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Influence of IL-2 and IL-4 on the IgE synthesis and the IgE-binding factor (sCD23) production by human lymphocytes *in vitro*

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SUMMARY

The influence of IL-2 and IL-4 on the mitogen-induced immunoglobulin E and IgG production *in vitro* was analysed. Furthermore the expression of FcεRII (CD23 antigen), as well as the release of its soluble products, the isotype-specific IgE binding factors (IgE-BF), was determined. Recombinant IL-2 (rIL-2) exerted opposite effects on the synthesis of IgE by human lymphocytes that were stimulated either by pokeweed mitogen (PWM) or *Staphylococcus aureus* Cowan I (SAC). rIL-2 induced a dose-dependent suppression of IgE and IgG synthesis in the presence of PWM. This effect was accompanied by a significant decrease of IgE-binding factor (BF), whereas the expression of FcεRII was not significantly modulated by rIL-2. A marked increase of IgE production was observed when lymphocytes, prestimulated with SAC for 48 hr, were further incubated with increasing amounts of rIL-2 for 6 days. In contrast, IL-4 in concentrations ranging from 500 to 4·9 U/ml did not lead to an enhancement of IgE synthesis in lymphocytes that were prestimulated with SAC. However, SAC-induced IgG secretion was significantly enhanced by 2·3 U/ml of rIL-4. A dose-dependent enhancement of IgE-BF was observed in SAC-prestimulated lymphocyte cultures in the presence of rIL-2 as well as rIL-4. These results demonstrate that the mitogen used for lymphocyte activation, T-cell-derived lymphokines such as IL-2 and IL-4, and IgE-specific binding factors (soluble CD23), are responsible for the induction of human IgE antibody production *in vitro*.

INTRODUCTION

Interleukins as well as isotype-specific lymphokines play an important role in the regulation of immunoglobulin E synthesis. Interleukin-2 (IL-2) as well as interleukin-4 (IL-4) have been reported acting as B-cell growth and differentiation factors in experimental animal and man. In the past, we have suggested the regulatory influence of IL-2 on mitogen-stimulated lymphocytes (Knöller *et al.*, 1988). Moreover, IL-4 is known to modulate IgE synthesis of activated mouse (Coffman *et al.*, 1986) as well as human lymphocytes (Pene *et al.*, 1988). Furthermore, IL-4 has been shown to induce the expression of the low affinity receptor for IgE (FCεRII, CD23 antigen) on B cells.

Recently, much emphasis has been directed on the role of CD23 and its soluble products (sCD23, IgE-binding factors) with regard to the IgE antibody regulation. It has been shown that FcεRII is present on T and B lymphocytes, monocytes, platelets and eosinophiles (Capron *et al.*, 1986). On B cells the *de novo* appearance of antigens such as CD23 at a distinct point during the B-cell programme suggests that these molecules are involved as foci for external signals in guiding the cells to the next phase of response. The availability of monoclonal antibodies allows the analysis of the kinetics of CD23 expression as well as the occurrence of soluble CD23.

The role of soluble CD23 as an autocrine growth factor has been suggested. With regard to the effects on IgE synthesis the IgE binding factors from different cell sources, as well as binding factors of various molecular weights, may exert different functions.

In rodents T-cell-derived IgE-binding factors, according to their glycosylation, potentiate or suppress IgE antibody formation (Ishizaka, 1985). In humans soluble products of CD23 (15,000 and 30,000–40,000 MW; Sarfati *et al.*, 1984) have been characterized from the lymphoblastoid B-cell line RPMI-8866 as well as peripheral blood lymphocytes (Nakajima, Sarfati & Delespesse, 1987). Furthermore, IgE-BF with molecular weights of 60,000 and 25,000 were detected within the sera of normal donors whereas sera of patients with atopic dermatitis contained a 60,000 MW component (Bujanowski-Weber *et al.*, 1988).

Abbreviations: AET, 2-aminoethylisothiouronium bromide; FcεRII, CD23, low affinity receptor for IgE; FCS, fetal calf serum; IgE-BF, IgE binding factor; IL(2,4), interleukin 2,4; PBS, phosphate-buffered saline; PWM, pokeweed mitogen; SAC, *Staphylococcus aureus* Cowan I; sCD23, soluble CD23.

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It was the purpose of the present study to analyse the effect of various lymphokines (IL-2, IL-4) on mitogen-activated human lymphocytes *in vitro*. Peripheral blood lymphocytes were stimulated with pokeweed mitogen (PWM) and *Staphylococcus aureus* Cowan I (SAC). The synthesis of Ig(E,G), the expression of CD23 as well as the release of IgE-BF (sCD23) were analysed.

