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Expression of low-affinity receptor for IgE (FcεRII, CD23) and IgE-BF (soluble CD23) release by lymphoblastoid B-cell line RPMI-8866 and human peripheral lymphocytes of normal and atopic donors

J. BUJANOWSKI-WEBER, B. BRINGS, I. KNÖLLER, T. PFEIL & W. KÖNIG *Institut für Medizinische Mikrobiologie und Immunologie, Arbeitsgruppe für Infektabwehrmechanismen, Ruhr-Universität Bochum, 4630 Bochum, FRG*

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SUMMARY

The low-affinity receptor for IgE (CD23) as well as the soluble IgE-binding factors (IgE-BF, sCD23) are important factors in IgE antibody regulation. The CD23 expression and the concomitant release of CD23 were analysed from the lymphoblastoid B-cell line RPMI-8866 and from peripheral blood lymphocytes (PBL) of healthy volunteers as well as atopic patients. CD23 expression and sCD23 release of RPMI-8866 cells were dependent on the stage of culture. While CD23 expression decreased with increasing time of culture (Day 1–3), the sCD23 release was enhanced during the culture period. Cytokines such as IL-4, IL-2, TNFα and IFN-γ exerted various effects on the target cells depending on the culture period. CD23 expression on normal lymphocytes was lower compared with the expression on atopic cells. Lymphokines (IL-2, IL-4) as well as mitogens (PHA, Con A) enhanced CD23 expression and IgE-BF (sCD23) release. The degree of enhancement was always higher with atopic cells compared with the results obtained with cells of normal donors.

INTRODUCTION

The low-affinity receptor for IgE (FCεRII), which has been shown to be identical with the B-cell marker CD23 (Yukawa *et al.*, 1987; Bonnefoy *et al.*, 1987) and the BLAST-2 antigen (Thorley-Lawson, 1985) and its soluble fragments, the IgE-binding factors (IgE-BF; sCD23), play an important role in the regulation of IgE synthesis. CD23 was described as an early B-cell marker (Kikutani *et al.*, 1986a) and the soluble CD23 has been shown to possess autocrine growth activity (Gordon *et al.*, 1986).

Furthermore, CD23 has been implicated in a variety of functions (Gordon & Guy, 1987; König *et al.*, 1985; Geha, 1984). In addition to B lymphocytes, CD23 has been detected on human T lymphocytes (Prinz *et al.*, 1987; Thompson *et al.*, 1983; Kanowith-Klein & Saxon, 1986; Deguchi *et al.*, 1983), macrophages (Sarfati *et al.*, 1986), eosinophils (Capron *et al.*, 1981) and platelets (Joseph *et al.*, 1986). The expression of CD23 is enhanced in the presence of IL-4 (Gordon *et al.*, 1986; Defrance *et al.*, 1987) as well as on the surface of EBV-transformed cells (Thorley-Lawson, 1985). Recently, the CD23 molecule has been characterized by cloning and sequence techniques (Kikutani *et al.*, 1986b; Lüdin *et al.*, 1987; Ikuta *et al.*, 1987).

It has been suggested that the soluble fragments of CD23 (IgE-BF) are important factors for the IgE regulation in rodents (Ishizaka, 1985) as well as in the human system (Geha, 1984; König *et al.*, 1985). Several groups have described IgE-BF derived from T cells after stimulation with IgE (Kanowith-Klein & Saxon, 1986; Deguchi *et al.*, 1983; Huff & Ishizaka, 1984; Young, Leung & Geha, 1984; Ishizaka & Sandberg, 1981). IgE-BFs have also been identified within the supernatants of T-cell lines (Huff & Ishizaka, 1984), of FcεRII-bearing peripheral B cells (Gonzales-Molina & Spiegelberg, 1977) and of the lymphoblastoid B-cell line RPMI-8866 (Jensen, Sand & Spiegelberg, 1984; Sarfati *et al.*, 1984a, b, c). The supernatant of T cells contains IgE-BF with a MW of 25,000–30,000 and 15,000 (Huff & Ishizaka, 1984), whereas within the B-cell supernatants factors with 20,000–25,000 and 45,000 MW were detected (Ikuta *et al.*, 1987). Sarfati *et al.* (1984b) described two IgE-BFs with molecular sizes in the range of 15,000 MW and 30,000–40,000 MW within the RPMI-8866 supernatant. Furthermore, IgE-BFs with MW of 60,000 and 15,000 were detected within the plasma of patients with a hyper-IgE syndrome (Leung *et al.*, 1986; Sarfati *et al.*, 1986). Recently, IgE-BFs with 25,000 and 45,000 MW were analysed within the sera of normal donors

Abbreviations: Con A, concanavalin A; BCGF, B-cell growth factor; EBV, Epstein–Barr virus; FcεRII, low-affinity receptor for IgE; FCS, fetal calf serum; IFN-γ, interferon-gamma; IgE-BF, IgE-binding factor; IL-4, interleukin-4; IL-2, interleukin-2; LPS, lipopolysaccharide; mAb, monoclonal antibody; MW, molecular weight; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; PHA, phytohaemagglutinin; RIA, radioimmunoassay; sCD23, soluble CD23, IgE-BF; TNFα, tumour necrosis factor α.

