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In vitro synthesis of IgE by human lymphocytes

I. THE SPONTANEOUS SECRETION OF IGE BY B LYMPHOCYTES FROM ALLERGIC INDIVIDUALS: A MODEL TO INVESTIGATE THE REGULATION OF HUMAN IGE SYNTHESIS

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Summary. In view of the controversial data in the literature regarding the *in vitro* IgE synthesis by human lymphocytes, the conditions for culture of lymphocytes and the methodology for measurement of the IgE produced are described in detail. In the absence of any added mitogen, enriched B cell preparations derived from 70% of allergic donors actively secreted 100 to 3200 pg/ml of IgE after culture for 7 days, at which time the cell viability was higher than 85%. In comparable B cell cultures derived from non-allergic donors, only trace amounts of *de novo* synthesized IgE were detected in 20% of the cases. All B cell cultures actively secreted IgG, IgA, IgM and there was no apparent relationship between the secretion of IgE and that of the other classes of Ig. By contrast, the synthesis of IgE by unfractionated peripheral blood mononuclear cells of allergic individuals, which were stimulated with pokeweed mitogen (PWM) under several experimental conditions, was not consistently reproducible, i.e. the spontaneous synthesis of IgE in such cultures was either suppressed or enhanced by PWM. The most important finding was that the secretion of IgE was selectively enhanced by supplementing the B cell cultures with cell-free supernatants (CFS) of cultures of neonatal lymphocytes which had been preincubated with 10 μg/ml IgE. It is, therefore, concluded that B cell cultures from allergic individuals constitute an appropriate model for investigations of the mechanisms underlying the regulation of human IgE synthesis.

Abbreviations: CBMC, cord blood mononuclear cells; CFS, cell-free supernatant; FCA, Freund's complete adjuvant; HBSS, Hanks' balanced salt solution; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; sIgA, secretory IgA.

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INTRODUCTION

Several studies in animal models have demonstrated the existence of Ig class-specific mechanisms capable of either potentiating or suppressing the production of IgE antibodies without altering the IgG antibody response (Katz, 1982; Kishimoto, 1982; Ishizaka, 1983). Clearly, if these findings could be reproduced with human cells, such observations would constitute the basis for the development of new prophylactic and therapeutic approaches to combat allergic diseases.

For obvious ethical reasons, most of the knowledge regarding the regulation of IgE synthesis in man has been derived from *in vitro* studies employing peripheral blood mononuclear cells (PBMC). The physiological relevance of the results of these studies has to be evaluated with caution, since it is not known if the cells involved in the *in vivo* regulation of IgE synthesis are present in circulation, particularly in view of the suggestion that, in individuals with respiratory allergy, IgE antibodies to air-borne allergens were produced locally in the respiratory mucosa or in its draining lymph nodes (Platts-Mills, 1979). The first reports on the *in vitro* IgE synthesis by human lymphocytes were published by Geha *et al.* (1975) and Patterson *et al.* (1975). The investigation by Geha *et al.* (1975) showed that, upon stimulation with the ragweed pollen antigen E (AgE), T lymphocytes of ragweed allergic patients released soluble factors into the culture supernatants which were capable of inducing the synthesis of anti-AgE antibodies of the IgE and IgG classes in B cell cultures of ragweed-sensitive individuals upon stimulation with AgE. By contrast, Patterson *et al.* (1975) reported that PBMC of a patient with very high serum IgE concentration spontaneously released IgE when cultured in the absence of any stimulant. Whereas the latter observation has been confirmed in several laboratories (Buckley & Becker, 1978; Fiser & Buckley, 1979; Tjio, Hull & Gleich, 1979; Saxon & Stevens, 1979; Pryjma *et al.*, 1980; Romagnani *et al.*, 1980; Hemady *et al.*, 1983), the possibility of inducing *de novo* IgE synthesis in cultures of lymphocytes from allergic or non-allergic individuals by soluble antigens or polyclonal B cell activators is still controversial (Buckley & Becker, 1978; Fiser & Buckley, 1979; Tjio *et al.*, 1979; Saxon & Stevens, 1979; Pryjma *et al.*, 1980; Romagnani *et al.*, 1980; Zuraw *et al.*, 1981; Nonaka *et al.*, 1981a, b; Sampson & Buckley, 1981; Hemady *et al.*, 1983). From an analysis of the results of all these studies it may be concluded that the lack of concordance among different investigations of the *in vitro* IgE synthesis by human lymphocytes may be attributable not only to the difficulty of determining accurately the very small amounts of IgE secreted into the culture medium, but also to the passive release of the cell bound and intracellularly preformed IgE which must be differentiated from the *de novo* synthesized IgE (Buckley & Becker, 1978; Romagnani *et al.*, 1980; Sampson & Buckley, 1981).

