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In vitro production of IgE by human peripheral blood mononuclear cells. III. Demonstration of a circulating IgE-bearing cell involved in the spontaneous IgE biosynthesis

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SUMMARY

The presence of surface membrane IgE (SmIgE)-bearing cells in the peripheral blood (PB) of atopic patients was investigated by the use of isotype-specific rosettes of human red blood cells coupled to immunosorbent-purified rabbit or monoclonal mouse antibodies against human IgE (R or M anti-ε-HRBC). After dissociation of cell bound IgE by treatment with acid buffer, 2·1 ± 0·3% and 1·2 ± 0·3% circulating non-T, non-phagocytic, cells from atopic patients were still capable of forming rosettes with R or M anti-ε-HRBC, respectively. IgE molecules detectable on cells after dissociation of cytophilic IgE were quite resistant, like surface membrane IgM (SmIgM), to treatment with proteolytic enzymes, but they were removed under capping conditions by soluble anti-IgE antisera. All SmIgE-bearing (IgE^+) cells also bore DR determinants, but many of them lacked SmIgM. Depletion of IgE^+ cells strongly reduced the ability of PB lymphocyte suspensions from atopic patients to produce spontaneously IgE protein *in vitro*. Likewise, depletion of cells bearing DR determinants (DR^+ cells) resulted in a marked decrease of the spontaneous IgE biosynthesis, whereas depletion of SmIgM-bearing (IgM^+) cells had no effect. These data suggest that cells mainly implicated in the spontaneous IgE production *in vitro* seen in atopic patients are DR^+ IgE^+ IgM^- circulating lymphocytes.

INTRODUCTION

In the last few years some laboratories, including our own, have reported *in vitro* systems for the study of production of human IgE (Patterson *et al.*, 1975; 1976; Buckley & Becker, 1978; Fiser & Buckley, 1979; Saxon & Stevens, 1979; Tjio, Hull & Gleich, 1979; Saxon, Morrow & Stevens, 1980b; Saxon, Kaplan & Stevens, 1980a; Romagnani *et al.*, 1980a, 1980c; Ghory *et al.*, 1980). The data in these papers presented evidence for at least two functional populations of lymphocytes that may produce IgE *in vitro*. The first subset is represented by B cells bearing either surface IgM or surface IgE, which need the presence of T cells for being activated by pokeweed mitogen (PWM) to produce IgE (Saxon & Stevens, 1979; Saxon *et al.*, 1980a, 1980b). The second one comprises spontaneously IgE producing cells that we and other investigators have been able to detect mainly in atopic people (Fiser & Buckley, 1979; Tjio *et al.*, 1979; Saxon *et al.*, 1980b; Romagnani *et al.*, 1980a, 1980c; Ricci *et al.*, 1981). In previous papers we presented evidence that most cells involved in the spontaneous

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IgE production *in vitro* are mitomycin C-resistant mononuclear cells equipped, at least in part, with surface immunoglobulins, but lacking either surface membrane IgM (SmIgM) or complement receptors (CR) (Romagnani *et al.*, 1980a, 1980c; Ricci *et al.*, 1981). Thus, it was suggested that surface membrane IgE (SmIgE)-bearing B cells and/or surface immunoglobulin free plasma cell precursors are the cells mainly implicated in the spontaneous IgE production *in vitro* seen in atopic patients (Romagnani *et al.*, 1980a; Ricci *et al.*, 1981).

The present study was undertaken in order to demonstrate the presence of SmIgE-bearing (IgE^+) cells in the peripheral blood (PB) of atopic patients and to ascertain their role in the spontaneous production of IgE *in vitro*.

MATERIALS AND METHODS

Cell donors. Peripheral blood (PB) was obtained from atopic patients, non-atopic normal volunteers and patients with chronic lymphocytic leukaemia (CLL). Umbilical cord blood (UCB) was collected immediately after delivery of the baby but before the expulsion of the placenta.

