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IgE synthesis *in vitro* induced by T cell factors from patients with elevated serum IgE levels

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SUMMARY

The effect of unstimulated T cell culture supernatants (TCS) from patients with atopic dermatitis and high serum IgE levels on the IgE production *in vitro* by B cell rich suspensions from normal individuals or grass pollen sensitive patients with mild atopy was evaluated. TCS from patients with raised IgE enabled B cell suspensions from normal individuals to produce detectable amounts of IgE and potentiated the spontaneous IgE synthesis *in vitro* by B cell suspensions from grass sensitive patients. In contrast, the addition of TCS from normal subjects with low serum IgE levels did not increase or even reduced IgE synthesis by B cell cultures. When the same B cell cultures were analysed for their ability to produce IgG or IgM protein, no significant differences were observed. These findings indicate that T lymphocytes from patients with high serum IgE levels can release soluble factor(s) possessing isotype (IgE) specific potentiating activity.

INTRODUCTION

In the last few years some laboratories, including our own, have reported success in establishing *in vitro* methods that could be of potential use in studies of human IgE immunoregulation. The results of these investigations have supported evidence that peripheral blood lymphocytes (PBL) from atopic patients are able to produce spontaneously *in vitro* detectable amounts of IgE protein and IgE antibody (Fiser & Buckley, 1979; Tjio, Hull & Gleich, 1979; Saxon, Morrow & Stevens, 1980; Romagnani *et al.*, 1980b). Some phenotypic and functional characteristics of these spontaneously IgE producing cells have also been described. The IgE producing cells are lighter than the majority of PBL (Saxon *et al.*, 1980), possess DR (Ia like) antigens and surface membrane (Sm)IgE, but not SmIgM (Romagnani *et al.*, 1980c, 1982a), and their ability to synthesize spontaneously IgE can be negatively influenced by addition to the cell culture of T lymphocytes, polyclonal activators or minute concentrations of the specific allergen (Fiser & Buckley, 1979; Saxon *et al.*, 1980; Romagnani *et al.*, 1980c, 1982b). In contrast, some disagreement appears to exist as to the ability of PBL from either normal or atopic individuals to produce IgE *in vitro* after stimulation with polyclonal agents, such as pokeweed mitogen, or on stimulation by specific antigens to which individuals were known to be sensitive, some groups reporting success (Saxon & Stevens, 1979; Urena *et al.*, 1979; Nonaka *et al.*,1981; Zuraw *et al.*, 1981) and others failure (Fiser & Buckley, 1979; Tjio *et al.*, 1979; Romagnani *et al.*, 1980b; Ohta *et al.*, 1980) in this regard.

We report here results indicating that T lymphocytes from patients with elevated serum IgE levels can spontaneously release soluble factors which possess the ability to stimulate IgE production *in vitro* by PBL from normal individuals and to potentiate the spontaneous IgE synthesis by PBL from patients with mild atopy.

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MATERIALS AND METHODS

Preparation of T cell supernatants (TCS). Mononuclear cells (MNC) were isolated from the peripheral blood of either normal subjects or patients with atopic dermatitis, some of whom showing elevated serum IgE levels, by Ficoll-Hypaque differential sedimentation. Purified T lymphocytes were prepared by a double E rosetting procedure with neuraminidase treated sheep red blood cells (SRBC) as previously reported (Romagnani *et al.*, 1980c). Five million purified T cells were cultured for 36 h at 37°C in 1 ml of RPMI 1640 tissue culture medium (DIFCO, Detroit, Michigan, USA), supplemented with 5% fetal calf serum (FCS; GIBCO, New York, USA). T cell supernatants (TCS) were collected by centrifugation, filtered through millipore filters and stored at −80°C until used.

Cell cultures. Cell suspensions enriched in B lymphocytes were prepared from MNC of four non-atopic subjects and four patients suffering from allergic rhinitis and/or bronchial asthma, who had been found sensitive to grass pollen allergens by skin testing and RAST, as detailed elsewhere (Romagnani *et al.*, 1980c). B cell rich suspensions were cultured for 7 days at 37°C in a humidified atmosphere of 5% CO_2 in air at the concentration of $0{\cdot}2 \times 10^6$/ml in RPMI 1640–10% FCS in the absence or in the presence of the appropriate dilution of TCS, which had been selected on the basis of previous titration experiments. Parallel 7 day B cell cultures were carried out in the presence of cycloheximide (Sigma; 100 μg/ml) to ascertain that immunoglobulins found 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, IgG and IgM protein. IgE protein was detected in reconstituted culture supernatants by a solid phase sandwich test previously described in detail (Romagnani *et al.*, 1980b, 1982a). IgG and IgM were measured by solid phase radioimmunoassays performed in microtitre plates. The wells of plates were filled with purified polyclonal IgG or IgM at a concentration of 10 μg/ml. After overnight incubation, the wells were washed and incubated again with 10% bovine serum albumin to saturate any remaining protein binding surface. The wells were then incubated overnight at 4°C with samples to be assayed and monospecific ^{125}I-labelled anti-γ or anti-μ chain antibodies, prepared in rabbit and purified as detailed elsewhere (Romagnani *et al.*, 1980a). After washings, the individual wells were cut apart and the bound radioactivity was determined. A 10 point standard curve of IgG and IgM was performed in parallel with the culture samples on each plate.