In the present report, we describe culture conditions and a radioimmunoassay for the measurement of *de novo* IgE synthesis by human lymphocytes. The results indicated that PWM failed to induce IgE synthesis reproducibly in PBMC cultures derived from normal or atopic individuals. By contrast, when purified B lymphocytes derived from the same donors were cultured in the absence of mitogen, a spontaneous synthesis of the three major classes of Ig was detected in all the cultures and a measurable secretion of IgE was present in the majority of cultures derived from allergic donors. Most importantly, it was possible to selectively potentiate the secretion of IgE without interfering with the production of the other immunoglobulin classes by supplementing B cell cultures with culture supernatants of neonatal lymphocytes, which had been preincubated with IgE.

MATERIALS AND METHODS

Reagents and materials

Polyvinyl microtitre plates were purchased from Dynatech Lab. Inc., Alexandria, VA; some batches of foetal calf serum (FCS), RPMI-1640, L-glutamine, Hanks' balanced salt solution (HBSS), penicillin-streptomycin, and Linbro multi-well tissue culture plates were obtained from Flow Lab., McLean, VA; other batches of FCS were purchased from Gibco, Grand Island, NY, and from Microbiological Associates, Bethesda, MD; cycloheximide and puromycin were purchased from Sigma Chemical Co., St., Louis, MO; radiolabelled anti-IgE antibodies (specific for $D\varepsilon_2$ determinant), Ficoll and sodium metrizoate were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden; 2-aminoethylisothiouronium hydrobromide (AET) was ordered from Aldrich Chemical Co., Milwaukee, WI; Diaflo XM50 (mol. wt. cut off 50,000) membranes were purchased from Amicon Corp., Lexington, MA, U.S.A. Pokeweed mitogen was ordered from Gibco, Grand Island, NY, and from Microbiological Associates, Bethesda, MD; HB 101 serum-free medium was from Hana Biologics Inc., Berkeley, CA; dextran (mol. wt. 200,000) was ordered from Baker Chemicals Co., Phillipsburg, NJ.

Cell lines

Human cell lines secreting IgG (κ and λ), IgA λ and IgM (κ and λ) were kindly provided by Dr A. Saxon (UCLA, Los Angeles, CA) and Dr I. Berczi of this Department. The U266 cell line secreting IgE ND, was obtained from Dr K. Nilsson, Uppsala, Sweden.

Cell preparations

PBMC were isolated from heparinized venous blood of adult volunteers by centrifugation on Ficoll-metrizoate gradient and were washed four times in HBSS (Boyum, 1968). B cells were separated from T cells by rosetting with AET-treated sheep red blood cells, and by separating the rosette-forming cells on Ficoll-metrizoate (Kaplan & Clarke, 1974). Non-rosetting cells were depleted of monocytes by adherence to plastic dishes by a modification of the method of Mosier & Coppleson (1968) as previously described (Gausset *et al.*, 1976). Such preparations, hereafter named B cells, contained 1–4% residual T cells forming rosettes with AET-sheep red blood cells. PBMC were obtained either from healthy young adults or from allergic individuals with a documented diagnosis of asthma, atopic dermatitis, hayfever or a combination of these. All the patients had clear-cut positive skin tests to common airborne allergens. They were not treated by corticosteroids nor by hyposensitization therapy. Cord blood was collected prior to expulsion of the placenta; it was sedimented 45 min at 37° with dextran (10% v/v) and the leucocyte rich plasma was layered on Ficoll-metrizoate.

Preparation of cell-free culture supernatants containing IgE-potentiating factors

CBMC were depleted of monocytes by adherence to plastic dishes and cultured for 48 hr in complete culture medium at the concentration of 3×10^6 cells/ml in the presence or in the absence of 10 μg/ml IgE PS. Cells were then successively washed three times in HBSS, resuspended in HB 101 serum-free medium for 60 min at 37°, washed three times in HBSS medium and cultured for 24 hr in HB 101 culture medium. The supernatant was collected by centrifugation, passed through Amicon XM50 membrane (mol. wt cut off 50,000) dialyzed and filtered through 0·22 μm Millipore membrane. Such supernatants contained no IgE, detectable by radioimmunoassay.

