

Human IgE Synthesis *in vitro*

Detection with Monoclonal Antibodies

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Using monoclonal anti-human IgE antibodies recognizing the De1 or De2 epitope, we have developed a sandwich radioimmunoassay (RIA) to determine IgE in human cell cultures. With the help of this assay, various methods of measuring an actual *de novo* IgE synthesis were compared. It was necessary to subtract preformed IgE from released IgE in the culture supernatant and the IgE associated to the cultured cells, in order to determine a net IgE synthesis which would reflect *de novo* synthesized IgE. Using this differential *in vitro* IgE assessment, no net IgE synthesis could be demonstrated in culture conditions which lead to strong IgG synthesis. Actual net *in vitro* IgE antibody production was only found in approximately 30% of cell cultures from atopic donors. This spontaneous IgE synthesis did not correlate to the serum IgE levels of the patients. However, correlations were found between serum IgE levels and amount of preformed, released or cell-associated IgE of the cultures.

Key words: *in vitro* IgE synthesis; monoclonal anti-human IgE antibodies.

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Most studies dealing with the *in vitro* synthesis and regulation of human IgE antibodies used peripheral blood leucocytes (PBL) from normal or atopic patients as source of IgE-producing B cells (7, 18, 21, 22, 26, 27). These cells were usually cultured for 7-10 days in the presence or absence of polyclonal B-cell activators such as Pokeweed mitogen (PWM) (18, 21, 22, 26, 27). Subsequently, the IgE content was determined in the culture supernatants, supposedly reflecting the IgE protein synthesis. However, it has already been described how such procedures do not allow dissociation between release of cell-associated IgE and actual *de novo* synthesis of IgE (28). Because of the large amounts of cytophilic or preformed IgE and the very low frequency of IgE B cells, it seems crucial to evaluate the levels of IgE present at onset of culture. However, dissociation between preexisting IgE and actual *de novo* synthesis differs among different laboratories. It has been reported that freeze-thawing at onset of culture can liberate preformed IgE (20).

Furthermore, it has been shown by Turner et al. (28) that acid extraction of the cells at the beginning of culture liberated preformed IgE completely. Other groups used as control protein synthesis inhibitors, such as cycloheximide, in order to differentiate between preformed IgE and *de novo* synthesized IgE (18, 21). Many of these reports imply that a number of results in the literature on the human *in vitro* IgE synthesis might be based on artefacts of technical nature. Therefore, we compared various methods of discriminating preformed IgE from *de novo* synthesized IgE. The measurement of IgE synthesis as described in the literature was mostly performed by assays using solely specific polyclonal anti-IgE antisera or anti-IgE sera in conjunction with one monoclonal antibody, which might have caused some of the conflicting results (13, 16, 17, 18, 21, 22). Therefore, we have developed a sensitive radioimmunoassay based on two monoclonal antibodies and used this assay to measure human *in vitro* IgE synthesis.

MATERIAL AND METHODS

Production and purification of mouse monoclonal anti-human IgE antibodies

Balb/c mice were sequentially immunized at monthly intervals with purified myeloma IgE (PS, gift from Dr. K. Ishizaka, Baltimore), two other myeloma proteins semi-purified from plasma (GB and Udine), and then with semi-purified IgE from a patient with a hyper-IgE syndrome (serum RAST 4 positive for multiple allergens). Spleen cells from immunized mice were adoptively transferred into irradiated syngeneic mice and boosted with IgE PS. Four days later, cells were fused as described earlier (25). Supernatants of growth-positive hybridomas were screened using the dot-immunobinding assay (10, 11) as described below. From a first fusion, anti-IgE monoclonal antibodies were selected with a high capacity to bind human IgE. One monoclonal antibody termed "Le" was arbitrarily chosen and iodinated by the chloramine T method (8, 12). All other monoclonal antibodies from this fusion were selected for inhibition of labelled Le binding to paper disks coupled with IgE PS. This procedure enabled selection of anti-IgE monoclonal antibodies recognizing a similar epitope.

However, as all Le antibodies recognized the same epitope, they could not be used in a sandwich RIA as will be shown elsewhere. Therefore, in additional fusions, we have screened for monoclonal antibodies recognizing human IgE but without the capacity to inhibit the binding of Le antibodies. As for Le antibodies, the new BS antibodies were selected by inhibition assays for antibodies with high affinity for human IgE.

The monoclonal antibodies used throughout these experiments were Le 61 and BS 17. They were isolated from ascitic fluid by ammonium sulfate precipitation and subsequent DEAE Sephacel chromatography as described earlier (25).

