

Biomolecular regulation of the IgE immune response

I. Cell-associated IgE and *in vitro* IgE synthesis

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Several cell types, including mast cells, basophils, macrophages/monocytes, lymphocytes, platelets and eosinophils, may bind or contain IgE. To investigate the source of cell-associated IgE and its relation to spontaneous IgE synthesis, peripheral blood mononuclear cells from allergic and non-allergic donors were examined. Using a combination of different cell fractionation techniques and varying methods for extraction of cell-associated IgE, data were obtained indicating that monocytes constitute a major source of cell-associated IgE in human blood. The amount of cell-associated IgE in peripheral blood mononuclear cells from allergic and non-allergic donors varied more than 100-fold but correlated closely to the level of serum IgE ($r = 0.84$, $p < 0.001$, $n = 38$). Spontaneous and mitogen-induced *in vitro* syntheses of IgA, IgE and IgG were compared for allergic and non-allergic donors. Only one donor with very high serum IgE demonstrated spontaneous or mitogen-induced *in vitro* IgE synthesis despite synthesis of IgA and IgG.

L. K. Poulsen^{1,2}, L. Baron^{1,2},
J. H. Heinig¹, P. Stahl Skov¹,
K. Bendtzen^{2,3}

¹ Laboratory of Medical Allergology,
² Biotechnological Centre for Research in
Signalling Peptides, and ³ Laboratory of Medical
Immunology, National University Hospital,
Copenhagen, Denmark

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Lars K. Poulsen, PhD
Laboratory of Medical Allergology
National University Hospital, TTA 7542
20 Tagensvej, DK-2200 Copenhagen N
Denmark

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IgE is known to play a key role in the allergic reactions elicited by mast cells and basophils. Other cell types, including macrophages/monocytes, lymphocytes, platelets and eosinophils, also have receptors for IgE (1, 19), but the biological function of these receptors is little known. Moreover, the existence of intracellular IgE in monocytes has been indicated (26). In spite of a short serum IgE half-life of about 2 days (28), IgE sensitivity can be very long-lasting. Patients with seasonal allergy may become sensitized after limited exposure to the offending allergens, and after re-exposure, the allergic reaction may be elicited several months later. In patients with insect venom allergy, this may be even more pronounced, as anaphylactic symptoms may be evoked years after the latest exposure (22).

Several hypotheses could explain this long-lasting sensitivity despite lack of allergen stimulation and the short serum half-life of IgE: 1) this labile immunoglobulin could be stabilized as cell-associated IgE, 2) an ongoing IgE-synthesis could occur, involving only terminally differentiated IgE-producing B cells or plasma cells (8, 11), or 3) an ongoing recruitment of memory B cells by interleukin 4 (IL-4), producing T-helper cells, could take place (12, 27).

Recently, the role of IL-4 in the IgE-synthesis has

been addressed by several groups. It is still not clear, however, whether T cells without any allergen stimulation may be activated so as to produce IL-4 and provide help for B cells. Thus, the present study addressed the former 2 possibilities. The first step was an investigation of the distribution of the cell-associated IgE in basophils, monocytes and lymphocytes in mononuclear cell suspensions from allergic and non-allergic individuals. To identify candidates for IgE-associated cells, varying methods of isolation and extraction of cells were compared. Further, in order to examine a possible ongoing IgE synthesis in allergics and non-allergics, the spontaneous or mitogen-induced synthesis of IgE *in vitro* was compared with that of the isotypes IgA and IgG.