Culture condition

Cells were cultured in RPMI–1640 medium supplemented with sodium bicarbonate (2 g/litre), 5 mM L-glutamine, penicillin G (50 IU/ml), streptomycin (50 μg/ml) and 10% FCS. Eighteen batches of FCS were tested for their ability to sustain IgE production by PBMC stimulated with PWM employed at the final concentrations of 1/10, 1/50, 1/100 and 1/200. The assays were performed either in tissue culture tubes (Falcon, 12 × 75, Cat. no. 2003) in 96 microtitre plate wells or in 24-well Linbro tissue culture plates; cells were tested at concentrations ranging from 0·5 to $1{\cdot}5 \times 10^6$/ml in culture medium containing 10, 15 and 20% FCS. None of these conditions were adequate for the induction of a reproducible synthesis of IgE by PBMC stimulated with PWM. B cells ($1{\cdot}5 \times 10^6$ in 1·5 ml) were cultured in flat-bottomed wells of Linbro 24-well tissue culture plates for 7 days at 37° in a humidified incubator with 8% CO_2 and 92% air. 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. All B cell cultures were performed with the same batch of FCS (29k9517, from Flow Lab).

Anti-immunoglobulin antisera

The sheep antisera, their IgG fractions and affinity chromatography purified antibodies specific to human α, γ and μ chains were prepared as described previously (Gausset *et al.*, 1976). Sheep antiserum specific to ε chain was prepared by immunization with IgE PS protein received from Dr K. Ishizaka (Johns Hopkins University, Baltimore, MD). One mg of IgE PS was emulsified in Freund's complete adjuvant (FCA, Difco Lab., Detroit, MI) and administered in multiple subcutaneous injections. Intramuscular booster injections of 1 mg IgE PS in FCA were given twice at 2-weekly intervals and then at intervals ranging from 6 to 12 weeks for 16 months. Several bleedings from two animals were collected during and subsequent to this immunization schedule. After removal of anti-light chain activity by absorption with human IgG $F(ab')_2$-Sepharose 4B, the samples were tested individually for their content in high affinity anti-IgE antibody. This was performed in a coprecipitation assay by determining the highest dilution of the antiserum capable of precipitating 50% of the radioactivity bound to 5 ng of ^{125}I-IgE PS. Samples with titres ranging from $2{\cdot}5 \times 10^5$ to $7{\cdot}5 \times 10^5$ were then tested in a RAST inhibition assay. RAST was performed by employing a Phadebas RAST kit (Pharmacia Fine Chemicals, Uppsala, Sweden) and a pool of human reaginic sera demonstrating a high titre of IgE antibodies to grass pollen. The sheep anti-IgE antisera were tested for their ability to inhibit the binding of the kit's radiolabelled anti-IgE to paper discs coated with grass pollen allergen and IgE antibodies to grass pollen. Serial dilutions of the sheep antisera were first incubated for 2 hr at room temperature with the allergen discs coated with IgE antibodies; subsequently the discs were washed, reacted overnight with the Phadebas ^{125}I-anti-IgE,

washed and counted. Four samples of sheep antisera which were capable of inhibiting the Pharmacia anti-IgE by 90% were pooled and the IgG fraction was isolated by ammonium sulfate precipitation. To render this preparation ε chain-specific, the IgG fraction was further absorbed with umbilical cord serum coupled to Sepharose 4B; the cord serum had previously been selected for its lack of IgE.

Specificity tests

The specificity of all antisera was assessed by at least two methods, i.e. double diffusion in agarose and indirect immunofluorescence on human cell lines secreting IgG (κ and λ), IgA (λ) and IgM (κ and λ). The anti-IgE antibody prepared as described above did not react with any of these cell lines; similarly the anti-IgA reacted only with the IgA cell line.

