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In vitro synthesis of IgE by human lymphocytes

III. IGE-POTENTIATING ACTIVITY OF CULTURE SUPERNATANTS FROM EPSTEIN–BARR VIRUS (EBV) TRANSFORMED B CELLS

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Summary. Seven Epstein–Barr virus (EBV)-transformed B cell lines were derived from circulating lymphocytes of two atopic and two non-atopic individuals, two preparations of cord blood lymphocytes and one tonsillar lymphocyte preparation. All the cell lines contained a significant proportion of cells expressing FcεR as detected by rosette formation with IgE-coated bovine erythrocytes (E-IgE) and by flow cytometry using IgE-linked to fluorescent microspheres. None of the cell lines displayed FcR for IgA, IgM or IgG. The cell-free supernatants (CFS) of EBV-transformed cells contained IgE-binding factors (IgE-BFs) detected by their ability to inhibit the binding to RPMI 8866 cells of either E-IgE or IgE-linked to microspheres. Whereas these CFS enhanced the synthesis of IgE and suppressed the synthesis of IgG by purified B lymphocytes isolated from the blood of allergic donors and cultured in the absence of stimulant, their effect on the synthesis of IgA or IgM was not predictable. CFS significantly enhanced the secretion of IgE by the U266 myeloma cell line without interfering with secretion of IgM, IgG or IgA by EBV-transformed cells. These data are in accord with similar properties of RPMI 8866 cells and suggest that B lymphocytes might play a regulating role in the IgE synthesis.

Abbreviations: AET, 2-aminoethylisothiouronium hydrobromide; CFS, filtered and 10-fold concentrated cell-free culture supernatants of EVB-cell line; EBV, Epstein–Barr virus; E-IgE, bovine erythrocytes coated with IgE; FcεR, receptor for the Fc region of IgE; FMS-IgE, fluorescent microspheres coated with IgE; IgE-BFs, IgE binding factors; sIgA, secretory IgA.

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INTRODUCTION

The role of lymphocytes bearing Fc receptors for either γ, α or ε chains in the isotypic regulation of the antibody response has been well documented (Ishizaka, 1983; Kishimoto, 1982; Yodoi *et al.*, 1983; Löwy *et al.*, 1983; Revillard & Le Thi Bich-Thuy, 1983). It was shown that IgE antibody production in rodents is controlled by class-specific regulatory T cells capable of expressing $Fc_\varepsilon R$ and of secreting IgE-BFs. A unique feature of this system is that the same cell may either enhance or suppress IgE synthesis depending on the influence of other T cells controlling the glycosylation of IgE-BFs (Ishizaka, 1983). Thus, whereas glycosylated IgE-BFs potentiate IgE synthesis, unglycosylated IgE-BFs suppress it (Yodoi, Hirashima & Ishizaka, 1982). The operation of similar mechanisms regulating the production of IgE antibodies in man is suggested by several observations, such as the finding

of $Fc_\varepsilon R$-bearing lymphocytes in the blood of normal and atopic individuals (Gonzalez-Molina & Spiegelberg, 1977; Hellstrom & Spiegelberg, 1979; Spiegelberg *et al.*, 1979) and of IgE-BFs in the serum of the latter (Sandberg, Provost & Ishizaka, 1983). Moreover, as shown in the preceding paper in this series, the culture supernatants of human lymphoblastoid RPMI 8866 B cells contained IgE-BFs capable of potentiating the synthesis of IgE by B lymphocytes cultured in the absence of stimulant (Sarfati *et al.*, 1984b). The same supernatants not only potentiated IgE synthesis but also suppressed IgA synthesis by the same B cells, and it is interesting to note that these two reciprocal activities could not be dissociated. On the basis of these observations it was inferred that B cells might play a role in the isotypic regulation and that factors affecting IgE secretion might concomittantly influence the production of IgA.

