines	IgM	IgG	IgA	IgE
		μg/ml	*μg/ml*	*μg/ml*	*pg/ml*
SAC (costimulation)	none	0.3	0.1	0.05	<156
	IL-13	0.6	0.1	0.05	<156
	IL-2	5.0	14.8	0.8	<156
Anti-CD40	none	0.2	0.2	0.05	<156
	IL-13	0.4	0.3	0.06	300
	IL-2	0.5	1.0	0.3	<156
SAC + anti-CD40	none	0.8	0.4	0.05	<156
	IL-13	1.3	0.4	0.1	<156
	IL-2	5.9	13.0	1.0	<156
SAC (restimulation)	none	0.3	0.1	0.03	<156
	IL-13	0.5	0.1	0.05	<156
	IL-2	3.6	10.9	0.2	< 156

Purified B cells were seeded at 3×10^5 cells/well and activated by SAC (0.005%, vol/vol), by the anti-CD40 mAb G28-5 (100 ng/ml) or by the combination of SAC and anti-CD40 in the presence or absence of optimal concentrations of IL-13 (100 ng/ml) or IL-2 (10 U/ml). When SAC was used as the stimulating agent, cytokines were either added together with SAC at the onset of the culture (costimulation assay) or 2 d after establishment of the culture (restimulation assay). Supernatants were harvested at day 10 and Ig levels were determined by ELISA. Results are means of quadruplicate determinations. SD never exceeded 10% of the mean values. IgM, IgG, and IgA levels are expressed in μg/ml, IgE levels are expressed in pg/ml. This table is representative of four separate experiments.

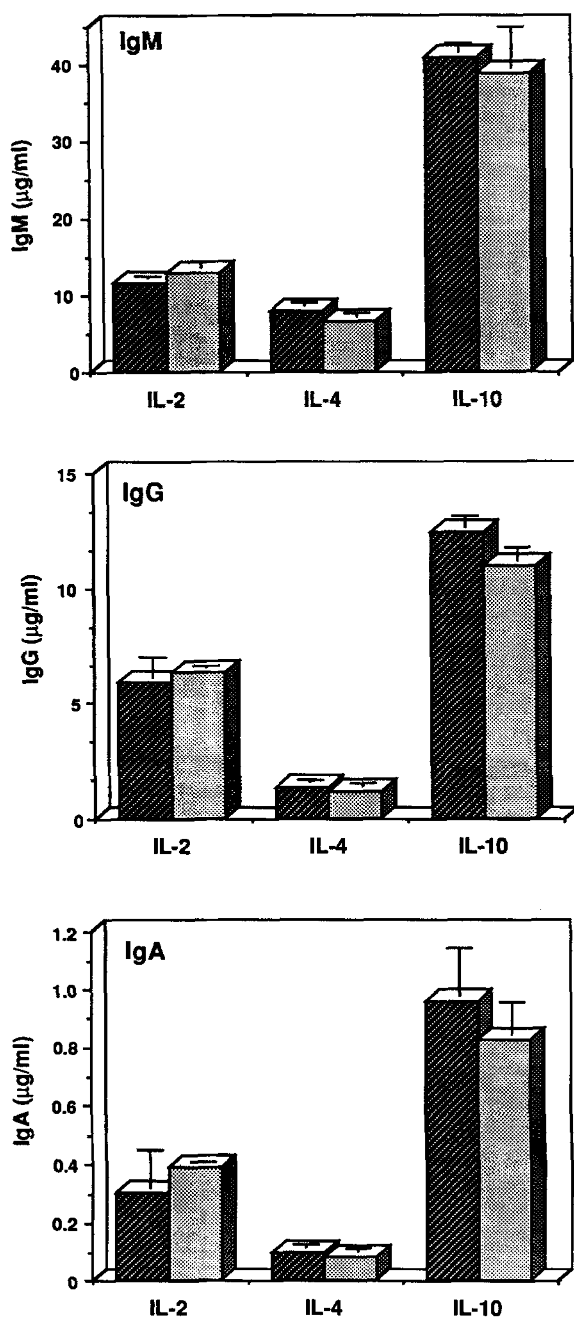

Figure 6. Influence of IL-13 on the IgM, IgG, and IgA response elicited by B cell stimulatory cytokines. B cells were dispensed in microtiter trays at the density of 3×10^5 cells per well, together with SAC (0.005%, vol/vol), under a final culture volume of 0.25 ml. IL-2 (10 U/ml), IL-4 (100 ng/ml), and IL-10 (100 ng/ml) were added alone or in the presence of IL-13 (100 ng/ml) on the second day of the culture. Ig levels were tested in 10-d culture supernatants by specific ELISAs. Results are expressed in µg/ml and correspond to the mean ± SD values calculated on eight culture replicates. ▨, −IL-13; ▧, +IL-13.