Dot-immunobinding assay for anti-IgE

1 µl of purified IgE PS at a concentration of 200 µg/ml was dotted on 4 × 4 mm nitro-

cellulose squares (Millipore HAWG, Millipore Corporation Bedford, Massachusetts, USA) and air dried. Nitrocellulose squares were put into TC 96 culture plate wells (Falcon 3070; Becton Dickinson, Oxnard, California, USA) and blocked with 100 µl of 20% heat-inactivated horse serum in Tris-buffered saline (TBS) pH 7.4 for 1 h at room temperature. The blocking solution was then removed and the square overlaid with 50–100 µl hybridoma culture supernatants for 18 h at room temperature. Nitrocellulose squares were then washed with TBS and incubated with 100 µl of anti-mouse immunoglobulin (diluted 1:200) horse radish peroxidase conjugate (NEN, New England Nuclear, Dreieich, Germany) for 6 h at room temperature. Nitrocellulose squares were washed with TBS and incubated with H₂O₂. The reaction was developed using 4-chloro-1-naphthol (E. Merck, Darmstadt, Germany) as a chromogen (10).

Isotype specificity of monoclonal anti-IgE antibodies

Isotype specificity of monoclonal antibodies was tested using the dot-immunobinding assay (1, 10). Myeloma proteins (temporary WHO-IUIS reference preparations obtained from Dr. Squaril, Tiefenauhospital, Berne, Switzerland) of the following immunoglobulin isotypes were used: IgG₁, IgG₂, IgG₃, IgG₄, IgA, IgA₂, as well as two different IgM myeloma proteins.

The dot-immunobinding assay was performed as described above. All myeloma proteins were dotted at 0.1–1 mg/ml, then incubated with monoclonal anti-IgE antibodies. Four different IgE myeloma proteins (see above) were used as positive controls.

After developing with peroxidase-labeled anti-mouse immunoglobulin, all four IgE myeloma proteins gave strong blue positive dots while all other immunoglobulin isotypes remained negative.

Particle counting immunoassay

The specificity of monoclonal anti-human IgE antibodies was evaluated by their agglutinating activities in the particle counting immuno-

assay (PACIA) using various pepsin and papain fragments of IgE DES to coat latex particles (Magnusson et al., in preparation). Briefly, IgE DES and its fragments were coupled to latex particles (13) which were subsequently agglutinated by serial dilutions (0.1–100 µg/ml) of monoclonal antibodies. Agglutinations > 80% were considered positive and those < 5% negative. The principle of PACIA and the instrumentation used have been previously described in detail (14, 15). Preparation of papain ($6S_2$, Fc_e , Fab'_e and Fc'_e) and pepsin ($F(ab')_{2e}$, $5S_e$ and Fc''_e) fragments of IgE DES (29) will be described in detail elsewhere (Magnusson and Vaerman, in preparation).

Radioimmunoassay for measurement of human IgE and estimation of net IgE synthesis

CNBr-activation of paper disks (Whatman filter paper No. 50, Whatman Ltd., Maidstone, UK) was performed as described by others (1, 3). 10 µg/disk monoclonal anti-IgE antibody Le 61 was covalently coupled to such disks (3). Antibody-coated disks were incubated with 400 µl cell culture supernatants and 50 µl horse serum in flat-bottomed glass tubes for 18 h at room temperature with shaking. Disks were transferred to plastic tubes and washed 3 times at 10-min intervals with saline containing 0.2% Tween 20. Bound IgE was revealed, using the second monoclonal antibody BS 17 labelled with ^{125}I by the chloramine T method (8, 12). After incubation overnight at room temperature, disks were washed 3 times in saline containing 0.2% Tween 20 and counted in a γ -counter. For the construction of standard curves, we used commercially available IgE proteins (Pharmacia Fine Chemicals, Uppsala, Sweden) diluted in our complete culture medium. According to the obtained standard curves, all experimental values were expressed as pg/ml. For the assessment of net IgE synthesis, we used a modification of the method described by Turner et al. (28). Briefly, net IgE synthesis was defined as the amount of IgE in the supernatant of 7-day cultures minus the preformed IgE on day 0.

Cell cultures

Heparinized venous blood was obtained from normal individuals (serum IgE levels < 50 U/ml) and atopic patients with a mean serum IgE level of 1380 ± 1167 U/ml (range 400–5000 U/ml IgE).