MATERIALS AND METHODS

Isolation of lymphocytes and cell separation

Lymphocytes were obtained from healthy volunteers. Isolation was performed by centrifugation on a Ficoll–sodium metrizoate (Sigma, München) gradient according to Böyum (1976). Briefly, heparinized venous blood (200 ml) was layered over Ficoll–sodium metrizoate (density = 1·075 g/ml) and centrifuged at 375 *g* for 45 min. Cells at the interface above the Ficoll–metrizoate were removed and washed three times with RPMI-1640. These cells are referred to as 'unseparated lymphocytes'.

For isolation of B cells, adherent cells were removed by incubation of lymphocytes (5×10^6/ml in RPMI-1640 + 10% FCS) on sterile plastic petri-dishes. After 2 hr of incubation non-adherent cells were removed, washed and resuspended in RPMI-1640 + 10% FCS. T cells were removed by differential centrifugation after incubation with 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells (Biologische, Ribeibgemeinschafts, Lich) (Kaplan & Clark, 1974). This procedure was repeated twice. Non-rosetting cells are referred to as the 'B-cell-enriched fraction'.

Culture conditions

The basic culture medium was RPMI-1640 supplemented with 2 mM glutamine, 100 μg/ml streptomycin, 100 IU/ml penicillin, 10 mM HEPES and 20 mM sodium hydrogen carbonate. Medium containing 10% fetal calf serum is referred to as RPMI-1640 + 10% FCS.

Cell suspensions containing 1×10^6/ml viable cells in RPMI-1640 + 10% FCS were dispensed into each well of 24-sample multi-well plates. Stimuli (see results) were added and the cultures were incubated at 37° in a humidified atmosphere of 5% CO_2 in air. Cell suspensions containing 2×10^5/ml cells were transferred into flat-bottomed microtitre plates. Thymidine incorporation was measured 3 days later after a 4-hr pulse with 7·4 KBq/10 μl [^{3}H]thymidine (Amersham Buchler, Braunschweig). The IgE, IgE-BF and IgG content of culture supernatants were determined by radioimmunoassay.

Cell suspensions containing 2×10^5/ml cells were transferred into flat-bottomed microtitre plates. Thymidine incorporation was measured 3 days later after a 4-hr pulse with 7·4 KBq/10 μl [^{3}H]thymidine (Amersham Buchler).

Preparation of RPMI-8866 cell supernatant containing IgE-BF

RPMI-8866 cells (G. Delepesse) were incubated 48 hr in RPMI-1640 + 10% FCS at a concentration of 2×10^6 cells/ml. The supernatant was collected by centrifugation (10 min, 375 *g*) and concentrated 10 times by ultrafiltration through a YM5 membrane (Amicon, Witten).

Lymphokines and polyclonal stimuli

Purified recombinant DNA derived human IL-2 (rIL2; activity: 2×10^6 U/ml) was obtained from Boehringer (Mannheim). Recombinant IL-4 (specific activity: 1×10^8 U/mg) was obtained from Genzyme (IC Chemikalien, München).

Pokeweed mitogen (PWM), obtained from Sigma (München), was used at final concentrations of 0·16 and 0·8 μg/ml. Formalized *Staphylococcus aureus* Cowan I (SAC) was purchased from Calbiochem-Behring Corp. (Gießen). For stimulation a final dilution of 1:10000 (v/v) was used.

Radioimmunoassay for IgE and IgG

The IgE and IgG content of culture supernatants were determined by a solid-phase radioimmunoassay. Briefly, purified goat anti-human IgE (100 μg) and IgG (100 μg) antibodies (1·5 mg/ml; Medac, Hamburg) were labelled with 37 MBq Na^{125}I (specific activity: 520·2 MBq/μg of iodine; Amersham Buchler) as described by Klinman & Taylor (1969).

Removawell U-bottomed wells (Dynatech, Denkendorf) were coated with anti-IgE (0·1 ml, diluted 1:1000 in phosphate-buffered saline supplemented with 0·05% Tween 20) or anti-IgG (0·2 ml, 1:1500 in PBS, 0·05% Tween 20) for 4 hr. 0·1 ml (IgE-RIA) or 0·2 ml supernatant (IgG-RIA: 1:20–1:40 dilution) was added and incubated overnight at room temperature. [^{125}I]anti-IgE (100,000 c.p.m.) or [^{125}I]anti-IgG (100,000 c.p.m.), respectively, were added for additional 4 hr at 37°. In parallel, standard curves for IgE (0·2–200 ng/ml) and IgG (0·3–300 ng/ml) were performed. The detection limits for IgE and IgG were 0·4 ng/ml and 0·8 ng/ml, respectively. Specificity of the assays was confirmed by adding Ig of other isotypes to rule out the possibility of cross-reactivity.