failed to modulate the IL-4–mediated secretion of these isotypes. Since signals provided by activated T cells have been described to be instrumental for induction of IgE synthesis in response to IL-4 (20, 21), IL-13 was first tested for its IgE-inducing capacity in cultures in which B cells were activated by mitomycin-treated T cells, in the presence of PHA (1 µg/ml). Because activated T cells have been shown to induce B cells for DNA synthesis and Ig secretion in a class II unrestricted and nonantigen-specific fashion (22), T cells used for T/B cell co-cultures were isolated from peripheral blood, whereas B cells were purified from tonsils. The T/B cell ratio in these experiments was 6/1. As shown in Table 3, IL-13 did not significantly enhance the production of IgM, IgG, and IgA but markedly increased the IgE synthesis induced by activated T cells. To rule out the possibility that part or all of the IgE-inducing activity of IL-13 could be mediated through the endogenous release of IL-4, IL-13 was also tested for its capacity to induce IgE synthesis in a culture system devoid of potential IL-4–producing cells. Regarding that IL-4 can efficiently stimulate B cells for IgE secretion when they are activated by anti-CD40 antibodies presented by a mouse fibroblastic cell line transfected with the human IgG Fc receptor (23), we decided to assess the IgE-stimulatory effect of IL-13 in a similar experimental model. For this purpose, B cells were co-cultured with the anti-CD40 mAb G28-5, presented by the D1B1 cell line stably expressing the murine FcγRII, and IL-4 or IL-13, in the presence or absence of a rabbit anti-human IL-4 polyclonal antibody. As shown in Table 4, IL-4 and IL-13, both used at optimal concentrations, induced equivalent levels of IgE synthesis from B cells activated by immobilized anti-CD40 antibodies. IL-13 failed to stimulate any IgM or IgA production in that culture system, but marginal enhancements (two times the background levels) of IgG secretion were observed (data not shown). Neutralizing polyclonal anti-IL-4 antibodies, but not the preimmune rabbit serum, completely blocked the IgE-inducing activity of IL-4. In contrast, the anti-IL-4–neutralizing antiserum did not antagonize the IgE-stimulatory effect of IL-13. Taken together these results indicate that IL-13 stimulates IgE secretion in an IL-4–independent fashion.

Discussion

In the present report, we have demonstrated that human IL-13 is susceptible to act on three distinct stages of the B cell maturation process: (*a*) during the early activation phase, by enhancing expression of CD23 and of class II MHC antigens on resting B cells; (*b*) during the proliferation phase, by stimulating growth of activated B cells; (*c*) during the differentiation phase, by inducing IgE synthesis. Thus, the spectrum of biological activities of IL-13 on human B cells presents striking similarities with that previously ascribed to IL-4 (24), although the IL-13–mediated responses were generally of lower amplitude than those elicited by IL-4. For example, the levels of CD23 expressed on IL-13–stimulated B cells, after 48 h of culture, were approximately equivalent to those found on freshly isolated B cells. In contrast, IL-4 not only induced higher levels of CD23 expression but also recruited a larger proportion of tonsillar B cells to become CD23-positive. In view of the fact that CD23 rapidly disappears from the B cell membrane in the absence of stimulant

Table 3. *IL-13 Stimulates IgE Synthesis from B Cells Co-cultured with Activated T Cells*

	IgM	IgG	IgA	IgE
	μg/ml	*μg/ml*	*μg/ml*	*ng/ml*
B	0.4 ± 0.01	0.3 ± 0.01	0.3 ± 0.04	<0.1
B + T	26 ± 0.4	12.7 ± 0.2	2.1 ± 0.1	2.6 ± 0.1
B + T + IL-13	28 ± 0.7	9.8 ± 0.3	1.7 ± 0.07	11.8 ± 0.9
B + T + IL-4	35 ± 1.6	10.9 ± 0.6	2.2 ± 0.1	20.9 ± 1.2

Purified tonsillar B cells (5 × 10^5/well) were co-cultured for 10 d with mitomycin-treated peripheral blood T cells (3 × 10^6/well) and PHA (1 μg/ml) in the absence or in the presence of IL-4 (100 ng/ml) or IL-13 (100 ng/ml). Each culture point was performed in six replicates in 24-well plates under a final culture volume of 1.0 ml. Supernatants from the culture replicates were pooled and Ig levels were determined by ELISA. Results are expressed as means ± SD of quadruplicate ELISA determinations. IgM, IgG, and IgA levels are expressed in μg/ml, IgE levels are expressed in ng/ml.

(17), it can not be excluded that IL-13 acts by stabilizing the CD23 molecule on the cell surface (for example by preventing the proteolytic cleavage or shedding of its soluble form) rather than by activating the CD23 gene.