Material and methods

Allergic and non-allergic donors

For determination of cell-associated immunoglobulin, we used blood cells sampled from 19 patients with IgE-mediated asthma and/or rhinitis caused by one or more common Danish inhalant allergens (birch, grass and mugwort pollen, animal dander, house dust mites or moulds (*Alternaria*, *Cladosporium*) with a positive RAST (RAST class ≥ 2 , Phade-

bas RAST, Pharmacia, Uppsala, Sweden) and skin prick test (mean diameter ≥ 3 mm when tested with Pharmedgen 100 000 BU/ml (Pharmacia)) (5). Cells from some of the patients (see Results) were also used for *in vitro* IgE synthesis experiments. Four of the allergic patients were in the up dosing phase of immunotherapy. At the time of blood sampling they had received 140–340 SQ units of house dust mite extract (described below) accumulated during 2 weeks of treatment. Nineteen healthy, normal individuals, with no history of allergic symptoms, served as controls. Mean ages of the groups were 30.5 (range 17.4–55.1) and 38.3 (range 23.2–45.0) and the sex distribution was 11/8 (F/M) and 12/7, respectively.

Isolation of cells

Cells were isolated from heparinized blood using either LymphoPrep (Specific density: 1.077) (Nycomed, Oslo, Norway) or Ficoll-Hypaque density gradient (specific density: 1.080). Subsequently, loosely bound immunoglobulin was removed by washing cells 3 times in RPMI + 5% fetal calf serum (Gibco, Paisley, Scotland). Adherent cells were further isolated by adherence to plastic dishes for 2 h at 37°C (2). Adherent cells isolated by this method have been demonstrated by esterase staining to contain 90% monocytes (range 77–98%, $n = 8$) (2). The differential counts described in Table 2 were obtained using a Coulter Counter.

Extraction of cytophilic immunoglobulins

Different methods were applied for extraction of cell-associated immunoglobulin.

Acid extraction. The method of Turner et al. was used (25): the acidity of 1 ml of cell suspension was lowered to pH 2–2.3 by addition of 50 μ l of 1 mol HCl, immediately followed by 50 μ l of 1 mol NaOH. The exact volume of acid/base had to be adjusted according to the buffering capacity of the medium. It was ensured by control testing that neither IgE nor IgG/IgA deteriorated if kept at low pH for maximum 30 s.

Stripping of surface-bound IgE. IgE was removed from the cell surface by a modification of the method originally described for Ficoll-Hypaque isolated cells (24): the cells were suspended in 5 ml of buffer (pH 3.6) containing 145 mmol of NaH_2PO_4 , 5 mmol of potassium acetate and human serum albumin 0.3 mg/ml, and were incubated at 4°C for 5 min. After centrifugation (250 g for 6 min), the acid-treated cells, now devoid of IgE, were suspended in 5 ml of Pipes-AMC buffer, washed 3 times (250 g for 6 min) and resuspended in this buffer to a final volume of 1 ml.

This method has previously been demonstrated to deprive cells of surface IgE (15).

Freezing/thawing. Cell suspensions were frozen (-80°C) and thawed in 5 cycles.

Ultrasonication. Cell suspensions were ultrasonicated (Elgasonic, Bienne, Switzerland) at 30 W for 60 s.

Culture conditions and stimulations

For *in vitro* synthesis of IgE, cells were grown (2×10^6 /ml) in RPMI 1640 supplemented with glutamine 2 mmol and fetal calf serum 5% at 37°C, in an atmosphere containing 5% CO_2 . Pokeweed mitogen (PWM, Gibco), lipopolysaccharide (LPS, *E. coli* 055:B5, Westphal Method, Difco, Detroit, Michigan, USA), anti-IgE (Behringwerke, Marburg, Germany) and house dust mite (*Dermatophagoides pteronyssinus*, Aquagen No. 503, ALK, Copenhagen, Denmark) were used to stimulate the cells.

Determination of IgE

IgE was determined by a sensitive (detection limit: 50 pg/ml) immunoradiometric assay (14). Briefly, 300 μ l of sample was incubated in polystyrene test-tubes ("Maxisorp", NUNC, Roskilde, Denmark), onto which anti-IgE (Behringwerke) had been adsorbed. ^{125}I -labelled anti-IgE (Pharmacia, Uppsala, Sweden) was used for detection.