Ficoll-hypaque isolated peripheral blood mononuclear cells were washed 3 times in Hank's balanced salt solution (HBSS) and suspended at a concentration of 1.5×10^6 cells/ml in RPMI-1640 medium supplemented with 100 µl/ml penicillin G, 100 µg/ml streptomycin, 10% heat-inactivated fetal calf serum (FCS, Gibco, Grand Island, N.Y.), 2 mM glutamine, and 5×10^{-5} M 2-mercaptoethanol. 2 ml duplicate cell cultures were set up in 16-mm culture tubes (Falcon Nb 3033, Div. Becton Dickinson & Co., Oxnard, California) in presence or absence of 10 and/or 5 µl/ml pokeweed mitogen (PWM, Gibco Grand Island, N.Y.). Whenever PWM was used, it was always added at the beginning of culture and either left for the whole culture period or washed out after 3 days' incubation as indicated in the experiments. Cells were cultured for 7 days at 37°C in a humidified incubator containing 5% CO₂.

Freeze-thawing and acid treatment of the cells

To evaluate the amount of preformed IgE associated with the cells, cultures were subjected 5 times to freeze-thawing (-70°C to $+37^\circ\text{C}$). Cell lysates were then incubated for 7 days under normal culture conditions, as described by Sampson et al. (20). To extract the cell-associated IgE at onset and/or end of culture, we used the method described by Turner (28). Briefly, cell pellets were resuspended in 1 ml complete culture medium and treated with 50 µl 1M HCl (to pH 2.5). Cell suspensions were vortexed and immediately returned to pH 7.4 by addition of 50 µl of 1M NaOH. 900 µl culture medium were added to each tube (end volume 2 ml) and the cultures were then either incubated as described above or frozen at -20°C until determination of IgE protein content. All IgE determinations were performed in duplicate. To some cultures, cycloheximide (Sigma Chemical Company, St. Louis,

MO, USA) was added in different concentrations (100, 50 and 10 µg/ml) at the beginning of culture (18, 21).

Detection of IgE-forming cells by the reverse plaque-forming cell assay

Coupling of protein A to sheep red blood cells (SRBC) and determination of the number of IgE and IgM plaque-forming cells (PFC) was performed as described by Hammarström (9). Briefly, SRBC stored in Alsever's solution were washed 3 times in saline. One part of packed SRBC was mixed with one part of 0.5 mg/ml protein A and 10 parts of 2.5×10^{-4} M CrCl_3 . The mixture was incubated for 30 min at 37°C, washed once in saline and 2 times in HBSS. The SRBC were then kept for maximally 3 days at 4°C until use. For determination of the IgE antibody production, cells were washed twice in HBSS and resuspended in 1 ml HBSS before plating. 1 ml agrose (Indubiose A 37, Produits Biologiques de France) was mixed with 50 µl protein A coupled to SRBC and 50 µl anti-IgE (Dako, Immunoglobulins Ltd, Copenhagen, Denmark). The mixture was plated on a 9 cm diameter petridish and incubated for 3 h at 37°C. 1 ml guinea pig complement (diluted 1:30 in saline) was added and the petridish was incubated for an additional hour at 37°C before counting the number of plaques.

Table 1

Specificity of monoclonal anti-human IgE antibodies as tested by PACIA

Latex with ^a	Epsilon deter- minants ^b	Agglutinating activity of monoclonals	
		BS 17	Le 61
IgE DES	D1 + D2	+	+
6S _e	D1 + D2	+	+
Fc _e	D1 + D2	+	+
Fab _e	-	-	-
F(ab') _{2e}	D1	+	-
5S _e	D1	+	-
Fc _e '	D1	+	-
Fc _e ''	D1	+	-

^a Obtained by papain and pepsin digestion (see Material and Methods).

^b According to Dorrington & Bennich (6) and Magnusson et al. (in preparation).

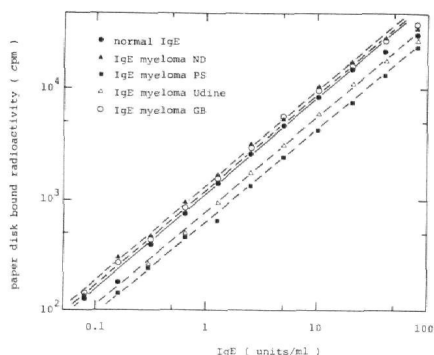


Fig. 1. Determination of different IgE myeloma proteins. Normal IgE was used as supplied in the commercial Pharmacia PRIST assay. (ND IgE was isolated from culture supernatants of the U266 cell line.) The IgE myeloma proteins PS, udine and GB were purified from plasma and then titrated to equal amounts using the commercial PRIST assay. All IgE samples were added to Le 61 antibodies bound to paper disks. Bound IgE was detected by ^{125}I -BS 17 monoclonal antibodies. The RIA was performed under the same standard conditions as the commercial Pharmacia PRIST. Background cpm: 157.