Radioimmunoassay

Immunoglobulins were measured in the culture supernatants buffered with 1 M Tris buffer containing 0·01% sodium azide by a solid-phase radioimmunoassay. For measurement of IgE, the wells of a 96-well polyvinyl microtiter plate were coated with the IgG fraction of ε chain-specific sheep antiserum at a concentration of 50 μg/ml in phosphate-buffered saline (PBS). In the last experiments reported here microplate wells were coated with 1 μg/ml of mouse monoclonal antibody specific to human IgE (Clone CIA/E/7·12; kindly donated by Dr A. Saxon, UCLA, Los Angeles, CA). After overnight incubation at room temperature in a humidified chamber, the plates were washed with PBS by means of a MASH II cell harvester (Microbiological Associates, Bethesda, MD). Ten percent FCS in HBSS was added to the wells for 2 hr to saturate the remaining protein-binding surface. After another wash, samples (200 μl of undiluted and buffered supernatants) were then placed into the wells and allowed to incubate overnight at room temperature in a humidified chamber. Following additional washes, the wells were reacted overnight with 200 μg of radiolabelled anti-IgE antibodies (specific for $D_{\varepsilon 2}$ determinant, Pharmacia Diagnostics, Uppsala, Sweden) diluted in PBS containing 0·5% Tween 20 and 0·1% BSA. Wells were then washed and counted in a Beckman Gamma Counter. The IgE standard used in this study consisted of a pool of human reaginic sera calibrated against the reference serum provided with the Phadebas kits. The standard was diluted in complete culture medium, buffered with 1 M Tris buffer containing 0·01% sodium azide, pH 7·2. The optimal sensitivity of the assay was 30 pg/ml, and in all assays standards containing 100 pg/ml were significantly different from the blank.

Cultures were performed in duplicate with each culture supernatant in turn tested in duplicate by RIA. The coefficient of variation of the resulting four measurements was most often less than 10%. IgA, IgM

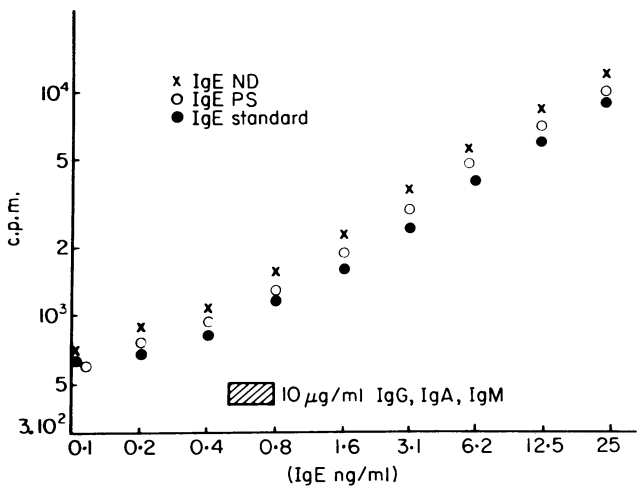

Figure 1. Radioimmunoassay for the measurement of IgE. The plot shows a representative standard curve and illustrates the sensitivity of the assay. The last dilution of the IgE standard (0·1 ng/ml) bound 620 ± 34 c.p.m. as compared to 432 ± 50 c.p.m. bound by the blank (buffered culture medium).

and IgG were measured in the culture supernatants by the same procedure but employing IgG fractions of sheep antisera to α, μ or γ chains to coat the microplate wells; affinity purified antibodies of the same specificity were radiolabelled according to Klinman & Taylor (1969) and used as the detection reagents. Culture supernatants were tested diluted 1/5 in phosphate buffer supplemented with 10% FCS.

RESULTS

Radioimmunoassays for Ig measurement

Figure 1 shows a typical titration curve obtained by serial two-fold dilutions of the IgE standard in buffered culture medium. The coefficient of variation between the three replicates was generally less than 5%. Standards containing 25 ng/ml of IgE bound 20–35% of the total radioactivity; the blanks ranged from 0·5 to 1·5% of the total radioactivity. It is to be noted that parallel titration curves were obtained by employing IgE PS or IgE ND myeloma proteins. The specificity of the IgE assay was further shown by demonstrating that the radioactivity bound by as much as 10 μg/ml of IgG, sIgA or IgM did not exceed that bound by the blank. The specificity of the radioimmonoassays for the measurement of IgG, IgM or IgA was similarly tested; the IgG and IgM assays were class specific to the same extent as the IgE assay, whereas, in the IgA assay, 10 μg/ml of IgG yielded the same binding of radiolabelled anti-IgA as 5 ng/ml of IgA. The sensitivity of the IgA and IgM assay was 0·4 ng/ml and that of the IgG assay was 0·8 ng/ml.

