

Suppression by relevant allergen of the spontaneous *in vitro* production of rye Group I specific IgE antibodies by human peripheral blood lymphocytes

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SUMMARY

The production *in vitro* of rye Group I specific IgE antibodies (rye I specific IgE Ab) by human peripheral blood lymphocytes (PBL) was examined. A sensitive enzyme-linked immunosorbent assay was developed which allowed the detection of rye I specific IgE Ab without interference from other immunoglobulin classes. A significant net synthesis of rye I specific IgE Ab by PBL from atopic subjects was detected only during the pollen season, which could not be observed with PBL from non-atopic donors whenever tested. This active synthesis *in vitro* of IgE antibodies by PBL from atopic patients in season was suppressed when PBL were pre-incubated with the relevant antigen. Furthermore, we are reporting data suggesting an inhibition of the passive release of preformed rye I specific IgE Ab from cells by the specific allergen.

Keywords specific IgE antibodies rye Group I suppression

INTRODUCTION

Atopic patients have an excessive production of specific IgE antibodies (Ab) which bind avidly to the mast cell surface and so are involved in the release of mediators responsible for allergic symptoms (Berg & Johanson, 1969; Ishizaka & Ishizaka, 1975). While trying to understand better the mechanisms regulating the IgE production and their putative defect in atopic patients, a number of investigators have reported a significant spontaneous synthesis *in vitro* of IgE protein by peripheral blood lymphocytes (PBL) from atopic donors (Saxon & Stevens, 1979; Urena *et al.*, 1979; Ohta *et al.*, 1980; Nonaka *et al.*, 1981; Fisher & Buckley, 1979; Romagnani *et al.*, 1980a; Sampson & Buckley, 1981; Hemady *et al.*, 1983; Turner *et al.*, 1985). Nevertheless, conflicting results were found regarding the ability of human PBL to produce IgE protein *in vitro* after stimulation with polyclonal agents (Patterson *et al.*, 1975; Buckley & Becker, 1978; Fisher & Buckley, 1979; Saxon, Marrow & Stevens, 1980; Romagnani *et al.*, 1980b; Turner, Holt & Holt, 1981). There is a growing body of evidence suggesting that IgE production is then controlled by suppressor T cell subsets (Saxon *et al.*, 1980).

However, allergic diseases are mediated by specific IgE Ab directed against relevant allergens which represent a portion of IgE protein. A deeper understanding of allergic diseases requires therefore a better knowledge of the regulation of production of specific IgE Ab. Assays sensitive enough to measure minute amounts of specific IgE Ab without interference from other classes of specific antibodies produced *in vitro* have lately become available.

Only a few studies reported a spontaneous synthesis *in vitro* of specific IgE Ab against mixed

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grass pollen extracts or ragweed (Tjio, Hull & Gleich, 1979; Romagnani *et al.*, 1980b; 1982) and its suppression in presence of free allergens. This study aims to assess the spontaneous production *in vitro* of purified rye I specific IgE Ab by PBL from atopic patients during and out of the pollen season. Also, the modulation of this synthesis by free specific allergen will be examined.

MATERIALS AND METHODS

Subjects

The allergic patients included in this study were selected from the allergy and immunology clinic of Centre Hospitalier de l'Université Laval. They had classical symptoms of allergic rhinitis to grass pollens and reactive skin tests to rye grass extracts. None of them had received any specific immunotherapy. Seventeen were studied in July during the pollen season and six of them were studied in December, out of the season. Six healthy non-atopic volunteers with no history of allergic rhinitis and negative skin tests were also studied.

Culture in vitro

Spontaneous synthesis of rye I specific IgE Ab. At the time of the study, serum was obtained for determining specific IgE Ab levels later and stored frozen until used. PBL were isolated by centrifuging over a density gradient as previously described (Hébert *et al.*, 1981). They were washed in RPMI 1640 (Gibco) containing 10% fetal calf serum, 1% L-glutamine, 100 U/ml of penicillin, and 100 µg/ml of streptomycin and resuspended in the same medium at a final concentration of 4×10^6 cells/ml. They were then cultured in 24-well flat-bottom microplates (Flow laboratories Inc.) (1 ml/well) in triplicate at 37°C, 7.5% CO₂. After the 7 day culture, cells were harvested and supernatants were lyophilized and reconstituted in 1/5 volume of PBS (pH 7.2, 50 mM) containing bovine serum albumin and 0.1% Tween-20. Viability was estimated by trypan blue exclusion test.

