Clin. exp. Immunol. (1980) **42.** 579–588.

In vitro production of IgE by human peripheral blood mononuclear cells

II. CELLS INVOLVED IN THE SPONTANEOUS IgE PRODUCTION IN ATOPIC PATIENTS

S. ROMAGNANI, G. F. DEL PRETE, E. MAGGI, R. TRONCONE,* GRAZIA M. GIUDIZI, F. ALMERIGOGNA & M. RICCI *Department of Clinical Immunology, University of Florence, Italy*

(Accepted for publication 4 July 1980)

SUMMARY

Spontaneous IgE production *in vitro* was investigated in 7-day cultures of unfractionated mononuclear cells (MNC) and MNC subpopulations from atopic patients. Depletion of either phagocytic or adherent cells decreased the amount of IgE detectable in 7-day culture supernatants, but this decrease was due, at least in part, to a loss of cytophilic IgE. Depletion of immunoglobulin-bearing cells (SIg^+) reduced significantly but did not abolish the spontaneous IgE production *in vitro*. On the other hand, depletion of IgM-bearing lymphocytes ($SIgM^+$), which virtually abolished the production of immunoglobulins of the IgM class, did not change significantly the spontaneous production of IgE. Similarly, no change in the spontaneous production of IgE was found when lymphocyte suspensions were depleted of complement receptor-bearing cells (CR^+). In contrast, spontaneous IgE production was significantly increased by depletion of T lymphocytes and this increase did not simply reflect the enrichment for IgE-producing cells caused by the fractionation procedure. No significant change in the spontaneous IgE production was found when small numbers of autologous T lymphocytes were added to B cell fractions, whereas the addition of higher concentrations of autologous T cells induced a marked inhibition of the spontaneous IgE production. On the other hand, the addition in culture of pokeweed mitogen (PWM) resulted in a marked reduction of the spontaneous IgE production by B cells, also in the presence of small concentrations of autologous T lymphocytes. Normal T cells were consistently effective in inducing a partial inhibition of the spontaneous IgE production by B cells from atopic patients, whereas T cells from a noticeable proportion of atopic patients were not. These data suggest that MNC responsible for the spontaneous IgE production in atopic subjects are SIgM- and CR-deficient well-differentiated lymphocytes which probably represent the result of an activation which has occurred *in vivo*. However, this spontaneous IgE production can still be influenced by *in vitro* manipulation, such as variations in T–B cell ratios or addition of PWM. The results here reported also indicate that normal T cells are generally more effective than T cells from atopic patients in regulating the activity of spontaneous IgE-producing cells present in the blood of atopic subjects.

INTRODUCTION

Some laboratories, including our own, have reported *in vitro* systems for the study of human IgE

* Visiting fellow from the Department of Pediatrics, 2nd School of Medicine, University of Naples, Italy.
Correspondence: Professor Mario Ricci, Cattedra di Immunologia Clinica, Istituto Clinica Medica 3, Policlinico Careggi, 50134 Firenze, Italy.

0099-9104/80/1200-0579$02.00

production. However, until now conflicting results have been reported with regard to the type of lymphocyte donors, the time-curve of IgE detection and the role of T lymphocytes and pokeweed mitogen (PWM) in stimulating and regulating IgE production (Patterson *et al.*, 1975; Fiser & Buckley, 1979; Tjio, Hull & Gleich, 1979; Saxon & Stevens, 1979; Romagnani *et al.*, 1980b).

Our laboratory has recently shown that mononuclear cells (MNC) from several grass-sensitive patients, studied during or immediately after the pollen season, are capable of spontaneously producing significant amounts of IgE protein and grass-specific IgE in 7-day cultures. The addition to these cultures of PWM did not increase or even decrease the amount of IgE recovered in the supernatants. Time-sequence studies showed that the IgE concentrations measured in the 7-day supernatants were due to a continuous release from the cells of IgE quantities progressively decreasing up to day 7. The maximum amout of IgE was released into the supernatant from days 2 to 4 of culture. The release into the supernatant of most IgE protein was dependent on protein synthesis and, therefore, clearly represented newly synthesized IgE (Romagnani *et al.*, 1980b).

