Clin. exp. Immunol. (1975) **20,** 265–272.

IN VITRO PRODUCTION OF IgE BY LYMPHOCYTES FROM A PATIENT WITH HYPERIMMUNOGLOBULINAEMIA E, EOSINOPHILIA AND INCREASED LYMPHOCYTES CARRYING SURFACE IgE

R. PATTERSON, IRENA M. SUSZKO, C. C. S. HSU,* MARY ROBERTS AND SOO H. OH

Department of Medicine, Northwestern University Medical School, Chicago, Illinois, U.S.A.

(*Received* 18 *June* 1974)

SUMMARY

The peripheral blood lymphocytes of a patient with massive hyperimmunoglobulinaemia E were used for *in vitro* studies. The serum IgE ranged from 140,000–210,000 u/ml. Peripheral blood lymphocytes had approximately 7% of cells staining for surface IgE. When these cells were cultured *in vitro*, IgE was produced as measured by the double antibody radioimmunoassay technique. The total IgE produced ranged from 140 to 484 units per 24 hr in different cultures. IgE production was greatest in the first 24 hr of culture and declined progressively thereafter. Some cultures still had measurable IgE at 48 hr. If the lymphocytes staining for surface IgE were the cells producing the IgE, it was estimated that between 1·7 and 2·8 molecules per cell per second were produced. No definite effect of concanavalin A, pokeweed mitogen or phytohaemagglutinin on *in vitro* IgE production could be demonstrated under the conditions of these experiments.

INTRODUCTION

A patient with a new syndrome was reported recently (Patterson *et al.*, 1975). Briefly, Patient E.M. is a 40-year-old man with massive serum hyperimmunoglobulinaemia E (200,000 u/ml), eosinophilia (13,000 cells/mm^3) and increased circulating lymphocytes bearing surface IgE (8·7%). The elevated serum $IgE_{E.M.}$ has been present for 4 years, is polyclonal as evidenced by presence of kappa and lambda chains, and has a half-life of 2·2 days in Rhesus monkeys (Patterson *et al.*, 1975). The abnormal concentrations of IgE, eosinophils and IgE staining lymphocytes exist in the absence of any apparent allergic, immunological, infectious or parasitic disease during a 4-yr period of observation

Correspondence: Dr R. Patterson, Department of Medicine, Northwestern University Medical School, Chicago, Illinois, U.S.A.

(Patterson *et al.*, 1975). It was not established whether the elevated serum IgE was the result of decreased catabolism or increased production of IgE, or both. If the elevated serum $IgE_{E.M.}$ is the result of increased production and is associated with the circulating lymphocytes which carry surface IgE, it appeared possible that the $IgE_{E.M.}$ might be produced *in vitro* in measurable amounts by peripheral blood lymphocytes from patient E.M. ($L_{E.M.}$). This question was studied using the sensitive double antibody radioimmunoassay for IgE of Gleich, Averbeck & Swedlund (1971) which can measure IgE as low as 1 ng of protein per millilitre.

MATERIALS AND METHODS

Cell donors

Samples of 100–150 ml of peripheral blood were drawn by venipuncture from patient E.M. (Patterson *et al.*, 1975) or two control subjects. The latter included subject A with IgE-mediated disease diagnosed by standard clinical diagnostic techniques (Patterson, 1972) as ragweed rhinitis and a serum IgE concentration of 800 u/ml. Subject B was an individual with no evidence of IgE-mediated disease by history or allergy skin testing and a serum IgE concentration of 40 u/ml.

Cell fractionation and culture techniques

The method of Wall *et al.* (1973) with slight modifications was used for the preparation of lymphocytes for cultures. Heparinized blood was mixed with two volumes of 6% dextran 75 in 0·15 M NaCl (Travenol Laboratories, Incorporated, Morton Grove, Illinois). After sedimentation of the red blood cells, the leucocyte-rich plasma–dextran was centrifuged at 400 *g* for 10 min. The centrifuged cells were resuspended in 40 ml of autologous plasma–dextran solution and introduced into a 50-ml sterile plastic syringe, loosely packed with cotton wool, and incubated for 30 min at 37°C. The lymphocytes were eluted by pushing of the plunger of the syringe and squeezing the cotton free of the plasma–dextran solution. Further elution of cells was done by flushing the cotton wool with additional plasma–dextran solution.

