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In vitro production of IgE by human peripheral blood mononuclear cells. IV. Modulation by allergen of the spontaneous IgE antibody biosynthesis

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SUMMARY

Peripheral blood lymphocytes (PBL) from a proportion of grass-sensitive patients, studied during or immediately after the grass pollination period, showed spontaneous production *in vitro* of grass-specific IgE antibody, whereas PBL from atopic patients sensitive to allergens other than grass pollens or non-atopic individuals did not. Pre-incubation of IgE antibody producing PBL from grass-sensitive patients with minute amounts of a mixed grass pollen (MGP) extract or Rye grass antigen Group I (Rye I) usually resulted in a reduction of the spontaneous production *in vitro* of IgE protein and in a marked inhibition of the spontaneous production *in vitro* of grass-specific IgE antibody. This antigen-specific inhibition was not mediated by T lymphocytes, but it was apparently due to a signal directly delivered by antigen to the spontaneously IgE antibody producing cells. The results support the concept that the activity of cells responsible for the persistent IgE antibody formation *in vitro* in atopic patients can be modulated by antigen.

INTRODUCTION

Recent studies have demonstrated the production of IgE protein by peripheral blood lymphocytes (PBL) *in vitro* and have examined the kinetics and control mechanisms of IgE biosynthesis (Buckley & Becker, 1978; Fiser & Buckley, 1979; Saxon & Stevens, 1979; Saxon, Kaplan & Stevens, 1980a; Saxon, Morrow & Stevens, 1980b; Romagnani *et al.*, 1980a, 1980c). IgE antibody biosynthesis *in vitro* was reported by Tjio, Hull & Gleich (1979) using PBL from patients allergic to grass and ragweed. We also showed *in vitro* production of grass-specific IgE antibody by PBL from grass-sensitive patients, studied during or immediately after the grass pollen season (Romagnani & Ricci, 1979; Romagnani *et al.*, 1980c). More recently, *in vitro* production of anti-mite IgE antibody by PBL from mite-sensitive patients has also been reported (Ohta *et al.*, 1980; Okudaira *et al.*, 1981).

In the accompanying paper (Romagnani *et al.*, 1982) evidence was presented that a DR^+ IgE^+ IgM^- circulating B cell is primarily implicated in the spontaneous production of IgE protein *in vitro* in atopic individuals. This cell shows similarities with the long-lived IgE antibody forming cell involved in persistent antibody formation in high responder mice (Okudaira & Ishizaka, 1981).

The experiments here reported were undertaken in order to evaluate the effect of interaction with grass allergens on the ability of IgE forming cells to produce IgE protein and grass-specific IgE antibody *in vitro*.

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MATERIALS AND METHODS

Cell donors. Blood was obtained from grass-sensitive patients examined at the Service of Allergology and Clinical Immunology of Florence in the last 3 years. All patients had rhinitis and/or bronchial asthma. None of the patients included in the study had received immunotherapy.

Allergenic extracts. A mixed grass pollen (MGP) extract obtained from the 10 most common grass species in Italy was a generous gift of Lofarma (Milan). A *Dermatophagoides pteronyssinus* (mite) extract was purchased from Diephuis (Groningen). Rye grass antigen Group I (Rye I) and ragweed antigen E (AgE) were kindly provided by National Institute of Allergy and Infectious diseases (Bethesda, Maryland, USA). Rye I was labelled with ^{125}I according to the technique described by Platts-Mills (1979).

Preparation of cell suspensions and fractionation procedures. The preparation of mononuclear cells (MNC) and fractionation procedures have been described in the accompanying paper (Romagnani *et al.*, 1982).

Cell cultures. The technique of cell culture has been detailed in previous papers (Romagnani *et al.*, 1980a, 1980c, 1982).

Measurement of IgE protein. IgE protein was detected in culture supernatants by a solid phase sandwich test, described in the accompanying paper (Romagnani *et al.*, 1982).