This study was undertaken in order to better characterize the MNC responsible for the spontaneous IgE synthesis seen in atopic patients and to establish whether or not IgE antibody-producing cells can be influenced by *in vitro* manipulation.

MATERIALS AND METHODS

Cell donors of mononuclear cells (MNC). Blood was obtained from 21 atopic individuals, five of whom were grass pollen-sensitive and 16 of whom were sensitive to either grass pollen or other common allergens, such as mites and danders. All patients had rhinitis and/or bronchial asthma. Five out of 21 patients had atopic dermatitis. None of the patients had received immunotherapy. Twelve non-atopic subjects were used as donors of normal T lymphocytes. MNC were obtained from peripheral blood of atopic and normal individuals by the Ficoll–Hypaque gradient procedure.

Depletion of monocytic cells. To remove phagocytic cells, MNC were rotated in plastic tubes with a suspension of carbonyl iron (20 mg/10^7 cells) in RPMI 1640–10% foetal calf serum (FCS; GIBCO) for 30 min at 37°C. Cells which had ingested the metal were immobilized by a magnet and the remaining cell suspension removed by careful pipetting. To remove adherent cells, MNC were incubated for 45 min at 37°C in a 75-cm^2 plastic flask (Falcon, Oxnard). Non-adherent cells were then collected by careful pipetting and incubated again for 45 min at 37°C in another plastic flask to remove residual adherent cells. Contaminating monocytes were identified by non-specific esterase staining (Koski, Poplak & Blaese, 1976). The number of esterase-staining cells found in MNC suspensions ranged between 10 and 30%. The number of esterase-staining cells found in suspensions depleted of phagocytic cells with carbonyl iron was consistently lower than 5% and in those depleted of adherent cells was consistently lower than 2%.

Depletion of surface immunoglobulin-bearing (SIg^+) and surface IgM-bearing ($SIgM^+$) lymphocytes. To remove SIg^+ or $SIgM^+$ lymphocytes, MNC suspensions, depleted of phagocytic cells by treatment with carbonyl iron, were rosetted with human red blood cells (hRBC) coated with immunosorbent-purified anti-human $F(ab')_2$ or anti-human μ chain rabbit antibodies respectively. Non-rosetted were then separated from rosetted lymphocytes by centrifugation on a density gradient. The methods for the preparation of immunosorbent-purified antibodies and conjugation to hRBC have been published elsewhere (Romagnani *et al.*, 1980a). Briefly, an antiserum specific for human $F(ab')_2$ was obtained by injecting rabbits with human $F(ab')_2$ prepared by pepsin digestion from normal human IgG, and then purified by Sephadex G-150 gel filtration and affinity chromatography with SpA-Sepharose CL-4B (Pharmacia, Uppsala) to remove undigested IgG. Purified anti-$F(ab')_2$ antibodies were prepared by absorption of the anti-human $F(ab')_2$ serum on an $F(ab')_2$ immunosorbent column. Bound molecules were then recovered by elution with glycine–HCl buffer, pH 2·5. An antiserum specific for human IgM was prepared by injecting rabbits with IgM obtained from pooled sera of macroglobulinaemia patients by euglobulin precipitation followed by Sephadex G-200 gel filtration. For the purification of anti-IgM (anti-μ) antibody, the anti-IgM serum was passed through umbilical cord serum, IgG, IgA, and IgD and $F(ab')_2$ immunosorbent columns to remove any contaminating antibody. The effluent was then passed over an IgM

immunosorbent column and bound molecules eluted by acidic elution, as described above. The specificity of the anti-μ serum was checked by double-diffusion in agarose, immunofluorescence on bone marrow smears and a paper-disc radioimmunoassay.