Eluted cells were centrifuged and washed five times with medium 199 (Hanks's Base) (Grand Island Biological Company, Grand Island, New York). Total and differential counts were made and cells divided equally between plastic sterile Petri dishes (100 × 20 mm) (Falcon Plastics). A portion of cells was kept for immunofluorescent staining. To each Petri dish enough medium 199 (Hanks's Base), containing 10% foetal bovine serum (Flow Laboratories, Incorporated, Rockville, Maryland) and 100 μg of streptomycin and 100 u of penicillin per millilitre (TCM), was added to provide a final suspension of 6,000,000 cells per millilitre of medium. Various mitogens were added to the medium of some dishes. Dishes were incubated at 37°C in a constant humidity incubator in an atmosphere of 5% CO_2 and 95% air. Every 24 hr or every 6 hr, supporting medium was removed and replaced with the fresh medium. Since some cells were also removed from the dish at the harvesting time, harvested medium was centrifuged and the cells returned to the original petri dish.

At the initiation of each culture, cells from one Petri dish in the appropriate amount of medium were frozen–thawed five times, homogenized and centrifuged. The supernatant was used for the determination of the amount of IgE that was present in or on the cells at the initiation of the experiment.

Mitogens

Bacto-phytohaemagglutinin P (PHA-P) (Difco Laboratories, Detroit, Michigan) was used at 25 μg/ml of medium. Pokeweed mitogen (PWM) (Grand Island Biological Company, Grand Island, New York) was used in a final concentration of 1·6 μg/ml. Concanavalin A (Con-A) (Sigma Chemical Company, St Louis, Missouri) was used at 25 μg/ml of medium.

Viability of cells

Cell viability was evaluated by exposure of cells to 0·2% trypan blue and determination of the percentage of cells which excluded the dye.

Immunofluorescent staining of lymphocyte surface immunoglobulins (Ig)

This was performed as previously described (Patterson *et al.*, 1975). Purified lymphocytes were suspended in Hanks's balanced salt solution at a concentration of 2×10^6 per millilitre. 0·025 ml of fluorescein-conjugated goat antiserum against human Ig (Meloy Laboratories) was added to 0·1 ml of the lymphocyte suspensions. Two antisera were employed. One was specific for the heavy chain fraction of IgE and the other was specific for the heavy chain determinants of IgG, IgA and IgM (anti-polyvalent Ig). The cell suspension was incubated in an ice bath for 30 min, washed twice with phosphate-buffered saline (300 *g* for 10 min), suspended in phosphate-buffered glycerine, and smears prepared for immunofluorescence examination. The cells were examined for fluorescence using a Zeiss ultraviolet microscope with Osram 200 mercury lamp. Two hundred cells were counted.

Measurement of IgE

The double antibody radioimmunoassay of Gleich *et al.* (1971) was used. Supporting medium (TCM) from cell cultures and TCM from extracted cells at the initiation of cultures was concentrated to 1 ml and the IgE content measured.

RESULTS

IgE production in vitro by lymphocytes from patient E.M.

Three successive cell culture preparations of lymphocytes from patient E.M. are shown in Fig. 1. The results show a marked increase in IgE in TCM from these preparations as compared with control TCM which contains the extract of homogenized cells. The minimum increase in IgE concentration in TCM from cultured cells was six times that of the comparable level. In contrast, there was no measurable IgE in TCM from cultured lymphocytes of the atopic patient or the normal subject (Fig. 1d). The results demonstrate that the maximum increase in IgE occurred within 24 hr of culture with slight elevations above control levels in only a few cultures after 48 hr of culture. The addition of PHA, Con A and PWM did not appear to have an effect which could be identified as either increasing or decreasing the IgE in the lymphocyte cultures of patient E.M.