Correspondence: Professor W. König, Lehrstuhl Med. Mikrobiologie und Immunologie, Arbeitsgruppe Infektabwehrmechanismen, Ruhr-Universität Bochum, 4630 Bochum, FRG.

whereas sera of patients with atopic dermatitis contained a 60,000 MW component (Bujanowski-Weber *et al.*, 1988). These data suggest that IgE-BFs (sCD23) from different sources as well as different molecular weights might also exert different functions. Quite recently the role of cytokines (IL-2, IL-4, IFN-γ) with regard to CD23 expression and IgE synthesis has been described. IL-4 was shown to enhance the CD23 expression on human B lymphocytes (Defrance *et al.*, 1987). In the presence of high amounts of IL-4 (10,000 U/ml) the IgE production of LPS-stimulated B cells (Snapper, Finkelman & Paul, 1988) was enhanced while IL-4 alone did not modulate the IgE secretion (Splawski, Jelinek & Lipsky, 1988). IFN-γ was shown to suppress the effect of IL-4 (Snapper *et al.*, 1988; Romagnani *et al.*, 1988) and IFN-α suppressed the release of IgE-BFs (sCD23) (Delespesse *et al.*, 1988). These data support the role of lymphokines with regard to IgE regulation. The purpose of our study was to compare CD23 expression with IgE-BF release from unstimulated and lymphokine-treated lymphoblastoid RPMI-8866 cells and peripheral blood lymphocytes (PBL) of healthy volunteers as well as of patients with atopic diseases.

MATERIALS AND METHODS

Preparation and culture conditions of human PBL and RPMI-8866 cells

RPMI-8866 cells (generous gift of Dr G. Delespesse, University of Montreal, Canada) were cultured in RPMI-1640 (Biochrom, Munich) medium supplemented with 10% fetal calf serum (FCS) as well as penicillin and streptomycin (Sigma, Munich). The cells were grown up to a density of $1-2 \times 10^6$ cells per ml and collected by centrifugation. Subsequently, the cells were washed three times with medium and a concentration of 1×10^7 cells/ml was adjusted for the receptor assay.

Human PBL were prepared from heparinized blood of healthy volunteers (clinically non-atopic; negative by skin test) or atopic patients (baker's asthma, pollinosis, house dust mite; characterized by skin test, histamine release and RAST) by centrifugation on a Ficoll–Metrizoate gradient (Boyum, 1976). For the receptor assay, PBL cell suspensions (5 ml) of 1×10^6 cells/ml were cultured up to 7 days in RPMI-1640 (supplemented with 10% FCS). After incubation the cells were collected by centrifugation and resuspended in 500 μl of medium. This cell suspension was used to perform the receptor assay. Each experiment was performed at least three times. The supernatants of the cells were stored at $-20°$.

Interleukins

Recombinant human IL-4, IFN-γ and TNFα were obtained from Genzyme (Munich). Recombinant human IL-2 was obtained from Boehringer (Mannheim). Interleukins were used in the cell culture as indicated for each experiment.

Radiolabelling

Antibodies (anti-IgE; mAb 135) at a concentration of 100 μg were labelled with ^{125}-Iodine (37 MBq; Amersham, Braunschweig) by the chloramine T method (Klinman & Taylor, 1969). The specific activity ranged from 5000 to 10,000 c.p.m./ng antibody.

Detection of the low-affinity receptor for IgE (FcεRII, CD23)

The FcεRII was detected with the monoclonal antibody mAb 135 (generous gift from Dr G. Delespesse, University of Montreal, Canada). Aliquots of 100 μl of the cell suspension were incubated for 60 min with radiolabelled mAb 135 (400,000 c.p.m. = 14·8 kBq per tube). After incubation the suspension was applied onto a FCS layer (minitubes; Greiner, Nürtingen, No. 100101). Cell-bound and unbound radioactivity were separated by centrifugation. The amount of unspecifically bound radioactivity attached to the tubes was lower than 0·5% of the total activity. Each sample was determined in at least triplicate. Only data with SD below 10% were used. If not indicated otherwise, the ratio of cell-bound radioactivity was calculated as the percentage of binding in relation to the total activity per 1×10^6 cells.

Scatchard-plot analysis (Rosenthal, 1967) of CD23 on the surface of RPMI-8866 cells was performed with samples containing 1×10^6 cells. Usually, five concentrations of the mAb 135, between 5×10^4 and $1{\cdot}5 \times 10^6$ c.p.m./sample were used. Each sample was determined in triplicate. The amount of binding sites was calculated by linear regression.

Radioimmunoassay for IgE-BF and IgE

The RIA for IgE-BF (sCD23) with the mAb specific for FcεRII (generous gift from Dr G. Delespesse, University of Montreal, Canada) cross-reacting with IgE-BF was performed as has been previously described (Bujanowski-Weber *et al.*, 1988). The total radioactivity added to the sample served as the control (100%). Results for IgE-BF were expressed in relation to the 100% value. The SD of quadruplicate samples ranged under 10%. There was no cross-reaction with IgE, IgG or the medium and its supplements (Bujanowski-Weber *et al.*, 1988).

IgE measurement was performed by RIA as has been described previously (Bujanowski-Weber *et al.*, 1988). The SD of duplicates was below 5%.