Anti-immunoglobulin (Ig) rabbit antisera. The preparation and characterization of affinity purified rabbit antibodies directed against human μ chain and human δ chain have been described in detail elsewhere (Romagnani *et al.*, 1980b). A rabbit anti-IgE antiserum was prepared against an IgE myeloma protein, DZA (Sala *et al.*, 1981), which was purified from the patient serum by repeated gel filtration on Sephadex G-200 followed by elution through a protein A–Sepharose CL-4B column (Pharmacia, Uppsala, Sweden). Analysis by sodium dodecyl-sulphate polyacrylamide gel electrophoresis demonstrated a single protein with a molecular weight of 180,000–200,000 daltons. Analysis by radioimmunoassay showed no IgG, IgM or IgA present. Purified IgE was digested with papain according to the technique described by Ishizaka, Ishizaka & Lee (1970), and the Fc(ε) fragment was separated from undigested IgE by gel filtration on Sephadex G-200. Rabbits were immunized with 1 mg of the Fc(ε) fragment in Freund's complete adjuvant (FCA) and boostered several times at 15 day intervals with 1 mg of antigen in Freund's incomplete adjuvant (FIA). Anti-Fc(ε) antibodies were then purified from rabbit antisera by immunoabsorption on an IgE–Sepharose CL-4B column.

Monoclonal antibodies. A BALB/c mouse was immunized at 20 day intervals with three subcutaneous injections of pure IgE myeloma protein DZA (100 μg each) in FCA. Four days after the last injection, spleen cells (5×10^7) and P3X63Ag8 U1 myeloma cells (a generous gift of Dr M.D. Scharff) ($0{\cdot}5 \times 10^7$) were fused with polyethylene glycol 1000 as described by Gefter, Margulies & Scharff (1977). After fusion the cells were resuspended in selective growth medium (HAT) in DME medium supplemented with 20% fetal calf serum (FCS), seeded in 48 wells of Costar plates (Cat. No. 3524) in 0·5 ml aliquots and cultured at 37°C in a humidified 5% CO_2 atmosphere in air. Fourteen days after fusion vigorously growing cell colonies were seen in all wells. Supernatants of the cultures were tested for anti-IgE activity by a solid phase binding assay (Damiani *et al.*, 1980) on IgE protein DZA. Eleven wells were found to be positive for anti-IgE activity. Cells from the wells showing anti-IgE antibody activity were repeatedly cloned in soft agar as described by Coffino *et al.* (1972) and subclones were used to produce ascitic fluid. Monoclonal proteins purified from ascites were tested with purified human IgG, IgA, IgM, IgD myeloma proteins either κ or λ in solid phase binding assay to check specificity. Four monoclonal antibodies were found to be specific for human ε chain.

Anti-μ monoclonal antibody was obtained similarly utilizing IgM protein purified from Waldenström's macroglobulinaemia for immunizations and a solid phase binding assay on IgM and the other human Ig classes to check specificity for the μ chain.

The monoclonal protein PTF 29.12, obtained and characterized as will be described elsewhere, recognizes a determinant common to all known DR specificities (Corte *et al.*, 1981). The monoclonal protein was purified from ascites and conjugated with fluoresein isothiocyanate (FITC) for direct immunofluorescence as reported by Ferrarini *et al.* (1975).

Coating of red blood cells (RBC) with rabbit (R) or monoclonal (M) antibody. Immunosorbent

purified R or M antibodies were coupled to human (H) or pigeon (P) RBC by chromium chloride, as detailed elsewhere (Romagnani *et al.*, 1980b).

Preparation of cell suspensions and fractionation procedures. Mononuclear cells (MNC) were obtained from PB or UCB by the Ficoll-Hypaque gradient separation procedure. MNC were then rotated in plastic tubes with a suspension of carbonyl iron (20 mg/10^7 cells) in TC 199 medium–10% FCS (GIBCO, New York, USA) for 1 hr at 37°C. Phagocytic cells were removed by magnetism. Suspensions virtually free from T cells were obtained by a double E-rosetting procedure with neuraminidase-treated sheep red blood cells (SRBC), as detailed elsewhere (Romagnani *et al.*, 1980b). These non-T, non-phagocytic, cell fractions primarily contained SmIg-bearing cells and will be referred as B cell rich fractions.