In view of a recent report by Spiegelberg *et al.* (1983) demonstrating the contamination of some anti-IgE sera by anti-idiotype(s) crossreacting with determinants expressed on other classes of Igs, it should be underlined that the RIA employed in this study is based on the detection of the heat-labile antigenic determinant of the ε chain, i.e. the $D_{\varepsilon 2}$ determinant. This was shown by comparing the IgE and IgG levels measured in four culture supernatants incubated or

Table 1. IgE concentrations in culture supernatants of unfractionated PBMC

Donors of PBMC*	Passively released IgE (pg/ml)†	*De novo* synthesized IgE (pg/ml)		
		Control cultures¶	PWM-stimulated cultures**	
Non-atopics	2/24‡ (150; 200)	2/24 (150; 200)	4/24 (2100; 150; 180; 260)	
Atopics			Enhanced synthesis	Suppressed synthesis
	15/27 (373 ± 242)§	16/27 (506 ± 253)	8/27 (1251 ± 240)	12/27 (112 ± 27·5)

* PBMC from 24 non-atopic and 27 atopic patients were used in these experiments.

† The data in this column indicate the passively released IgE by PBMC in cultures containing cycloheximide and puromycin.

‡ Each ratio represents the number of culture supernatants containing IgE to total number of supernatants tested.

§ Mean ± SEM.

¶ The data in this column represent the spontaneous *de novo* IgE synthesis, i.e. the net concentrations of IgE synthesized after subtraction of IgE concentrations released in parallel cultures containing protein inhibitors.

** The data in this column correspond to IgE synthesis in PWM-stimulated cultures which, in the case of cells from atopics, were in excess or below the IgE synthesis measured in the control cultures.

not for 60 min at 56°. Untreated supernatants contained respectively 1200, 980, 450 and 320 pg/ml of IgE as compared to less than 100 pg/ml in the heat-inactivated aliquots of the same supernants; IgG levels were not affected by the incubation at 56°. These data in agreement with those of Spiegelberg *et al.* (1983) thus confirmed that the Pharmacia anti-$D_{\varepsilon 2}$ antibody was not cross-reacting with idiotypic determinants expressed on other Ig classes.

Secretion of IgE by PBMC on stimulation with PWM

Several attempts were made to induce secretion of IgE by stimulating PBMC with PWM. Eighteen batches of foetal calf serum tested at different concentrations in cultures stimulated with various concentrations of PWM from four different batches. Cultures were carried out at various cellular densities either in tissue culture tubes, 96-well microplates or in Linbro 24-well culture plates. Table 1 summarizes the results obtained by culturing PBMC ($1{\cdot}5 \times 10^6$ in 1·5 ml) in Linbro 24-well tissue culture plates; in each of these experiments a minimum of two different batches of FCS were tested. The supernatants of cultures containing protein synthesis inhibitors were used to determine the passive release of cell-bound or intracytoplasmic preformed IgE. As shown in Table 1, small but significant amounts of passively released IgE were detected in the supernatants of 15/27 and 2/24 such cultures derived, respectively, from atopic and nonatopic donors. Spontaneous synthesis of IgE was found in 2/24 control cultures from normal donors and in 16/27 cultures from allergic subjects. PWM induced a small but significant IgE synthesis in four cultures from non-allergic donors, whereas in the cultures from allergic patients PWM augmented the spontaneous IgE synthesis in eight cases and suppressed it in 12 cases. It must be noted that the same PWM-stimulated cultures secreted 1500 ± 370 ng/ml IgG (m ± SEM) and that the secretion of IgG was comparable in the cultures derived from the two groups of donors.