Characteristics of cell populations cultured and the rate of IgE production in vitro

The numbers of lymphocytes $_{\text{E.M.}}$ cultured in the three experiments illustrated in Fig. 1, and an additional experiment, are shown in Table 1. These numbers were comparable with those of two other subjects studied as controls. The percentage of lymphocytes $_{\text{E.M.}}$ staining

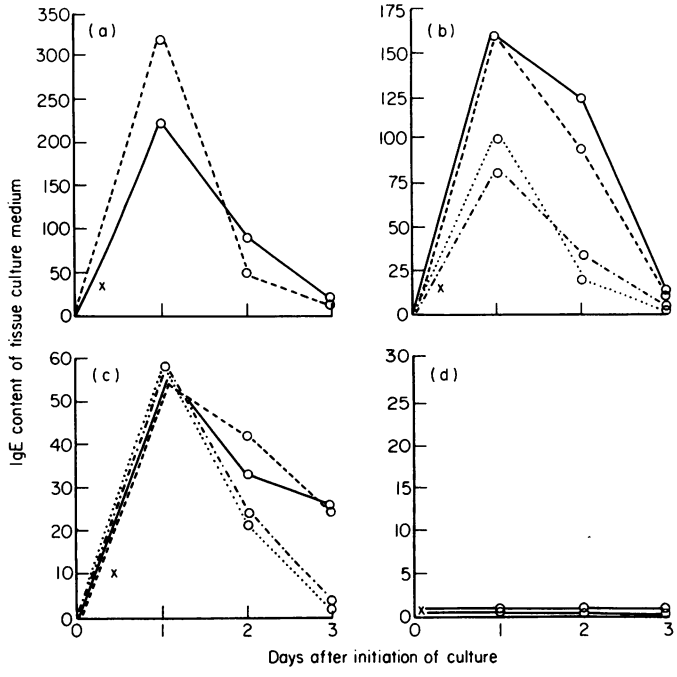

FIG. 1. IgE determination of TCM from cultures of lymphocytes of (a), (b) and (c) patient E.M. and (d) from cultures of an allergic patient and normal subject. Cultures with TCM alone (———), phytohaemagglutinin (·–·–·), concanavalin A (· · · ·) and pokeweed mitogen (– – –). The control level of IgE obtained by extract of $L_{E.M.}$ is shown by ×.

TABLE 1. Summary of data from tissue cultures of the peripheral blood lymphocytes of patient E.M. and the determinations used for estimations of the rate of production of IgE molecules by lymphocytes staining for IgE

	Experiment number			
	1	2	3	4
Volume of blood drawn (ml)	100	100	150	150
Total number of lymphocytes isolated ($\times 10^6$)	104	249	346	140
Number of cultures	2	2	4	4
Number of cells/sample ($\times 10^6$)	35	100	70	40
Percentage of cells which are lymphocytes	99	83	99	70
Number of lymphocytes cultured ($\times 10^6$)	69	166	277	112
Percentage of lymphocytes staining for IgE ($L_{E.M.}$-IgE)	6	9	5	8
Number of $L_{E.M.}$-IgE in cultures ($\times 10^6$)	4·16	14·9	13·9	8·9
IgE produced in 24 hr (u) (IgE in TCM after 24 hr-IgE in CE)*	140	484	446	187
IgE produced in 24 hr (molecules $\times 10^{12}$)	1·03	3·54	3·26	1·36
Molecules of IgE/$L_{E.M.}$-IgE/24 hr ($\times 10^5$)	2·47	2·37	2·35	1·53
Mean number of molecules of IgE/$L_{E.M.}$-IgE/sec	2·88	2·74	2·73	1·78

* TCM = tissue culture medium; CE = cell extract.

for surface IgE (L-IgE) are also shown in Table 1. In all samples of lymphocytes from patient E.M. there was a higher percentage of lymphocytes staining for surface IgE than there was in the lymphocytes from either the allergic patient or the normal subject which was 3% and 2% respectively. The amount of IgE produced by an $L_{E.M.}$-IgE was estimated as follows:

$$\text{Number of molecules per unit of IgE} = \frac{\text{Weight of 1 u of IgE} \times \text{Avogadro's number}}{\text{Gram molecular weight of IgE}}$$

$$= \frac{2{\cdot}3 \times 10^{-9} \text{ g}^* \times 6{\cdot}06 \times 10^{23}}{1{\cdot}9 \times 10^{5} \text{ g}}$$

$$= 7{\cdot}3 \times 10^{9}$$

$$\text{Number of IgE molecules produced by an } L_{E.M.}\text{-IgE per sec} = \frac{(\text{Units of IgE produced in 24 hr})/(86{,}400 \text{ sec}/24 \text{ hr})}{\text{Number of } L_{E.M.}\text{-IgE cultured}}$$

The number of estimated molecules of IgE produced per $L_{E.M.}$-IgE by this analysis are shown in Table 1. There is a relative consistency of results of the four cultures.