Rosette assay with anti-Ig RBC and Ficoll-Hypaque separation of rosetted lymphocytes. One volume of the lymphocyte suspension (2×10^6/ml) was mixed with 1 vol of 1% antibody coated RBC and centrifuged at 200 ***g*** for 5 min at 4°C. The pellet was resuspended by pipetting, stained with toluidine blue, and the percentage of rosettes was determined by microscopic examination of 1,000 cells. In order to separate non-rosetted from rosetted lymphocytes, 1 vol of the rosetted mixture was layered on 2 vol of Ficoll-Hypaque and centrifuged at 400 ***g*** for 30 min at 4°C. After centrifugation, non-rosetted cells recovered at the interface of the gradient were collected.

Rosette assay on fluorescent cells. For simultaneous detection of DR antigens and SmIgE, B cell rich fractions (2×10^6/ml) were incubated for 30 min at 4°C in the presence of 20 mM sodium azide with FITC-conjugated anti-DR monoclonal antibodies, washed twice at 4°C and mixed with 1% HRBC coupled to anti-ε monoclonal antibodies. Stained cells capable of forming rosettes were then scored on a fluorescent Leitz microscope.

Mixed rosettes. Mixed rosetting techniques were employed for simultaneous detection of SmIgE and SmIgM on the same cells. In such mixed rosette assay, two types of indicator cells were used that could be distinguished microscopically. Fifty microlitres of a lymphoid cell suspension (2×10^6/ml) in TC 199 medium with 20% FCS were incubated with equal volumes of 1% anti-ε-HRBC and 1% anti-μ-PRBC, and rosetting cells were examined in the same manner as described above. PRBC were easily recognized for their oval form and the presence of nucleus. A mixed rosette was scored when the lymphocyte was surrounded by at least two indicator cell types.

Treatment of cells with acid pH. To obtain dissociation of cell bound IgE, cells were treated for 4 min at 0°C with glycine-HCl buffer, pH 3·0 as reported by Kulczycki & Metzger (1974). After this treatment, viability of cells, as detected by the trypan blue staining, was consistently greater than 90%.

Enzymatic treatment of the cells. Cells were resuspended at a concentration of 10×10^6/ml in TC 199 medium containing different amounts of pronase (Serva, Heidelberg, West Germany) or trypsin (Sigma, St Louis, Missouri, USA), incubated at 37°C for 30 min, and washed three times with TC 199 medium supplemented with 20% FCS.

Staining for basophil granulocytes. Basophilic granulocytes were stained by toluidine blue solution prepared according to the method of Kimura, Moritani & Tanizaki (1973).

Cell cultures. The technique of cell culture has been detailed elsewhere (Romagnani *et al.*, 1980a, 1980c). Briefly, cells were cultured in 1 ml at a concentration of 1×10^6/ml at 37°C in a humidified atmosphere of 5% CO_2 in air. The tissue culture medium consisted of RPMI 1640 (GIBCO), 10% FCS, 100 units/ml penicillin G, 100 μg/ml streptomycin and 2·0 mM L-glutamine. Parallel 7 day cultures were carried out in the presence of cycloheximide (Sigma Chemical Co., 100 μg/ml) to ascertain that the IgE measured at the end of the 7 day culture period represented *de novo* synthesized IgE. After 7 days, the supernatants were centrifuged, lyophilized and reconstituted in 1/10 volume of distilled water.

Measurement of IgE protein. IgE protein was detected in the reconstituted supernatants by a sensitive solid phase sandwich test, specific for IgE protein and capable of measuring as little as 50 pg/ml of IgE, previously described in detail (Romagnani *et al.*, 1980a, 1980c). Briefly, solid phase anti-IgE was prepared by reaction of CNBr-activated Sepharose CL-4B (Pharmacia, Uppsala) with an anti-human Fc(ε) rabbit antiserum. The solid phase was incubated with the IgE under test, washed and then incubated again with a ^{125}I-labelled anti-IgE antibody. A calibration curve was always prepared with the help of appropriate dilutions of the purified IgE myeloma protein mentioned above.