Analysis of cellular proliferation

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

RESULTS

Expression of CD23 and IgE-BF (sCD23) release by unstimulated RPMI-8866 cells

In a first series of experiments, RPMI-8866 cells were studied for Fcε receptor II expression. RPMI-8866 cells were cultured for 72 hr. Aliquots of 1×10^6 cells per 100 μl were incubated with five concentrations of the radiolabelled mAb 135 ranging from 5×10^4 up to 1×10^6 c.p.m./100 μl at room temperature. Calculation of Fcε receptors was carried out by Scatchard plot analysis which led to $4-6 \times 10^5$ binding sites of mAb 135 per cell. When the receptor assay was performed at 37° a reduced binding of the mAb 135 to the cells was obtained; however, incubation of the cells at room temperature or on ice did not show significant differences in binding (data not shown). For the following experiments, the receptor assays were performed at room temperature.

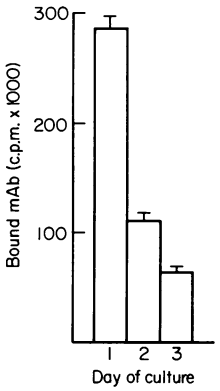

Figure 1. Kinetics of CD23 expression on RPMI-8866 cells. RPMI-8866 cells (5×10^5 cells/ml) were cultured for 24, 48 and 72 hr. $n = 5$ experiments were carried out in triplicate. One representative experiment is shown. SD of triplicates ranged under 10%.

Experiments were then carried out to study the influence of culture duration on CD23 expression. Therefore, RPMI-8866 cells (5×10^5 cells/ml) were cultured for 24, 48 and 72 hr. The cells were harvested and adjusted to 1×10^6 cells/100 μl. Subsequently, the ratio of bound mAb 135 was determined for each culture stage using the same dilution of the radiolabelled mAb 135 (400,000 c.p.m./100 μl). Figure 1 shows the kinetics of CD23 expression on RPMI-8866 cells. The kinetics clearly demonstrate that the percentage of bound monoclonal antibody decreases with increasing time of culture, whereas the amounts of soluble IgE-BF (sCD23) within the supernatant of RPMI-8866 cells increased over time (Bujanowski-Weber *et al.*, 1988).

Effect of lymphokines on RPMI-8866 cells

Evidence has been provided that various lymphokines such as IL-4 and IFN-γ modulate CD23 expression on human lymphocytes (Defrance *et al.*, 1987). Experiments were performed to analyse the effects of IL-4, TNFα, IFN-γ and IL-2 on CD23 expression and IgE-BF (sCD23) release at various days of culture using RPMI-8866 cells as target cells. The proliferation of the cells was analysed with [^{3}H]thymidine. An optimal response was obtained at Day 3. CD23 expression as well as IgE-BF (sCD23) release of stimulated cells were calculated as percentage of the control (unstimulated cells) (Table 1).

At Day 1 of culture the addition of IL-4 (1–100 U/ml) suppressed the CD23 expression with a concomitant release of IgE-BF (sCD23). In contrast, at Day 3 of culture an increase in CD23 expression was observed at concentrations of IL-4 from 1 to 10 U/ml, while sCD23 release was not affected.

TNFα (10 U/ml) slightly increased CD23 expression and sCD23 release (1–100 U/ml) at Day 1 of culture. At Day 3 the CD23 expression was reduced in a dose-dependent manner while sCD23 release was not affected.

At low concentrations IFN-γ (1 U/ml) reduced CD23 expression and enhanced sCD23 release. Addition of increasing concentrations of IFN-γ led to an up-regulation of CD23 (Day 1). When Day 3 was studied IFN-γ-revealed no significant effects.

IL-2 at concentrations of 0·1–10 U/ml reduced CD23 and increased sCD23 release. At Day 3 0·1 U/ml of IL-2 enhanced

Table 1. Stimulation of RPMI-8866 cells by various lymphokines

		RPMI-8866 cells			
		Day 1		Day 3	
Lymphokine (U/ml)		CD23 (% control)	BF (% control)	CD23 (% control)	BF (% control)
Control		100·0	100·0	100·0	100·0
IL-4	1	73·4	151·0	143·7	99·2
IL-4	10	107·5	123·8	148·8	94·6
IL-4	100	46·2	151·4	111·9	85·0
TNFα	1	83·8	134·8	122·7	92·5
TNFα	10	136·7	153·4	85·0	96·7
TNFα	100	89·0	116·2	59·2	106·6
IFN-γ	1	40·7	132·1	85·7	95·5
IFN-γ	10	72·3	107·6	118·9	89·0
IFN-γ	100	83·2	157·6	70·5	101·4
IL-2	0·1	93·2	71·0	134·8	73·8
IL-2	1	86·9	107·2	85·1	101·3
IL-2	10	65·0	127·6	92·7	104·0

RPMI 8866 cells (5×10^5 cells/ml) were cultured in the presence of IL-4, TNFα, IFN-γ and IL-2. CD23 expression as well as sCD23 release were determined after Days 1 and 3 of incubation. The experiments were carried out three times in triplicate. The data are indicated as percentage of the control (without lymphokine) to demonstrate the stimulatory effects.

CD23 expression with a concomitant reduction of IgE-BF (sCD23) release.

These data suggest that lymphokines apparently affect both parameters (CD23 and sCD23) depending on the growth specific characteristics of the cell.