Preparation and purification of grass-specific IgE antibody. Five hundred millilitres of pooled sera from 100 grass-sensitive patients were passed through a column of CNBr-activated Sepharose CL-4B (Pharmacia, Uppsala, Sweden), sensitized with anti-ε immunosorbent purified rabbit antibodies (10 mg of protein/1 gr of Sepharose) (Romagnani *et al.*, 1982). After extensive washings with phosphate-buffered saline, pH 7·2 (PBS), IgE protein was eluted with 0·2 M glycine-HCl buffer, pH 2·5. Grass-specific IgE antibodies were then isolated by passing the purified IgE protein solution through a column of CNBr-activated Sepharose CL-4B sensitized with a MGP extract (10 mg of dried powder/1 gr of Sepharose), followed by extensive washings with PBS and elution with the glycine-HCl buffer. The amount of grass-specific IgE present in the solution was evaluated by radioimmunoassay, using a purified IgE myeloma protein as a standard (Romagnani *et al.*, 1982).

Measurement of grass-specific IgE antibody. Grass-specific IgE antibody was measured by a calibrated radioallergosorbent test (RAST), as previously reported (Romagnani *et al.*, 1980c). Briefly, CNBr-activated paper discs were coated with MGP extract and incubated for 48 hr with concentrated cell culture supernatants. The discs were washed and incubated with ^{125}I-labelled immunosorbent purified rabbit anti-IgE for an additional 48 hr. After additional washings, the discs were counted in a gammacounter. Simultaneous standard curves were always prepared using immunosorbent purified grass-specific IgE antibody.

Assay for DNA synthesis. Assay for DNA synthesis was performed as detailed elsewhere (Romagnani *et al.*, 1980b). Briefly, cells were cultured for 6 days in microplates and pulsed for 16 hr before harvesting with 0·5 μCi of ^{3}H-thymidine. Cultures were harvested by a multiple harvesting machine (Skatron, Lierbyen, Norway) and incorporation of ^{3}H-thymidine was measured in a Packard scintillation counter.

RESULTS

Production of grass-specific IgE antibody by MNC from grass-sensitive patients

Seven day culture supernatants from MNC of grass-sensitive patients studied immediately after or during the grass pollen season usually contained detectable amounts of grass-specific IgE antibody. In most cases the IgE antibodies measured at the end of culture period represented *de novo* synthesis *in vitro* since their concentration was greater than in parallel cultures in which an inhibitor of protein synthesis (cycloheximide, 100 μg/ml) was added. When MNC from patients were tested during the winter, detectable amounts of grass-specific IgE antibody were found in culture supernatants from very few grass-sensitive patients (Fig. 1). This finding was consistent in studies performed in more than 150 grass-sensitive patients during three consecutive years (1979, 1980, 1981). Synthesis of

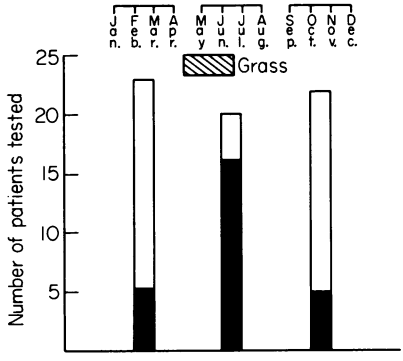

Fig. 1. Seasonal variation of spontaneous production *in vitro* of grass-specific IgE antibody. Columns represent the number of patients tested. The black part of the column represents the number of patients whose MNC were able to release in 7 day culture supernatants an amount of grass-specific IgE antibody at least two times greater than that found in parallel cultures performed in the presence of cycloheximide (100 μg/ml). (▨ = grass pollination in Florence).

grass-specific IgE antibody was never observed in cultures of MNC from non-atopic individuals or patients sensitive to allergens other than grass pollens.

Grass-induced inhibition of the spontaneous production in vitro *of grass-specific IgE antibody*
To evaluate whether spontaneous production *in vitro* of grass-specific IgE antibody could be influenced by the specific allergen, MNC from seven grass-sensitive individuals were incubated for 24 hr with different concentrations of MGP extract or Rye I. For control, the cells were also incubated with unrelated allergens, such as mite extract or AgE. The cells were extensively washed and cultured in fresh medium for an additional 6 days. The amount of IgE protein and grass-specific IgE antibody released in the supernatant of these cultures was then evaluated. As shown in Fig. 2, a marked inhibition of the spontaneous production of grass-specific IgE antibody was found in cultures pre-incubated with either MGP extract or Rye I, but not in those pre-incubated with mite extract or AgE. A partial inhibition of the spontaneous production of IgE protein in supernatants of