Radioimmunoassay for IgE-BF

The RIA for IgE-BF was performed as has been described by Delespesse, Sarfati & Rubio-Trujillo (1987). Briefly, Removawells were coated with monoclonal antibody (mAb) 176 (2–10 μg/ml; diluted in bicarbonate buffer, pH 9·0; a generous gift from Dr G. Delespesse, Hopital Notre Dame, University of Montreal, Canada) overnight at room temperature. After washing and saturation with PBS + 10% FCS, samples were added for 4 hr at room temperature. Subsequently, the wells were washed again and incubated with the ^{125}I-labelled mAb 135 (100 μl, 100,000–300,000 c.p.m.) overnight. Bound radioactivity was determined. The amount of IgE-BF was expressed as a percentage of total radioactivity (100%).

Detection of FcεRII on lymphocytes

FCεRII were determined by using mAb 135 (generous gift of Dr G. Delespesse). One-hundred microlitres of lymphocyte suspension (1×10^6/ml) were incubated with 100 μl of radiolabelled mAb 135 (400,000 c.p.m.) for 60 min. The cells were then applicated to an FCS layer (400 μl FCS per tube). Cell-bound and unbound radioactivity were separated by centrifugation (500 *g*). The amount of non-specific activity attached to the tubes was lower than 0·5% of total activity. Each sample was determined in triplicates at least. If not indicated otherwise, the ratio of cell-bound radioactivity was calculated as percentage of binding (of total activity) per 1×10^6 cells.

Statistics

The data were calculated as means ± SD. The significance was evaluated with Student's *t*-test for independent means. $P < 0{\cdot}05$ was considered significant.

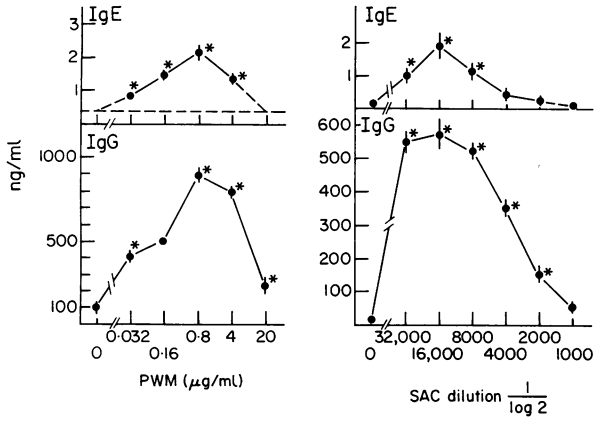

Figure 1. Effect of PWM and SAC as polyclonal stimuli for IgE (upper panel) and IgG (lower panel) induction of unfractionated blood lymphocytes. A PWM-induced IgE response could be demonstrated in only three out of nine experiments. The maximal response differed from donor to donor ranging from 1·2 to 2·2 ng/ml of IgE. Therefore a representative experiment in which an IgE response was obtained by stimulation with PWM is shown. The optimal proliferative response (stimulation index 1·24±0·12, $n=9$, data not shown) was obtained at a concentration of 0·8 μg/ml of PWM. The data representing the influence of SAC are mean values of three independent experiments ($n=3\pm$SD). Optimal proliferative response: stimulation index 1·6±0·3 in the presence of SAC at a final dilution of 1:10,000 (data not shown). * Significant enhancement compared with the controls.

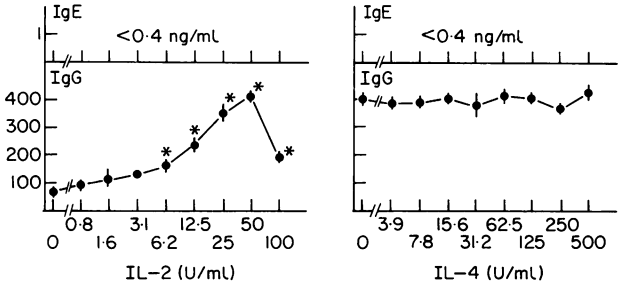

Figure 2. Effect of rIL-2 ($n=6$) and rIL-4 ($n=5$) on IgE (upper panel) and IgG (lower panel) production in unstimulated cultures of unfractionated peripheral blood lymphocytes. *Significant enhancement compared with the controls.