Determination of IgA and IgG

Radioimmunometric assays were developed for determination of IgA and IgG. To "Maxisorp" test-tubes was added 500 μ l of either rabbit anti-human IgA antibody diluted 10^3 times in PBS or F(ab)2-fragments of rabbit anti-human IgG antibody diluted 10^4 times in PBS (Code Nos. A262 and A407, Dakopatts, Copenhagen, Denmark), and the test-tubes were incubated at room temperature overnight. After coating, the test-tubes were washed 3 times (PBS + Tween 0.1%). The standard or the samples to be tested were diluted in RPMI medium containing EDTA 7.4 g/l and fetal calf serum 5%, and 500 μ l was added to the test-tubes and incubated at room temperature for 2 h. The test-tubes were then washed, and 500 μ l of mouse anti-human IgA/IgG monoclonal antibody (Code Nos. M 12011 or Mo 6014 from Oxoid, Basingstoke, Hampshire, UK) was added and incubated at 37°C for 2 h. The test-tubes were then incubated with 500 μ l of iodine-labelled rabbit anti-mouse IgG (see below). They were washed and counted in a gamma-scintillation counter the following day.

A serum pool containing 3.9 ± 0.1 mg IgA/ml and

11.6 ± 0.2 mg IgG/ml (5 determinations) by standard nephelometric methods (Department of Clinical Chemistry) served as standard. Measurement ranges of 4–500 ng IgA/ml and 1.5–500 ng IgG/ml were applied. When the control serum diluted to 26 ng IgA/ml was repeatedly tested for IgA in quadruplicate on 4 different days, the intra- and interassay variations were 5% and 12%, respectively. Dilution of the control serum to 20 ng IgG/ml produced intra- and interassay variations of 8% and 23% in the IgG assay.

The specificity of the monoclonal antibodies has previously been established (3). Possible cross-reactivity of the IgA, IgM or IgE isotypes in the IgG assay was tested by addition of up to 10 µg/ml of IgA or IgM standards, or 1 µg/ml of IgE myeloma (ND) to 10 ng/ml of IgG or dilution buffer standard; in no case did this affect the results. When serum dilutions were prepared by dilution of cell culture medium instead of PBS, identical dilution curves were obtained, indicating the absence of interference from the medium. For IgA, no interference from the cell culture medium or cross-reactivity from the IgG, IgM or IgE isotypes were observed for up to 10 µg/ml of IgG or IgM standards, or 1 µg/ml of IgE myeloma (ND). Rabbit anti-mouse immunoglobulins were obtained from Dakopatts (Code No. Z 109), and purified by acid elution from Sepharose-coupled murine IgG, followed by adsorption on Sepharose-coupled human IgG at neutral pH. The immunoglobulins were radiolabelled as described (3).

Statistics

The Mann-Whitney test for unpaired and the Wilcoxon test for paired observations were applied for testing differences between groups. Coefficient of correlation was calculated as Spearman's rho.

Results

Acid extraction of cell-associated IgE

We have previously developed a method for stripping IgE bound to the cell-surface (cf. Material and methods), but with the aim of extracting intracellular IgE, different extraction methods were compared for LymphoPrep-isolated cells from the blood of allergic and non-allergic individuals. The yield of IgE was consistently higher for the acid extraction (pH 2.0) procedure, as compared with ultrasonication (Table 1, exp. I) or 5 cycles of freezing and thawing (results not shown). Neither the cell number nor the original level of cellular IgE influenced the recovery (Table 1, exps. II and III). If cultures were kept at 37°C after acid treatment, the level of IgE decreased, but the same tendency could be observed for IgE kept at 37°C in a cell-free medium (Table 1, exp.

