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In vitro IgE formation by peripheral blood lymphocytes from normal individuals and patients with allergic bronchopulmonary aspergillosis

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SUMMARY

Peripheral blood lymphocytes from five normal donors and five individuals with allergic bronchopulmonary aspergillosis (ABPA) were used for measurement of *in vitro* IgE formation in tissue culture preparations. IgE was measured by a sensitive radioimmunoassay using concentrated tissue culture medium (TCM) and expressed as ng IgE/ml of TCM. The results demonstrated that IgE was formed by unstimulated lymphocytes from one of five normal donors (range 1·5–2·4 ng/ml) and in two of five ABPA donors (range 2–4 ng/ml). However, when different dilutions of pokeweed mitogen were added to the tissue culture, three of five normal donors and three of five ABPA donors in remission showed enhanced IgE formation. At the time of an ABPA exacerbation in one individual, there was a definite increase in IgE synthesis up to 40 ng/ml which was suppressed by pokeweed mitogen to 17·3 ng/ml. Clear differences between normals and ABPA patients in remission are not apparent.

INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a disease characterized by asthma, immediate skin reactivity to *Aspergillus fumigatus* (Af), peripheral blood eosinophilia, fleeting pulmonary infiltrates, proximal bronchiectasis, increased total serum concentrations of IgE, and elevated serum IgE and IgG antibody against Af (Wang *et al.*, 1978). Total serum concentrations of IgE in patients with ABPA have been shown to vary markedly in different individuals and in the same individual depending on the disease activity and thus can be used as a guide in clinical management (Rosenberg, Patterson & Roberts, 1977).

Previous investigators have demonstrated *in vitro* IgE production from normal persons (Saxon & Stevens, 1979) and in patients with disease states characterized by greater than normal formation (Buckley & Becker, 1978; Patterson *et al.*, 1975; Patterson *et al.*, 1976).

The present study was done to determine if *in vitro* formation of IgE by peripheral blood lymphocytes (PBL) of patients with ABPA was similar to normal subjects or if the elevated IgE in patients with ABPA might, in part, result from formation by PBL.

MATERIALS AND METHODS

Cell donors. Peripheral blood lymphocytes were used from five non-atopic normal controls and

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Table 1. Characteristics of donors used in *in vitro* studies

Cell donor	Serum IgE (ng/ml)	Clinical characteristic	Prednisone therapy* (mg)	Reason for prednisone
1	205	Normal control	None	—
2	229	Normal control	None	—
3	405	Normal control	None	—
4	43	Normal control	None	—
5	63	Normal control	None	—
6	6,462	ABPA† in remisson	10	Control of asthma
7	601	ABPA in remisson	10	Control of asthma
8	735	ABPA in remisson	40	Control of asthma
9	391	ABPA in remisson	10	Control of asthma
10E‡	21,786	ABPA exacerbation	12·5	Control of asthma
10R§	6,464	ABPA in remisson	20	Reduction phase for control of ABPA

* Given as a single alternate-day morning dose.
† Allergic bronchopulmonary aspergillosis.
‡ Blood drawn on 3 December 1978.
§ Blood drawn on 17 May 1979.

five subjects with known ABPA. Criteria for diagnosis of ABPA were those described in the Introduction and previously reviewed in detail (Wang *et al.*, 1978). A remission of ABPA is considered present when the patient has had a stable serum IgE concentration, no infiltrate on chest X-ray, and no other evidence of uncontrolled ABPA for several months. An exacerbation is a recurrence of chest X-ray infiltrates, a rise in total serum IgE with varying degrees of symptoms (Wang *et al.*, 1979). Characteristics of cell donors and medication at the time of studies are listed in Table 1. If the donor was receiving corticosteroids, the blood was drawn at least 36 hr after the last dose of prednisone.