Effect of exogenous IgE on RPMI-8866 cells

Since RPMI-8866 cells bear low-affinity receptors for IgE on their surface, experiments were performed to study the role of exogenous IgE. RPMI-8866 cells (1×10^6 cells/ml) were incubated with IgE (41·5–188 ng/ml) derived from a human IgE-secreting myeloma cell line, the U266 cell line (Nilsson *et al.*, 1970). After 24 hr of culture, CD23 expression and sCD23 release were determined (Fig. 2). In the presence of exogenous myeloma IgE, a dose-dependent enhancement of CD23 expression was obtained. A maximal expression was observed with 188 ng/ml of IgE. For sCD23 no significant modulation above control levels was obtained. These data suggest that the exogenous addition of the ligand increases the binding sites.

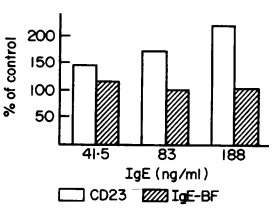

Figure 2. Stimulation of RPMI-8866 cells by exogenous IgE. Experiments were carried out twice in triplicate. The data are indicated as percentage of the control values (samples without IgE). SD of triplicates ranged under 10%.

Table 2. Evaluation of total serum IgE, CD23 expression and sCD23 release

	IgE (serum) (ng/ml)	CD23 (% total/1 × 10^6 cells)	IgE-BF (serum) (% total)
Normal donors	478·8 ± 548·5 (n = 82)	0·55 ± 0·19 (n = 22)	0·43 ± 0·33 (n = 92)
Atopic donors	1116·2 ± 1823·5 (n = 16)*	1·7 ± 1·18 (n = 16)†	0·6 ± 0·92 (n = 29)*

Total IgE (ng/ml) and sCD23 (% total activity) levels were detected as described by RIA. Isolated lymphocytes were analysed by receptor assay. All parameters were determined at least two times (in triplicate) for each volunteer. CD23 expression is indicated as percentage of bound mAb per 1 × 10^6 cells.

* Patients with wheat flour allergy.

† Donors with pollinosis or house dust mite allergy.

CD23 expression and sCD23 release from PBL

Further experiments were carried out to analyse CD23 expression and sCD23 release from PBL isolated from normal as well as atopic donors. Table 2 represents the mean values and SD for the data obtained from freshly prepared lymphocytes, the serum levels for total IgE as well as for sCD23. Obviously, the mean values for the expression of CD23 (P = 0·9995) and IgE (P = 0·9) are significantly higher in atopic donors compared with the healthy control group. The differences for the concentrations of sCD23 within the sera of both donor groups were not significant (P = 0·9), as determined by the two-tailed Student's t-test.

Effects of mitogens on CD23 expression and sCD23 release

PBL (1 × 10^6 cells/ml) from normal as well as atopic donors were stimulated with either PHA (1, 5, 10 μg/ml) or Con A (1, 5, 10 μg/ml) for either 1, 2, 5, 6 or 7 days. An optimal response was obtained with 10 μg/ml of the mitogens. Proliferation, CD23 expression and sCD23 release were analysed. Under the experimental conditions Con A revealed a fivefold and PHA a 25-fold stimulation index. It is apparent (Fig. 3) that the mitogens enhanced CD23 expression and sCD23 release in both groups. With PHA the expression of CD23 on atopic cells exceeded that on normal lymphocytes at Days 1, 2 and 5 of culture, while the Con A the value at Day 6 was still higher compared with the value of PHA-stimulated cells. In both groups CD23 values at Day 7 were below those obtained from the lymphocytes of normal donors. sCD23 release increased from Day 2 of culture and reached a plateau of 5% binding at Days 5–7 after PHA treatment; with Con A a maximum of sCD23 release was obtained at Days 5 and 6, with a significant decline at Day 7.

These data indicate that with cells of atopic donors, CD23 expression and sCD23 release are enhanced by PHA and Con A to a higher extent than normal human lymphocytes.

Regulation of CD23 expression and sCD23 release on human lymphocytes by cytokines

In the subsequent experiments the expression of CD23 and sCD23 release were analysed with human lymphocytes from normal and atopic donors (1 × 10^6 cells/100 μl). The cells were cultured for 7 days in the presence of IL-4 (15–250 U/ml), IL-2 (1·2–100 U/ml, IFN-γ (1–100 U/ml) and TNFα (1–100 U/ml). After various days of culture (Days 1, 2, 5, 6, 7) CD23 and sCD23 release were studied. It is apparent that IL-4 (50 U/ml) increases CD23 on normal lymphocytes over time (Fig. 4). The highest value was obtained after Day 7. With the lymphocytes of atopic donors a pronounced increase is obtained after Days 2 and 5 with a decline after Day 7. sCD23 in the culture of normal as well as atopic lymphocytes increased over time. The amount of sCD23 obtained in the presence of IL-4 from cells of atopic donors exceeded that from normal donors.

PBL of normal donors revealed, on incubation with either IL-2 or IL-4, a different pattern to sCD23 release (Fig. 5a, b). IL-2 increased the sCD23 release with a maximal value at 5–10 U/ml. Addition of higher concentrations (50–200 U/ml) of IL-2 led to a decrease. In contrast, increasing concentrations of IL-4 led to a dose-dependent increase of sCD23 from the cells.