Immunosorbent-purified anti-F(ab')$_2$ or anti-μ antibodies were coupled to hRBC by chromic chloride according to the technique of Gold & Fudenberg (1967). For this purpose, one volume of washed and packed hRBC and one volume of immunosorbent-purified antibody (1 mg/ml) in 0·15 M NaCl were mixed. Then, one volume of $CrCl_3 \cdot 6H_2O$ (1 mg/ml) in 0·15 M NaCl was added. The three components were mixed and allowed to react at room temperature for 5 min. The coated hRBC were washed three times with 0·15 M NaCl and resuspended to 1% in 0·15 M NaCl. One volume of 1% hRBC coated with affinity chromatography-purified antibodies was mixed with one volume of the MNC suspension (2×10^6/ml) 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 at least 300 cells. In order to separate non-rosetted from rosetted lymphocytes, one volume of the rosetted mixture was layered on two volumes of Ficoll–Hypaque and centrifuged at 400 ***g*** for 30 min at 4°C. After centrifugation, non-rosetting cells recovered at the interface were collected. hRBC coated by chromic chloride with human serum albumin (HSA) were used as control.

Depletion of complement receptor-bearing (CR^+) cells. To remove CR^+ lymphocytes, phagocyte-depleted MNC suspensions were rosetted with 1% ox erythrocytes coated with purified anti-ox erythrocyte rabbit IgM antibodies and human serum as a complement source (EAC). Non-rosetted were then separated from rosetted cells by centrifugation on a density gradient.

Depletion of E rosette-forming cells (E-RFC). Suspensions virtually free from T lymphocytes were obtained by a double E rosetting procedure with neuraminidase-treated sheep red blood cells (SRBC), previously reported in detail (Romagnani *et al.*, 1980a). Briefly, non-T cells obtained from the interface after the initial density-gradient separation of E rosetting cells were deprived of residual T cells by rosetting again with neuraminidase-treated SRBC and density-gradient separation. This double-step procedure gave suspensions consistently containing less than 1% E-RFC. The number of esterase-staining cells found in non-T cell populations obtained from MNC suspensions initially depleted of phagocytic cells with carbonyl iron ranged between 3 and 10% esterase-staining cells. These suspensions contained primarily SIg^+ lymphocytes and will subsequently be referred to as B cell fractions.

Preparation of purified T lymphocytes. Purified T lymphocytes were prepared from MNC of atopic patients or normal donors as detailed elsewhere (Romagnani *et al.*, 1980a). Briefly, MNC were depleted of phagocytic cells by treatment with carbonyl iron and E-RFC were separated from non-E-RFC by centrifugation on a gradient of Ficoll–Hypaque. The pellet was resuspended and further centrifuged on a second density gradient. SRBC were lysed by 0·87% NH_4Cl. These suspensions contained consistently more than 95% E-RFC and less than 1% SIg^+ cells.

Cell cultures. Either unfractionated, fractionated or fractionated and recombined cell suspensions were cultured in RPMI 1640 medium buffered with $NaHCO_3$ and supplemented with L-glutamine (2 mM), 10% FCS, 100 u/ml penicillin G and 100 μg/ml streptomycin. All cultures were done in a final volume of 1 ml in 17-mm-diameter round-bottomed tissue culture tubes (Falcon, Oxnard). The tubes were incubated in a humidified atmosphere at 37°C with 5% CO_2 for 7 days. After 7 days the supernatants were collected, centrifuged, lyophilized and reconstituted in 1/10 distilled water.

Cell extracts. Day-0 cell pellets were saved from all experiments in order to evaluate the amount of preformed IgE in and/or on the cells at initiation of culture. For this purpose, cell pellets were lysed by freezing and thawing five times, following which the lysates were resuspended to the same volume as 7-day supernatants and centrifuged to remove cell debris. Cell extracts were then lyophilized and reconstituted, like supernatants, in 1/10 distilled water.

Measurement of IgE protein. IgE protein was detected by a sensitive solid-phase sandwich test specific for IgE protein and capable of measuring as little as 100 pg/ml of IgE, previously reported in detail (Romagnani *et al.*, 1980b). Briefly, solid-phase anti-IgE was prepared by reaction of CNBr-activated Sepharose 4B (Pharmacia, Uppsala) with an anti-human Fc (ε) sheep 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 a reference IgE serum containing 2,000 ng of IgE/ml.

