

Seasonal variation of IgE synthesis *in vitro* by human peripheral blood mononuclear cells

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Seasonal variations in IgE antibody synthesis *in vitro* were studied in cultures of blood mononuclear cells (MNC) from 11 pollen allergic individuals. The IgE levels were significantly higher in two summer seasons than in the winter and spring between them. Net synthesis was confined to the summer in all but one of the patients. All the IgE in the cultures outside the pollen season represented preformed IgE which was present mainly (59%) in the monocyte fraction. Thus, preformed IgE seems to persist in monocytes at times when there is little *de novo* synthesis of IgE.

Key words: allergy; IgE; *in vitro*, seasonal variation

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Increased IgE antibody formation is a hallmark of atopic allergy. Over the past few years much has been learnt about the immunological mechanisms controlling IgE synthesis in experimental animals and IgE antibody formation *in vitro* by human cells. It has been shown that peripheral mononuclear cells (MNC) from at least some atopic individuals with increased serum IgE levels have the capacity to synthesize IgE *in vitro* (4-7, 9). In contrast, MNC from non-atopic donors with normal IgE levels cannot easily be stimulated to make IgE (4-7, 9).

In this study, we have analysed, prospectively, over 1 year the IgE antibody formation *in vitro* by MNC from pollen allergic individuals, comparing MNC obtained during the peak of the pollen season and during other seasons when the patients have been symptom-free for several months.

MATERIAL AND METHODS

Patients

A total of 11 pollen allergic adolescents aged 12-19 years and nine healthy non-atopic con-

trols of similar age were studied. The allergic patients all had a history of allergic rhinoconjunctivitis to grass pollen and most of them also to deciduous trees, but no history of allergic symptoms outside the pollen season. The diagnosis was confirmed by skin prick tests (PhazetTM, Pharmacia, Uppsala, Sweden). Their medication during the pollen season included locally administered sodium cromoglycate and in some cases the antihistamine drug terfenadine, but no corticosteroids. None of them had had any medication for at least 2 months at the time of blood sampling outside the pollen season.

Cell cultures

Heparinized blood was obtained in July 1987 and 1988, i.e. during two grass pollen seasons, in the autumn (October 1987), in the winter (December 1987) and early spring (March and April 1988). Mononuclear cells were isolated by Ficoll-Paque (Pharmacia AB, Uppsala, Sweden)

Table 1

Seasonal variations in IgE (ng/ml) levels in cultures of MNC from atopic individuals.
The table shows total levels and net synthesis

Patient No. and serum IgE (kU/l)	Summer		Winter		Spring		Summer	
	Total	Net	Total	Net	Total	Net	Total	Net
1. 1400	4.0	3.0	4.8	2.3	4.4	1.6	-	-
2. 150	1.5	0.7	0.5	<0.1	0.2	<0.1	2.3	0.2
3. 500	2.9	2.1	0.8	<0.1	0.5	<0.1	1.0	4.0
4. 260	2.2	1.9	1.0	<0.1	0.9	<0.1	1.2	0.4
5. 280	2.0	1.7	0.4	<0.1	0.5	<0.1	1.0	0.6
6. 280	5.3	4.7	0.7	<0.1	0.5	<0.1	1.7	1.3
7. 1100	4.2	3.1	0.8	<0.1	0.8	<0.1	-	-
8. 130	1.3	0.6	1.0	<0.1	0.4	0.1	1.8	<0.1
9. 330	5.5	5.2	1.0	<0.1	-	-	-	-
10. 700	1.6	0.6	1.4	<0.1	1.5	<0.1	-	-
11. 140	4.6	2.0	1.2	0.2	0.4	<0.1	0.9	0.9

gradient centrifugation under sterile conditions. The cells were washed in Hanks' balanced solution (HBSS) and then in RPMI 1640 (Dutch modification, Flow laboratories, UK) and resuspended in RPMI 1640 with 10% heat inactivated fetal calf serum, penicillin 100 µ/ml, streptomycin 50 µg/ml and 2 mM L-glutamine.

In some experiments T- and B-lymphocyte enriched cell populations were obtained by a rosetting technique using 2-amino-isothronium bromide hydrobromide (Sigma Chemical Co., St Louis, Mo.) treated sheep red blood cells (8). Mononuclear phagocytic cells were isolated from the MNC suspensions by adherence to plastic in tissue culture flasks (No. 3002, Falcon, Oxnard, CA) pretreated for 30–60 min at 4°C with a medium consisting of 50% RPMI and 50% AB negative serum (2). After rinsing the flasks MNC suspensions in RPMI with heat inactivated (56°C, 30 min) AB negative serum at a concentration of 10% were added and incubated at 37°C for 1–2 h. The flasks were vigorously rinsed with medium to remove non-adherent cells. Cold RPMI with 5% AB negative serum and 0.2% EDTA was added and the flasks were held for 20–30 min at 4°C. The macrophages were then removed by shaking and repeated pipetting, washed twice and counted.