Table 1. Methods for extraction of IgE

Experiment I. Comparison: acid extraction versus ultrasonication (amount of IgE recovered in pg/ml)

	Donor A	Donor B
Acid extraction (pH 2.0)	1350	787
Ultrasonication	169	192

Experiment II. Recovery of exogenously added IgE (amount of IgE recovered in pg/ml)

	Donor A	Donor B
a acid extraction of cells with no IgE added	1350	712
b amount of IgE added	787	787
a+b	2137	1499
c acid extraction of cells with addition of IgE	2137	1312
recovery: c/(a+b)	100%	88%

Experiment III. Recovery, dependency of cell number¹

Number of cells extracted	IgE (pg/ml)	IgE (pg/10 ⁶ cells)
2 × 10 ⁶	285	142
3 × 10 ⁶	436	145
4 × 10 ⁶	615	154

Experiment IV. Recovery after acid extraction day 0 (amount of IgE recovered in pg/ml)

	Day 0	Day 3	Day 7
Donor A	169	134	150
Donor B	315	246	292
Donor C	916	N.D.	886
IgE+cell-free medium	423	425	375

Cells were isolated on LymphoPrep, washed 3 times and resuspended at 2 × 10⁶ cells/ml. After extraction (details of the procedures are given in Material and methods), IgE in supernatants was determined by immunoradiometric assay.

¹ Freezing/thawing of the cells after acid extraction did not produce any IgE.

IV). The IgE recovered by freezing/thawing and by acid extraction might originate from different intracellular compartments. However, freezing/thawing of the acid-treated cell suspension did not increase the level of detectable IgE. Based on these results, the acid extraction procedure, followed by storage at –20°C, was used for all further experiments.

Location of cell-associated IgE

In a preliminary attempt to elucidate the cellular origin of cell-associated IgE, a comparison was made between 2 gradients for cell isolation in combination with 2 different procedures for extraction of cell-associated IgE. Variable levels of cellular IgE could be recovered, depending on both of these factors. Table 2 shows the cellular IgE recovery from the Ficoll or LymphoPrep cell preparation combined with either cell extraction (pH 2.0) or surface-Ig stripping (pH 3.6) of cells from 4 donors.

Table 2. Preparation of cells and extraction of IgE

Donor No.	1	2	3	4
Donor allergic?	-	-	+	+
IgE (in pg/10 ⁶ cells) recovered when using different methods of cell preparation followed by acid extraction (pH 2.0) or surface Ig stripping (pH 3.6).				
Ficoll/Stripping	104	136	377	381
Ficoll/Extraction	317	371	735	999
LymphoPrep/Stripping	96	96	170	263
LymphoPrep/Extraction	128	134	389	328
Differential counts (%) for Ficoll				
Lymphocytes	92.4	90.0	96.7	86.3
Monocytes	2.4	6.0	0.3	7.6
Neutrophils	4.5	1.9	2.9	3.2
Basophils	0.4	0.2	0.1	0.3
Differential counts (%) for LymphoPrep				
Lymphocytes	97.2	96.4	97.6	93.2
Monocytes	0.9	0.9	0.6	1.8
Neutrophils	1.5	2.5	1.3	4.3
Basophils	0.1	<0.1	<0.1	0.1

Regardless of the extraction method, the Ficoll isolation improved the yield of cellular IgE, as compared with LymphoPrep. Differential counts demonstrated higher fractions of monocytes (3 donors) and basophils (4 donors) in Ficoll-isolated cell suspensions. Stripping of cells with pH 3.6 – a procedure developed to remove surface-bound IgE from basophils – produced IgE levels correlating to the level of serum IgE rather than to the number of basophils. Extraction of cells with pH 2.0 unequivocally gave a higher yield than stripping of the cells. The additional yield obtained by extraction paralleled the number of monocytes in the 8 cell suspensions prepared with the 2 gradients, indicating that monocytes rather than basophils might be the major source of cell-associated IgE.