RESULTS

Demonstration and characterization of PB cells bearing SmIgE (IgE⁺ cells)

When B cell rich fractions of PB from either atopic or non-atopic subjects were tested for ability to form rosettes with HRBC coupled to immunosorbent purified rabbit or monoclonal mouse antibodies against human IgE (R or M anti-ε-HRBC) a small, but significant, number of cells was found capable of binding both reagents. The number of rosettes was usually greater with R than with M anti-ε-HRBC (Table 1). The majority of IgE⁺ cells still maintained the ability to form rosettes with anti-ε-HRBC even after dissociation of cell-bound IgE by incubation at 37°C in IgE free medium or treatment with acid buffer (Table 1). Virtually no cells forming rosettes with anti-ε-HRBC (<0·1%) were found in B cell rich fractions from UCB or in PB from patients with CLL.

The treatment of B cell rich fractions with trypsin or pronase, which abolished their capacity to form rosettes with anti-δ-HRBC, did not induce any decrease of the number of rosettes formed by cells with anti-μ-HRBC and usually resulted in a slight increase in the number of IgE⁺ cells (Table 2). After incubation in capping conditions (30 min at 37°C) with soluble antibodies, cells lost the ability to react with either anti-ε- or anti-μ-HRBC. However, no re-synthesis of either SmIgM or SmIgE was observed after a 12–48 hr incubation at 37°C.

To better characterize IgE⁺ cells, the presence on their surface of DR antigens and immunoglobulins of the IgM class was investigated. The results of these experiments are summarized in Table 3. All IgE⁺ cells had on their surface DR antigens but only a minor proportion of them showed SmIgM. In addition, the removal from B cell rich fractions of SmIgM-bearing (IgM⁺) cells by rosetting with HRBC coated with anti-μ antibodies, led to increased percentage of rosettes formed by cells with anti-ε-HRBC (Fig. 1).

IgE⁺ cells are implicated in the spontaneous production of IgE in vitro

The role of circulating IgE⁺ cells in the spontaneous production of IgE *in vitro* was then investigated. For this purpose, IgE⁺ cells were removed from B cell rich fractions by rosetting with HRBC coupled to either R or M anti-ε-HRBC. The amount of IgE protein found in the 7 day supernatants of IgE⁺ cell-depleted cultures was compared with that made by cell suspensions of the same donors obtained at the interface of density gradient after rosetting with HRBC coated with normal rabbit or mouse IgG. The amount of IgE protein found in parallel cultures performed in the

Table 1. Detection of cells reacting with monoclonal (M) or rabbit (R) anti-ε-antibodies in non-T, non-phagocytic, cell fractions of PB from non-atopic or atopic individuals.

Cell fractions	No. of cases	% of rosettes with HRBC coated with M anti-ε antibody	% of rosettes with HRBC coated with R anti-ε antibody
Non-atopic	10	1·13±0·2	n.d.*
Atopic	22	1·57±0·2	2·44±0·2
Untreated†	8	1·78±0·3	2·47±0·2
Acid-treated†		1·28±0·3	2·10±0·3

* n.d. = not determined.

† Non-T, non-phagocytic cell fractions from eight atopic patients were tested for ability to form rosettes with either M or R anti-ε-HRBC prior to and after treatment for 4 min at 0°C with glycine-HCl buffer, pH 3·0.

Table 2. Effect of treatment with trypsin or pronase on the ability of non-T, non-phagocytic, cells from PB of atopic patients to form rosettes with anti-ε HRBC.*

		% of rosettes with HRBC coated with		
Enzyme	μg/ml	anti-ε	anti-μ	anti-δ
Trypsin	0	1·3	29	16
	250	2·8	31	7
	2,500	1·9	30	< 1
Pronase	0	2·3	41	27
	250	4·0	41	1
	2,500	2·7	38	< 1

* Cells (10×10^6/ml) were incubated for 30 min at 37°C with different concentrations of trypsin or pronase, washed and then tested for ability to form rosettes with HRBC coated with anti-ε, anti-μ or anti-δ chain immunosorbent purified rabbit antibodies. Each value represents the mean of three separate experiments.

Table 3. Detection of DR antigens and SmIgM on IgE^+ cells from PB of atopic patients

	% of IgE^+ cells having	
Exp. No.	DR antigens*	SmIgM†
1	100	20
2	100	40
3	100	10

* Simultaneous detection of DR antigens and SmIgE was performed by incubation of B cell rich fractions with fluorescein-conjugated monoclonal anti-DR antibodies, washing and further incubation with HRBC coupled to anti-ε antibodies. Stained cells capable of forming rosettes were then scored on a fluorescent Leitz microscope.