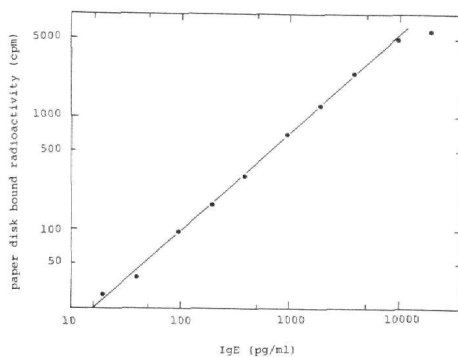


Fig. 2. Typical standard curve obtained with our modified sandwich RIA based on monoclonal antibodies. Background 124 cpm was subtracted from all values.

Statistical evaluation

Pearsons coefficients were used for calculations. Similar values were obtained when using Spearman rank correlation coefficients.

RESULTS

Sandwich RIA for the assessment of low levels of human IgE

The mouse monoclonal anti-human IgE antibodies were only of use in a sandwich radioimmunoassay if the solid phase antibody was different from the revealing antibody, suggesting that two distinct epitopes on the F_c part had to be recognized. To study this question, we prepared digests of IgE and tested the fragments with the particle counting immunoassay.

Table 1 shows that Le 61 and BS 17 monoclonal anti-IgE antibodies were without agglutinating activity towards latex-Fab, but strongly agglutinated latex coated with intact IgE DES, $6S_e$ - and F_c -fragment. Further, BS 17 reacted also with the $F(ab')_{2e}$, $5S_e$ - F_c' - and F_c'' -fragment, which was not the case for Le 61. Taking the location of the D1 and D2 determinants of the F_c -fragment into consideration, it seems that BS 17 has a specificity for an epitope of the D1 region, whereas Le 61 is directed against an epitope pertaining to the D2 region (6, Magnusson et al., in preparation). When we used Le 61 coupled to paper disks as the solid phase antibody and iodinated BS 17 as the revealing antibody in a PRIST-like assay under standard conditions as prescribed by the supplier (Pharmacia Fine Chemicals), our antibodies detected four different IgE myeloma proteins as well as normal IgE (Fig. 1). The obtained standard curves plotted on a log-log scale were parallel and linear over a wide range of IgE concentrations. IgG_1 , IgG_2 , IgG_3 , IgG_4 , IgA , IgA_2 as well as two different IgM myeloma

proteins (see Material and Methods) were also added to assays (Fig. 1) in concentrations from 0.1-50 μ g/ml. No elevation of background cpm was observed, suggesting the isotype specificity of this monoclonal tandem radioimmunoassay, as described above.

To improve the sensitivity of this RIA, we modified the standard PRIST procedure as described in Material and Methods. Fig. 2 shows that our modification allowed measurement of IgE between 50 and 20,000 pg/ml. Fig. 2 represents a typical standard curve used for the estimation of IgE in human culture supernatants as determined for each individual experiment.

IgE levels in supernatants of PBL cultures from normal and atopic blood donors

To determine IgE levels in supernatants of human PBL, we cultured cells at a density of 3×10^6 cells/2ml. Table 2 shows that supernatants from unstimulated normal PBL cultures contained 200 pg/ml protein. Stimulation of PBL by 10 μ l PWM (or 5 μ l, results not shown) did not elevate the IgE concentration in the supernatants. However, PWM stimulated the IgG (and IgM, data not shown) antibody response in these cultures, as determined by the reverse plaque-forming cell assay. The supernatants of unstimulated cultures from atopic blood donors contained higher levels of IgE. Stimulation of PBL with 10 or 5 μ l/ml PWM did not elevate the IgE culture supernatant levels, but seemed to reduce them. Again, the IgE antibody response was clearly enhanced by PWM stimulation. Fig. 3 shows that the individual IgE antibody response

Table 2
Determination of IgE and IgG in supernatants of PWM-stimulated PBL cultures

Stimulation	Immunoglobulin concentration in PBL culture supernatants of:			
	Normal donors ($n = 7$)		Atopic donors ($n = 20$)	
	IgE (pg/ml) ^a	IgG (PFC/10 ⁶ c) ^b	IgE (pg/ml) ^a	IgG (PFC/10 ⁶ c) ^b
-	203 $\pm$ 103	238 $\pm$ 257	1240 $\pm$ 853	992 $\pm$ 80
PWM 10 μ l/ml	185 $\pm$ 55	1906 $\pm$ 632	735 $\pm$ 615	2077 $\pm$ 1565

^apg/ml determined by sandwich RIA. Mean $\pm$ SD.