Cell survival, as determined by trypan blue exclusion at 3 days in cultures of $L_{E.M.}$ and L from control subjects, ranged from 85 to 93%.

Rate of production of IgE in vitro determined at 6-hr intervals

Because the *in vitro* appearance of IgE described above occurred primarily in the first 24 hr of culture with lower production thereafter (Fig. 1), it was questioned whether the majority of IgE might not be released from cells in the first few hours or even minutes of culture. For this reason, $L_{E.M.}$ were cultured and tissue culture medium changed at intervals of 6 hr. The results showing IgE production by $L_{E.M.}$ in cultures done in triplicate are seen in Fig. 2. These results demonstrate the reproducibility of IgE appearance in the culture preparations. The greatest production of IgE occurred during the first 6 hr and declined relatively slowly but consistently over the next two successive 6-hr periods. This indicates that the average production of IgE by cells as estimated in Table 1 would not be as grossly in error as they would be if all *in vitro* production of IgE occurred in the first few minutes or hours of culture. The 24-hr production of IgE shown in Fig. 2 is less than that shown in Fig. 1. This may be because the additional manipulation of the cultures decreased production or because the $L_{E.M.}$ were producing less IgE at the time they were collected for culture. In support of the latter explanation is that the patient's serum IgE had fallen to 140,000 u/ml as compared with prior levels which were in excess of 200,000 u/ml when other cell samples were obtained for culture.

DISCUSSION

These studies demonstrate that IgE may be produced *in vitro* from this patient's peripheral blood lymphocytes in amounts which are measurable by direct quantitative techniques. The amounts of IgE appearing in the TCM were significantly higher than in the cell extracts of the same number of cells used as controls. For this reason, the source of the IgE is not

* The value of $2{\cdot}3 \times 10^{-9}$ g per unit of IgE was determined experimentally in this laboratory. Other reports of this determination include $2{\cdot}2 \times 10^{-9}$ g/unit (Bazaral, Orgel & Hamburger, 1971) and $2{\cdot}4 \times 10^{-9}$ g/unit (Bazaral & Hamburger, 1972).

FIG. 2. IgE content of tissue culture medium determined at 6-hr intervals after initiation of the culture of lymphocytes$_{E.M.}$. The range and the average of three separate cultures are shown. Control IgE content of lymphocytes$_{E.M.}$ extracted are shown by ×.

considered to be release of cell-bound IgE from dead cells *in vitro* or inadequate washing of serum IgE from cell preparations. *In vitro* production of IgE could not be demonstrated in comparable studies with cells from an allergic and normal subject. Thus the abnormality of patient E.M. which is manifested clinically by his massive increase in serum IgE is reflected *in vitro* by the production of IgE by his peripheral blood lymphocytes. This abnormality could be in part due to a quantitative difference in the number of IgE-producing cells present in the peripheral blood of patient E.M. compared with those in atopic or normal subjects. It has been shown clearly that the serum immunoglobulins can be produced by circulating B cells bearing the same classes of Ig on their surfaces (Cooper, Lawton & Kincade, 1972; Wu, Lawton & Cooper, 1973; Walters & Wigzell, 1970). Therefore the increased number of IgE-bearing lymphocytes in the patient's peripheral blood may account for at least a part of the increase in serum IgE level. In addition to Ig-bearing B lymphocytes, plasma cells and blast cells which differentiate from B lymphocytes following antigenic and mitogenic stimulations are known to secrete immunoglobulins (Moore & Schoenberg, 1968; Douglas & Fudenberg, 1969). This patient's peripheral blood smears failed to show any plasma cells or blast cells on repeated examinations. Therefore it seems reasonable to assume that the patient's IgE produced *in vitro* was synthesized by IgE-bearing B lymphocytes and not by plasma cells or blast cells. With regard to the latter, the localization of plasma cells staining for IgE has been in such areas as the tonsil or respiratory tract (Ishizaka & Ishizaka, 1971).