Measurement of IgM protein. The IgM secreted into the culture medium of 7-day supernatants from MNC cultures was detected by a paper-disc radioimmunoassay using solid phase-coupled anti-human μ antibodies and immunosorbent-purified ^{125}I-labelled anti-μ antibodies. Paper discs (diameter 5·5 mm; weight 2·3 mg) were prepared from ashless paper and activated with CNBr according to the method of Ceska & Lundkvist (1972). The IgG fraction obtained by DEAE chromatography from rabbit antiserum against human μ chain was coupled to the discs according to the method reported by Ceska & Lundkvist (1972). Immunosorbent-purified anti-μ antibodies were labelled with ^{125}I by the chloramine T method. The assay was performed in two steps. During the first incubation period (at least 12 hr) the IgM present in the test sample or in the standard was allowed to bind to the antibody-coated disc. During the second incubation period (at least 12 hr) radioactive antibodies were allowed to react with the bound IgM. After three washings, the radioactivity on the disc was counted in a gamma counter. A standard curve was obtained using human IgM purified as described above. Either standard IgM or test samples were diluted in phosphate-buffered saline, pH 7·2 (PBS), containing 2% FCS.

RESULTS

Spontaneous production of IgE by unfractionated MNC from atopic individuals

Significant amounts of IgE protein were found in the supernatant of 7-day cultures of MNC from 21 atopic individuals. In all these cases the amount of IgE detected in 7-day culture supernatants was significantly higher than the amount detected in time-0 frozen-thawed cell supernatants, thus suggesting that most of the IgE was produced *in vitro* (Table 1). The maximum production of IgE

Table 1. Detection of IgE protein in time-0 frozen-thawed cell pellets and in supernatants of 7-day cultures of MNC from atopic patients

Patient	Serum IgE level (iu/ml)	IgE protein (pg/ml)	
		0-day cell pellet	7-day supernatant
P.A.	1,300	1,950	5,400
M.B.	121	800	2,100
R.L.	880	1,800	5,640
N.D.	360	970	2,750
G.L.	84	880	2,040
S.T.	430	1,200	4,500
F.L.	800	760	3,800
E.M.	1,000	910	21,000
G.F.	930	440	1,310
S.F.	860	700	4,800
M.S.	470	860	2,230
I.M.	1,700	790	3,125
M.A.	113	820	2,020
D.A.	400	1,500	3,400
S.A.	1,520	1,300	2,400
G.E.	560	1,800	3,800
M.E.	1,000	1,600	3,200
B.S.	192	920	1,950
B.R.	49	420	1,940
C.W.	53	385	1,600
L.G.	225	580	2,750

protein was found in MNC cultures from patients with high serum IgE levels, positive skin tests to several allergens and/or atopic dermatitis.

Effect of depletion of phagocytic or adherent cells

The role of monocytes in the spontaneous IgE production was first investigated. This was done by attempting to remove as many monocytes as possible by treatment of MNC with carbonyl iron or adherence to plastic surfaces. As expected, either treatment with carbonyl iron or adherence to plastic surfaces depleted the monocytes, which were quantitated by a non-specific esterase stain, from approximatively 10–30% in unfractionated suspensions to less than 5% in either non-phagocytic or non-adherent populations. IgE concentrations were then measured in the 0-day cell extracts and in the 7-day culture supernatants of either unfractionated MNC or MNC depleted of monocytes. As presented in Fig. 1, monocyte depletion resulted in a decrease in the amount of IgE protein detected in 7-day supernatants. A significant decrease in the amount of IgE present in the time-0 cell extracts was also observed.

Effect of depletion of B lymphocytes

To determine the nature of non-T cells responsible for the spontaneous production of IgE in atopic subjects, phagocyte-depleted MNC suspensions were further depleted of SIg^+ or $SIgM^+$ B lymphocytes by rosetting with hRBC coated with immunosorbent-purified anti-human $F(ab')_2$ or anti-human μ chain rabbit antibodies respectively. Non-rosetted were then separated from rosetted lymphocytes by centrifugation on a density gradient. As control, other cell aliquots were rosetted with hRBc coated with HSA and subjected to the same procedures. CR^+ lymphocytes were also removed from cell suspensions of the same donors by rosetting phagocyte-depleted MNC with