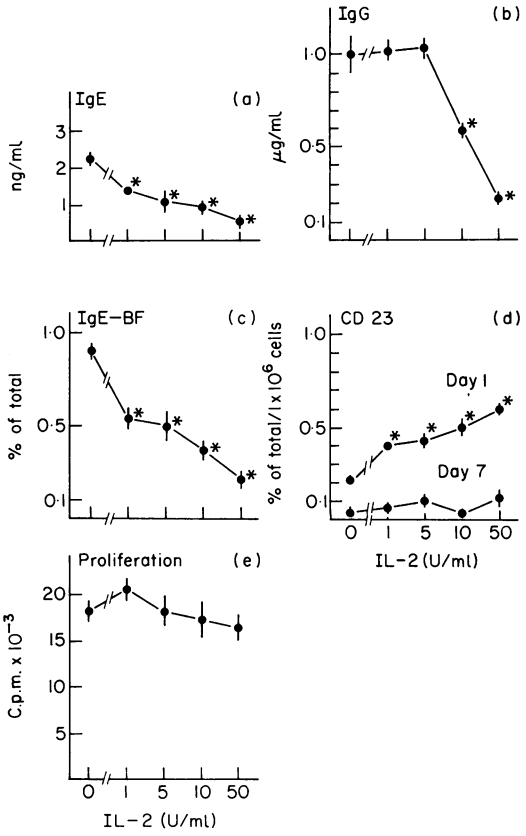

Figure 3. Influence of rIL-2 on PWM-driven IgE (a) and IgG (b) synthesis as well as proliferative response (e) of unseparated peripheral blood lymphocytes. The release of IgE-BF (c) and expression of CD23 antigen (d) were determined by the use of monoclonal antibodies. These data represent an experiment in which an IgE response was obtained by stimulation with PWM ($n=3/9$). *Significant compared with the control cultures stimulated by PWM only.

RESULTS

Induction of IgE and IgG synthesis by PWM

Peripheral blood lymphocytes (1×10^6/ml) of apparently non-atopic donors were incubated with PWM or SAC in various concentrations for 7 days (Fig. 1).

Stimulation of lymphocytes with PWM at a concentration of 0·8 μg/ml and 0·16 μg/ml, respectively, induced an IgE response up to 2·2 ng/ml in three out of nine independent experiments. In all experiments ($n=9$) PWM led to a dose-dependent increase in IgG synthesis; maximal responses were obtained in cultures containing 0·16–0·8 μg/ml PWM (stimulation index: 6·6–14).

IgE antibody production was consistently observed when lymphocytes were incubated with SAC bacteria at final concentrations from 1:8000 to 1:32000 (Fig. 1). In these experiments ($n=10$) IgE secretion ranged from 0·50 ng/ml to 2·21 ng/ml. The increase in IgE production was accompanied by a significantly enhanced IgG synthesis. Similar results were obtained when lymphocytes were preincubated with SAC (1:10000 v/v) for 2 days only, followed by a 6-day culture period without the stimulus.

Influence of recombinant IL-2 or IL-4 on the Ig(E,G) synthesis of unstimulated lymphocytes

To investigate the effects of either IL-2 or IL-4 on IgE production *in vitro*, peripheral lymphocytes were incubated with rIL-2 or rIL-4 at various concentrations for 7 days (Fig. 2). Neither rIL-2 nor rIL-4 in concentrations ranging from 100 to 1 U/ml and 500 to 10 U/ml, respectively, were able to induce detectable IgE secretion from unstimulated lymphocytes. However, in four out of 11 experiments IgE secretion up to 1·2 ng/ml was observed in the presence of 200 U/ml of rIL-2 (data not shown). Secretion of IgG was significantly enhanced when unstimulated lymphocytes were incubated with 6·25 U/ml of rIL-2. Increasing concentrations of rIL-2 induced an enhanced

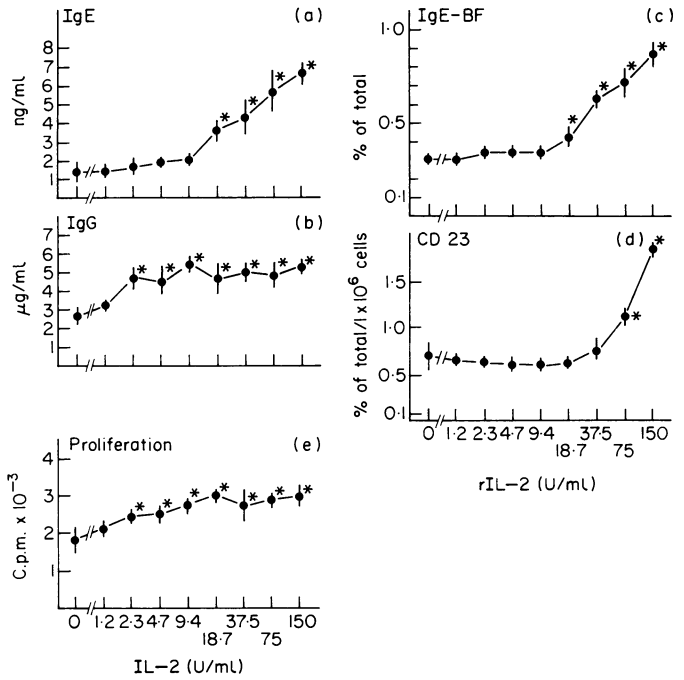

Figure 4. Influence of rIL-2 on SAC-prestimulated unseparated lymphocytes: synthesis of IgE (a), IgG (b), IgE-BF (c) as well as expression of CD23 (d) and proliferative response (e) were analysed. Mean ± SD of triplicate samples of a representative experiment is shown. *Significant compared with the control cultures stimulated by SAC only.