The kinetics of IgE synthesis *in vitro* may be of importance. The synthesis occurred without addition of any mitogens and was greatest at 24 hr after initiation of the culture. This pattern is in contrast to the usual kinetics of other Ig synthesis by peripheral blood lymphocytes which reaches a peak after the 5th or 6th day of culture in the presence of B-cell mitogens (Greaves & Roitt, 1968; Forbes & Lawton, 1969; Moore & Schoenberg, 1968). Of particular interest in this regard is the fact that the spleen cells from chickens pre-immunized with antigens had a similar pattern of antibody production *in vitro* as the IgE synthesis pattern demonstrated by the patient's lymphocytes (Patterson, Suszko & Pruzansky, 1964).

The chicken spleen contained increased number of plasma cells and it was thought that the plasma cells were responsible for this pattern of Ig production. In patient E.M., it is possible that there was a stimulatory factor for IgE synthesis which caused B cells in circulation to differentiate to a point where spontaneous early *in vitro* IgE production could be demonstrated, as in primed chicken spleen cells. However, these cells are still classifiable under light microscopy as lymphocytes, rather than fully differentiated plasma cells or blast cells.

Assuming that the IgE-bearing lymphocytes were responsible for the *in vitro* IgE synthesis, the molecules of IgE produced by the cells can be estimated as in Table 1. There are few studies that are comparable to those reported here either because of species difference, Ig class difference or because a majority of studies measure antibody production from antigen-stimulated animals, rather than continuing production of Ig from unstimulated cells. One study that appears particularly relevant reported the measurement of IgM production *in vivo* and *in vitro* using cells from a murine plasmacytoma. The *in vitro* release of IgM after 30 min was approximately 76 molecules/cell/sec. Twenty-four-hour *in vivo* preparations showed a release rate of about three to six molecules/cell/sec (Ghanta *et al.*, 1974), a rate of release similar to that estimated for $IgE_{E.M.}$ at 24 hr in the current study (Table 1).

The production of IgE by lymphocytes from patient E.M. suggests that the markedly elevated serum IgE in this patient is due, at least in part, to increased production of IgE rather than decreased catabolism. That the major portion of serum IgE is from circulating $L\text{-}IgE_{E.M.}$ does not seem likely on the basis of the calculations in Table 2. It would appear that there are cells in patient E.M. producing IgE which are sequestered outside of the circulation.

TABLE 2. Relationship of IgE production by lymphocytes *in vitro* to intravascular content of IgE of patient E.M., indicating that all IgE probably is not produced by circulating lymphocytes staining for IgE

Estimated total blood volume of patient E.M.	5600 ml
White blood cell count	$2{\cdot}41 \times 10^7$/ml
Percentage lymphocytes	36
Total $L_{E.M.}$	$48{\cdot}5 \times 10^9$
Percentage $L_{E.M.}$-IgE (see Table 1)	7 (average)
Total $L_{E.M.}$-IgE	$3{\cdot}4 \times 10^9$
IgE content of serum of patient E.M.	210,000 u/ml
In vitro production of IgE by $4{\cdot}16 \times 10^6$ $L_{E.M.}$-IgE in 24 hr	140 u
On the basis of the *in vitro* rate of IgE production (see Table 1) $3{\cdot}4 \times 10^9$ $L_{E.M.}$-IgE would produce:	115,000 u
Total intravascular content of IgE of patient E.M. (plasma volume estimated at 2800 ml)	$5{\cdot}8 \times 10^8$

The mitogens known to stimulate lymphocytes did not have a definite effect on $lymphocytes_{E.M.}$. It was considered possible that the mitogens (PHA and con A) known to stimulate T lymphocytes might have a stimulating or suppressing action on $IgE_{E.M.}$ production. This could occur either as a result of stimulation of a T-cell factor which stimulates B cells, or stimulation of a factor which suppresses B cells, respectively. PWM might stimulate B cells directly. Neither a stimulatory nor an inhibitory effect on IgE production by these mitogens

could be shown consistently in these studies. This may been have because *in vitro* production of $IgE_{E.M.}$ occurred during the first 24 hr of culture and the three mitogens are known to have maximal stimulatory activity after 3 days (Ling, 1968). Their effect thus may not be evident under the condition of these experiments.