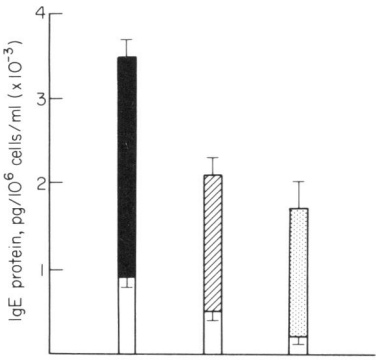

Fig. 1

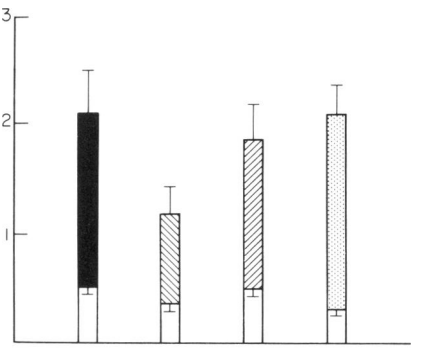

Fig. 2

Fig. 1. Effect of depletion of monocytes on the spontaneous IgE production in atopic subjects. MNC were depleted of phagocytic cells by treatment with carbonyl iron or of adherent cells by incubation on plastic surfaces. After these treatments, MNC suspensions contained less than 5 and 2% non-specific esterase-staining cells respectively. Culture supernatants from unfractionated MNC (■), MNC depleted of phagocytic cells (▨) and MNC depleted of adherent cells (▣) were assayed on day 7 for IgE. The open part of the columns represents the amount of IgE detected in 0-day cell extracts. The mean values ± s.e. of five separate experiments are reported.

Fig. 2. Effect of depletion of SIg^+, $SIgM^+$ or CR^+ cells on the spontaneous production of IgE in atopic patients. SIg^+ and $SIgM^+$ cells were removed by rosetting phagocyte-depleted MNC with hRBC coated with immunosorbent-purified anti-human $F(ab')_2$ or anti-human μ chain respectively. As control, the cells were rosetted with hRBC coated with HSA. CR^+ cells were removed by rosetting phagocyte-depleted MNC with EAC. Culture supernatants from suspensions depleted of cells forming rosettes with hRBC coated with HSA (■), anti-F $(ab')_2$ (▩), anti-μ chain (▨) or with EAC (▣) were assayed on day 7 for IgE. The open part of the columns represents the amount of IgE detected in 0–day cell extracts. The mean values ± s.e. of five separate experiments are reported.

EAC followed by centrifugation on a density gradient. Almost all the SIg^+ or the $SIgM^+$ cells were removed from lymphocyte suspensions collected at the interface of the density gradient after rosetting with hRBC coated with anti-$F(ab')_2$ or anti-μ chain respectively. Similarly, virtually no CR^+ cells could be found in lymphocyte suspensions depleted of EAC-RFC. The effect of these depletions on the IgE production is shown in Fig. 2. Depletion of SIg^+ cells decreased significantly, but did not abolish, the amount of IgE detected in 7-day culture supernatants. In contrast, after depletion of $SIgM^+$ lymphocytes, which virtually abolished spontaneous production of immunoglobulins of the IgM class, the amount of IgE found in the 7-day culture supernatants was not significantly different from that found in supernatants of control cell cultures. Similarly, no significant change in the spontaneous IgE production was found in cultures of lymphocyte suspensions which had been depleted of CR^+ cells.

Effect of depletion of T lymphocytes

The necessity for T cell help in the IgE production was also investigated. This was done by attempting to remove as many T lymphocytes as possible from MNC suspensions by the E rosetting technique. To ensure the completeness of this removal, multiple sequential E rosette depletions were performed. After two cycles of E-RFC depletion almost all of the T cells had been eliminated from the B cell preparation ($< 1\%$ E-RFC). As shown in Fig. 3, despite this exhaustive depletion, the amount of IgE detected in the supernatants of 7-day cultures was significantly greater than that found in the supernatants of unfractionated MNC cultures. The increase in the amount of IgE found in the culture supernatants of T cell-depleted suspensions did not simply reflect the enrichment for non-T cells caused by the fractionation procedure. In fact, $0{\cdot}2 \times 10^6$ non-T cells synthesized significantly greater amounts of IgE than did the corresponding number of non-T cells presumably present in 1×10^6 unfractionated cell suspensions (Table 2).