IgG response with a stimulation index of 8·43 ± 3·78 ($n = 6 \pm$ SD). Addition of rIL-4 to unstimulated lymphocyte cultures did not modulate IgG synthesis.

Effect of lymphokines (rIL-2, rIL-4) on mitogen-stimulated lymphocytes

Experiments were carried out to study the role of IL-2 on the PWM-driven IgE synthesis. Lymphocytes (1×10^6/ml) stimulated with PWM (0·16 μg/ml) were incubated with rIL-2 at various concentrations (1–100 U/ml). As is demonstrated in Fig. 3a, increasing concentrations of IL-2 led to a dose-dependent suppression of IgE production. IL-2 at a concentration of 100 U/ml completely suppressed IgE production (detection limit of the RIA for IgE: 0·4 ng/ml), while 10 U/ml or rIL-2 diminished IgE synthesis between 12·2% and 48·1%. However, the suppression of IgE, which was observed in all experiments of the PWM-induced IgE production ($n = 3$), was not isotype specific. In nine independent experiments it was shown that rIL-2 (10–50 U/ml) led to a dose-dependent suppression of the PWM-induced IgG synthesis (Fig. 3b). A mean reduction of the IgG synthesis up to 81·3% ± 13·9% ($n = 9 \pm$ SD) compared with the control values (PWM-induced IgG secretion without rIL-2) was obtained.

Under the experimental conditions ($n = 8$) DNA synthesis was not altered in the presence of 50 U/ml of rIL-2 (Fig. 3e). Addition of 100 U/ml of rIL-2 led to a 30% reduction of the proliferative response in three experiments (data not shown).

In subsequent experiments the influence of rIL-2 or rIL-4 was analysed on SAC prestimulated lymphocytes. As was described above, IgE synthesis was induced by prestimulating lymphocytes with SAC (1:10,000) for 48 hr. A significant enhancement of IgE production was observed when SAC prestimulation was followed by a 6-day incubation with increasing amounts of rIL-2 (Fig. 4a). As was demonstrated in 12 independent experiments, IL-2 enhanced IgE production up to 12·6 ± 5·2 ng/ml ($n = 12 \pm$ SD).

Experiments were then carried out to analyse the effect of rIL-2 on activated B cells. B lymphocytes (1×10^5/ml) were incubated with SAC (1:10,000) for 48 hr, washed three times and cultured in the presence of rIL-2 (3·12–100 U/ml). rIL-2 did not induce IgE synthesis in SAC-prestimulated B cells ($n = 5$). Furthermore, IgG production was not significantly modulated by rIL-2 ($n = 4$), whereas proliferation was markedly enhanced in the presence of 12·5 U/ml of rIL-2 ($n = 5$) (data not shown).

In contrast to rIL-2, prestimulation of peripheral unseparated blood lymphocytes with SAC ($n = 6$) and subsequent addition of rIL-4 (4·9–500 U/ml) did not lead to an enhanced IgE synthesis (Fig. 5a). The IgG secretion induced by SAC was modulated by both lymphokines: 2·3 U/ml of rIL-2 (Fig. 4b) as well as rIL-4 (Fig. 5b) induced a significant enhancement of IgG production compared with the control cultures (SAC induced Ig secretion without addition of interleukins). Increasing concentrations of either rIL-2 (up to 150 U/ml) or rIL-4 (up to 150 U/ml) did not lead to a further increase of the IgG synthesis.

In contrast to rIL-4, increasing concentrations of rIL-2 induced a slight enhancement of lymphocyte proliferation (Fig. 4e), which was 1·35 ± 0·22-fold at 100 U/ml ($n = 5 \pm$ SD).

Effect of mitogens and lymphokines on the expression of FcεRII (CD23), release of IgE-BF (sCD23) and Ig(E,G) synthesis

Since it has been suggested that IgE-BF (soluble CD23) may modulate IgE synthesis, the expression of FcεRII on lymphocytes as well as the secretion of IgE-BF in PWM- and SAC-stimulated lymphocytes was studied.