ACKNOWLEDGMENTS

This research was supported by U.S.P.H.S. grant number AI 11403 and the Ernest S. Bazley grant. C.C.S.H. was the recipient of U.S.P.H.S. grant number RR-05370.

REFERENCES

BAZARAL, M., ORGEL, H.A. & HAMBURGER, R.N. (1971) IgE levels in normal infants and mothers and an inherent hypothesis. *J. Immunol.* **107,** 794.

BAZARAL, M. & HAMBURGER, R.N. (1972) Standardization and stability of immunoglobin E (IgE). *J. Allergy,* **49,** 189.

COOPER, M.D., LAWTON, A.R. & KINCADE, P.W. (1972) A two-stage model for development of antibody-producing cells. *Clin. exp. Immunol.* **11,** 143.

DOUGLAS, S.D. & FUDENBERG, H.H. (1969) *In vitro* development of plasma cells from lymphocytes following pokeweed mitogen stimulation: a fine structural study. *Exp. Cell. Res.* **54,** 277.

FORBES, I.J. & LAWTON, J.W.M. (1969) Autoradiographic analysis of proteins synthesized by human lymphocytes. I. Multiple myeloma and macroglobulinemia: evidence relevant to clonal selection theory. *Aust. Ann. Med.* **18,** 12.

GHANTA, V.K., MCGHEE, J.R., HAMLIN, N.M. & HIROMOTO, R.N. (1974) Measurements of antibody release from single cells. III. Rate of release of IgM from MOPC 104E plasmacytoma. *J. Immunol.* **112,** 266.

GLEICH, G.J., AVERBECK, A.K. & SWEDLUND, H.A. (1971) Measurement of IgE in normal and allergic serum by radioimmunoassay. *J. Lab. clin. Med.* **77,** 690.

GREAVES, M.F. & ROITT, I.M. (1968) The effect of phytohaemagglutinin and other lymphocyte mitogens on immunoglobulin synthesis by human peripheral blood lymphocytes *in vitro. Clin. exp. Immunol.* **3,** 393.

ISHIZAKA, K. & ISHIZAKA, T. (1971) The significance of immunoglobulin E in reaginic hypersensitivity. *Ann. Allergy,* **28,** 189.

LING, N.R. (1968) *Lymphocyte Stimulation.* North-Holland, Amsterdam.

MOORE, R.D. & SCHOENBERG, M.D. (1968) Restimulation of antibody synthesis by antigen in cultures of lymphocytes. *Nature (Lond.),* **219,** 297.

PATTERSON, R. (1972) *Allergic diseases. Diagnosis and Management,* p. 63. Lippincott, Philadelphia.

PATTERSON, R., OH, S.H., ROBERTS, M. & HSU, C.C.S. (1975) Massive polyclonal hyperimmunoglobulinemia E, Eosinophilia and increased IgE-bearing lymphocytes. *Amer. J. Med.* (In press.)

PATTERSON, R., SUSZKO, I.M. & PRUZANSKY, J.J. (1964) *In vitro* production of chicken globulins and precipitating antibody. *Immunology,* **7,** 440.

WALL, J.R., GOOD, B.F., FORBES, I.J. & HETZEL, B.S. (1973) Demonstration of the production of the long acting thyroid stimulator (LATS) by peripheral lymphocytes cultured *in vitro. Clin. exp. Immunol.* **14,** 555.

WALTERS, C.S. & WIGZELL, H. (1970) Demonstration of heavy and light chain antigenic determinants on the cell-bound receptor for antigen. Similarities between membrane-attached and humoral antibodies produced by the same cell. *J. exp. Med.* **132,** 1233.

WU, L.Y.F., LAWTON, A.R. & COOPER, M.D. (1973) Differentiation capacity of cultured B lymphocytes from immunodeficient patients. *J. clin. Invest.* **52,** 3180.