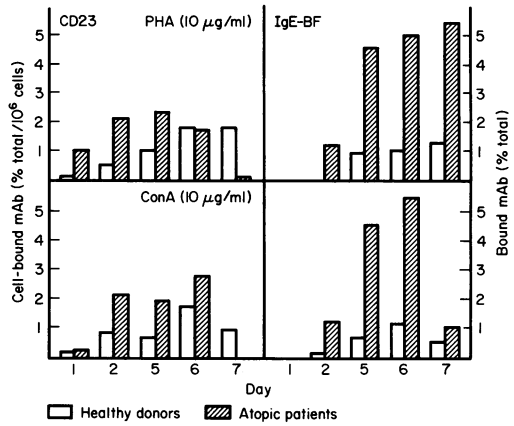

Figure 3. Stimulation of PBL with PHA and Con A. PBL of either cell group (atopics, wheat flour allergy; normals) were incubated with PHA (10 μg/ml) or Con A (10 μg/ml). CD23 expression as well as IgE-BF release was analysed. The experiment was carried out with two different donors. Each experiment was performed in triplicate.

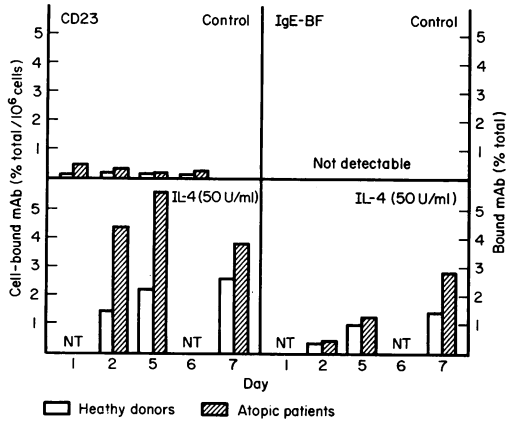

Figure 4. Stimulation of PBL with IL-4—analysis of CD23 expression and sCD23 release. Lymphocytes from atopic (wheat flour allergy; n = 3) and healthy donors (n = 2) were stimulated by adding IL-4 (50 U/ml) and incubated up to 7 days. Analysis of CD23 expression and sCD23 release was performed by RIA. The experiments were carried out in triplicate. A representative experiment is shown. NT, not tested.

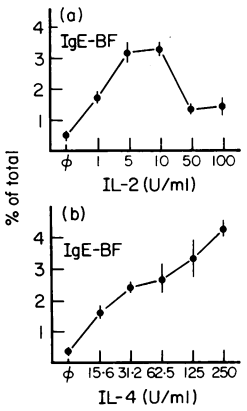

Figure 5. Effect of IL-2 on IgE-BF release from PBL. PBL of normal donors ($n=3$; 1×10^6 cells/ml) were stimulated with (a) IL-2 (1–100 U/ml) or (b) IL-4 (15–250 U/ml) and cultured for 7 days. sCD23 release was determined by RIA. Experiments were carried out in triplicate; a representative experiment is shown.

TNFα ($n=2$) increased CD23 expression. In the presence of low concentrations (1 U/ml) a maximal value was obtained at Day 1 of incubation (+200% of control). Addition of increasing amounts of TNFα (10–100 U/ml) required longer incubation periods (3 days) to reach a maximal value (+220% and +80% of control). The sCD23 release was not affected in the presence of TNFα (data not shown).

Treatment with 10 U/ml of IFN-γ reduced the CD23 expression (−25%) of atopic lymphocytes with increasing time of culture, while there was no influence of IFN-γ (10 U/ml) observed with regard to IgE-BF release. When cellular proliferation was analysed IFN-γ induced a dose-dependent enhancement of proliferation at Day 2 (+100%) compared with control values (e.g. cells in the absence of IFN-γ). At Day 5 an increased spontaneous thymidine uptake was observed compared with Day 2; while significant differences between control and stimulated cells were not apparent. In the presence of TNFα and IL-4 no proliferation compared with the control values was observed (data not shown). These data suggest that sCD23 release is not due to cellular proliferation.

DISCUSSION

In the present study a comparison of CD23 expression as well as secretion of sCD23 from (i) unstimulated and lymphokine-treated, lymphoblastoid RPMI-8866 cells, and (ii) PBL of healthy volunteers as well as of patients with atopic diseases (wheat flour allergy, pollinosis, house dust mite) was carried out. It became apparent that cytokines, e.g. IL-4, IL-2, TNFα and IFN-γ, modulate the above parameters. Cells of atopic donors in this regard expressed CD23 after stimulation with IL-4, PHA and Con A to a higher degree and showed enhanced sCD23 release as compared to cells of non-atopic donors.

(i) Low-affinity receptors for IgE are present on various cell types (B cells, T cells, monocytes, eosinophils, platelets, RPMI-8866 cells). Spiegelberg & Melewicz (1980) and Ishizaka (1980) have demonstrated that cells of the lymphoblastoid cell line RPMI-8866 bear on their surface $2–3 \times 10^5$ receptor sites, as determined by the binding of [^{125}I]IgE. The receptor assay based on the binding of the mAb revealed $4–5 \times 10^5$ binding sites/cell as has been confirmed by binding studies performed with radiolabelled IgE (data not shown). Incubation at 37° showed a lower binding rate (data not shown) compared with the incubation at 4° or 20°, respectively, suggesting a shedding of CD23, induced by the antibody.