Cell cultures. A modification of the method previously described (Patterson *et al.*, 1976) was used. Heparinized venous blood (100 ml) was drawn for each culture and a Ficoll–Hypaque gradient was used for separation of lymphocytes. The lymphocyte fraction was removed and washed five times with medium 199 (Hanks' base). Total and differential cell counts were done. Supporting tissue culture medium (TCM) consisted of medium 199 (Hanks' base) containing 100 units penicillin per ml and 100 μg streptomycin. Aliquots of cells were distributed equally in sterile plastic petri dishes and 1 ml of TCM was added for every 5 million cells. At the initiation of each culture, cells were removed and extracted from one dish for 0-time (control) cell content of IgE.

Cells from the 0-time control were frozen and 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. Cells were incubated at 37°C in a constant humidity incubator in a 5% CO_2 atmosphere. The supporting media was removed and replaced at 24-hr intervals.

Mitogen. Pokeweed mitogen (PWM) (Grand Island Biological Company, Grand Island, New York) was diluted with TCM in order to obtain a final concentration of 1:16 or 1:64 and was added to selected TCM cultures.

Measurement of IgE. The double-antibody radioimmunassay of Gleich, Averbeck & Swedlund (1971) was used to measure IgE in the culture medium. Supporting medium (TCM) from cell cultures was pooled at the end of the 6-day incubation period. Both the pool of TCM from cultures and the extracted (control) cells at the initiation of cultures were concentrated × 5 using Minicon B-15 concentrators (Amicon Corporation, Lexington, Massachusetts) and the IgE content was measured.

Determination of in vitro IgE formation. In order to determine if *in vitro* IgE formation had occurred, the value obtained at 0 time was subtracted from TCM. If this value was greater than 0·5

ng, this was considered evidence of *in vitro* IgE formation. In order to determine the effect of PWM, if the value obtained from the PWM culture was greater than 0·5 ng over the TCM value irrespective of the 0 time, this was considered evidence of *in vitro* IgE formation.

RESULTS

Figs 1 and 2 depict the results of *in vitro* IgE formation in normal persons and individuals with ABPA. One of five normal individuals had evidence of minimal *in vitro* IgE formation. Total range

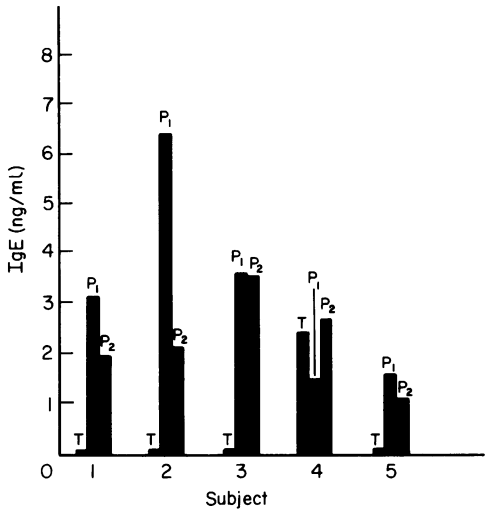

Fig. 1. *In vitro* IgE determination from cultures of lymphocytes from normal donors numbered 1 to 5. T is tissue culture medium, P_1 is pokeweed mitogen 1:16 and P_2 is pokeweed mitogen 1:64.

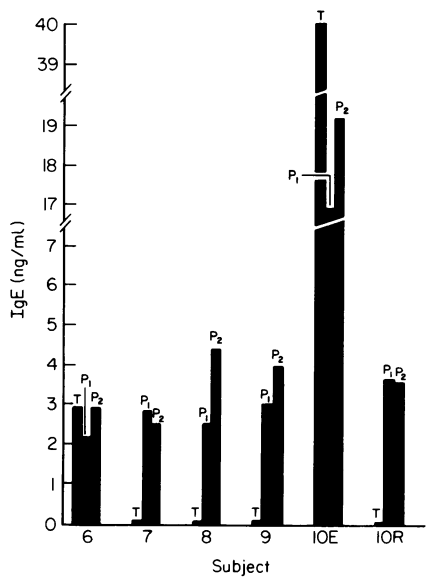

Fig. 2. *In vitro* IgE determination from cultures of lymphocytes from ABPA donors numbered 6–10. 10E represents donor at the time of a clinical exacerbation and 10R when this donor is in remission, T is tissue culture medium, P_1 is pokeweed mitogen 1:16 and P_2 is pokeweed mitogen 1:64.

of IgE with TCM alone was 1·5–2·4 ng/ml. However, when PWM was added to the culture, three of five normal donors showed IgE formation at one or both dilutions of PWM with a total IgE range from 1·05–6·4 ng/ml.