Quantification of preformed rye I specific IgE Ab. In order to assess the amount of preformed rye I specific IgE Ab, PBL were selectively cultured in the presence of cycloheximide (50 µg/ml) (Sigma, Chemical Co) as protein synthesis inhibitor (Hébert *et al.*, 1981). Preformed IgE Ab were also estimated by cell disruption as previously described (Sampson & Buckley, 1981). PBL were frozen and thawed until complete disruption, and cell supernatants were taken immediately at initiation of the culture (day 0) or after the 7 day culture to determine rye I specific IgE Ab.

Effect of addition of rye I allergen on specific IgE Ab production. To study the modulation of rye I specific IgE Ab production by the allergen, PBL were first incubated with various concentrations of rye I (10 or 1 ng/ml) for 6 h (Romagnani *et al.*, 1982), washed extensively and cultured for 7 days as described. In some experiments, PBL were incubated with ¹²⁵I-labelled rye I for 6 h, then washed and the radioactivity counted in a LKB Gamma counter.

Purification of rye I allergen

Rye I was purified by a reverse immunosorbent column as described previously (Mourad *et al.*, 1986). Briefly purified anti rye I monoclonal antibodies were crosslinked to cyanogen bromide-activated Sepharose CL4B beads (Pharmacia Fine Chemical, Sweden) using standard procedure (Cuatrecasas, 1970) and packed in a column; then crude extracts of rye grass were passed over the column and after extensive washing, the bound materials were eluted and dialysed.

Determination of specific IgE Ab levels

Phadezym Rast. Serum specific IgE Ab to rye grass extracts were measured by enzyme assay using cellulose filter paper discs (Phadezym RAST, Pharmacia). Briefly, 50 µl of serum were incubated with rye grass-coated discs for 3 h at 22°C. Unbound immunoglobulins were washed off with phosphate-buffered solution (PBS, pH 7.2, 100 mM) containing 0.1% Tween-20. The discs were further incubated with α-galactosidase labelled anti-human IgE for 18 h at 22°C. After incubation with substrate, the reaction was stopped and the absorbance was read at 415 nm with an automatic spectrophotometer (Titertek). The results were expressed in units (absorbance × (1/dilution)).

ELISA procedure. Rye I specific IgE Ab from serum and culture supernatants were measured by

ELISA (Mourad *et al.*, 1986). Briefly, 96-well microtitre plates were coated with purified rye I allergen (200 ng/well), saturated with 3% bovine serum albumin and then incubated with different dilutions of either the serum or the culture supernatants. After three washes, purified monoclonal anti-human IgE myeloma (UCB, DES_κ IgE_κ myeloma Belgium) antiserum (1:500) was added. This antiserum is an IgG₁ with κ light chain that reacts only with the thermostable part of the heavy chain of the IgE and not with purified IgM, IgG or IgA. Its dissociation constant value is of 3.8×10^{-9} mol/l. After washing, peroxidase-labelled anti-mouse IgG (1/1000) was added for 2 h, followed by the substrate (*o*-phenylenediamine 0.4 mg/ml and 0.006% H₂O₂). All incubations were performed at room temperature. The reaction was stopped 30 min later by the addition of H₂SO₄ 4 M and the absorbance was read at 492 nm with an automated spectrophotometer (Titertek). The results were expressed in units (absorbance $\times$ (1/dilution)). Positive and negative controls (pool of sera from atopic and non-atopic donors respectively) were run each time. In our hands, this assay had a good reproducibility with a coefficient of variation of less than 10%.

RESULTS

Comparison between phadezym RAST and ELISA for determining serum specific IgE Ab during pollen season. Specific IgE Ab could not be detected in sera from all non-atopic donors but were so in the sera of all atopic subjects. The levels measured ranged from 0.103 to 1.89 units with phadezym RAST and from 0.120 to 7.38 units for ELISA. The correlation between the two techniques for measurement of specific IgE Ab was highly significant (Fig. 1). Since ELISA detected a wider range of values and specific IgE Ab in culture supernatants (not shown) than phadezym RAST, it was used throughout the study.