The aim of the present study was to test these possibilities by investigating the culture supernatants of a series of EBV-transformed B cells for their content in IgE-BFs and the effect(s) of these factors on the *in vitro* synthesis of immunoglobulins by B cells from allergic individuals and by a human myeloma B cell line secreting IgE. The findings demonstrated that EVB-transformed cells expressed $Fc_\varepsilon R$ and released IgE-BFs into culture supernatants, which enhanced IgE synthesis, suppressed IgG synthesis but had no significant effect on IgA and IgM synthesis by lymphocytes cultured in the absence of stimulant.

MATERIALS AND METHODS

Reagents and materials

The reagents and materials, as well as the anti-immunoglobulin antisera and their purified fractions specific to human ε, α, γ and μ chains were the same as those employed in a previous study (Sarfati *et al.*, 1984a). Secretory sIgA (sIgA) was isolated from human colostrum (Johnstone & Thorpe, 1982). Cyclosporin A (10 μg/ml) was donated by Sandoz Ltd., Basel, Switzerland; radiolabelled anti-IgE (specific for $D_{\varepsilon 2}$ determinant) was obtained from Pharmacia Diagnostics, Uppsala, Sweden; OKT3 monoclonal antibody was obtained from Ortho Diagnostics (Raritan, NJ, U.S.A.).

Preparation of B cells

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation from fresh heparinized venous blood (Boyum, 1968). The B cell fraction was separated from cells forming rosettes with AET-sheep red blood cells by centrifugation on Ficoll metrizoate (Kaplan & Clarke, 1974). The non-rosetting fraction, named B cells hereafter, were depleted of adherent cells by incubation in plastic petri-dishes (Mosier & Coppleson, 1968). B cells contained from 1 to 4% of residual T cells forming rosettes with AET-sheep erythrocytes and were obtained from normal or from allergic individuals.

Epstein–Barr virus

A stock of EBV was prepared from cultures of EBV-transformed marmoset cells, strain B95–8, kindly provided by Dr L. Perelmutter, Ottawa, Canada. Supernatants from cultures of 1×10^6 cells/ml, propagated in complete culture medium, were centrifuged at 1000 ***g*** for 15 min and passed through 0·22 μm Millipore filter. The virus was titrated for its mitogenic activity of cultures of cord blood mononuclear cells (CBMC), and aliquots of the titrated viral suspensions were frozen at $-70°$.

Preparation of lymphoblastoid cells and of their cell-free supernatants (CFS)

For the production of lymphoblastoid cell lines, one volume of B cells (1×10^6/ml) was cultured with 1 volume of titrated EBV for 1 week in the presence of 10 μg/ml cyclosporin A. After 1 week, the cells were washed and resuspended in fresh culture medium at a concentration of 2×10^5 cells/ml. Ten millilitres of such suspensions were cultured in tissue culture flasks (Falcon 3013); after 2–3 days when the cellular density reached 1×10^6 cells/ml, the cultures were split and replated in fresh culture medium. For the preparation of the cell-free supernatants, cells were grown as indicated above in HB 101 serum-free medium. The supernatants of $2–3{\cdot}5 \times 10^8$ cells were collected by centrifugation. Viability at the time of harvest was higher than 90% as estimated by trypan blue exclusion test. Supernatants were successively passed through Diaflo XM50 membrane (in order to remove the Ig secreted by the transformed cells), concentrated 10-fold by filtration through Diaflo YM5, dialysed against Hanks' balanced salt solution (HBSS) and complete culture medium and finally passed through 0·22 μm Millipore filter. These processed supernatants are referred to hereafter as CFS.

The U266 myeloma cell line secreting IgE ND was kindly donated by Dr K. Nilssen, Uppsala, Sweden; the RPMI 8866 cells, derived from a patient with acute

myelocytic leukaemia (Finegold, Fahey & Granger, 1967) have been previously characterized (Sarfati *et al.*, 1984b). THe CEM, the MOLT4 and the Raji cell (CCL85) lines were obtained from the American type culture collection.