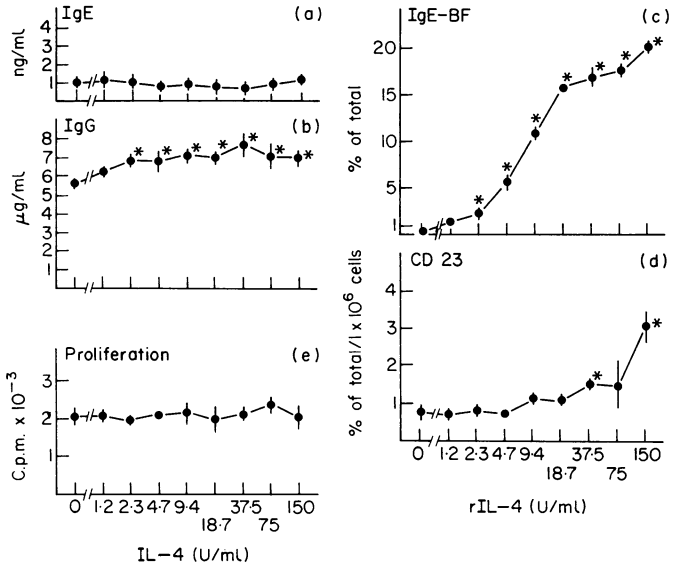

Figure 5. Effect of rIL-4 on SAC prestimulated unseparated lymphocytes: synthesis of IgE (a), IgG (b), IgE-BF (c) as well as expression of CD23 (d) and proliferative response (e) were analysed. Means ± SD of triplicate samples of a representative experiment are shown. * Significant as compared to the control cultures stimulated by SAC only.

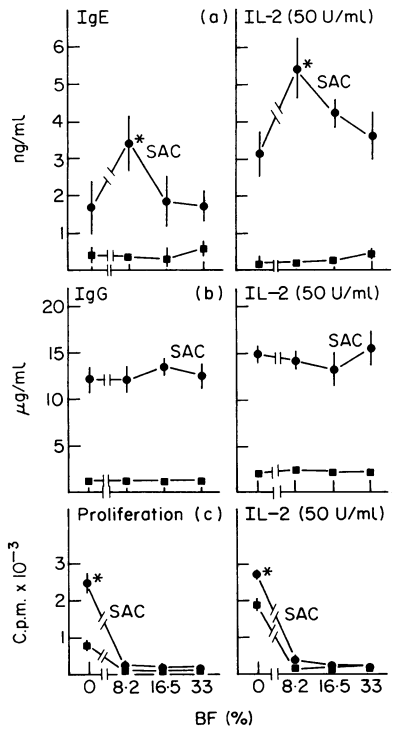

Figure 6. Influence of IgE-BF on lymphocytes which were either unstimulated (squares) of prestimulated with SAC (circles) in the absence (left panels) or presence (right panels) of rIL-2 (50 U/ml). Secretion of IgE (a), IgG (b) as well as proliferative response (c) were analysed. Mean ± SD of triplicate samples of a representative experiment is shown. * Significant as compared to the unstimulated or prestimulated control cultures without addition of IgE-BF.

As is apparent from Fig. 3c, IgE-BF production in PWM-stimulated cultures was suppressed by increasing concentrations of rIL-2 in parallel to IgE synthesis. FcεRII expression (Fig. 3d) was enhanced up to 0·58 ± 0·03% at Day 1 of culture in the presence of rIL-2. At Day 7 FcεRII expression was minimal (0·04 ± 0·01%–0·1 ± 0·02%) at each concentration of rIL-2 (Fig. 3d).

A significant enhancement of IgE-BF was observed when SAC prestimulated lymphocyte cultures were analysed in the presence of rIL-2 (Fig. 4c). As was shown for the IgE synthesis 18·7 U/ml of rIL-2 induced a significant increase in IgE-BF up to 0·8 ± 0·3% ($n = 5 \pm$ SD). Fcε receptor II expression also increases from 0·7 ± 0·18% to 1·8 ± 0·1% at a concentration of 150 U/ml of rIL-2 (Fig. 4d).

The effect of rIL-4 on IgE-BF production as well as FcεRII expression of SAC-prestimulated lymphocyte cultures is demonstrated in Fig. 5c and d. At a concentration of 150 U/ml of rIL-4 IgE-BF production increased up to 22% ± 2% binding. FcεRII expression increased from 0·87 ± 0·2% to 3·12 ± 0·38% after 7 days of culture.

We next studied the influence of IgE-BF on lymphocytes that were prestimulated with SAC in the absence or presence of rIL-2 (50 U/ml). Unseparated lymphocytes (1×10^6/ml), either unstimulated or stimulated with SAC (1 : 10,000) for 48 hr, were further incubated for 7 days with the culture supernatant of RPMI-8866 cells which contained IgE-BF. As is demonstrated (Fig. 6a) addition of RPMI-8866 supernatant at a concentration of 8·2% led to a significant enhancement of IgE synthesis by SAC-stimulated lymphocytes, which further increased in the presence of 50 U/ml of rIL-2. In contrast, IgE production of unstimulated lymphocytes was not significantly modulated by IgE-BF in the presence or absence of rIL-2.