Synthesis in vitro of rye I specific IgE Ab. Net synthesis of rye I specific IgE Ab *in vitro* could be seen with PBL from atopic patients when studied during pollen season (Table 1). The correlation between this net production *in vitro* and serum IgE Ab levels is weak but significant ($r=0.53$, $P<0.0125$, not shown). Six of these patients were studied out of the pollen season. Their net synthesis during the pollen season (0.358 ± 0.15 units) was not different from that of the whole group (0.33 ± 0.08 units). In this group of subjects, no significant net synthesis *in vitro* of IgE Ab was seen out of the pollen season (Table 2). On the other hand, PBL from non-atopic donors did not produce detectable amounts of IgE Ab *in vitro* whenever tested. Finally, IgE Ab levels measured in supernatants after complete cell disruption by successive cycles of freezing and thawing at the beginning or end of culture were always significantly lower than levels obtained in presence of protein inhibitors (not shown). This could be due to the fact that in some cases, 15 cycles of freezing and thawing were needed to disrupt the cells completely which could alter to a certain degree the binding site of the IgE Ab. Therefore, only IgE Ab detected in culture in presence of cycloheximide were taken into consideration as performed IgE Ab.

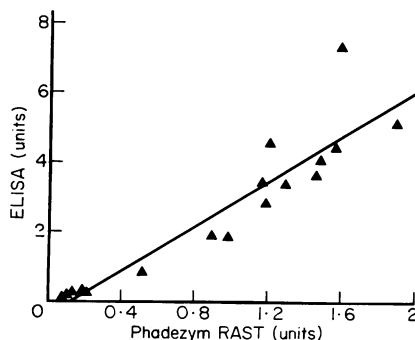


Fig. 1. Serum specific IgE antibody levels: phadezym RAST vs enzyme-linked immunosorbent assay (ELISA). Each point represents the mean value of three determinations. Units are defined as absorbance $\times$ 1/serum dilution. $r=0.82$, $P<0.0005$.

Table 1. Spontaneous synthesis of rye I IgE Ab during pollen season by PBL of non atopic and atopic patients

	No. of cases	IgE Ab in culture without protein inhibitor (units $\pm$ s.e.m./culture)	IgE Ab in culture with protein inhibitor (units $\pm$ s.e.m./culture)	Net IgE* Ab synthesis (units $\pm$ s.e.m./culture)
Atopic	17	0.56 $\pm$ 0.11† (0.01 – 1.42)‡	0.22 $\pm$ 0.04 (0 – 0.84)	0.33 $\pm$ 0.08 (0.01 – 1.2)
Non-atopic	6	ND§	ND	ND

* Net IgE Ab synthesis was calculated by subtracting the IgE Ab in culture with protein inhibitor from the IgE Ab in culture without protein inhibitor.

† Mean $\pm$ s.e.m. (cultures were done in triplicate).

‡ Extreme values are indicated in parentheses.

§ Not detectable.

Table 2. Effect of free allergen on specific IgE Ab production *in vitro* by PBL of atopic patients

	Levels (units $\pm$ s.e.m.) of IgE Ab in presence of free allergen		
	0	1 ng/ml	10 ng/ml
During season (17 subjects)			
IgE Ab in culture without protein inhibitor	0.56 $\pm$ 0.11†	0.31 $\pm$ 0.09 (40.7 $\pm$ 8.56)‡	0.28 $\pm$ 0.06 (41.2 $\pm$ 4.85)
Net IgE Ab* synthesis	0.33 $\pm$ 0.08	0.12 $\pm$ 0.07 (79.5 $\pm$ 3.4)	0.05 $\pm$ 0.07 (92.9 $\pm$ 20.8)
Out of season (six subjects)			
IgE Ab in culture without protein inhibitor	0.43 $\pm$ 0.08	0.13 $\pm$ 0.02 (71.9 $\pm$ 3.4)	0.08 $\pm$ 0.06 (79.2 $\pm$ 4.8)
Net IgE Ab synthesis	ND§	—	—

* Net IgE Ab synthesis was calculated by subtracting the IgE Ab in culture with protein inhibitor from the IgE Ab in culture without protein inhibitor.

† Mean $\pm$ s.e.m. (cultures were done in triplicate).

‡ % of suppression = $\left[\left(1 - \frac{\text{IgE Ab in presence of allergen}}{\text{IgE Ab in absence of allergen}} \right) \right] \times 100$.

§ Not detectable.