Culture conditions

B cells ($1{\cdot}5 \times 10^6$ in 1·5 ml) were cultured in 24-well Linbro tissue culture plates for 7 days at 37° in a water saturated atmosphere consisting of 92% air and 8% CO_2. The culture medium consisted of RPMI-1640 medium supplemented with 5 mM L-glutamine, 10% FCS, 50 IU penicillin and 50 μg/ml streptomycin. Some cultures were supplemented with cycloheximide (50 μg/ml) and puromycin (10 μg/ml) in order to evaluate the passive release of preformed IgE into the culture supernatants.

Radioimmunoassays

Immunoglobulins were measured in the culture supernatants by a solid-phase sandwich radioimmunoassay (Sarfati *et al.*, 1984a). Briefly, buffered culture supernatants were incubated overnight in microplate wells coated with either mouse monoclonal antibody specific to human IgE (clone CIA/E/7.12, kindly given by Dr A. Saxon, UCLA, Los Angeles, CA) or with sheep IgG antibodies specific to human γ, μ or ε chains. The non-specific binding sites of the wells were saturated by HBSS containing 10% FCS; 200 μl of buffered test supernatant was placed into each well. Following overnight incubation and washing, the wells were filled with 200 μl of radiolabelled and affinity-purified sheep antibodies specific to ε, α, μ or γ chains; 18 hr later, the wells were washed and their radioactivity determined in a Beckman gamma counter. The sensitivity of the assay was 0·1 ng/ml for IgE, 0·4 ng/ml for IgM and IgA and 0·8 ng/ml for IgG.

Detection of cells bearing receptors for the Fc region of Ig

FcR(+) cells were identified by a rosette assay performed as described by Yodoi & Ishizaka (1980). Lymphoblastoid cells were harvested 3 days after passage in culture and reacted with bovine erythrocytes (E) coated with IgE PS or with sIgA. Bovine erythrocytes were sensitized by employing solutions containing 200 μg/ml sIgA or IgE PS. To correct for non-specific rosette formation, red cells coated with 200 μg/ml BSA were employed. Receptors for IgG or IgM were detected as described by rosetting with bovine erythrocytes sensitized with a subagglutinating dose of rabbit IgG or IgM antibodies (Moretta *et al.*, 1979). Acridine orange (10 μl) was added to each cell preparation before counting the number of rosette-forming cells (RFC) under a fluorescent microscope. The percentages of RFC were determined on 500–600 cells; rosettes were counted in coded duplicate or triplicate preparations, and the experimentator was not informed of the codes.

Detection of IgE-binding factors

IgE binding factors were detected by their ability to inhibit the binding of E-IgE to RPMI 8866 cells as described by Ishizaka & Sandberg (1981). In the rosette inhibition assay, bovine erythrocytes were coated with 10 times less IgE PS (0·02 mg/ml) or BSA than in the rosette formation test described above. Briefly, 15 μl of 2% IgE-coated erythrocytes (E-IgE) in HBSS containing 3% BSA (HBSS-BSA) was incubated 60 min at 4° with 30 μl of the test sample with intermittent agitation; 15 μl of RPMI 8866 cells (10^7 cells/ml in HBSS-BSA) was added and after 15 min at 4°, the mixture was centrifuged (90 ***g***, 5 min at 4°) and kept at 0° for 2 hr. Ten microlitres of acridine orange and 200 μl of HBSS-BSA were added to the pellet and the cells were gently resuspended before being examined under a fluorescent microscope. The RFC background determined with E-BSA was subtracted for the calculation of the percentages of E-IgE RFC. Rosettes were counted as described above; the experimental error was less than 12%.

Identification of $Fc_\varepsilon R$ and of IgE-binding factors by flow-cytometry

In some experiments reported in the present study, $Fc_\varepsilon R$ were detected concomittantly by the rosette assay described above or by means of IgE-coated microspheres and flow cytometric analysis using the EPICS V™ dual laser fluorescence-activated cell sorter (Coulter Electronics Inc., Hialeah, FL). Flow cytometry was also employed to detect IGE-BFs in the culture supernatants of EBV-transformed cell lines. These procedures will be described in detail elsewhere.