^bplaque-forming cells (PFC) per 10⁶ cells determined by reverse plaque assay. Mean $\pm$ SD.

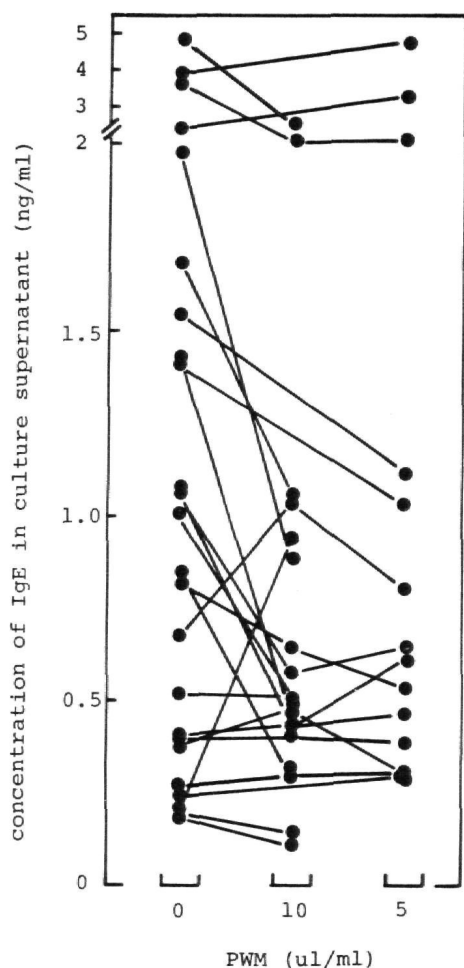


Fig. 3. Determination of IgE levels in culture supernatants of cells from atopic blood donors. PBL were stimulated with 5 or 10 μ l/ml PWM and culture supernatants harvested after 7 days.

of 22 supernatants from cell culture of atopic blood donors followed three tendencies: Some cultures remained unaffected by the addition of PWM, while most culture supernatants contained less IgE and only four supernatants contained slightly more IgE than the supernatants of unstimulated cultures. These data suggested that we were not able to induce a primary IgE

response using polyclonal activators even though the same preparations were excellent stimulators of an IgG response. Therefore, throughout the next experiments, we focused on the spontaneous IgE release by cells from atopic patients.

Determination of net in vitro IgE synthesis

To study whether the increased IgE levels in culture supernatants of atopic patients represented released IgE (cytophilic or cytoplasmic), or an actual *de novo* IgE synthesis, we evaluated the amount of preformed IgE either by freeze-thawing cells 5 times before culture or by treating cells with acid to remove cytophilic IgE. Table 3 shows seven representative experiments. The acid extraction procedure in our culture system allowed detection of higher levels of preformed IgE than the freeze-thawing method. It has been reported that cell lysates after freeze-thawing (F/T) liberated more IgE when incubated for 7 days at 37°C than when kept at -20°C (20). Therefore, we also incubated our acid-treated cell cultures in the same way and compared their IgE levels with IgE concentrations of untreated or F/T cultures. As shown in Table 3, higher levels of IgE were found after treatment of cell cultures. If acid-treated cultures were main-

Table 3
Assessment of preformed IgE in atopic PBL cultures by freeze-thawing (F/T) or acid extraction

Exp.	IgE (pg/ml)		
	F/T, +37°C ^a	Acid, -20°C ^b	Acid, +37°C ^c
1	2577	5900	5820
2	566	1965	2390
3	970	2400	2311
4	82	1315	901
5	143	396	566
6	188	715	895
7	560	2650	2450
$\bar{x}^d$	727 $\pm$ 873	2191 $\pm$ 1836	2190 $\pm$ 1792

^aCells frozen and thawed 5 times and cultured for 7 days at 37°C.

^bpH of cultures was lowered to pH 2.5 with 1N HCl and then immediately neutralized with 1N NaOH. Supernatants were stored at -20°C and measured at end of experiment.