IgE is associated with adherent cell fraction

To test whether IgE was associated with the monocytes, an experiment was performed in which LymphoPrep-isolated mononuclear cells from allergics and non-allergics were further separated into adherent and non-adherent fractions. In each of these fractions and in the total mononuclear cell suspension, the cell-associated IgE, IgG and IgA were extracted at pH 2.0 and determined. In most cell suspensions IgA was below detection limit (40 ng/ml). IgE and IgG in adherent and non-adherent fractions of cells are shown in Fig. 1. For IgE, it was clearly demonstrated that a higher amount was associated with the adherent (monocyte-enriched) than with the non-adherent (monocyte-

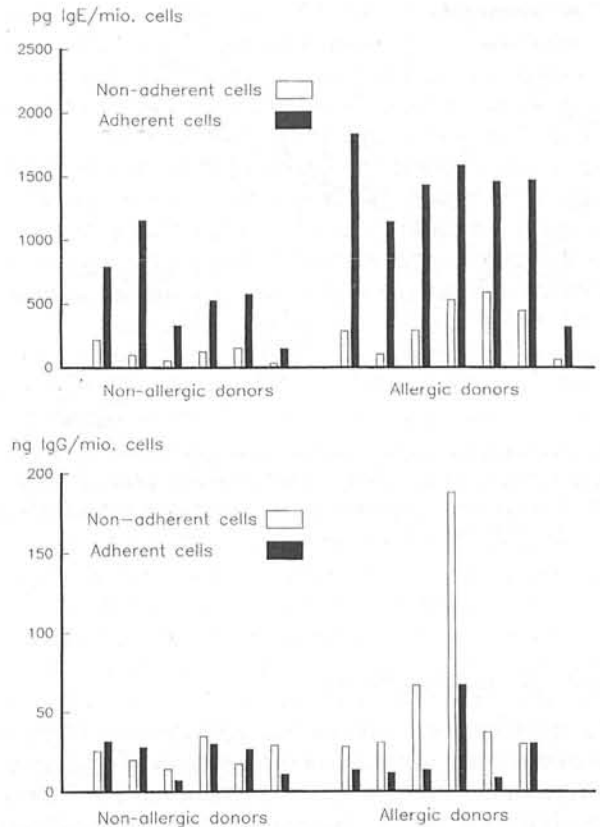


Fig. 1. Cell-associated IgE and IgG in adherent and non-adherent fractions of mononuclear cells. Cells were isolated on Lympho Prep and adherent cells removed by adherence to a plastic surface. Immunoglobulin was determined in acid extracts of the cells and expressed/million cells.

deprived) fraction when calculated on a mio. cell basis ($p = 0.02$ for allergic donors and $p = 0.03$ for non-allergic donors). For IgG, there was no difference between the 2 fractions.

Cell-associated IgE in normal and allergic individuals

As the results presented in Fig. 1 indicated that allergic individuals had more cell-associated IgE, this was compared with the level of serum IgE (Fig. 2). Even though both cellular and serum IgE varies within several decades, a correlation ($r = 0.84$, $p < 0.001$) between the 2 parameters was seen. However, at high levels of serum IgE, saturation of the cells seemed to occur, i.e., $r_{\text{non-allergic}} = 0.79$, $r_{\text{allergic}} = 0.55$. Based on rough estimates of the number of cells/ml blood, the geometric means of serum-to-cell-IgE-ratios were 1312 (range 257–8144) and 372 (range 257–1312) for the allergic versus the normal donor group. The cells were prepared by means of LymphoPrep, implying that lymphocytes and monocytes but very few basophils (cf. Table 2) would appear in the preparations. These ratios cannot therefore be considered true indicators of the *in vivo* ratios of free/cell-bound IgE.