Table 2. Spontaneous secretion of IgE (a) and of the three major classes of Ig (b) in B lymphocyte cultures from normal and atopic individuals

(a)	Donors of B cells	Spontaneous IgE secretion		Cultures with protein inhibitors: IgE (pg/ml)	Control cultures: IgE (pg/ml)	Net IgE synthesis* (pg/ml)
		+	−			
	Atopics (49)	35†		1586 ± 1109§ (50–3800)¶	2650 ± 1593 (310–7500)	1064 (100–3300)
			14‡	2523 ± 1812 (100–6200)	1887 ± 1258 (110–5500)	[−]636 ([−]3800–0)
	Non-atopics (29)	6		361 ± 187 (50–530)	545 ± 325 (100–960)	184 (50–460)
			23	530 ± 548 (0–1800)	460 ± 479 (0–1600)	[−]70 ([−]230–0)

(b)	Ig class	Cultures with protein inhibitors	Control cultures	Net Ig synthesis* (ng/ml)
	IgG (ng/ml)	68·6 ± 95§ (0–340)¶	289 ± 199 (55–725)	220·4 (15–685)
	IgM (ng/ml)	3·4 ± 8·8 (0–45)	40·3 ± 37·3 (4–150)	36·9 (5–180)
	IgA (ng/ml)	2·3 ± 8·2 (0–22)	55·3 ± 33 (2·4–250)	53 (2·4–245)

* Net Ig synthesis was calculated by subtracting the Ig in the presence of protein inhibitors from that in the control cultures.

† Number of cultures with spontaneous IgE secretion.

‡ Number of cultures without spontaneous IgE secretion.

§ Mean ± SD.

¶ Extreme values are indicated in parentheses.

PBMC from four allergic donors responding to PWM stimulation by an increased synthesis of IgE were retested after 3–7 weeks; the PWM-induced IgE secretion was reproduced in only one case.

Spontaneous secretion of immunoglobulins in B lymphocyte cultures

In initial studies, B cells ($1{\cdot}5 \times 10^6$ in 1·5 ml) were cultured in 24-well culture plates for 3, 5 and 7 days. As the results (not shown) indicated that the net IgE synthesis increased from day 3 to day 7, in all subsequent experiments cultures were harvested after 7 days. The viability of the cells in such cultures was $86{\cdot}5 \pm 2{\cdot}1\%$ (m ± SEM of 15 cultures) and the recovery of viable cells corresponded to $63{\cdot}3 \pm 4{\cdot}2\%$ of the number of cells initially cultured. Table 2(a, b) summarizes the results of B cell cultures derived from 49 atopic and 29 non-atopic donors. Passively released IgE was detected in the majority of cultures from both normal and allergic donors, although it was much higher in the latter. In the group of allergic donors, a spontaneous synthesis of IgE was observed in 35 cases (71%); the values ranged from 100 to 3300 pg/ml and a secretion greater than 500 pg/ml was found in 23 such cultures (Fig. 2). The cellular viability was the same in cultures secreting or not secreting IgE. A small but significant *de novo* IgE synthesis ranging from 50 to 460 pg/ml was found in six out of 29 cultures from normal individuals. As shown in Table 2(b), *de novo* synthesis of IgG, IgA and IgM was present in all of the 78 B cell cultures; there was no difference between the two groups of donors. Cultures spontaneously secreting IgE produced similar amounts of IgG, IgA and IgM as those which did not secrete IgE (not shown).

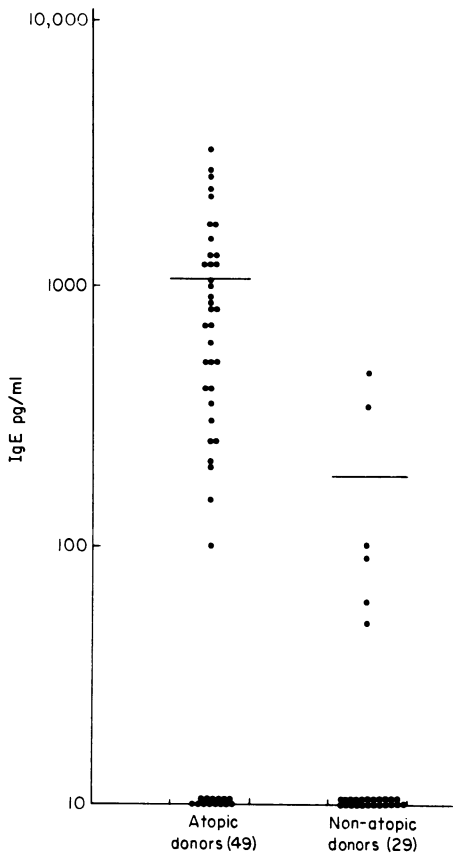

Figure 2. *De novo* synthesis of IgE in B lymphocyte cultures of atopic and non-atopic individuals. The figure shows the net IgE synthesis measured in 49 B cell cultures of atopic subjects and in 29 B cell cultures of normal individuals. Log scale; bars indicate mean values of each group.