^cas in b, but cultured for 7 days at 37°C.

^d $\bar{x}$ = mean $\pm$ SD.

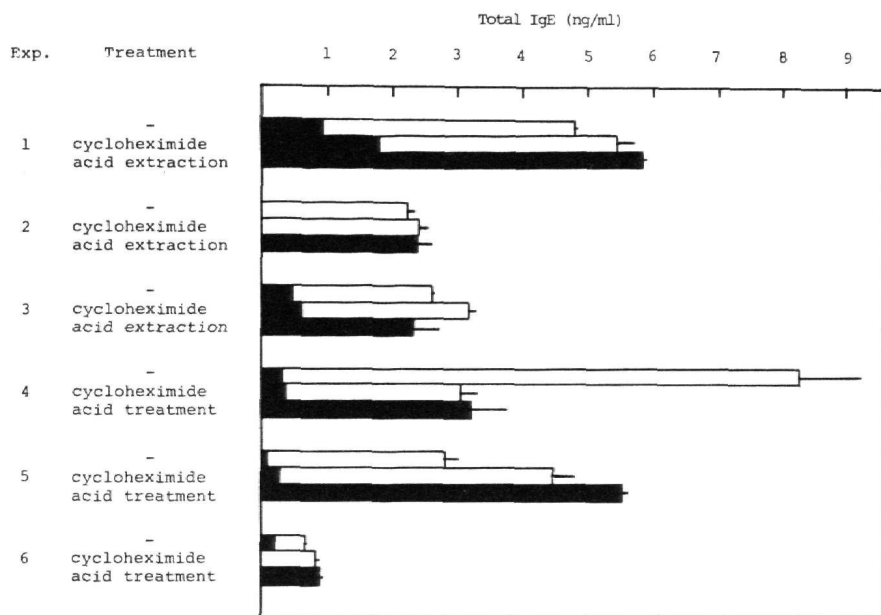


Fig. 4. Determination of cell-associated (closed bars) or cell culture supernatants (open bars) IgE levels in cultures of atopic blood donors. PBL were either left untreated or cultured with 50 μ g/ml cycloheximide. Additionally, cultures were treated with acid (acid extraction) at the onset of culture to determine the preformed IgE. Untreated and cycloheximide treated cultures were acid-treated after 7 days to determine cell-associated IgE at the end of culture.

tained for 7 days at 37°C, the supernatants contained the same amounts of IgE as in the supernatants kept frozen. Furthermore, these experiments showed that cells from different atopic individuals stored extremely variable amounts of IgE in or on the cells. Thus, as already shown by others (28), supernatant IgE levels *per se* did not reflect an actual *de novo* IgE synthesis, but in many instances only represented the release of preformed IgE. We therefore decided to use the acid extraction method with further incubation at 37°C as a standard procedure to define the amount of preformed IgE. Thus, net *in vitro* synthesis of IgE was defined as the amount of IgE found at the end of culture to be associated with the cells plus the IgE in the culture supernatant minus the amount of IgE obtained by the acid extraction method on day 0.

Several reports in the literature on the human *in vitro* IgE synthesis showed the use of cyclohex-

imide as a control to differentiate between supernatant IgE content and *de novo* IgE synthesis (18, 21). Therefore, we compared the two methods, using either cycloheximide or the acid extraction procedure as a control. Fig. 4 shows that the results obtained by the acid extraction procedure or cycloheximide treatment (50 μ g/ml) were comparable. However, when the acid extraction procedure was also applied to the cultures treated with cycloheximide, some residual IgE was still occasionally associated with the cell pellets of cells blocked by the protein synthesis inhibitor (Fig. 4). Thus, we favoured the acid extraction method as it yielded higher levels of background IgE and produced fewer false positive IgE synthesis results.

Kinetic studies on the *in vitro* IgE synthesis have shown that preformed IgE was released into culture medium mainly during the first 3–4 days of culture (18, 20, 23). There are