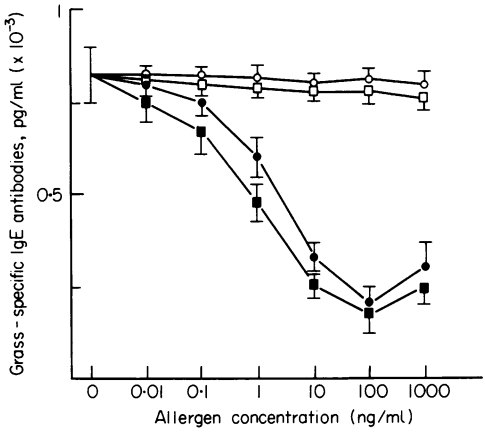

Fig. 2. Inhibition of the spontaneous production *in vitro* of grass-specific IgE antibody induced by MGP extract and Rye I. MNC from grass-sensitive patients were incubated for 24 hr with different concentrations of MGP extract (●), Rye I (■), mite extract (□) or AgE (○), extensively washed and then cultured for an additional 6 days. The amount of grass-specific IgE antibody was measured in the culture supernatant by RAST. The mean values (±s.e.) of 15 separate experiments are reported.

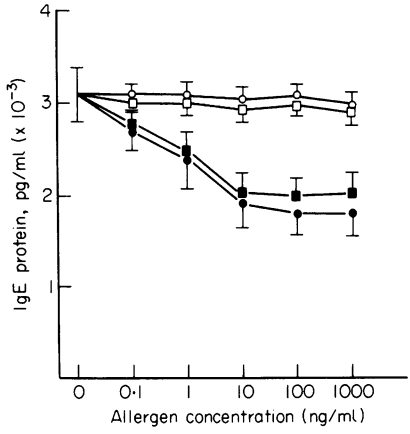

Fig. 3. Inhibition of the spontaneous production *in vitro* of IgE protein induced by MGP extract and Rye I. MNC from grass-sensitive patients were incubated for 24 hr with different concentrations of MGP extract (●), Rye I (■), mite extract (□) or AgE (○), extensively washed and then cultured for an additional 6 days. The mean values (±s.e.) of 10 separate experiments are reported.

MGP extract or Rye I pre-incubated cultures compared with that of mite extract or AgE pre-incubated cultures was also observed (Fig. 3). The reduction of IgE antibody concentrations found in allergen pre-incubated cultures was not due to a saturation of released IgE by residual allergen present in the supernatant. In fact, the last washing supernatant did not show any inhibitory effect in the RAST assay. On the other hand, no radioactivity was recovered on cells pre-incubated with ^{125}I-Rye I after washings and before culturing in fresh medium.

In another series of experiments the incubation time necessary to achieve inhibition of the spontaneous production *in vitro* of grass-specific IgE antibody was evaluated. For this purpose, MNC from three grass-sensitive patients were incubated for different periods of time (12, 24, 48 and 72 hr) with 1 μg of MGP or mite extract, extensively washed and then tested for ability to produce grass-specific IgE antibody in 7 day culture supernatants. As shown in Fig. 4, a 12 hr incubation

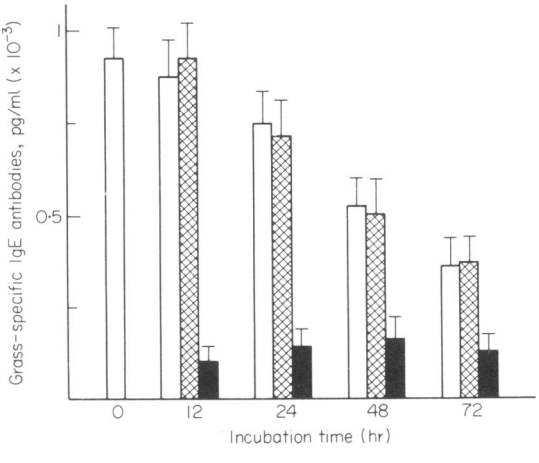

Fig. 4. Effect of incubation time on the inhibitory activity of MGP extract on the spontaneous production of grass-specific IgE antibody by MNC from grass-sensitive patients. MNC were incubated for 12, 24, 48, or 72 hr with medium (□), MGP (■) or mite (▩) extract, washed and cultured for additional 156, 144, 120 and 96 hr, respectively. The amount of grass-specific IgE antibody found in the supernatants was measured by RAST. The mean values (±s.e.) of four separate experiments are reported.