RESULTS

EBV-transformed cells express $Fc_\varepsilon R$ and release IgE-BFs into the culture medium

The B cell lines were established by infecting with EBV two preparations of cord blood mononuclear cells (CV_1, CV_2), four preparations of PBMC—two from normal donors MA and JG, and two from the allergic

Table 1. Characterization of B cell lines expressing $Fc_\varepsilon R$ and releasing IgE-BFs into the culture supernatant

		Cell lines						
		CV_1	CV_2	MA	HC	JG	RCA	WL
Secretion of Ig* (ng/ml)	IgG	—	100±11	122±130	90±23	120±10	15±8	30±12
	IgA	—	—	—	—	—	42±5	—
	IgM	28±2·1	24±3	23±15	280±45	3·6±3·7	—	>500
	IgE	—	—	—	—	—	—	—
FcR†	γ	2	2	2·8	3·6	0	1	ND
	μ	1	4·7	6·6	ND	0·8	2	ND
	α	0	0	0	ND	0	0·7	ND
$Fc_\varepsilon R$‡	E-IgE-RFC	16·9±3 (2)	41·6±17 (3)	40·3±22 (3)	67±14 (2)	67·3±14 (2)	35±25 (3)	37±30 (2)
	FMS-IgE(+)	ND	89±1·7 (1)	52·6±23·3 (3)	79·9±1·18 (1)	53·8±32 (2)	59·8±31·4 (3)	48·2±32 (3)
IgE-BF§ (% Inhibition)		31·7 (1)	34·6±18 (3)	51±0·7 (2)	24·7±20 (2)	38·2 (1)	21·3±6·5 (3)	37·4±14 (3)

* Ig were measured in the supernatants of 72 hr cultures of 10^5 cells in 1·5 ml culture medium; mean±SD of triplicate cultures.

† The results are expressed as percentages of the number of cells forming rosettes with E-sIgA, E-IgG or E-IgM. Mean values of two experiments.

‡ The percentages of cells bearing $Fc_\varepsilon R$ were determined either by rosetting with E-IgE or by reacting the cells with IgE linked to fluorescent microspheres; the proportions of cells forming rosettes were determined in duplicate by counting 200 cells, whereas the proportion of cells reacting with FMS-IgE were determined in duplicate by screening 20,000 cells. The number of experiments is indicated in brackets. Numbers correspond to mean±SD.

§ IgE-BFs were detected by virtue of their ability to block the rosetting of RPMI 8866 cells with E-IgE; results are expressed as mean±SD of percentages of inhibition; the number of experiments are indicated in brackets.

patients WL and HC—and one preparation of tonsillar lymphocytes RCA. At the time of testing, the cells had been maintained for a minimum of 2 months in standard culture medium and they did not contain detectable T cells as tested by both rosette formation with AET-treated sheep red blood cells and by membrane fluorescence employing OKT3 antibody. As indicated in Table 1, four cell lines derived from adult atopic and nonatopic donors secreted both IgG and IgM, one cord blood cell line secreted only IgM and the tonsillar B cells secreted both IgA and IgG. It is interesting to note that none of the cell lines secreted IgE. Two to 3 days after passage in culture, the cells were tested for their expression of surface FcR specific to γ, μ, α and ε chains.

None of the cell lines contained significant proportions of cells bearing FcR for μ, γ or α chains. All the cell lines contained significant proportions of cells expressing $Fc_\varepsilon R$ as documented by two different methods. It should be mentioned that when the two methods were employed simultaneously on the same preparations of cells, higher proportions of $Fc_\varepsilon R(+)$ cells were detected with the immunofluorescent than with the rosette assay. In each method negative controls were included to estimate the non-specific binding of either BSA-coated erythrocytes or microspheres to the B cells; the background values were comparable in the two assays and ranged from less than 1% to 6%. The specificity of the assays was further demonstrated by the observation of negative results with cell lines known to lack $Fc_\varepsilon R$ (MOLT 4, Raji, CEM) and of low values in four preparations of normal peripheral blood mononuclear cells (0·8–2·4%). Finally, the binding of either E-IgE or IgE-linked microspheres was inhibited by soluble IgE but not by IgG, IGM or sIgA employed even at the high concentrations in the range of 500–1000 μg/ml. In the rosette tests, the intra-assay variation was less than 15%, whereas it was less than 3% in the immunofluorescent method based on the analysis of 20,000 cells.