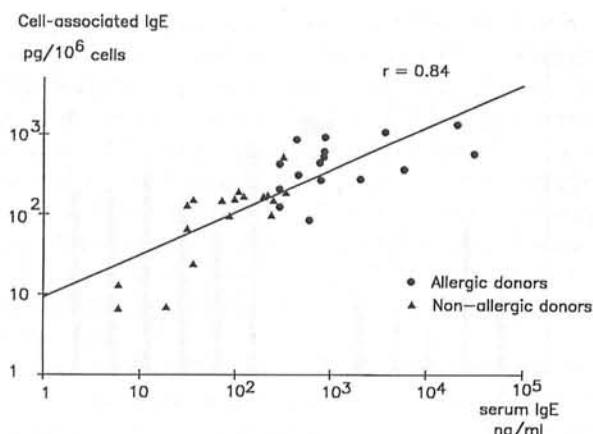


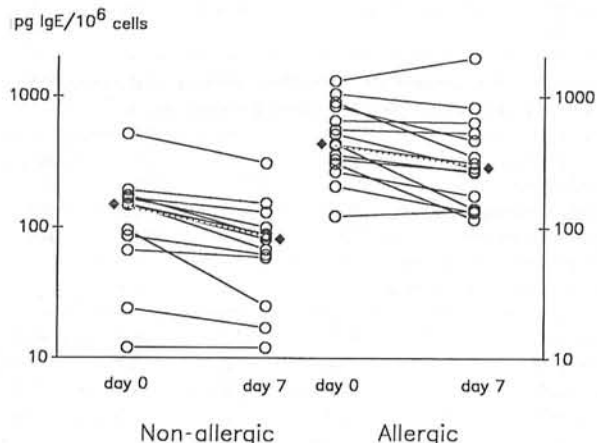
Fig. 2. Cell-associated IgE versus serum IgE. Lymphocytes from 18 non-allergic and 16 allergic individuals were isolated by LymphoPrep. IgE was determined in acid extracts and compared with the level of serum IgE.

Spontaneous IgE synthesis

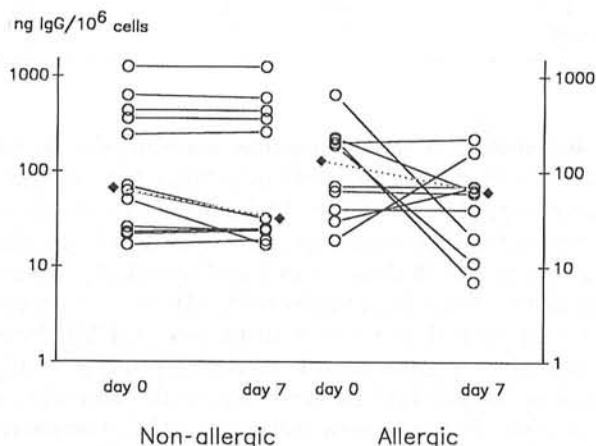
To investigate the spontaneous synthesis of IgE in mononuclear cells, LymphoPrep-isolated cells from allergics and non-allergics were cultured for 7 days. The IgE obtained by acid extraction of the cultures (i.e. total amount of IgE in cultures) was compared with control extractions made on day 0 (Fig. 3a). The level of IgE in the cultures decreased significantly (normal group: $p = 0.002$, $n = 12$; allergic group: $p = 0.01$, $n = 15$) during the culture period. The only donor showing a spontaneous synthesis (or increase of IgE during the culture period) was an allergic patient with the highest level of serum IgE (i.e. 13000 U/ml). Four donors in the early phase of immunotherapy presented no ongoing *in vivo* IgE production.

The IgE production was compared with that of IgA and IgG. The levels of cellular IgG and IgA were generally 100–1000 times above those of IgE, and levels remained constant or increased during the 7-day culture period. For IgG (Fig. 3b), there was no significant difference between days 0 and 7. Conversely, a significant increase was found in the IgA level ($p = 0.002$) (Fig. 3c), indicating spontaneous synthesis in the cultures. However, when the non-allergic and allergic groups were separated and evaluated statistically, cell cultures of the non-allergic group ($p = 0.006$), but not those of the allergic group ($p = 0.2$), demonstrated a significant increase in the IgA level. The marked difference in cellular IgE between the non-allergic and the allergic group was not evident when comparing day 0 IgG and IgA levels. A significant correlation ($r = 0.46$, $p < 0.001$) was found between the cellular IgG and IgA level on day 0.