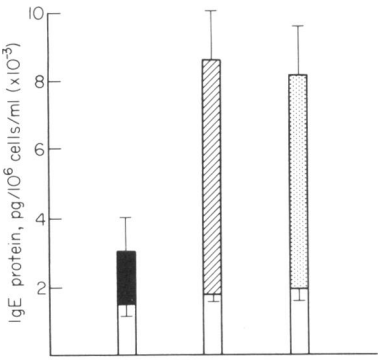

Fig. 3

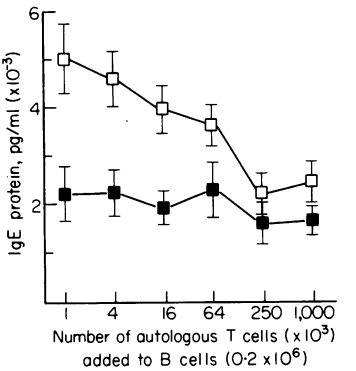

Fig. 4

Fig. 3. Effect of depletion of T lymphocytes on the spontaneous IgE production in atopic subjects. T lymphocytes were removed by two cycles of separate incubations with neuraminidase-treated SRBC and centrifugation through a density gradient. After one cycle B cell fractions comprised 1–8% E-RFC and after two cycles less than 1% E-RFC. Culture supernatants from unfractionated lymphocytes (■), B cell fractions obtained after one cycle (▣) and B cell fractions obtained after two cycles (▣) were assayed on day 7 for IgE. The open part of the columns represents the amount of IgE detected in 0-day cell extracts. The mean values ± s.e. of 10 separate experiments are reported.

Fig. 4. Effect of different concentrations of autologous T lymphocytes on the IgE production by B cell fractions ($0{\cdot}2 \times 10^6$/culture) of atopic patients in the absence (□) or in the presence (■) of PWM (5 μl/culture). Culture supernatants were assayed on day 7 for IgE. The results represent the mean values ± s.e. of four separate experiments.

Table 2. Detection of IgE protein in 0-day cell extracts and 7-day culture supernatants of unfractionated and B cell fractions from atopic patients

Cell population	IgE protein (pg/culture) 0-day cell extract	7-day supernatant
Unfractionated		
1×10^6/culture	1,670 ± 188	4,050 ± 789
B cells*		
1×10^6/culture	1,885 ± 477	8,887 ± 918
$0{\cdot}2 \times 10^6$/culture	512 ± 122	6,020 ± 710

* B cell fractions were obtained by a double E rosetting procedure with neuraminidase-treated SRBC and contained less than 1% E-RFC. The results represent the mean value ± s.e. of five separate experiments.

Effect of autologous T lymphocytes, in the presence or absence of PWM, on spontaneous IgE production by B cells from atopic patients

The effect of autologous T lymphocytes on spontaneous IgE production by B cell fractions was also evaluated. For this purpose different concentrations of autologous T lymphocytes were added to B cell fractions from atopic individuals in the presence or absence of PWM. There was no change in the spontaneous IgE production when a small number of T lymphocytes was added to B cells, whereas the addition of higher T cell concentrations induced a decrease in the amount of IgE recovered in the 7-day culture supernatants. The addition of PWM caused consistently a reduction

Table 3. Detection of IgE production in 7-day supernatants of co-cultures of B cells from atopic patients with 'normal' or 'atopic' allogeneic T cells*

Expt no.	Patient B cells + normal T cells (IgE, pg/culture)	Per cent of expected	Patient B cells + patient T cells (IgE, pg/culture)	Per cent of expected
1	3,300	89	2,700	73
2	2,550	68	2,500	67
3	960	81	1,500	127
4	405	34	1,180	101
5	3,600	73	2,105	43
6	3,200	66	3,310	68
7	780	69	1,220	108
8	4,360	87	4,880	98
9	3,500	87	3,720	93
10	2,700	77	3,940	112
11	3,320	86	3,960	102

* The amount of IgE found in the supernatant of cultures of patient B cells (1×10^6) with allogeneic T cells (1×10^6) was compared with that found in cultures of patient B cells (1×10^6) with autologous T cells (1×10^6).

in the amount of IgE produced by B cell fractions even in the presence of small concentrations of autologous T lymphocytes (Fig. 4).