Kinetic studies performed with unstimulated RPMI-8866 cells showed that the CD23 expression declined after 2–3 days of culture, while sCD23 release increased in the supernatant (Bujanowski-Weber *et al.*, 1988). Addition of exogenous IgE as a ligand increased the CD23 expression in a dose-dependent manner while the release of sCD23 remained nearly constant. It is unclear whether an enhanced endocytosis of an IgE–IgE-BF complex may account for this phenomenon or a masking of BF within the complex. For monocytes it was shown that the stimulation with exogenous IgE reduced the IgE-BF release (Delespesse, Sarfati & Rubio-Trujillo, 1987). A quantification of surface-associated CD23 was not carried out in those studies.

In view of the fact that soluble CD23 may act in an autocrine fashion on IgE-synthesis it became important to analyse the effects of cytokines on CD23 expression and sCD23 release. With the lymphoblastoid cell line RPMI-8866, the effect of cytokines appears to depend on the cell cycle and the duration of the interaction. In this regard IL-4 (100 U/ml) initiated a suppression of CD23 at Day 1 of culture and led to an increase of sCD23 in the supernatant, while at Day 3 of culture CD23 expression was enhanced and sCD23 release remained constant. IFN-γ (1 U/ml) significantly reduced CD23 expression to about 40% and enhanced IgE-BF release. TNFα reduced (100 U/ml) CD23 expression at Day 3 without an increase in sCD23. These results demonstrate that depending on the cell growth or receptor expression various cytokines may up- or down-regulate the expression of CD23 on RPMI-8866 cells. Furthermore, our data suggest that a decrease in CD23 expression does not necessarily induce an increased sCD23 release. This may be due to an altered fragmentation of soluble CD23 or masking of epitopes in a way that the monoclonal antibodies do not detect these components.

(ii) Recently, it was shown that T cells of B-venom allergic patients showed an increased expression of CD23 on addition of the specific allergen PLA_2 (Prinz *et al.*, 1987). In our experiments atopic patients revealed an enhanced expression of CD23 compared with peripheral lymphocytes of healthy volunteers. This could be due to the fact that atopic patients either possess more CD23 expressing cells or that the individual cells showed an enhanced CD23 expression. Spiegelberg, O'Connor & Simon (1979) demonstrated by rosette technique a higher amount of low-affinity receptors in atopics than in normals. However, sCD23, total IgE and CD23 were not correlated with each other in these studies. A correlation has been shown recently in patients with chronic lymphatic leukaemia (Sarfati *et al.*, 1988), asthma and rhinitis (Delespesse & Sarfati, 1987).

The up-regulation of CD23 by IL-4 and its inhibition by IFN-γ has been described in mice and humans (Snapper *et al.*, 1988; Romagnani *et al.*, 1988). Our results demonstrate that in the presence of IL-4 the lymphocytes of atopics express CD23 to a higher extent than normal cells. Concomitantly with the expression of CD23 the release of IgE-BF occurred which revealed a maximum after 7 days of culture in the absence of a proliferative response. The expression exceeded that obtained with cells of healthy volunteers. IL-2 also led to an increase of IgE-BF release. However, the kinetics were different compared with the IL4-induced IgE-BF release. TNFα and IFN-γ

enhanced or suppressed CD23 expression. It is unclear whether different receptor-mediated membrane biochemical events may account for these results. The various cytokines could use different enzymatic pathways to express CD23 as well as to cleave sCD23. Therefore, the extent of CD23 expression is not necessarily linked to the amount of sCD23 released.

Quite recently it was shown that the biochemical characteristics of radiolabelled sCD23 obtained from the supernatant of normal B cells cultured with IL-4 were identical to those isolated from the cell line RPMI-8866 (Bonnefoy *et al.*, 1988). Furthermore it was suggested that sCD23 acted synergistically with suboptimal concentrations of IL-4, indicating an important role of sCD23 for IgE synthesis (Pene *et al.*, 1988).

In this regard mitogens lead to a higher release of sCD23 from the cells of atopic donors compared with normal cells. T cells isolated from PHA-stimulated cultures expressed more Fcε receptors than those isolated from control cultures (Delespesse *et al.*, 1987). It is evident from our experiments that the sCD23 release induced by mitogens exceeded that obtained after addition of IL-4. These results suggest that the mitogen-activated cells may either trigger enzymatic cascades for the turnover and release of the CD23 molecule or initiate an additional factor for the release of CD23 (sCD23, IgE-BF). One also may suggest that the various cleavage products of sCD23 may serve as regulatory factors with regard to CD23 expression. They could provide either positive or negative signals in the course of B-cell induction and IgE synthesis. Under this assumption the amount of total sCD23 may not necessarily reflect the capacity of an atopic donor to secrete IgE. The fact that cells of atopic donors express a higher sensitivity towards cytokine- and mitogen-induced expression of, CD23 and sCD23 release suggests a key role of these molecules during allergic disease processes. Future studies are directed to compare CD23 expression, IgE-BF (sCD23) release as well as IgE synthesis (Knöller *et al.*, 1988) after cytokine, antigen and mitogen stimulation of the cells in order to understand the crucial role of this (these) molecule(s) during cellular activation and IgE-antibody synthesis.

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REFERENCES

Bonnefoy J.Y., Aubry J.P., Peronne C., Wijdenes J. & Banchereau J. (1987) Production and characterization of a monoclonal antibody specific for the human lymphocyte low affinity receptor for IgE: CD23 is a low affinity receptor for IgE. *J. Immunol.* **138,** 2970.