IgE-BF in the presence or absence of rIL-2 (50 U/ml) did not induce a significant modulation of the IgG synthesis in SAC

stimulated or unstimulated control cultures (Fig. 6b), whereas the proliferative response was markedly suppressed in the presence of the IgE-BF containing supernatant (Fig. 6c).

DISCUSSION

Our results clearly demonstrate that IL-2 has opposite effects on the synthesis of IgE and IgG when PWM- and SAC-stimulated human lymphocyte cultures are analysed. rIL-2 induced a dose-dependent suppression of imunoglobulin synthesis in the presence of PWM, whereas a significant enhancement of both IgE and IgG synthesis in SAC-stimulated cultures was obtained. Addition of IL-4 up to 150 U/ml did not influence IgE antibody production in SAC-stimulated lymphocytes.

The regulatory function of IL-2 regarding the PWM-induced IgM, IgG and IgA production is suggested by different observations:

Tac antigen expression was demonstrated on B cells that were activated by incubation with PWM (e.g. Jung, Hara & Fu, 1984; Waldmann *et al.*, 1984). Furthermore, the T-cell dependent PWM-induced IgM, IgG as well as IgA production by B cells was inhibited by different monoclonal antibodies against the IL-2 receptor (Depper *et al.*, 1983; Ceuppens & Stevens, 1986).

The role of $CD4^+$ cells or helper cell-derived soluble mediators in the PWM-driven IgM, IgG and IgA synthesis has been suggested by several groups (e.g. Miedema *et al.*, 1985; Nakagawa *et al.*, 1987). It has been shown that the PWM-induced IL-2 production is responsible for the secretion of various B-cell growth and differentiation factors which regulate the development of B cells. Our studies are supported by the observations of Pryjma *et al.* (1986) who demonstrated a dose-dependent reduction of Ig-secreting cells in lymphocyte cultures stimulated with PWM. The suppressive influence of IL-2 was obviously dependent on the functional activation of $CD8^+$ suppressor T cells by exogenous IL-2 (Pryjima *et al.*, 1986). These suppressor cells apparently regulated the secretion of T-cell derived B-cell differentiation factors.

Although the *de novo* synthesis of IgE by PWM from normal donor cells has been controversially discussed (e.g. Saxon & Stevens, 1979; Romagnani *et al.*, 1980) and is by no means consistent in our experiments, it has been suggested that the PWM-induced IgE synthesis may be due to the release of soluble mediators (Saxon, Morrow & Stevens, 1980). In our experiments three out of nine donors without an apparent history of atopy showed IgE generation after stimulation with PWM. The release of preformed or cell-bound IgE from the cells as an explanation in contrast to the *de novo* IgE synthesis appeared to be unlikely; our data showed that the addition of IL-2 suppresses IgG as well as IgE synthesis. In these experiments the stimulation index is hardly affected by the addition of rIL-2. A cross-reaction of the IgE measurement with IgG was also excluded. An ongoing spontaneous IgE synthesis (patients with atopic dermatitis) in the absence of PWM was modulated to a variable degree after addition of rIL-2. Several donor cells ($n=4$) were responsive while others ($n=3$) remained unaffected as to the synthesis of IgE and IgG.

The IL-2-induced suppression of the PWM-induced Ig(G,E) synthesis indicates that similar mechanisms are involved in the IL-2-induced regulation of both isotypes. The activity of IgE-specific suppressor cells was demonstrated in various cell culture systems. As is shown by our results the suppression of IgE synthesis is accompanied by a reduction of IgE binding factor (sCD23) secretion. Therefore, a decrease of IgE-BF production mediated by an IL-2 dependent activation of $CD8^+$ T cells might be responsible for the suppression of IgE synthesis. Since IgE-BF is the soluble product of the low affinity receptor for IgE (sCD23), the expression of the FcεRII was determined at the first day and at the end of the culture period. As became apparent, no significant modulation of FcεRII expression was observed in cultures stimulated with PWM and rIL-2. Thus, it appears that a decrease in IgE-BF (sCD23) secretion is not coupled to an enhanced number of residual CD23 antigen on lymphocytes after 7 days of culture. However, time kinetics have to be performed to further clarify the interdependent interaction of cell-bound and soluble CD23.