† Cells bearing both SmIgE and SmIgM were detected by a mixed rosette technique with HRBC coated with anti-ε antibodies and PRBC coated with anti-μ antibodies.

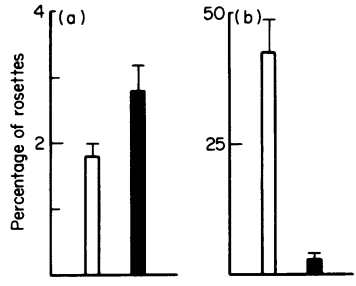

Fig. 1. Enrichment for IgE$^+$ cells by rosetting B cell rich fractions from PB of atopic patients with HRBC coupled to anti-μ chain antibodies followed by centrifugation on density gradient. The percentage of cells able to form rosettes with anti-μ-HRBC (b) and anti-ε-HRBC (a) was evaluated in B cell rich fraction prior (□) and after (■) depletion of IgM$^+$ cells. Results represent the mean value ($\pm$s.e.) of 12 separate experiments.

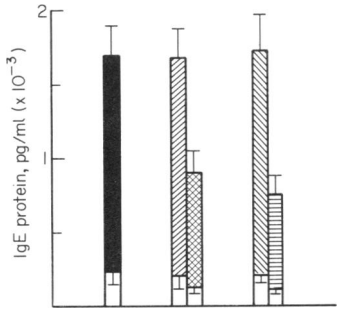

Fig. 2. Effect of depletion of IgE$^+$ cells on the spontaneous production *in vitro* by PB lymphocytes from atopic patients. Depletion was performed by rosetting PB lymphocytes with HRBC coupled to immunosorbent purified rabbit (■) or monoclonal mouse (▩) anti-IgE antibodies, followed by centrifugation on density gradient. The cells collected at the interface of the gradient were then cultured for 7 days and the amount of IgE protein released in the supernatants was measured by a radioimmunoassay. The amount of IgE protein present in parallel cultures containing cycloheximide (100 μg/ml) (□) was also evaluated. The amount of IgE found in the supernatants of cultures from cell suspensions depleted of IgE$^+$ cells was compared with that released from undepleted suspensions (■) or suspensions collected at the interface of the gradient after rosetting with HRBC coupled to normal rabbit (▨) or normal mouse (■) gammaglobulins. The mean value ($\pm$s.e.) of four separate experiments is reported.

presence of cycloheximide was also evaluated. As shown in Fig. 2, depletion of IgE$^+$ cells with either R or M anti-ε-HRBC led to a strong decrease of the amount of IgE found in 7 day culture supernatants in which the inhibitor of protein synthesis was not added.

In another series of experiments the effect of depletion of IgE$^+$ cells on the spontaneous IgE production *in vitro* was compared with that obtained by depletion of cells bearing DR antigens (DR$^+$ cells) and IgM$^+$ cells. Depletion of IgM$^+$ cells had no effect compared to negative control depletion with normal mouse Ig rosettes. By contrast, both IgE$^+$ and DR$^+$ cell depletions led to a marked reduction of IgE biosynthesis (Table 4).

DISCUSSION

In the present study IgE$^+$ cells were detected in the PB of atopic patients by the use of isotype-specific rosettes of HRBC coupled to either immunosorbent-purified rabbit or monoclonal anti-IgE antibody. A small proportion of non-T, non-phagocytic, cells formed rosettes with R

Table 4. Effect of depletion of DR^+, IgM^+ or IgE^+ cells on the spontaneous production of IgE protein *in vitro* by PB lymphocytes from atopic patients*

	IgE protein detected in 7 day supernatants (pg/ml)			
Cell suspensions	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Undepleted	2,120	14,000	3,200	8,300
Depleted of				
DR^+ cells	640	5,700	n.d.	3,700
IgM^+ cells	2,100	12,000	3,550	12,500
IgE^+ cells	510	1,650	1,200	2,200