Table 1. Effect of pre-incubation with MGP extract on DNA synthesis by PBL from grass-sensitive patients*

Exp. No.	Incubation with	DNA synthesis (c.p.m.)	IgE detected in 7 day supernatants: IgE protein (pg/ml)	IgE detected in 7 day supernatants: Grass-specific IgE antibody (pg/ml)
1	Medium alone	1,451	1,400	610
	MGP (1 μg/ml)	6,127	625	< 50
2	Medium alone	912	2,400	400
	MGP (1 μg/ml)	3,971	2,000	100

* Mononuclear cells were incubated for 12 hr with medium alone or MGP extract (1 μg/ml), washed thrice and then cultured for 6 days. Sixteen hours before harvesting, cultures were pulsed with ^{3}H-thymidine. Results are expressed as mean value of c.p.m. obtained in triplicate cultures. The amount of IgE protein and grass-specific IgE antibody found in 7 day supernatants of parallel cultures was also evaluated.

period with MGP extract was sufficient to induce a marked reduction of the spontaneous production of grass-specific IgE antibody, whereas incubation with mite extract had no effect. To exclude the possibility that MGP extract was toxic, the ability of MNC which had been pre-incubated with MGP extract, to synthesize DNA was also evaluated. After incubation for 12 hr with MGP extract, washed MNC were still able to incorporate significant amounts of ^{3}H-thymidine (Table 1).

Grass-induced inhibition of the spontaneous production in vitro *of grass-specific IgE antibody is not mediated by T lymphocytes*

To establish whether or not the inhibitory activity of MGP extract was mediated by T lymphocytes, the effect of incubation with MGP extract on the spontaneous production of grass-specific IgE antibody by MNC before and after depletion of E-rosette forming cells (T cells) was evaluated. As shown in Fig. 5, exhaustive depletion of T lymphocytes did not influence the inhibitory activity exerted by MGP extract on the spontaneous production *in vitro* of grass-specific IgE antibody.

In another series of six experiments, purified suspensions of T lymphocytes were incubated for 24 hr with MGP extract or medium, washed and then added to suspensions of autologous non-T cells, and cultured for an additional 6 days. The amount of grass-specific IgE antibodies found in supernatant of cultures containing grass pre-incubated T cells (638 ± 145 pg/ml) was not significantly different from that found in cultures containing T cells which had not been pre-incubated with MGP extract (680 ± 158 pg/ml).

DISCUSSION

The development of new diagnostic procedures in allergology has shown that in the serum of pollen-sensitive patients IgE antibodies can account for high percentages of the total IgE protein (Gleich & Jacob, 1975). IgE antibody serum levels peak immediately after the maximal pollen exposure and then decline until the following pollen season (Henderson, Larson & Gleich, 1975; Gleich *et al.*, 1977). However, the reasons why IgE antibody levels persist so high for several months after pollen exposure in atopic subjects, while non-atopic individuals fail to produce detectable amounts of IgE antibodies in response to pollen inhalation are at present unclear.

The present experiments showed spontaneous *in vitro* production of grass-specific IgE antibody by PBL from grass-sensitive patients studied during or immediately after the grass pollination

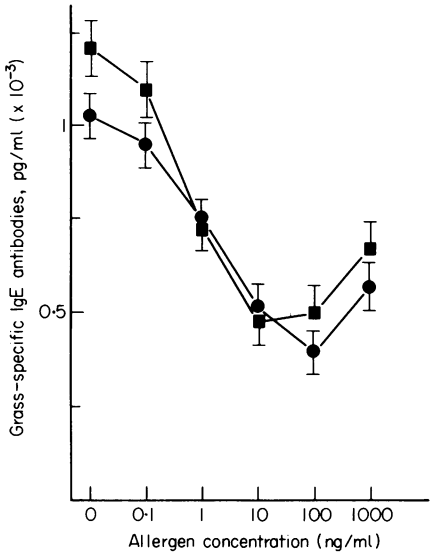

Fig. 5. Effect of T cell depletion on the inhibitory effect of MGP extract on the spontaneous production *in vitro* of grass-specific IgE antibody. MNC from grass-sensitive patients were incubated for 24 hr with MGP extract prior to and after exhaustive depletion of T lymphocytes by a double E-rosetting procedure with neuraminidase-treated SRBC and centrifugation on density gradient. The amount of grass-specific IgE antibody found in the supernatants of cultures from unfractionated (●) or T cell depleted (■) suspensions was measured by RAST. The mean values (±s.e.) of 12 separate experiments are reported.