Presence of IgE-potentiating factors in the cell-free supernatants (CFS) of cord blood mononuclear cell (CBMC) cultures exposed to IgE

In these experiments CBMC, depleted of adherent cells, were first cultured for 48 hr in the presence or in the absence of 10 μg/ml IgE PS. The cells were then successively washed three times with HBSS, resuspended in HB 101 medium for 60 min at 37°, washed three times in HB 101 medium and cultured for 24 hr in the same culture medium (3×10^6 cells/ml). Cells were centrifuged, CFS was filtered through Diaflo XM50 membrane and finally passed through 0·22 μM Millipore membrane. It is to be stressed that none of the CFS preparations contained any detectable amounts of IgE. Table 3 summarizes the results obtained by supplementing B lymphocytes cultures with three CFS preparations, derived from CBMC exposed to IgE, and employed at the final concentration of 20%. Control cultures were included containing either protein inhibitors or 20% of CFS from control cultures of CBMC. The data in Table 3 corresponds to *de novo* synthesized IgE, calculated by subtracting the IgE concentrations measured in cultures supplemented with protein inhibitors. The results clearly showed that CFS from IgE-treated neonatal lymphocytes increased significantly the spontaneous IgE synthesis without altering the production of IgG, IgA or IgM. It is to be noted that CFS had a small effect on the IgE synthesis by B lymphocytes from a normal individual donor (Donor I).

Table 3. Influence of cell-free supernatants from cultures of cord blood mononuclear cells, which had been exposed to IgE, on IgE secretion by B lymphocytes

		I*				II*				III*				IV*			
Origin of CFS†	B cell cultures in the presence of	IgE‡	IgA	IgM	IgG	IgE	IgA	IgM	IgG	IgE	IgA	IgM	IgG	IgE	IgA	IgM	IgG
CBMC-1	HB 101	0	73	15·5	210	900	83	26	130								
	CFS	0	93	12·5	200	1800	85	23	80								
CBMC-2	HB 101	0	73	15·5	210	900	83	26	130	520	95	23	1550				
	CFS	150	80	14·5	300	1400	87	33	130	1200	110	19	2100				
CBMC-3	HB 101	0	73	15·5	210	900	83	26	130					2600	57	110	1500
	CFS	150	78	11·5	190	3800	85	31	150					5100	42	100	625

* B lymphocyte donors: donor I was a non-allergic individual; donors II, III and IV were allergic patients.
† The CFS was collected from cultures of the three lots of CBMC indicated by the corresponding numbers.
‡ The results are expressed in pg/ml of *de novo* synthesized IgE in duplicate cultures, and in ng/ml of the three other immunoglobulins. The differences between duplicate cultures was less than 15%.

DISCUSSION

The present report describes both the culture conditions and a specific and sensitive radioimmunoassay for the measurement of the spontaneous secretion of IgE in cultures of B lymphocytes isolated from allergic individuals. The specificity of the RIA for ε chain was documented by the following three observations: (i) parallel dilution curves were obtained with two different IgE myelomas and reaginic serum, (ii) the lack of crossreactivity with a 10,000-fold excess of polyclonal IgG, IgA or IgM, (iii) the heat sensitivity of the material measured in the IgE but not in the IgG assay. In accord with previous publications, inhibitors of protein synthesis were added to some cultures in order to differentiate *de novo* synthesis from passive release of cell-bound or intracytoplasmic preformed Ig (Buckley & Becker, 1978; Romagnani *et al.*, 1980; Sampson & Buckley, 1981). Under these conditions, *de novo* synthesis of IgE was detected in 70% of the lymphocyte cultures derived from atopic individuals, whereas small amounts of IgE often at the limit of detection of the assay (100 pg/ml) were found in some cultures derived from non-allergic donors. It is interesting to note, however, that comparable levels of IgG, IgA and IgM were secreted in the two groups of cultures.