IgE, IgG and IgM protein were also measured in TCS and in the supernatants of B cell cultures carried out in the presence of cycloheximide.

RESULTS

Table 1 summarizes our findings. In absence of TCS, virtually no IgE was detected in 7 day cultures of B cell enriched suspensions from non atopic subjects, whereas *de novo* synthesized IgE was found in culture supernatants of B cell enriched suspensions from atopic donors. No detectable amounts of IgE were found in TCS from either normal subjects or patients with raised IgE. The addition of TCS from patients with high serum IgE levels enabled B cell suspensions from normal individuals to produce detectable amounts of IgE and potentiated the spontaneous IgE production *in vitro* by B cell suspensions from atopic patients. In contrast, the addition of TCS from subjects with low serum IgE levels did not increase or caused a fall in the amount of IgE synthesized by the B cell suspensions. When the same culture supernatants were analysed for their IgG content, no significant differences were observed irrespective of whether B cell enriched suspensions had been cultured with TCS showing stimulating or suppressive activity on IgE biosynthesis (Table 1). Likewise, no differences were found with regard to the production of IgM protein (data not shown). These results indicate that soluble factors are released by T lymphocytes from patients with elevated serum IgE levels, which possess IgE, but not IgG or IgM, potentiating activity.

Table 1. IgE and IgG production *in vitro* by B cell rich cultures from normal individuals and grass sensitive patients induced by TCS from patients with elevated serum IgE levels

TCS*	Serum IgE level† of TCS donor (IU/ml)	IgE production (pg/ml)‡ 'Normal' B cells	IgE production (pg/ml)‡ 'Patient' B cells	IgG production (ng/ml)‡ 'Normal' B cells	IgG production (ng/ml)‡ 'Patient' B cells
None	—	<50	301±97	110±17	105±25
T 52	11,000	184±64	856±93	98±28	107±12
T 55	5,200	214±96	786±62	79±18	121±29
T 46	2,200	194±68	389±83	102±25	106±21
T 53	980	<50	316±78	118±28	112±19
T 51	520	75±20	289±62	97±28	120±20
T 54	80	<50	300±88	99±25	89±27
T 85	60	<50	163±47	109±31	96±18
T 86	40	<50	100±32	117±48	102±33
T 87	40	<50	141±38	90±16	98±26

* Five million purified T cells were cultured for 36 h in 1 ml of RPMI 1640–5% FCS without stimulants. The cell suspension was centrifuged and the T cell supernatant (TCS) collected.

† IgE levels were measured in the serum of TCS donors by the radioimmunosorbent test (Phadebas IgE test, Pharmacia).

‡ B cell rich suspensions from four normal subjects and four grass sensitive patients were cultured for 7 days at the concentration of $0{\cdot}2 \times 10^6$/ml in RPMI 1640–10% FCS in the absence or in the presence of TCS (1/5 final dilution). IgE and IgG concentrations were measured in the supernatants by radioimmunoassays. The results represent the mean value (±s.e.) of the newly synthesized immunoglobulin (Ig) (difference between Ig amounts found in supernatants of cultures performed without cycloheximide and of parallel 7 day cultures containing 100 μg/ml of cyclohexamide).

DISCUSSION

The present findings are in agreement with the results of studies carried out in experimental animals, demonstrating that immunocompetent cells involved in the regulation of IgE response are different from those involved in the IgG response (Kishimoto & Ishizaka, 1973). They are also consistent with the observations showing production by T cells from rodents of soluble factors capable of enhancing IgE synthesis (Katz *et al.*, 1979; Suemura & Ishizaka, 1979; Hirashima, Yodoi & Ishizaka, 1981). In the animal models evidence was obtained that the IgE potentiating factor had affinity for IgE and its target was IgE bearing cells (Yodoi, Hirashima & Ishizaka, 1980). Interestingly, experimental immunization usually resulted in the formation of a mixture of IgE potentiating and IgE suppressive factors (Hirashima *et al.*, 1981). The IgE-binding affinity of the IgE potentiating factor(s) described in this paper was not investigated. However, TCS possessing IgE potentiating activity did not show any significant effect on the synthesis of immunoglobulins of the IgG or IgM class. Thus, although a potentiating effect on the production of IgA or of single IgG subclasses (i.e. IgG4) could not be at the present excluded, it is reasonable to suggest that T cells from patient with raised serum IgE levels are able to release factor(s) possessing isotype (IgE) specific potentiating activity. The results here reported also showed that TCS from subjects with low serum IgE levels possessed no or suppressive activity. Based on the findings in experimental animal models, we suggest that the activity of TCS from subjects with raised IgE reflects the prevalent production by their T cells of IgE potentiating factors, whereas T cells from non-atopic individuals

primarily produce factors exerting suppressive activity on the synthesis of IgE. Whether the exaggerated IgE antibody production, which represents the main characteristics of atopic patients, is the result of excessive production of IgE potentiating factors, reduced formation of IgE suppressive factors, or of both, remains matter for future investigations. Recently, a deficiency of T lymphocytes capable of suppressing the spontaneous IgE production *in vitro* has been reported in patients with hyperimmunoglobulin E (Geha *et al.*, 1981). However, the present demonstration that also in humans T cell derived factors showing regulatory activity on the IgE synthesis do, indeed, exist and are biologically active provides a potential avenue to explore as a means for manipulating IgE antibody responses in IgE-mediated allergic diseases.

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