Stimulation of B lymphocytes with SAC leads to a progression of the resting stage into the G1 stage of the B-cell cycle, which is characterized by cell enlargement. RNA synthesis and expression of cell surface proteins such as the IL-2 receptor (CD25) (Mingari *et al.*, 1984; Mittler *et al.*, 1985; Muraguchi *et al.*, 1985; Tsudo, Uchiyama & Uchino, 1984). The appearance of new surface proteins suggests that these molecules may serve as receptors for soluble growth and differentiation factors which might regulate the next phase of B-cell differentiation.

As was demonstrated by our results, stimulation of lymphocytes with SAC for 48 hr followed by the incubation with rIL-2 led to an enhancement of IgE as well as IgG synthesis, even in the absence of the mitogen. These results are obviously dependent on the presence of T cells as well as monocytes since no Ig production was observed in the cultures of isolated B cells, although the proliferation was enhanced in both culture systems.

The ability of IL-2 to act as a growth factor for SAC activated B cells has been described by several groups (Mingari *et al.*, 1984, 1985; Muraguchi *et al.*, 1985; Tsudo *et al.*, 1984; Teranishi *et al.*, 1984).

Whether IL-2 directly induces B-cell differentiation via IL-2 receptors on B lymphocytes is still contradictory, although this hypothesis is supported by studies using B-cell hybridomas (e.g. Kishi *et al.*, 1985) and isolated B lymphocytes (Muraguchi *et al.*, 1985; Romagnani *et al.*, 1986; Ralph *et al.*, 1984). In these studies different concentrations of rIL-2 (1·5 U/ml, Romagnani *et al.*, 1986, to 10,000 U/ml, Ralph *et al.*, 1984) influenced Ig (M,G,A) synthesis.

Our results indicate that incubation of SAC-activated lymphocytes with rIL-2 does not only enhance IgG production but also leads to an augmented IgE secretion. In addition, we have demonstrated an increase in isotype-specific IgE-BF (sCD23) as well as FcεRII expression which parallels IgE synthesis in cultures stimulated with SAC and rIL-2. Several possibilities are likely: IL-2 may regulate the secretion of T-cell derived lymphokines that may exert a modulatory function on B-cell differentiation. This consideration is also supported by studies using isolated B cells which were co-cultured with different numbers of T lymphocytes (Pryjma *et al.*, 1986; Teranishi *et al.*, 1984). Using monoclonal antibodies against the

IL-2 receptor, Kehrl *et al.* (1986) demonstrated that IL-2 among other differentiation factors plays an important role in B-cell differentiation. Furthermore, Ishizaka *et al.* (1985) demonstrated that SAC is responsible for the activation of $CD4^+$ T-helper cells. Since the secretion of IgE-BF (sCD23) by $CD4^+$ T-helper cells has already been described (Young, Leung & Geha, 1984a, b), rIL-2 may be involved in the cleavage of CD23 antigen into its biological active fragments. Thus, rIL-2 might induce the secretion of B-cell differentiation factors in SAC-stimulated cell cultures; among those IgE-specific lymphokines could be responsible for the differentiation of IgE-secreting plasma cells.

Recently, evidence has been provided that IL-4 modulates IgE synthesis in mice (Coffman *et al.*, 1986) and enhances the expression of CD23 on the surface of human B cells (Defrance *et al.*, 1987; Rousset *et al.*, 1988). The IL-4-induced IgE synthesis from normal human B cells was also recently analysed. Concentrations of up to 2560 U/ml were applied (Pene *et al.*, 1988), which were five-fold higher compared with the amounts in our experiments. The mechanism of IgE synthesis, CD23 expression and soluble CD23 (IgE-BF) release at suboptimal concentrations of IL-4 has also been reported. The IL-4 induced IgE production by peripheral blood lymphocytes and tonsil cells was enhanced in a dose-dependent fashion by IL-5. Furthermore, a role of soluble CD23 at suboptimal concentrations of IL-4 was proposed, suggesting a synergism for the modulation of IgE (Pene *et al.*, 1988).

These results imply that various lymphokines in addition to IL-4 are involved in IgE synthesis, which might also be released from the cells by high concentrations of IL-4.

Our data suggest that IgE production in SAC-prestimulated lymphocyte cultures after incubation with IL-4 alone at the concentrations studied does not induce IgE synthesis in unseparated cells. A marked increase, however, in IgE-BF (sCD23) secretion as well as CD23 expression was observed. IL-4 enhanced the IgE-BF (sCD23) production and CD23 expression to a higher extent compared with IL-2. Thus, the up-regulation of IgE-BF (sCD23) production does not necessarily induce an enhanced IgE synthesis. It is not clear whether the different molecular forms of IgE-BF (sCD23) (Bujanowski-Weber *et al.*, 1988) may exert opposite effects. Our results suggest that various soluble mediators derived from co-operating T-helper cell populations are necessary to induce human IgE synthesis *in vitro*.

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