The absence of IgE synthesis in approximately one-third of the cultures derived from allergic individuals could not be ascribed to inadequate experimental conditions as indicated by the cell viability studies and by the measurement of a normal synthesis of IgG, IgA or IgM in these cultures. The most likely explanation is that these negative cultures were derived from allergic donors who had no circulating activated IgE B cells at the time of testing. Indeed, previous studies strongly suggested that the B cells spontaneously secreting IgE *in vitro* had been preactivated *in vivo* and that they were present only in individuals with an ongoing IgE antibody response (Romagnani *et al.*, 1980; Saxon, Morrow & Stevens, 1980). For example, lymphocytes from hayfever patients sensitive to grass pollens were capable of secreting *in vitro* IgE, or specific IgE antibodies, only when tested during or immediately after the pollen season (Romagnani *et al.*, 1980; Hemady *et al.*, 1983). The cellular basis of the Ig synthesis observed in the B cell cultures has not been investigated and the possibility exists that it was due to B cell activation by some undefined stimulant present in the FCS. However, in spite of a systemic investigation of various culture conditions and of several batches of foetal calf serum, it has not been possible to obtain a reproducible secretion of IgE in PBMC cultures stimulated with PWM. A similar observation has been made in several laboratories and the reasons why some investigators succeeded in inducing IgE synthesis by PWM stimulation of PBMC are still unclear (Katz, 1982). For these reasons it was decided to employ the spontaneous secretion of IgE in B lymphocyte cultures of atopic individuals as a model for investigation of the regula-

tion of human IgE synthesis. Such an approach has been successfully utilized by Saryan, Leung & Geha (1983) and Romagnani *et al.* (1983) who reported that cell-free supernatants of T lymphocyte cultures from allergic individuals with high levels of serum IgE were capable of potentiating the synthesis of IgE without affecting that of IgG.

On the basis of previous personal observations on the ability of CBMC to synthesize IgE *in vitro* (Delespesse *et al.*, 1983), it was decided to investigate the potential regulatory activity of CBMC on IgE synthesis by adult B lymphocytes. The present data clearly demonstrated that upon incubation which IgE, CBMC can be induced to release soluble factors capable of selectively potentiating IgE synthesis by B lymphocytes from allergic individuals without interfering with the secretion of IgG, IgM or IgA. The absence of detectable IgE in the processed culture supernatants of IgE-treated neonatal cells ruled out the possibility of a carry-over effect accounting for an apparent IgE-enhancing activity. It has been reported that, following incubation with IgE, human T lymphocytes are capable of releasing IgE-binding factors (IgE-BFs) (Ishizaka & Sandberg, 1981). In addition, Spiegelberg (1981) reported that the frequency of lymphocytes bearing receptors for IgE is three times higher in human neonatal lymphocyte preparations than in lymphocytes obtained from adults. Hence, we consider that the IgE-potentiating activity observed in the present study may be due to IgE-BFs.

The observations that neonatal lymphocytes were capable of providing IgE-specific helper activity are in keeping with previous reports indicating that IgE synthesis occurs early during the ontogeny of the immune system. IgE synthesis was detected in cultures of lung and liver tissues of 11-week-old human foetuses and in the spleen of 21-week-old foetuses (Miller, Hirvonen & Gitlin, 1973). The presence of IgE in human cord blood has been well substantiated (Bazarel, Orgel & Hamburger, 1971; Stevenson, Orgel & Hamburger, 1971; Kjellman, 1976; Kjellman & Johansson, 1976; Michel *et al.*, 1980; Hamburger, 1981) and, in a limited number of well documented cases, specific IgE antibodies to a variety of antigens, including cow's milk, grass pollen, penicillin and the 2,4-dinitrophenyl group, have been found in newborn but not in maternal sera (Michel *et al.*, 1980; Levin, Altman & Sela, 1971; Kaufman, 1971). More recently, Weil *et al.* (1983) reported the presence of high levels of IgE and of filaria-specific IgE antibodies in umbilical cord sera of neonates born to parasite-infected mothers; the authors provided evidence that the cord blood IgE was derived from the foetus and not from placental antibody transfer.

The finding of a consistent spontaneous synthesis of IgE in a significant proporton of B lymphocyte cultures derived from atopic individuals and the present observation, that this IgE synthesis could be specifically modulated by immunologic manipulations, indicate that it is now possible to investigate in detail the mechanisms regulating the *in vitro* synthesis of human IgE.

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