The cell cultures, containing 1.5–2 ml volumes of the cell suspensions, were placed in 16

mm diameter flat bottom tissue culture wells (Costar 3524, Cambridge, MA) and were cultured for 7 days at 37°C in a humidified incubator containing 5% CO₂. The concentration of cells was 1 × 10⁶/ml in the T-lymphocyte suspensions.

Determination of IgE antibodies

The serum IgE levels of the MNC donors were determined with the Phadebas IgE PRIST® (Pharmacia Diagnostics AB, Uppsala, Sweden) as recommended by the manufacturer. For the determination of supernatant IgE a modified IgE PRIST assay was employed (4). Briefly, standard curves were obtained by diluting the commercial standard at a concentration of 20 kU/ml with medium to 2 kU/l, corresponding to approximately 4.8 ng IgE/ml. A reference curve was then obtained by diluting the standard to 2.4, 1.2, 0.6, 0.12 and 0.06 ng/ml. The paper discs were placed in polystyrene tubes with round bottoms and 300 µl of the standards or culture supernatants were added to the tubes. Incubation was then carried out at room temperature for 20 to 24 h under continuous shaking at 300 rpm. The tubes were washed three times with 0.9% NaCl and 0.5% Tween 20 with a dispenser aspirator DA 300 (Pharmacia Diagnostics, Uppsala, Sweden). For the second incuba-

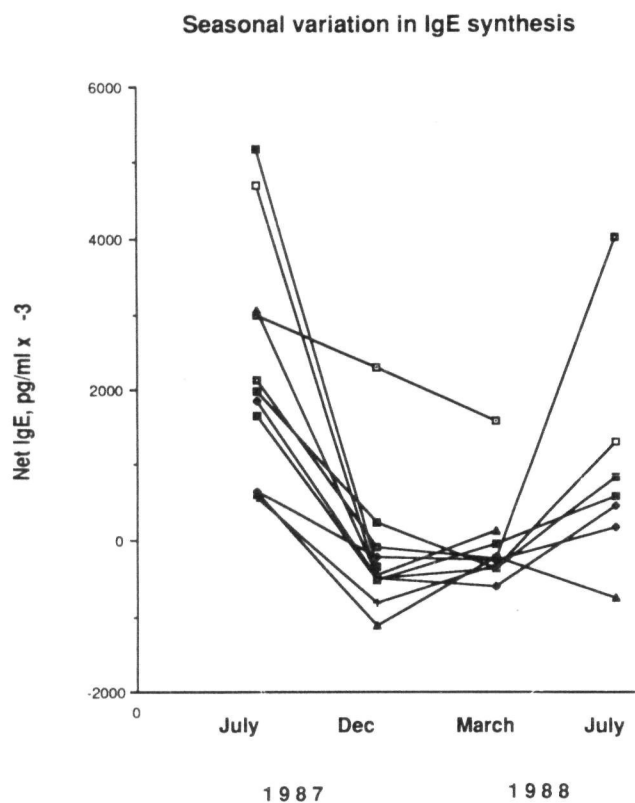


Fig. 1. Net IgE synthesis (pg/ml) in MNC cultures from 11 pollen allergic patients during and outside pollen season.

tion, Phadebas RAST tracer was diluted four times with phosphate buffered saline containing 0.1% Tween 20, and 200 µl of diluted tracer added to each tube. The tubes were again continuously shaken for 20–24 h at room temperature and then washed with 0.9% NaCl and 0.5% Tween 20 three times. The radioactivity in the tubes was counted in a gamma counter for 10 min, or until a 2% error level was reached. The IgE levels were then calculated from the reference curve, plotted on log-log paper.

Differentiation of net synthesis of IgE in the cultures from preformed IgE present in the cell cultures from the beginning was done by acid extraction as recommended by Turner et al. (10). Briefly, cell pellets were adjusted to pH 2.0–2.5 with 1-N HCl thoroughly mixed and then immediately returned to pH 7.4 with 1-N NaOH. Synthesis of IgE in the cultures was then calculated according to the formula: Net IgE synthesis = IgE level in supernatant on day 7 + IgE level in cell pellet acid treated on day 7 – IgE in acid treated cells day 0.