Spontaneous IgE synthesis



Spontaneous IgG synthesis



Spontaneous IgA synthesis

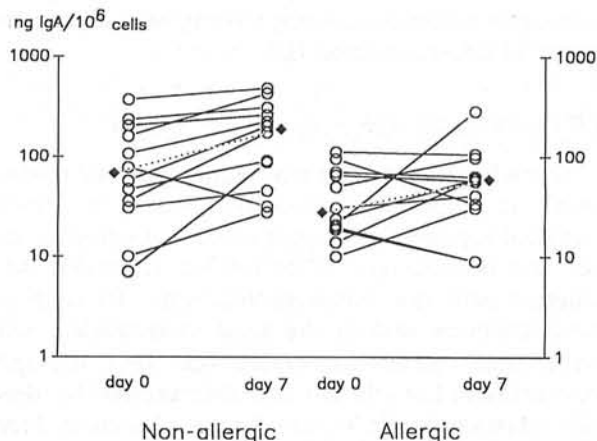


Fig. 3. Spontaneous synthesis of IgE (a), IgG (b) and IgA (c) in cells from 15 allergic and 12 non-allergic individuals. After 7 days of culture, cell suspensions were acid extracted. Suspensions of washed cells, extracted on day 0 and kept at -20°C , served as controls. Filled diamonds represent geometric means.

Stimulation with mitogen, allergen or anti-IgE

In an attempt to activate B cells to Ig production, cells from 4 non-allergic and 4 allergic individuals were stimulated with PWM (Table 3). Synthesis of IgA and IgG was strongly induced by PWM, indicating that the culture medium provided sufficient nutritional factors for immunoglobulin production. Only 1/8 persons showed a marginal net synthesis of IgE. Cells from 4 non-allergic and 4 house dust mite-allergic individuals were stimulated for 24 h with varying doses of allergen or anti-IgE antibody. The cells were then washed and cultured for 6 days, and subsequently the IgE was extracted and determined as described. For calculation of net IgE synthesis, IgE was extracted from parallel cultures (to which an equal amount of anti-IgE antibody or allergen had been added) after washing on day 1. No net IgE synthesis ($= \text{IgE}_{\text{day } 7} - \text{IgE}_{\text{day } 1}$) was observed in these cases.

Discussion

Several investigators have demonstrated the pitfalls of determining *in vitro* net IgE synthesis (9, 18, 25). First, the assay for IgE has to be validated carefully (6, 14). Second, the presence of preformed, cell-associated IgE in the cultures must be taken into account when estimating net IgE synthesis. Sampson & Buckley convincingly demonstrated that freezing/thawing of a cell suspension failed to release all IgE to the supernatant. Indeed they found significant release of IgE from frozen/thawed cells if kept at 37°C in culture medium (18). Turner et al. demonstrated that a brief acid extraction of cell suspensions significantly improved the IgE yields; this permitted a more reliable correction for preformed IgE in cell cultures (25). We have confirmed the higher

yields obtained by this method, as compared with ultrasonication or freezing/thawing.

The cellular sources of the cell-associated IgE may be several. The preliminary findings demonstrated that in 3 of 4 donors, more IgE was found in cell preparations with high monocyte content, and for all 4 donors the difference between IgE obtained by surface Ig stripping and by cellular extraction was found to increase with a higher number of monocytes. Differential counts of the cell suspensions showed <0.1% basophils. Assuming 10^5 occupied Fcε-receptor molecules on 0.1% basophilic cells, we can calculate that around 30 pg of IgE/ 10^6 cells would originate from basophils. Taken together, our data seem to indicate that the monocyte fraction is the major source of the cellular IgE: 1) the number of basophils cannot account for the level of cell-associated IgE (not even if we assume that all high-affinity receptors are occupied); 2) no spontaneous IgE synthesis takes place, which suggests the absence of IgE-containing plasma cells; and 3) the adherent cell fraction contains a high amount of IgE/cell. This parallels reports from others (7, 26), and suggests a physiological role of the low-affinity IgE receptor which is found on a large fraction of monocytes (4). As mentioned in Material and methods, the procedure for isolation of monocytes produces an average of 90% pure monocytes. Thus another cell type may still contribute to the amount of IgE found in adherent cell fractions.