* Ficoll-Hypaque purified mononuclear cell suspensions from PB of atopic patients were depleted of phagocytic cells by treatment with carbonyl iron and then of DR^+, IgM^+ or IgE^+ cells by rosetting with HRBC coated with monoclonal antibodies against DR antigens, μ chain and ε chain, respectively, followed by centrifugation on density gradient. The cells collected at the interface of the gradient were then cultured for 7 days and culture supernatants tested for the presence of IgE protein by radioimmunoassay. For control, cells obtained at the interface of density gradient after rosetting with HRBC coated with normal mouse IgG were used (undepleted).

anti-ε-HRBC. A slightly lower number of IgE rosettes was usually found with M anti-ε-HRBC. A first possibility is that these IgE^+ cells represent B lymphocytes or contaminating monocytes having cytophilic IgE absorbed on their surface via Fc (ε) receptors (Gonzalez-Molina & Spiegelberg, 1977; Spiegelberg & Melewicz, 1980). This possibility, however, can be excluded since the affinity of monomeric IgE to the Fc(ε) receptors of these cells is relatively low and the IgE is easily removed by washings (Spiegelberg & Melewicz, 1980). Another possibility is that cells forming rosettes with anti-ε-HRBC are basophilic granulocytes. In fact, our T cell depleted fractions contained from 1 to 5% basophils, as determined by toluidine blue staining. Data presented herein, however, argue against this possibility. In fact, the great majority of IgE^+ cells still maintained the ability to react with anti-ε-HRBC even after treatment for 4 min with glycine-HCl buffer, pH 3, which had been shown to induce dissociation of 90% cell-bound IgE from basophil granulocytes without significant loss of cell viability (Kulczycki & Metzger, 1974).

To provide direct evidence that IgE molecules, still detectable on PB cells after acid treatment, represented autochthonous SmIg, we tried to demonstrate their re-synthesis after stripping with pronase or incubation of cells in capping conditions with soluble anti-IgE antisera. However, either IgE molecules or SmIgM detected by rosetting technique were not removed by enzyme concentrations as high as those which could be used without significant loss of cell viability. Quite the contrary, the number of IgE^+ cells was increased by enzyme concentrations as low as those sufficient to remove SmIgD, thus suggesting enzyme-induced exposure of normally cryptic or buried Fc(ε) determinants. On the other hand, after incubation under capping conditions with soluble anti-IgE antibodies, all IgE^+ cells lost the ability to form rosettes with anti-ε-HRBC. Unfortunately, no re-synthesis of either SmIgE or SmIgM was observed at least for a 48 hr incubation period after treatment of cells with anti-IgE or anti-IgM antisera, respectively. This is in agreement with previously reported data showing inability of human PB lymphocytes to re-synthesize SmIg after treatment with anti-Ig antisera under capping conditions (Ault & Unanue,

1977). A better characterization of IgE^+ cells was then attempted by techniques employing simultaneous use of immunofluorescence and rosetting or mixed rosette techniques. Thus, we could demonstrate that virtually all IgE^+ cells also bear DR determinants. This data does not provide, however, direct evidence of the B cell nature of IgE^+ cells, since it is well known that DR antigens are also expressed on monocytes or activated T cells (Winchester & Kunkel, 1980). To our knowledge, until now no data showing the presence of DR antigens on basophilic granulocytes has been reported.

More clear evidence of the B cell nature of the DR^+ IgE^+ IgM^- circulating cell was provided by the study of spontaneous IgE production *in vitro* after selective depletion of DR^+, IgE^+ or IgM^+ cells, respectively. In agreement with results previously reported (Romagnani *et al.*, 1980a), depletion of IgM^+ cells had no effect on the *in vitro* spontaneous IgE production. In contrast, IgE biosynthesis was markedly reduced by depletion of either DR^+ or IgE^+ cells. These data indicate that spontaneous IgE production *in vitro* is mainly due to circulating lymphocytes bearing DR antigens and SmIgE, but lacking SmIgM. These cells seem to be distinct from those responsible for PWM-driven IgE production *in vitro*, which have been demonstrated to possess both SmIgM and SmIgE (Saxon *et al.*, 1980a).