Unstimulated cells from two of five ABPA individuals who were clinically stable and in remission demonstrated *in vitro* IgE formation with a total range from 2–4 ng/ml. PWM at either dilution stimulated IgE formation in two cases and enhanced total formation in three of five ABPA individuals who were in remission with the total range from 2·15–4·6 ng/ml (Fig. 2).

Marked *in vitro* IgE formation occurred in cells from the ABPA individual whose blood was obtained at the time of a clinical exacerbation of ABPA (Fig. 2). This increased IgE formation to 40 ng/ml was suppressed by PWM at either dilution to 17 ng/ml. This same individual was re-evaluated after a remission of ABPA and there was a marked decrease in the *in vitro* IgE formation which was enhanced by the addition of PWM (Fig. 2). The total serum IgE correspondingly decreased following treatment with corticosteroids.

DISCUSSION

The present results are in agreement with those showing that *in vitro* IgE synthesis is minimal using PBL of normal individuals (Saxon & Stevens, 1979) without the addition of PWM, a known stimulator of both B and T lymphocytes (Oppenheim & Schecter, 1976). Cells from patients with disorders associated with increased serum IgE concentrations have been found to have maximal IgE synthesis in unstimulated cultures of peripheral blood mononuclear cells and PWM has a suppressive effect (Buckley & Becker, 1978). Patients with ABPA have a dramatic rise and fall in total serum IgE concentrations depending on their clinical status. IgE values are high in newly diagnosed cases, lower after prednisone-induced remission and rise dramatically during exacerbations of ABPA (Rosenberg *et al.*, 1977). The alternate-day dose of prednisone may have affected the *in vitro* formation of IgE by PBL. However, these cells were obtained at least 36 hr after the last dose of prednisone. Other studies from the laboratory (Chiang *et al.*, 1979) have demonstrated that T lymphocyte function as measured by phytohaemagglutinin responsiveness returned to normal within 24 hr after the last dose of prednisone. We consider it unlikely that these moderate to low doses of prednisone affected the *in vitro* lymphocyte function in terms of IgE formation.

One of the characteristics of untreated ABPA is a definite increase in total serum IgE. This declines with prednisone therapy (Rosenberg *et al.*, 1978) but may remain significantly increased following remission (case 6, Table 1). The explanation for the increased concentrations of IgE in ABPA is not known, but much of this IgE is not antibody against antigens of Af (Patterson, Rosenberg & Roberts, 1977). One of the objectives of this study was to determine whether circulating PBL of patients with ABPA form increased amounts of IgE *in vitro* as compared with normal donor PBL. PBL of ABPA patients in remisson either with modest increases in serum IgE (cases 7–9, Fig. 2) or with greater increases in serum IgE (cases 6 and 10R, Fig. 2) did not form IgE significantly different than normals. This suggests the PBL of ABPA are not forming excess IgE. In those patients (6 and 10R, Fig. 2) in which there is an excess of IgE PBL may reach the circulation from IgE-forming cells in tissues, probably pulmonary tissue. The opportunity to culture PBL of a patient during an acute exacerbation of ABPA was a fortunate occurrence since the diagnosis of the exacerbation was made subsequent to obtaining PBL. In this patient (case 10E, Fig. 2), PBL formed significantly larger amounts of IgE than normals, ABPA patients in remission or PBL from the same patient in remisson (Fig. 2). This suggests that during the exacerbation of ABPA, IgE-forming cells are released into the systemic circulation, most likely from the lung since this is the single organ involvement in ABPA. It is possible that these IgE-forming lymphocytes, presumptively stimulated by *Aspergillus fumigatus*, are a subset of IgE-forming cells usually restricted to the lung.

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