Effect of allogeneic T lymphocytes from non-atopic and atopic subjects on spontaneous IgE production by B cells from atopic patients

The effect of allogeneic T lymphocytes derived from either atopic patients or normal donors on spontaneous IgE production was also assessed. The addition of normal T lymphocytes consistently decreased the spontaneous IgE production by patient B cell fractions (Table 3). In contrast, T cells from atopic patients induced an increase of spontaneous IgE production in five out of 11 experiments (Table 3).

DISCUSSION

In a recent paper we have shown that *in vitro* spontaneous IgE production can be detected in cultures from some normal and several atopic subjects and that cells from the latter usually produce significantly more IgE than cells from the former (Romagnani *et al.*, 1980b). Other laboratories have also reported *in vitro* production of human IgE, but the conditions leading to increased production appear to be in conflict. Saxon & Stevens (1979) showed that PWM is capable of enhancing significantly IgE synthesis in cultures of either normal donors or atopic individuals. In contrast, Fiser & Buckley (1979) and ourselves (Romagnani *et al.*, 1980b) found that PWM does not cause significant augmentation of IgE synthesis by either normal or atopic subjects and Tjio *et al.* (1979) showed that PWM has an inhibitory effect on the IgE production by MNC from grass-sensitive and ragweed-sensitive individuals. The reasons for these discrepancies are still unknown. However, our studies on the kinetics of IgE production in atopic patients showed that it is maximal from days 2 to 4 and declines progressively thereafter (Romagnani *et al.*, 1980b). On the other hand, the results of the present paper show that B cells equipped with SIgM and/or CR are not involved in the spontaneous IgE production by MNC from atopic patients and that these spontaneous IgE-producing cells are at least in part apparently lacking in SIg. This finding, together with the finding of the precocity of the IgE release into culture supernatants, strongly suggests that SIgE-bearing memory cells and/or SIg-free plasma cell precursors are the cells mainly implicated in the spontaneous IgE production. Since this early IgE production probably represents the result of an activation which has occurred *in vivo*, it is unlikely that the spontaneous IgE-producing cells can be further activated by a second signal, like that of PWM. On the other hand, in our previous paper (Romagnani *et al.*, 1980b), we showed that the treatment of cells with mitomycin C was able to decrease significantly the amount of IgE released in the supernatant after the 3rd day of culture. Thus, in order to explain this finding, as well as the results reported by Saxon & Stevens (1979), it may be suggested that there are two functionally distinct subpopulations of IgE-producing cells. The first subset could be represented by B cell precursors which are present also in normal people, need proliferation and can be activated by PWM to produce IgE in particular experimental conditions. The second subset comprises the well-differentiated spontaneous IgE-producing cells that we and other investigators (Fiser & Buckley, 1979; Tjio *et al.*, 1979) have been able to detect mainly in atopic patients.

In the present paper the possibility of influencing the activity of the spontaneous IgE-producing cells was investigated. Thus, it was shown that spontaneous IgE-production can be reduced by depletion of phagocytic or adherent cells. However, since after depletion of these cells there was also a marked decrease in the amount of IgE detectable in time-0 cell extracts, the reduction of IgE concentration in 7-day culture supernatants probably reflects, at least in part, the loss of cytophilic IgE due to incubation of cells at 37°C and longer cell handling. Fc receptors for IgE have been shown recently on lymphocytes and monocytes (Gonzalez-Molina & Spiegelberg, 1977; Yodoi & Ishizaka, 1979) and it has been found that IgE binds to these cells with a lower affinity than it does to mast cells and basophilic granulocytes (Spiegelberg & Melewicz, 1980). On the other hand, it is well known that treatment of cells with carbonyl iron or adherence to plastic surfaces can also lead to a depletion of cells other than monocytes. For these reasons, it is not yet possible to establish whether

the decrease in IgE production is due to the removal of monocytic cells which help IgE-producing cells or reflects the loss of some IgE-producing cells.