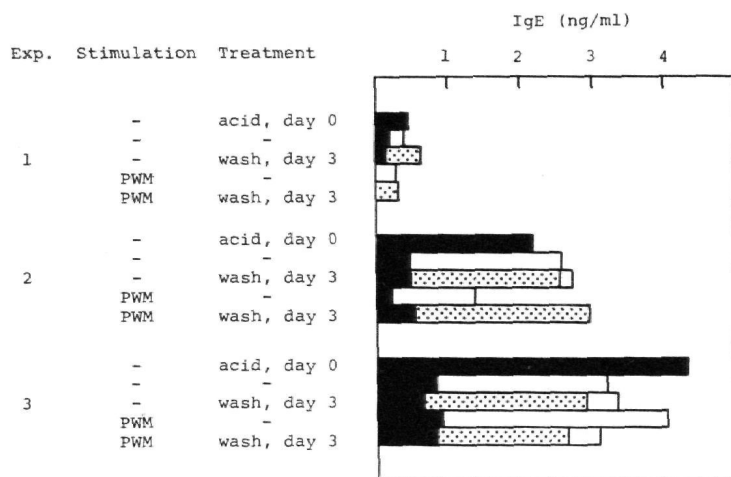


Fig. 5. Effect of medium replacement on *in vitro* IgE formation. PBL from atopic patients were cultured for 7 days with or without 5 μ l PWM. Bars indicate measured IgE levels. Black bars represent cellular associated IgE recovered by acid extraction either at the onset (day 0) or the end culture (day 7). Dotted fraction of the bars represents IgE in supernatants after 3 days of culture, while open fraction represents IgE in the supernatant harvested on day 7.

also reports showing that exchanging culture medium after 3 or 4 days made it possible to stimulate (with PWM) IgE synthesis in cell cultures from normal and atopic patients (16, 30). Fig. 5 shows three typical experiments with cells containing different concentrations of IgE at the onset of culture. In experiment 2, the figure shows that washing procedure appears to slightly enhance the IgE synthesis. However, in a series of 10 additional experiments, we never observed a significant induction of IgE synthesis by PWM (results not shown). Fig. 5 also shows that more than 80% of the IgE was usually released after 3 days of culture. In conclusion, our results show that when stringent controls were applied, net IgE synthesis very rarely took place in cultures of atopic blood donors. So far, we have not been able to induce *in vitro* IgE synthesis by stimulation with PWM. It seems crucial that the preformed IgE levels are precisely determined as well as the IgE which is associated with cells at the end of the culture period.

Correlation between serum and culture IgE levels

To study whether there might be a correlation between serum IgE levels and the net *in vitro* IgE synthesis, we cultured cells of atopic blood donors with serum IgE levels between 400 and 5000 U/ml. As shown in Fig. 6, panel A, we found a correlation between serum IgE levels and the IgE associated with the cells on day 0. Similar correlations were found between serum IgE levels and IgE concentrations associated with cells at end culture (panel B), or with the total IgE present in the culture after 7 days of incubation (panel C). However, we could not observe a correlation between serum IgE levels and the net IgE synthesis (panel D). Furthermore, it can also be seen in panel D that only seven of 20 patients showed a spontaneous net IgE synthesis. It has been reported previously that a correlation between serum IgE level and net IgE synthesis does indeed exist (28). However, in our study, we used donors with lower serum IgE levels, which suggests that marginally elevated serum

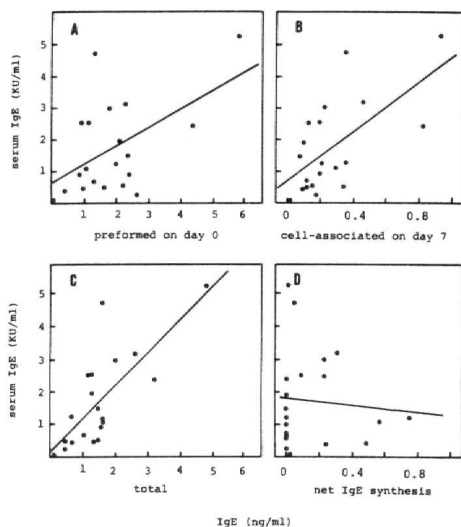


Fig. 6. Correlation coefficients between the following parameters: Panel A) correlation between serum IgE levels and cell-associated IgE on day 0, ($r = 0.54$); Panel B) correlation between serum IgE levels and cell-associated IgE on day 7, ($r = 0.64$); Panel C) correlation between serum IgE levels and total IgE on day 7 (IgE associated with cells plus culture supernatant IgE), ($r = 0.74$); Panel D) correlation between serum IgE levels and net IgE synthesis ($r = -0.08$).

IgE levels are not predictive of an eventual *in vitro* net IgE synthesis.