The high interassay variation could not be ascribed to variations in the culture conditions nor of the cellular viability which was always higher than 90%. As it is known that the expression of cellular receptors

may be influenced by the cell cycle, it is likely that part of the variation was due to the fact that cells were not tested at the same stage of growth.

The finding that each of the seven EBV-transformed cell lines expressed $Fc_\varepsilon R$ together with previous observations that RPMI 8866 B cells bearing $Fc_\varepsilon R$ were capable of releasing IgE-binding factors (IgE-BFs) in the culture supernatant (Sarfati *et al.*, 1984b), prompted us to test the possibility that the culture supernatants of the EBV cell lines also contained IgE-BFs. For this purpose, cells were grown in serum-free medium and their culture supernatants were prepared as described under 'Materials and Methods'. CFS preparations were tested for their ability to block the rosetting of RPMI 8866 cells with E-IgE. Controls, consisting of concentrated and filtered serum-free culture medium were included in each assay. As shown in Table 1, all the EBV cell lines released IgE-BFs into the culture supernatants. In several cases the presence of IgE-BFs in the supernatants was confirmed by showing their ability to block the binding of IgE-coated microspheres to RPMI 8866 cells; these observations as well as a detailed description of the immunofluorescence test to detect $Fc_\varepsilon R$ and IgE-BFs will be reported elsewhere. Further analysis of the results indicated that there was no correlation between the proportions of $Fc_\varepsilon R^+$ cells and the rosette-inhibiting activity of the corresponding cell-free supernatant.

Immunoregulatory activity of cell-free supernatants of cultures of EBV-transformed B cells

Cell-free supernatants of five B cell lines were added to cultures of B lymphocytes isolated from the blood of allergic individuals. After 7 days, the supernatants of these cultures were tested for their content in Ig. As illustrated in Table 2, showing five representative

Table 2. Influence of CFS from B cell lines on the spontaneous Ig production by B lymphocytes

		Ig secreted into the culture supernatants*			
Origin of CFS	Added to cultures	IgE(pg/ml)	IgG(ng/ml)	IgA(ng/ml)	(IgM(ng/ml)
HC	—	3750	221	130	17
	Cyclo-puro†	850	41	0	0
	CFS 5%	5850	120	130	7·5
	10%	7000	52	145	15
MA	—	1500	380	35	31
	Cyclo-puro	1300	40	0	4
	CFS 5%	1900	50	74	30
	10%	2350	40	52	20
	20%	2100	35	32	16
WL	—	1500	380	35	31
	Cyclo-puro	1300	40	0	4
	CFS 5%	1800	180	66	52
	10%	1900	130	54	44
	20%	3000	110	52	50
RC	—	1600	125	7·5	20
	Cyclo-puro	1300	40	0	0
	CFS 5%	2000	100	8	13·5
	10%	2700	40	17	14
JG	—	1600	125	7·5	20
	Cyclo-puro	1300	40	0	0
	CFS 5%	2000	60	6·5	14
	10%	2150	55	6·5	13·5
	20%	2400	35	6	13

* The results correspond to mean values of duplicate cultures; the difference between the duplicates was less than 15%.