In this paper we could also show that spontaneous IgE production was significantly increased by depletion of T lymphocytes and that this increase did not simply reflect the enrichment for B cells caused by the fractionation procedure. These results are consistent with those of Fiser & Buckley (1979), but are apparently at variance with those of Saxon & Stevens (1979), who showed inability of B cell cultures alone to produce significant amounts of IgE either in normal or atopic individuals. The addition to B cell fractions of increasing concentrations of autologous T cells resulted in a significant inhibition of the spontaneous IgE synthesis. On the other hand, the addition of PWM induced a marked inhibition in the spontaneous IgE production by B cells also in the presence of low concentrations of autologous T cells. These data clearly indicate that spontaneous IgE production can be altered by *in vitro* manipulation, such as variations in T–B cell ratios or addition of PWM. They also suggest that PWM stimulation has a suppressive rather than a helper effect on the spontaneous IgE production from cells of atopic patients and that a few PWM-stimulated autologous T cells are able to suppress this IgE production to the same extent as many unstimulated autologous T cells as those existing in unfractionated MNC suspensions. Thus, the present results are consistent with those previously reported by studying IgE production in unfractionated MNC suspensions stimulated with PWM and showing inhibitory or no effect of PWM on the IgE production in atopic patients (Fiser & Buckley, 1979; Tjio *et al.*, 1979; Romagnani *et al.*, 1980b).

Further evidence for the possibility of influencing spontaneous IgE production was obtained in another series of experiments in which B cells from atopic patients were co-cultured in the presence of normal or atopic allogeneic T lymphocytes. In fact, it was shown that normal T cells are consistently effective in inducing a partial inhibition of spontaneous IgE production by patient B cells, whereas T cells from a sizeable proportion of atopic patients are not. In this respect, our data are consistent with those of Fiser & Buckley (1979), who also showed that T lymphocytes from atopic patients are generally less effective than control T cells in inhibiting the spontaneous IgE production by atopic B cells. Thus, it may be suggested that in a noticeable proportion of atopic patients there is a functional deficiency of regulatory T cells responsible for providing suppressor activity on spontaneous IgE production. However, the most impressive finding emerging from this study is the demonstration in the blood of atopic patients of spontaneous IgE-producing cells which, even though they probably represent the result of an activation which has occurred *in vivo*, can still be influenced by the regulatory activity of T cells or PWM.

This work was supported by funds from CNR, Progetto finalizzato Medicina Preventiva, Sottoprogetto BPC (contratto n. 79.01098.83). The authors wish to acknowledge Importex (Trieste), distributor for Pharmacia Diagnostic (Uppsala), who very kindly supplied the anti-human Fc(ε) sheep antiserum. Dr A. Sehon (Winnipeg) is also thanked for helpful discussion.

REFERENCES

CESKA, M. & LUNDKVIST, U. (1972) A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry*, **9**, 1021.

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.

GOLD, E.R. & FUDENBERG, H.H. (1967) Chromic chloride: a coupling reagent for passive hemagglutination reactions. *J. Immunol.* **99**, 859.

GONZALEZ-MOLINA, A. & SPIEGELBERG, H.L. (1977) A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. clin. Invest.* **59**, 616.

KOSKI, I.R., POPLAK, D.G. & BLAESE, R.M. (1976) A nonspecific esterase stain for the identification of monocytes and macrophages. In In Vitro *Methods in Cell-Mediated and Tumor Immunity* (ed. by B. R. Bloom and J. R. David), p. 359. Academic Press, New York.

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 hyperimmunoglobulinaemia E, eosinophilia and increased lymphocytes carrying surface IgE. *Clin. exp. Immunol.* **20**, 265.

ROMAGNANI, S., GIUDIZI, M.G., ALMERIGOGNA, F. & RICCI, M. (1980a) 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. (1980b) *In vitro* production of IgE by human peripheral blood mononuclear cells. I. Rate of IgE biosynthesis. *Clin. exp. Immunol.* **42**, 167.

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

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

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

YODOI, J. & ISHIZAKA, K. (1979) Lymphocytes bearing Fc receptors for IgE. I. Presence of human and rat T lymphocytes with Fcε receptors. *J. Immunol.* **122,** 2577.