DISCUSSION

The monoclonal antibodies Le and BS do not recognize immunoglobulin classes other than IgE, and normal IgE is qualitatively and quantitatively recognized in the same fashion as all other tested IgE myeloma proteins. The observed differences in the absolute cpm obtained by using the different IgE myeloma proteins are due to the experimental variation introduced by titration with the commercial PRIST assay. We have used the very sensitive dot-immunobinding assay for specificity testing. The protein concentrations of the different isotypes (0.1–1 $\mu\text{g}/\text{dot}$) are several logarithms more concentrated than whatever

could be expected to be present in culture supernatants. Furthermore, our sandwich RIA can be used to determine rather elevated IgE concentrations, even in undiluted serum samples, suggesting also no cross-reaction of the monoclonal antibodies with other serum proteins. Thus, it seems that this tandem of monoclonal antibodies recognizing different epitopes in the Fc part of IgE is very suitable for the measurement of *in vitro* IgE synthesis.

To increase sensitivity for the detection of human IgE, it was essential to incubate culture supernatants with the solid phase antibody on the disk for 18 h. Longer incubation did not improve the sensitivity (data not shown). This is in contrast to some reported RIAs based on heteroantisera, which require incubation times of up to 6 days for maximal sensitivity (20). The sample volumes were usually 400 μl , as smaller volumes decreased the assay sensitivity and reproducibility. However, it should be noted that equal sensitivity in our RIA was achieved when different commercially available specific anti-IgE sera were used as revealing antibodies (5, data not shown).

Several culture supernatants containing IgE levels $> 100 \text{ pg}/\text{ml}$ were assayed in the RIA at different dilutions. Such dilution curves displayed the same slope as the standard curve using myeloma protein.

Using this RIA for the determination of IgE in human PBL cultures, we found data similar to that reported by many other laboratories, namely that cells of normal individuals contain or secrete very small or unmeasurable amounts of IgE, and that cells from atopic patients contain elevated IgE levels or sporadically show an ongoing elevated IgE synthesis (16, 21, 26, 30). Based on our data, it seems essential to determine the preformed IgE in order to measure a net *in vitro* IgE synthesis. In our hands, the acid extraction procedure gave the most reliable control values for the estimation of net IgE synthesis. Our finding that PWM did not induce IgE antibody production, but rather inhibited it, is in agreement with some laboratories (7, 20, 21, 26, 30), but not with others (2, 22, 23).

We have used different batches of PWM and FCS, suggesting that negative results are not due to the lectin or serum supplementation. Studies are presently underway to evaluate whether manipulations on the cellular level (B-cell enrichment, T-cell irradiation [7, 9, 19, 30]), or the addition of other stimulators (Cowan 1 [24]) might be suitable procedures for the stimulation of IgE synthesis.

It has been reported that serum IgE levels do correlate with the net *in vitro* IgE synthesis (28). We find acceptable correlations between serum IgE levels and cell-associated IgE or released IgE. However, we do not find a correlation between serum IgE levels and the net *in vitro* IgE synthesis. Such a correlation might eventually be obtained with cells of patients with strongly elevated serum IgE concentrations. Upon retesting of individual allergic patients for the capacity to spontaneously produce IgE *in vitro*, we have found that only patients with serum IgE levels in the range of or greater than 1000 U/ml are reproducibly showing a spontaneous net *in vitro* IgE synthesis. The source of the preformed IgE released in the culture supernatants has not yet been studied. It is not known whether this IgE is derived from cytoplasmic pools or from IgE bound to Fc receptors. The short-time acid extraction (as described in Material and Methods) lyses cells immediately and could therefore release both cytoplasmic and cytophilic IgE. The acid treatment procedure gave higher IgE supernatant levels than the freeze-thawing method, suggesting that a substantial amount of IgE is most likely associated with cell surfaces.

In conclusion, our data show that monoclonal antibodies can be used for the measurement of *in vitro* IgE synthesis. The sensitivity of our assay seems to be similar to the sensitivity reported by others (18, 20, 22, 27). The advantage of a tandem of monoclonal antibodies is that reagents remain stable and are available in large quantities that could be shared with many other laboratories. Such an approach might also help to avoid contradictory data on the *in vitro* IgE synthesis as reported in the past. It seems to be established

that supernatant IgE levels do not represent the actual cellular capacity to produce IgE. Thus, appropriate controls to determine the preformed IgE are mandatory. However, in order to use such *in vitro* IgE systems for the assessment of isotype-specific IgE regulation, it will be crucial to develop culture conditions which would allow a polyclonal IgE stimulation, even in cell cultures of normal donors. As only about 30% of atopic blood donors showed a spontaneous IgE synthesis, it might also be necessary to understand better the preexisting *in vitro* regulation of IgE antibody production.

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