† Cultures containing 50 μg/ml cycloheximide and 10 μg/ml puromycin.

experiments out of 20, CFS induced a significant dose-related increase of IgE synthesis, an inhibition of the IgG synthesis and had no consistent effect on IgA and IgM synthesis. Moreover, it was found that CFS was capable of potentiating IgE synthesis in cultures displaying a *de novo* spontaneous IgE synthesis, whereas they had no such activity in cultures in which there was no spontaneous IgE synthesis (results not shown). By contrast, their IgG-inhibiting activity was observed in all the B cell cultures. In some of these experiments B cell cultures were supplemented with 5, 10 and 20% of serum-free HB 101 culture medium which had been concentrated and filtered in parallel with the CFS from the B cell lines; the Ig synthesis in such cultures was not different from that of the control cultures. Cellular viability was comparable in cultures supplemented or not with CFS and was greater than 80% after 7 day cultures.

On the basis of previous studies indicating that the secretion of IgE by either human myeloma cells (Hassner & Saxon, 1983) or by mouse B cell lines (Suemura *et al.*, 1983) could be specifically inhibited by T cell factors, CFS were tested for their ability to interfere with the secretion of IgE by U266 cells. In these assays U266 (2×10^5 cells in 1·5 ml) were cultured either for 3 hr or overnight with various concentrations of CFS or of concentrated and filtered HB 101 culture medium. As shown in Fig. 1, CFS increased moderately but significantly the secretion of IgE by U266 cells after 18 hr but not after 3 hr of culture. Finally, addition of CFS to cultures of RCA or JG EBV-transformed cells, had no significant effect on the secretion of IgA, IgG or IgM (data not shown).

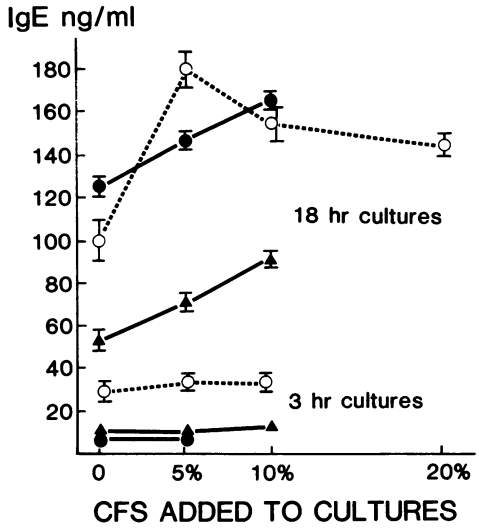

Figure 1. Influence of CFS from EBV-transformed cell lines on IgE secretion by U266 cells. CFS from three cell lines (O- - - -O, MA; ●——●, HC; ▲——▲ WL) were added to cultures of U266 cells at the final concentration of 5, 10 or 20%; IgE was measured in the supernatants collected after 3 or 18 hr of culture. The data correspond to the mean ± SD of triplicate cultures.

DISCUSSION

As expected from earlier observations, the EBV-transformed cell lines investigated in the present study contained a significant proportion of cells expressing $Fc_\varepsilon R$ and none of them expressed Fc_α, Fc_γ or Fc_μ receptors. The expression of $Fc_\varepsilon R$ on human lymphocytes of the B cell lineage has been well documented on normal lymphocytes from atopic or non-atopic individuals (Gonzalez-Molina & Spiegelberg, 1977; Hellstrom & Spiegelberg, 1979), on cultured B cell lines as well as on leukemic B cells (Gonzalez-Molina & Spiegelberg, 1976; Spiegelberg, 1981). The latter co-expressed Fc_ε and Fc_γ receptors by opposition to EVB-transformed B cells which, as confirmed in this study, were $Fc_\varepsilon R$ single-bearing cells (Gonzalez-Molina & Spiegelberg, 1976). The present results, showing that the culture supernatants of EBV-cell lines were capable of inhibiting the binding of IgE to RPMI 8866 cells, strongly suggested that EBV-transformed cells, like RPMI 8866 cells, released IgE-BFs in the culture supernatants. This suggestion was supported by the subsequent demonstration of the IgE-potentiating activity of such supernatants, when added to cultures of both B cells or U266 myeloma cells. The IgE-potentiating activity was observed only in B cell cultures from allergic donors displaying a spontaneous synthesis of IgE but not in the cultures derived from non-allergic donors. This finding was similar to that made with the supernatant of RPMI 8866 cells and suggested that the IgE-potentiating factors were acting on *in vivo* pre-activated IgE B cells. The latter interpretation is in agreement with the results obtained with U266 myeloma cells secreting IgE. It is of interest to note that the potentiating effect of CFS on IgE secretion by U266 cells was not detected after 3 hr of culture and that an overnight incubation was necessary to demonstrate the IgE-enhancing activity. This is in contrast with the optimal duration of such cultures for the detection of IgE