As observed for CD23 induction, the growth-stimulatory effect of IL-13 on B cells activated either through cross-linking of sIgs or ligation of CD40 was less pronounced than that of IL-4. Examination of the B cell proliferative response to IL-13 used in combination with other B cell-stimulatory cytokines revealed one point of discrepancy between IL-4 and IL-13. Indeed, although IL-4 and IL-13 shared the capacity to enhance the growth-response induced by IL-10 (25), IL-13 potentiated the DNA synthesis elicited by IL-2, whereas there is published evidence that IL-4 antagonizes the human B cell responses to this cytokine (26, 27). We suspect that the positive interplay between IL-2 and IL-13 on B cell proliferation could relate to the fact that the two molecules have distinct cellular targets. IL-13 predominantly induced growth of $sIgD^+$ B cells, whereas, in accordance with a previous published report (28), IL-2 had a preferential impact on the $sIgD^-$ subset which is enriched for GC-derived B cells. This finding is consistent with the hypothesis that a larger B cell population is recruited to proliferate when IL-2 and IL-13 are combined, than when each cytokine is used alone. Additionally, the enhanced responsiveness of virgin $sIgD^+$ B cells to IL-13 raises the possibility that this cytokine could be involved in the establishment of the primary humoral response rather than in the development of the secondary immune response.

Table 4. *The IL-13–mediated Induction of IgE Synthesis Is IL-4 Independent*

	IgE secretion		
	Med*	+ anti-IL-4	+ control antiserum
		ng/ml	
Med	<0.15	ND	ND
IL-4	11 ± 0.7	<0.15	8.5 ± 0.4
IL-13	12 ± 0.8	11 ± 0.4	9 ± 0.3

Purified tonsillar B cells (5 × 10^5/well) were activated by the anti-CD40 mAb G28-5 (100 ng/ml) presented by the irradiated fibroblast cell line D1B1 stably expressing the murine FcγRII (2 × 10^5/well). Anti-CD40–activated B cells were cultured with purified recombinant IL-4 (100 U/ml) or IL-13 (100 ng/ml) in the presence or absence of: (*a*) a rabbit anti–human IL-4 polyclonal antibody (10 μg/ml); (*b*) a control rabbit anti-serum (10 μg/ml). Each culture point was performed in quadruplicates in 24-well plates. Supernatants from the culture replicates harvested on day 10 were pooled and IgE concentrations were measured by ELISA. Results are expressed as means ± SD of quadruplicate ELISA determinations. IgE levels are expressed in ng/ml.
* Med, Medium.

Despite the fact that IL-13 behaved as a B cell growth-factor, it was virtually unable to induce IgM, IgG, and IgA synthesis from activated B cells in different assay systems, thus implying that IL-13 does not play a major role in the regulation of the production of these Ig isotypes. However, IL-13 selectively stimulated IgE secretion from B cells co-cultured with activated T cells or with an anti-CD40 mAb presented by a fibroblastic cell line expressing the murine FcγRII. The observation that neutralizing anti-IL-4 antibodies failed to antagonize the IgE-stimulatory effect of IL-13, in a T cell–free culture system demonstrates that IL-13 operates through an IL-4–independent mechanism. Since the biological effects of IL-13 are preferentially directed at $sIgD^+$ B cells it is likely that the IgE-inducing activity of IL-13 results from isotype switching rather than from differentiation of IgE-committed precursors.

Based on the observation that IL-4 and IL-13 have a highly overlapping set of biological activities on human B cells, it is tempting to speculate that these two cytokines, despite limited protein sequence homology, could share receptor signaling pathways. Experimental evidence has been provided that this is the case for other cytokines such as IL-5, IL-3, and GM-CSF which share a common receptor subunit responsible for signal transduction (29). Until recently, the IL-4 receptor was believed to consist of a single chain binding IL-4

with high affinity and capable of transmitting a growth signal when transfected in various cell lines (24, 29). However, two lines of evidence now support the existence of two IL-4 receptors of different affinities, possibly coupled to separate signal transduction pathways. First, Rigley et al., (30) have shown that the IL-4–mediated enhancements of sIgM and CD23 expression on human B cells are regulated independently through distinct second messenger cascades. Second, an additional IL-4 binding protein of low affinity has recently been identified on human pre-B cells (31). Thus, the possibility exists of a multisubunit IL-4 receptor including potential IL-13 signal transducing molecules. It can not be excluded that the IL-13 receptor may also be coupled to a unique signaling pathway, as suggested by the observation that IL-4 and IL-13 display opposite effects on the IL-2–mediated proliferation of activated B cells.

Although IL-13 would appear to contribute an additional redundancy in the cytokine regulation of the B cell maturation process it may be unique in its capacity to preferentially induce growth of virgin sIgD$^+$ B cells. It does not significantly affect the production of the IgM, IgG, and IgA isotypes but stimulates IgE synthesis and as such might contribute to the development of allergic diseases. In keeping with this, although IL-4 has been implicated by gene-deletion experiments (32, 33) as the major, if not the unique switch factor for IgE in the mouse, the existence of an additional IgE-inducing activity, produced by T cells from atopic patients, has been recently demonstrated (34). This observation raises the possibility that an IL-4–independent mechanism of IgE regulation, possibly mediated by IL-13 might operate in atopic individuals.

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