Bonnefoy Y.J., Defrance T., Peronne C., Menetrier C., Rousset F., Pene J., De Vries J.E. & Bachereau J. (1988) Human recombinant interleukin 4 induces normal B cells to produce soluble CD23/IgE-binding factor analogous to that spontaneously released by lymphoblastoid B cell lines. *Eur. J. Immunol.* **18,** 117.

Boyum A. (1968) Separation of leukocytes from blood and bone marrow. *Scand J. Lab. Invest.* **21** (suppl. 97), 77.

Bujanowski-Weber J., Knöller I., Brings B., Pfeil T. & König W. (1988) Detection and characterization of IgE-binding factors (IgE-BF) within supernatants of cell line RPMI 8866, normal sera and sera from atopic patients. *Immunology*, **65,** 53.

Capron M., Capron A., Dessaint A., Torpier G., Gunnar S., Johansson O. & Prin L. (1981) Fc receptors for IgE on human and rat eosinophils. *J. Immunol.* **126,** 2087.

Defrance T., Aubry J.P., Rousset B., Vanbervliet B., Bonnefoy J.Y., Arai N., Takebe Y., Yokota T., Lee F., Arai K., de Vries J. & Banchereau J. (1987) Human recombinant IL4 induces FcE receptors (CD23) on normal human B lymphocytes. *J. exp. Med.* **165,** 1459.

Deguchi H., Ishizaka A., Ozaki Y., Kishimoto S., Yamamura Y. & Kishimoto T. (1983) IgE class-specific suppressor T-cells and factors in humans. *J. Immunol.* **131,** 2751.

Delespesse G. & Sarfati M. (1987) Measurement of serum IgE-binding factors (IgE-BFs) in patients with allergic asthma and rhinitis. *J. All. Clin. Immunol.* **79,** 130.

Delespesse G., Sarfati M., Kolb J.P. & Suter U. (1988) Influence of IFN-α on IgE-binding factor (IgE-BF) by human B cells. *FASEB J.* A1249, Abs. 5543.

Delespesse G., Sarfati M. & Rubio-Trujillo M. (1987) *In vitro* production of IgE-binding factors by human mononuclear cells. *Immunology*, **60,** 103.

Geha R.S. (1984) Human IgE. *J. All Clin. Immunol.* **74,** 109.

Gonzales-Molina A. & Spiegelberg H.L. (1977) A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. clin. Invest.* **59,** 616.

Gordon J. & Guy G.R. (1987) The molecules controlling B lymphocytes. *Immunol. Today*, **8,** 339.

Gordon J., Webb A.J., Walker L., Guy G.R. & Rowe M. (1986) Evidence for an association between CD23 and the receptor for a low molecular weight B cell growth factor. *Eur. J. Immunol.* **16,** 1627.

Huff T.F. & Ishizaka K. (1984) Formation of IgE-binding factors by T-cell hybridomas. *Proc. natl. Acad. Sci. U.S.A.* **81,** 1514.

Ikuta K., Takami M., Kim C.W., Honjo T., Miyoshi T., Tagaya Y., Kawabe T. & Yodoi J. (1987) Human lymphocyte Fc receptor for IgE: sequence homology of its cloned cDNA with animal lectins. *Proc. natl. Acad. Sci. U.S.A.* **84,** 1987.

Ishizaka K. (1985) Immunoglobulin E (IgE). *Meth. Enzymol.* **116,** 76.

Ishizaka K. & Sandberg K. (1981) Formation of IgE binding factors by human T lymphocytes. *J. Immunol.* **126,** 1692.

Ishizaka T. (1980) IgE receptors and their interference. In: *Advanced Allergology and Clinical Immunology* (eds A. Ochling, J. Glazer, E. Methor and C. Arkesman), p. 586. Pergamon Press, New York.

Jensen J.R., Sand T.T. & Spiegelberg H.L. (1984) Generation of IgE-binding and IgG-binding factors from human lymphoblastoid cell lines. *Immunology*, **53,** 1.

Joseph M., Capron A., Ameisen J.C., Capron M., Vrong H., Pancre V., Kusinierz J.-P. & Auriault C. (1986) The receptor for IgE on blood platelets. *Eur. J. Immunol.* **16,** 306.

Kanowith-Klein S. & Saxon A. (1986) Regulation of ongoing IgE-synthesis by human T-cell supernatants derived from atopic and non atopic donors. *Int. Arch. Allergy appl. Immun.* **80,** 3.

Kikutani H., Inui S., Sato E., Barsumian E.L., Owaki H., Yamasaki K. *et al.* (1986a) Molecular structure of the human lymphocyte receptor for immunoglobulin E. *Cell*, **47,** 657.

Kikutani H., Suemura M., Barsumian E.L., Hardy R.R. & Kishimoto T. (1986b) Fcε receptor, a specific differentiation marker transiently expressed on mature B cells before isotype switching. *J. exp. Med.* **164,** 1455.

Klinman N.R. & Taylor R.B. (1969) General methods for the study of cells and serum during the immune response; The response to dinitrophenyl in mice. *Clin. exp. Immunol.* **4,** 473.

Knöller I., Bujanowski-Weber J., Brings B., Delespesse G. & König W. (1988) Modulation of IgE-synthesis by IgE-binding factors and interleukins. *FASEB J.* **2,** A1442, Abs. 6654.

König W., Pfeiffer P., Rauschen I., Schönfeld W., Knöller J. & Theobald K. (1985) The role of immunoglobulin E receptors in allergic disease. *Drug Res.* **35,** 1983.