suppressive activity (Hassner & Saxon, 1983; Suemura *et al.*, 1983). The supernatants of EBV cell lines, unlike those of RPMI 8866 cells (Sarfati *et al.*, 1984b), had no inhibitory activity on IgA synthesis, whereas they generally suppressed the synthesis of IgG and had inconsistent effects on IgA or IgM synthesis. The mechanism underlying the suppression of IgG has not been investigated. It is known that EBV-transformed cells are capable of secreting several kinds of immunologically active molecules such as interferon (Zajac, Henle & Henle, 1969), macrophage migration-inhibiting factor (Yoshida *et al.*, 1976), inhibitors of DNA synthesis (Beneke *et al.*, 1980) and lymphotoxins (Granger *et al.*, 1970); it is possible that in the present experimental conditions IgG precursor B cells were more sensitive to some of these factors.

The relatively high frequency of B cells or B cell lines expressing $Fc_\varepsilon R$ contrasts with the lack of such receptors on several T cell lines as well as with the low frequency of normal T cells expressing $Fc_\varepsilon R$ (Gonzalez-Molina & Spiegelberg, 1977; Helsstrom & Spiegelberg, 1979). This contrast is even more striking in the light of the demonstration that in experimental animals T cells play a major role in the isotypic regulation of the immune response. In humans, the existence of T cells secreting IgE-BFs and specifically regulating the synthesis of IgE was suggested by the following observations. First, Saryan, Leung & Geha (1983) and Romagnani *et al.* (1983) reported that the culture supernatants of T lymphocytes from patients with high serum IgE levels, were capable of specifically potentiating the *in vitro* spontaneous synthesis of IgE by B lymphocytes. Second, one group of investigators not only succeeded in inducing *in vitro* $Fc_\varepsilon R$ expression on activated human T cells but also demonstrated the presence of IgE-BFs in T cell culture supernatants (Yodoi & Ishizaka, 1980; Ishizaka & Sandberg, 1981). Third, Hassner & Saxon (1983) recently reported that under adequate experimental conditions human T cells or their culture supernatants could block the secretion of IgE by U266 myeloma cells without interfering with the synthesis of other classes of Ig by different B cell lines. Finally, and most importantly, in very recent observations, Deguchi *et al.* (1983) described a human T cell hybrid secreting soluble factors capable of selectively suppressing *in vitro* IgE synthesis. There is no incompatibility between the present findings on IgE regulation by cell-free supernatants of B cell lines and the above reports on the IgE-specific regulatory T cells. Indeed, the suggestion that B lymphocytes might participate in collaboration with T lymphocytes in the regulation of IgE synthesis has been substantiated in recent experiments by Katz (1983). This author has dissected the complex cellular interactions involved in the control of IgE synthesis in mice; his data clearly showed that upon interaction with IgE, B lymphocytes bearing $Fc_\varepsilon R$ initiated a series of cellular interactions leading to the activation of IgE-class specific suppressor and helper T cells. It must be pointed out that both the present data on EBV-transformed cells and the previous observations on RPMI 8866 cells suggest but do not demonstrate that normal human B cells also play a role in the regulation of IgE synthesis. Indeed, with the exception of the aforementioned studies by Katz, the physiological role of $Fc_\varepsilon R$ on normal B cells has not been investigated and the biological significance of the $Fc_\varepsilon R$ expressed on B cell lines or on leukemic B cells is still unknown. Possible interactions between human T and B lymphocytes for the *in vitro* generation of IgE-BFs are presently being investigated.

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