The functional role of the circulating IgE^+ IgM^- cell responsible for the spontaneous IgE production *in vitro* is still unclear. Recently, it was found that persistent antibody formation in high responder mice is mediated by long-lived, radio-resistant, antibody forming cells which produce IgE antibody requiring neither antigen nor T cells (Okudaira & Ishizaka, 1981). These cells are quite different from conventional memory B cells, which are radio-sensitive and need the presence of residual antigen and T lymphocytes (Tew *et al.*, 1973; Peeters & Carter, 1978).

In previous papers it was shown that the majority of spontaneously IgE producing cells *in vitro* are mitomycin C- and radio-resistant, even though the participation to *in vitro* IgE biosynthesis of mitomycin C- and radio-sensitive cells cannot be excluded (Romagnani *et al.*, 1980c; Okudaira *et al.*, 1981). Thus, it is reasonable to suggest that the DR^+ IgE^+ IgM^- cells mainly responsible for the spontaneous IgE production *in vitro* described herein are a heterogeneous population containing either conventional memory B lymphocytes or cells possibly similar to the long-lived IgE antibody forming cells. Further experiments are required to better characterize these cell populations and to elucidate the mechanisms for their inactivation.

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REFERENCES

AULT, K.A. & UNANUE, E.R. (1977) Failure of B lymphocytes in human blood to regenerate surface immunoglobulin after its removal by antibody. *J. Immunol.* **119,** 327.

BUCKLEY, R.H. & BECKER, W.G. (1978) Abnormalities in the regulation of human IgE synthesis. *Immunol. Rev.* **41,** 288.

COFFINO, P., BAUMAL, R., LASKOV, R. & SCHARFF, M.D. (1972) Cloning of mouse myeloma cells and detection of rare variants. *J. Cell. Physiol.* **79,** 429.

CORTE, G., DAMIANI, G., CALABI, F., FABBI, M. & BARGELLESI, A. (1981) Analysis of HLA-DR polymorphism by two dimensional peptide mapping. *Proc. Natl. Acad. Sci.* **78,** 534.

DAMIANI, G., FRASCIO, M., BENATTI, U., MORELLI, A., ZOCCHI, E., FABBI, M., BARGELLESI, A., PONTREMOLI, S. & DE FLORA, A. (1980) Monoclonal antibodies to human erythrocyte glucose 6-phosphate dehydrogenase. *FEBS Lett.* **119,** 169.

FERRARINI, M., BARGELLESI, A., CORTE, G., VIALE, G. & PERNIS, B. (1975) Comparative study of membrane and intracytoplasmic immunoglobulin classes in human lymphoid cells. *Ann. N.Y. Acad. Sci.* **254,** 243.

FISER, P.M. & BUCKLEY, R.H. (1979) Human IgE biosynthesis *in vitro*: studies with atopic and normal blood mononuclear cells and subpopulations. *J. Immunol.* **123,** 1788.

GEFTER, M.L., MARGULIES, D.H. & SCHARFF, M.D. (1977) A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. *Somat. Cell Genet.* **2,** 231.

GHORY, A.C., PATTERSON, R., ROBERTS, M. & SUSZKO, I. (1980) *In vitro* IgE formation by peripheral blood lymphocytes from normal individuals and patients with allergic bronchopulmonary aspergillosis. *Clin. exp. Immunol.* **40,** 581.

GONZALEZ-MOLINA, A. & SPIEGELBERG, H.L. (1977)

A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. clin. Invest.* **59,** 616.

ISHIZAKA, T. & ISHIZAKA, K. (1973) Biology of immunoglobulin E: molecular basis of reaginic hypersensitivity. *Progr. Allergy* **19,** 60.

ISHIZAKA, K., ISHIZAKA, T. & LEE, E.H. (1970) Biological function of the Fc fragments of E myeloma protein. *Immunochemistry*, **7,** 687.

KIMURA, I., MORITANI, Y. & TANIZAKI, Y. (1973) Basophils in bronchial asthma with reference to reaginic-type allergy. *Clin. Allerg.* **3,** 195.

KULCZYCKI, A., JR. & METZGER, H. (1974) The interaction of IgE with rat basophilic leukemia cells. II. Quantitative aspects of the binding reaction. *J. exp. Med.* **140,** 1676.