Leung D.Y., Frankel R., Wood N. & Geha R.S. (1986) Potentiation of human immunoglobulin E synthesis by plasma immunoglobulin E

binding factors from patients with hyper immunoglobulin E syndrome. *J. clin. Invest.* **77,** 952.

LÜDIN C., HOFSTETTER H., SARFATI M., LEVY C.A., SUTER U., ALAIMO D., KILCHHERR E., FROST H. & DELESPESSE G. (1987) Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO J.* **6,** 109.

NILSSON K., BENNICH H., JOHANSSON S.G.O. & PONTEN J. (1970) Established immunoglobulin producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient. *Clin. exp. Immun.* **7,** 477.

PENE J., ROUSSET F., BRIERE F., CHRETIEN I., WIDEMAN J., BONNEFOY J.Y. & DE VRIES J.E. (1988) Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen. *Eur. J. Immunol.* **18,** 929.

PRINZ J.C., ENDRES N., RANK G., RING J. & RIEBER E.P. (1987) Expression of Fcε receptors on activated human T lymphocytes. *Eur. J. Immunol.* **17,** 757.

ROMAGNANI S., DEL PRETE G.F., MAGGI E., PARRONCHI P., TIRI A., MACCHIA D. & RICCI M. (1988) IL4 is an essential factor for IgE-synthesis induced *in vitro* by human T cell clones and their supernatants. *FASEB J.* **2,** A1250, Abs. 5550.

ROSENTHAL H.E. (1967) A Graphic method for the determination and presentation of binding parameters in a complex system. *Analyt. Biochem.* **20,** 525.

ROSSI G., SALZANO S., PUCILLO C., PEPE S. & FORMISANO S. (1988) Regulation of the expression of FcεR in human monocyte-like cell line U937 by TPA and IgE. *FASEB J.* **2,** A1249, Abs. 5542.

SARFATI M., BRON D., LAGNEAUX L., FONTEYN C., FROST H. & DELESPESSE G. (1988) Elevation of IgE-binding factors in serum of patients with B cell derived chronic lymphatic leukemia. *Blood,* **71,** 94.

SARFATI M., NUTMAN T., FONTEYN C. & DELESPESSE G. (1986) Presence of antigenic determinants common to Fc IgE receptor on human macrophages, T and B lymphocytes and IgE-binding factors. *Immunology,* **59,** 569.

SARFATI M., RECTOR E., RUBIO-TRUJILLO M., WONG K., SEHON A.H. & DELESPESSE G. (1984a) *In vitro* synthesis of IgE by human lymphocytes. III. IgE-potentiating activity of culture supernatants from Epstein–Barr virus (EBV)-transformed B cells. *Immunology,* **53,** 207.

SARFATI M., RECTOR E., WONG K., RUBIO-TRUJILLO M., SEHON A.H. & DELESPESSE G. (1984b) *In vitro* synthesis of IgE by human lymphocytes. II. Enhancement of the spontaneous IgE synthesis by IgE-binding factors secreted by RPMI 8866 lymphoblastoid B cells. *Immunology,* **53,** 197.

SARFATI M., RUBIO-TRUJILLO M., WONG K., RECTOR E., SEHON A.H. & DELESPESSE G. (1984c) *In vitro* synthesis of IgE by human lymphocytes. I. Spontaneous secretion of IgE by B lymphocytes from allergic individuals: a model to investigate the regulation of human IgE-synthesis. *Immunology,* **53,** 187.

SNAPPER C.M., FINKELMAN F.D. & PAUL W.E. (1988) Differential regulation of IgG1 and IgE synthesis by interleukin 4. *J. exp. Med.* **167,** 183.

SPIEGELBERG H.L. & MELEWICZ F.M. (1980) Fc receptors specific for IgE on subpopulations of human lymphocytes and monocytes. *Clin. Immunol. Immunopathol.* **15,** 424.

SPIEGELBERG H.L., O'CONNOR R.D. & SIMON R.A. (1979) Lymphocytes with immunoglobulin E Fc receptors in patients with atopic disorders. *J. clin. Invest.* **64,** 714.

SPLAWSKI J., JELINEK D. & LIPSKY (1988) Immunomodulatory role of IL4 on the secretion of immunoglobulin by human B cells. *FASEB* 1988, 3378.

THOMPSON L.F., MELLON M.E., ZEIGER R.E. & SPIEGELBERG H.L. (1983) Characterization with monoclonal antibodies of T lymphocytes bearing Fc-receptors for IgE (T-cells) and IgG (T-cells) in atopic patients. *J. Immunol.* **131,** 2772.

THORLEY-LAWSON D. (1985) Early events in Epstein–Barr Virus infection provide a model for B cell activation. *J. exp. Med.* **162,** 45.

YOUNG M.C., LEUNG D.Y.M. & GEHA R.S. (1984) Production of IgE-potentiating factor in man by T-cell lines bearing Fc receptors for IgE. *Eur. J. Immunol.* **14,** 871.

YUKAWA A., KIKUTANI H., OWAKI H., YAMASAKI K., YOKOTA A., NAKAMURA H., BARSUMIAN E.L., HARDY R.R., SUEMURA M. & KISHIMOTO T. (1987) A B cell-specific differentiation antigen, CD23, is a receptor for IgE (FcεRII) on lymphocytes. *J. Immunol.* **138,** 2576.