period. In contrast, no production of grass-specific IgE antibody was found in non-atopic subjects or patients sensitive to allergens other than grass pollens, even though they were examined during or immediately after pollen exposure. These data are in agreement with those reported by Gleich (1981), who showed little or no *in vitro* production of ragweed IgE antibody in patients studied prior to the ragweed pollination period, whereas a peak of ragweed IgE antibody production occurred during pollination (mid-September), decreased by mid-October and was gone by early December. They are also consistent with the findings of Ghory *et al.* (1980), showing that PBL of allergic bronchopulmonary aspergillosis (ABPA) patients in remission either with modest increases in serum IgE or with greater increases in serum IgE did not form IgE *in vitro* significantly different than normals, whereas during an acute exacerbation of ABPA PBL formed significantly larger amounts of IgE than normals, ABPA in remission or PBL from the same patient in remission (Ghory *et al.*, 1980). Thus, cells responsible for exaggerated and persistent IgE antibody production in atopic patients appear to be released into systemic circulation, most likely from the sites of preferential IgE synthesis, such as respiratory tissues and gut.

Recent evidence suggests that spontaneous IgE production *in vitro* is primarily due to cells bearing DR antigens and surface IgE, but lacking surface IgM (Romagnani *et al.*, 1982). On the other hand, it had previously been shown that spontaneously IgE producing cells *in vitro* are radio- as well as mitomycin C-resistant (Romagnani *et al.*, 1980c; Iwamoto *et al.*, 1980; Okudaira *et al.*, 1981) and their ability to produce IgE protein can be inhibited by the addition in culture of T lymphocyte or pokeweed mitogen (Fiser & Buckley, 1979; Tjio *et al.*, 1979; Romagnani *et al.*, 1980a; Saxon *et al.*, 1980b). Some similarities between the spontaneously IgE producing cell present in the blood of atopic patients and that responsible for the persistent antibody formation in high responder mice (Okudaira & Ishizaka, 1981) suggest that atopic patients may have long-lived IgE antibody forming cells which are involved in the persistent antibody production.

The results reported in this paper show that spontaneous production of grass-specific IgE antibody *in vitro* can be inhibited by incubation of cells with a crude grass pollen extract or Rye grass antigen Group I. The inhibition was antigen-specific and it was not due to technical artifacts,

such as saturation of released IgE by residual amounts of the specific allergen present in the supernatant or absorbed on the cell membranes. Nor could it be related to a cytotoxic effect of grass pollen extract. Thus, it may be reasonable to assume that incubation of cells, even for a short time period, with very small amounts of the allergen results in a specific reduction or abrogation of the spontaneous production of IgE antibody *in vitro*. These data are in agreement with the findings of Iwamoto *et al.* (1980) and Perelmutter *et al.* (1981), who also reported inhibition of the spontaneous production *in vitro* of mite- or ragweed-specific IgE antibody induced by incubation of cells with minute amounts of mite antigen or ragweed AgE, respectively. In addition, we were also able to demonstrate that the inhibitory activity induced by allergen(s) is apparently not mediated by T lymphocytes. In fact, it was still demonstrable after exhaustive depletion of E-rosette forming cells and could not be induced by the addition to B cells of allergen pre-incubated autologous T lymphocytes. Thus, it is reasonable to suggest that the inhibitory signal by allergen is mediated by cells other than T lymphocytes or, alternatively, it is directly delivered to the IgE producing cell. The latter possibility is consistent with the findings of Schrader & Nossal (1974) and Okudaira & Ishizaka (1981). Schrader & Nossal (1974) showed that the production of anti-dinitrophenyl (DNP) antibody by plaque forming cells can be specifically inhibited by exposure of the cells *in vivo* or *in vitro* to multivalent DNP conjugates of polymeric flagellin and human gammaglobulin. This inhibitory mechanism has been termed effector cell blockade (Schrader & Nossal, 1974; Klaus & Humphrey, 1974). More recently, it has been shown that multivalent DNP-heterologous carrier conjugates are able to inactivate long-lived anti-DNP antibody forming cells for IgG and IgE isotypes (Okudaira & Ishizaka, 1981). If the inhibitory activity exerted *in vitro* by allergenic extracts on IgE producing cell reflects an effector cell blockade like phenomenon, this is additional evidence to support the similarity between the spontaneously IgE producing cell which has been found to circulate in the blood of atopic patients and that responsible for persistent IgE antibody formation in high responder mice.

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