OKUDAIRA, H. & ISHIZAKA, K. (1981) Reaginic antibody formation in the mouse. *Cell. Immunol.* **58,** 188.

OKUDAIRA, H., OHTA, K., OGITA, T. & MIYAMOTO, T. (1981) Human IgE antibody-forming cells. *Int. Archs. Allerg. appl. Immunol.* **65,** 162.

PATTERSON, R., SUSZKO, I.M., HSU, C.C.S., ROBERTS, M. & OH, S.H. (1975) *In vitro* production of IgE by lymphocytes from a patient with hyperimmunoglobulinemia E, eosinophilia and increased lymphocytes carrying surface IgE. *Clin. exp. Immunol.* **20,** 265.

PATTERSON, R., SUSZKO, I.M., METZGER, W.J. & ROBERTS, M. (1976) *In vitro* production of IgE by human peripheral blood lymphocytes: effect of cholera toxin and β adrenergic stimulation. *J. Immunol.* **117,** 97.

PEETERS, S.H. & CARTER, B.G. (1978) Regulation of the IgE antibody response in mice. I. Long-term production of IgE anti-ovalbumin antibody in irradiated recipients. *J. Immunol.* **121,** 1596.

RICCI, M., ROMAGNANI, S., MAGGI, E., DEL PRETE, G.F., TRONCONE, R., GIUDIZI, G.M. & ALMERIGOGNA, F. (1981) *In vitro* spontaneous IgE production by human lymphocytes from atopic patients. In *Induction, function and suppression of IgE antibodies* (ed. by A. H. Sehon), p. 61. Excerpta Medica, Amsterdam.

ROMAGNANI, S., DEL PRETE, G.F., MAGGI, E., TRONCONE, R., GIUDIZI, G.M., ALMERIGOGNA, F. & RICCI, M. (1980a) *In vitro* production of IgE by human peripheral blood mononuclear cells. II. Cells involved in the spontaneous IgE production in atopic patients. *Clin. exp. Immunol.* **42,** 579.

ROMAGNANI, S., GIUDIZI, M.G., ALMERIGOGNA, F. & RICCI, M. (1980b) Interaction of staphylococcal protein A with membrane components of IgM- and/or IgD-bearing lymphocytes from human tonsil. *J. Immunol.* **124,** 1620.

ROMAGNANI, S., MAGGI, E., DEL PRETE, G.F., TRONCONE, R. & RICCI, M. (1980c) *In vitro* production of IgE by human peripheral blood mononuclear cells. I. Rate of IgE biosynthesis. *Clin. exp. Immunol.* **42,** 167.

SALA, P.G., TONUTTI, E., RUSCIO, M., COLLE, R., ANTONUTTO, G. & FALCONIERI, G. (1981) IgE myeloma. Report of a new case and review of the literature. *Haematologica*, **66,** 787.

SAXON, A. & STEVENS, R.H. (1979) Stimulation and regulation of human IgE production *in vitro* using peripheral blood lymphocytes. *Clin. Immunol. Immunopathol.* **14,** 474.

SAXON, A., KAPLAN, M. & STEVENS, R.H. (1980a) Isotype specific human B lymphocytes that produce IgE *in vitro* when stimulated by pokeweed mitogen. *J. Aller. clin. Immunol.* **66,** 233.

SAXON, A., MORROW, C. & STEVENS, R.H. (1980b) Subpopulations of circulating B cells and regulatory T cells involved in *in vitro* immunoglobulin E production in atopic patients with elevated serum immunoglobulin E. *J. clin. Invest.* **65,** 1457.

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

TEW, J.G., SELF, C.H., HOROLD, W.W. & STAVITSKY, A.B. (1973) The spontaneous induction of anamnestic antibody synthesis in lymph node cell cultures many months after primary immunization. *J. Immunol.* **110,** 416.

TJIO, A.H., HULL, W.M. & GLEICH, G.J. (1979) Production of human immunoglobulin E antibody *in vitro*. *J. Immunol.* **122,** 2131.

WINCHESTER, R.J. & KUNKEL, H.G. (1980) The human Ia system. *Adv. Immunol.* **28,** 221.