Interestingly, the cellular level of IgE, i.e. the ratio of cell-associated IgE to serum immunoglobulin, is relatively higher than those of IgG and IgA. This indicates preferential concentration of certain isotypes in specific cell types. Remarkably, when the recovery of cells and immunoglobulins in adherent versus non-adherent cell fractions was calculated (Fig. 1), there was an overall cell recovery of $118\% \pm 43$ and an IgE recovery of $119\% \pm 40$, whereas only $45\% \pm 32$ of the IgG was recovered. There may be several reasons for the loss of IgG, one being that IgG is actively released from monocytes/macrophages during the adherence, and another being that IgG is only loosely cell-associated and therefore released during the experimental manipulations. The latter is less likely, however, as the cells are washed only once after separation, whereas during the preparation following the gradient centrifugation they are washed 3 times. The hypothesis has been advanced that intracellularly located IgE in monocytes/macrophages may contribute to the "arming" of mast cells in the tissues (7). So far our attempts to stimulate the monocytes into releasing IgE, using calcium ionophore, have not been successful (results not shown).

Long-lived and terminally differentiated IgE-producing B-cells have been demonstrated in

Table 3. Spontaneous and mitogen-stimulated immunoglobulin synthesis in cells from non-allergic or allergic subjects

Donor allergic status	Serum IgE (ng/ml)	Spontaneous net synthesis			PWM induced synthesis		
		IgE	IgA	IgG	IgE	IgA	IgG
-	36	neg	13	350	neg	38	6650
-	82	neg	135	530	neg	168	1310
-	122	neg	34	330	neg	233	16640
-	250	neg	105	50	neg	114	2160
+	290	neg	20	150	neg	59	2000
+	845	neg	172	900	neg	147	1890
+	1526	neg	8	990	neg	57	1050
+	31200	neg	76	120	105	110	870

Cells were isolated on LymphoPrep, cultured for 7 days and subjected to an acid extraction. Control cells (day₀) were extracted day 0. IgE (expressed as pg/ 10^6 cells) and IgA/IgG (ng/ 10^6 cells) were determined by immunoradiometric assays.

Net Ig synthesis calculated as day₇-day₀. Neg = net Ig synthesis <0.

rodents (8, 11). The existence of such a cell type in humans has not been demonstrated, but several authors have demonstrated spontaneous IgE synthesis in B-cell cultures from donors with high serum IgE levels (20, 21). In contrast, B cells from donors with low serum IgE levels do not produce IgE *in vitro* (9, 16). The results of our experiments are compatible with these findings, since only the donor with the highest level of serum IgE had a significant cellular IgE synthesis.

Earlier studies have demonstrated PWM-induced IgE production (10, 23) whereas other investigators have failed to show this effect (9, 17, 25). Part of this discrepancy could be explained by technical difficulties in determining net IgE synthesis. Indeed, in a multicentre study comparing different tests for IgE, 15 of 18 groups presented no spontaneous IgE synthesis when PWM-stimulated cultures were compared with control cultures (6). We were not able to demonstrate any significant PWM-induced IgE synthesis. This is not likely to be caused by poor culture media, since IgG and IgA responses developed readily. Recent investigations suggest that interleukin 4 is decisive in switching B cells into producing IgE (12). Animal (13) and human (27) experimental models indicate that a close interaction between B- and IL-4 producing T-helper lymphocytes is another prerequisite for the formation of IgE-producing B cells. The T/B interaction activates the T-helper cell into producing IL-4, whereas the B cell receives an activation signal necessary to become IgE producing. It thus seems likely that *in vitro* IgE synthesis can be induced in cellular systems devoid of monocytes. Recent experiments in our laboratory (to be published) have confirmed this. However, the monocyte-associated IgE may still possibly play a role in the regulation of the IgE synthesis. One interesting question in this respect is whether IgE possibly participates in antigen-presentation, perhaps inducing a distinct signal to T cells causing them to produce high levels of IL-4.

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