Effect of rye I on spontaneous synthesis in vitro of specific IgE Ab. The net synthesis *in vitro* of specific IgE Ab measured during the pollen season in atopic patients was almost completely abolished by preincubation of cells with two concentrations (10 and 1 ng/ml) of rye I (Table 2). This inhibition was not due to cell death since the viability of PBL after the incubation with rye I was over 95% (not shown). This phenomenon was not explained either by an interference on the measurement of specific IgE by free allergen carried over in the culture since no radioactivity could be detected on the cells under our experimental conditions when ^{125}I labelled rye I was used for preincubation (not shown).

No net synthesis of IgE Ab was observed out of the pollen season but IgE Ab measured in absence of cycloheximide were significantly reduced when PBL were preincubated with free antigen before culture (Table 2).

DISCUSSION

Serum specific IgE Ab to grass pollens represent a fraction of total IgE protein. They reach their peak at the end of pollination period after maximal pollen exposure and persist for several months before declining to a minimum before the following pollen season (Gleich & Jacob, 1975; Henderson, Larson & Gleich, 1975; Gleich *et al.*, 1977). Among these pollens, rye grass is an important source for airborne allergic diseases and rye I is the major allergenic component of it (Marsh, 1975; Mourad *et al.*, 1986). A number of investigations (Stenuis, Wide & Seymour, 1971; Norman, Lichtenstein & Ishizaka, 1973; Beaulieu *et al.*, 1986) have shown that the clinical symptoms and skin sensitivity to allergen correlate with the serum level of specific IgE Ab.

In the present study, determination of levels of rye I specific IgE Ab *in vitro* was done by ELISA because it showed a wider range of values than Phadezym RAST and still correlated very well with the latter ($P < 0.0005$). The results reported show net production *in vitro* of rye I specific IgE Ab by PBL from grass sensitive patients and only during the pollen season. This synthesis *in vitro* correlated to some degree with serum levels. A subgroup of six patients had a net synthesis comparable to the whole group during the season but no significant net IgE Ab production could be seen out of the season. In contrast, PBL from normal volunteers did not produce any detectable specific IgE Ab at any time they were tested. These data agree with those reported by Romagnani *et al.* (1982) in grass sensitive patients and Gleich (1981) in ragweed sensitive subjects. They both showed synthesis of grass and ragweed specific IgE Ab *in vitro* during or immediately after the pollen season. Together these observations suggest that a net synthesis of specific IgE Ab by PBL *in vitro* is possible only when cells are obtained during the pollination period when naturally stimulated by the allergen. Such secreting cells are probably released into the systemic circulation, most likely from the respiratory tract, upon active stimulation from the environment and they can continue to produce IgE Ab for a short time. But after the season, these cells will eventually cease to form IgE Ab; moreover, no newly stimulated secreting cells will be released from the respiratory tract. However, cells will continue to have preformed IgE Ab in their cytoplasm which, since they seem to be long-lived cells, may be an explanation for the persistence of cells with preformed IgE Ab out of the pollen season.

The results reported here show also that pre-incubation of PBL with the relevant allergen inhibits the spontaneous synthesis *in vitro* of specific IgE Ab. They confirm previous reports showing inhibition of synthesis *in vitro* of specific grass IgE Ab by rye I in patients allergic to grasses (Romagnani *et al.*, 1982) and by ragweed in ragweed-sensitive subjects (Perelmutter *et al.*, 1981). The suppression of this IgE Ab production could not be explained by carry over of antigen and interference in the assay for measuring IgE Ab synthesis. It is tempting to speculate that the inhibitory signal of the allergen is acting at the B cell level since the suppressive effect of the allergen on IgE Ab synthesis is maintained after T cell depletion (Romagnani *et al.*, 1982). Furthermore, a recent study (Urena *et al.*, 1985) seems to suggest that pre-incubation of PBL with the allergen inhibited more the spontaneous synthesis of IgE protein than its passive release. However, pre-incubation with the allergen (Rye I) of PBL obtained out of the pollen season, when no active synthesis could be detected, also decreased significantly the rye I specific IgE Ab measured in culture supernatants. This finding suggests an inhibition by free allergen of release of preformed IgE Ab as well. It is not clear whether or not this inhibition is mediated by activation of suppressor T cells or inactivation at the B cell level. Further studies are in progress to understand this phenomenon better.

In summary, active IgE Ab synthesis to rye I by PBL of grass sensitive patients could be observed only during the pollination period and correlation between this active synthesis and serum IgE Ab is significant. The pre-incubation of cells with specific allergen inhibits both IgE Ab synthesis and perhaps its release.

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