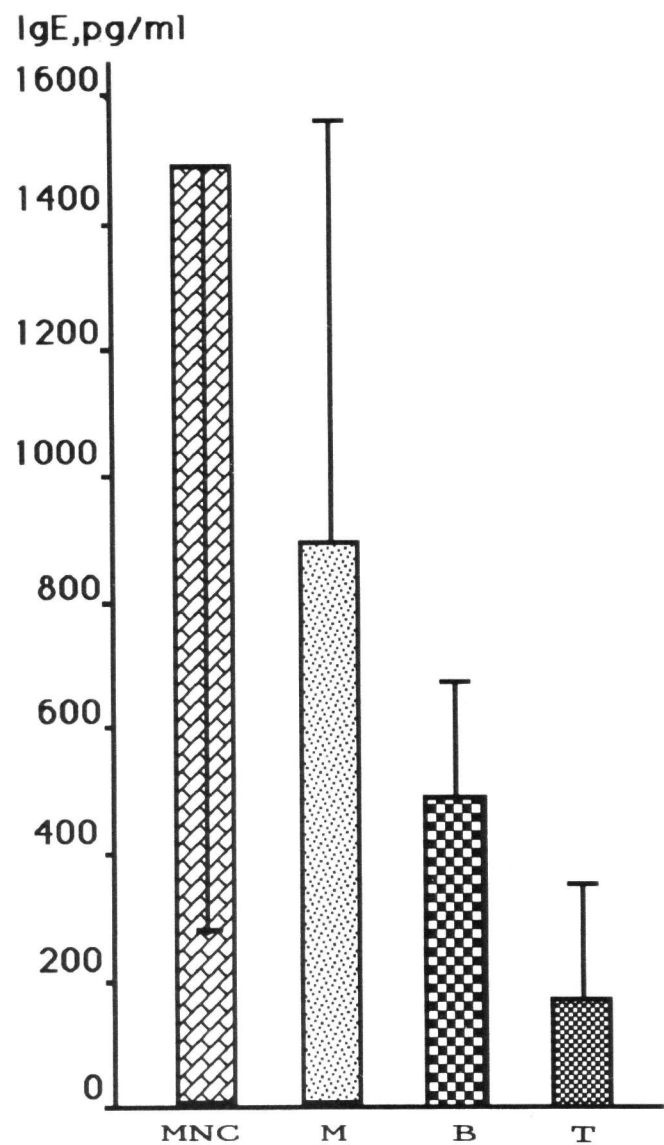


Fig. 2. IgE (mean and SD) after acid extraction in cultures of mononuclear cells (MNC) and enriched subpopulations of monocytes (M), B lymphocytes (B) and T lymphocytes (T).

IgE antibodies against timothy and birch pollen was determined by a modified procedure of the Phadesbas RAST by which the sensitivity was increased to 0.01 Phadebas RAST units (3).

Statistical methods. The cell culture experiments and IgE determinations were done in duplicate. The mean values after exclusion of occasional outliers were then used for the calculations. Wilcoxon two-sample test and sign test were employed for the statistical analysis.

Ethics. The study was approved by the Human Research Ethics Committee, Linköping University.

RESULTS

As shown in Table 1 the IgE levels in MNC cultures obtained from pollen allergic patients in the summer were higher than in cultures of cells obtained from the same patients in other seasons in all but one of the individuals. In none of the cultures of cells from the nine non-allergic individuals was the IgE above 200 pg/ml and all of it represented preformed IgE (data not shown).

Most of the IgE in the MNC cultures from the allergic individuals in July represented IgE antibody synthesis *in vitro* (Table 1 and Fig. 1) and all of them displayed net synthesis. Thus, 61 and 72 % of all IgE present in the supernatants represented new synthesis and 39 and 28 % preformed IgE in 1988 and 1987, respectively. In contrast, the IgE in MNC cultures in winter and spring represented preformed IgE, and only one individual had a significant ongoing IgE synthesis at that time.

Mononuclear cells were further separated into monocytes, B and T cells in experiments performed on cells from the allergic patients in the winter. As shown in Fig. 2, 59 % of the preformed IgE at day 0 was present in the monocyte population and 30 % in the B cells. The IgE levels in the cultures were similar on day 0 and after 7 days, showing that there was no net IgE synthesis (data not shown).

Analysis of specific IgE antibodies against timothy in the cultures revealed a kinetics similar to total IgE antibody levels (Table 2).

DISCUSSION

This study shows that pollen allergic patients have a net synthesis of IgE in MNC cultures obtained during the pollen season. In contrast, almost all the IgE present in the MNC cultures in the winter and spring represented preformed IgE and only one individual displayed a significant IgE synthesis at that time. The findings agree with and extend a recent Australian study of seasonal variations in rye pollen specific IgE antibody formation by MNC (11). This pronounced seasonal variation should be considered when selecting cell donors for *in vitro* experiments of IgE synthesis and it can explain differences in the literature regarding IgE synthesis *in vitro* by MNC from atopic individuals.

Most of the preformed IgE in the winter was present in the macrophage population. This supports the very recent findings by Turner et al. that monocytes may store IgE (12), similar to that previously reported for IgA (1).

The persistence of preformed specific IgE throughout the winter in the absence of any appreciable *de novo* synthesis is of interest and could at least in part explain clinical observations that the postseasonal fall in serum IgE is less pronounced than would be expected merely from the short half-life of IgE.

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Table 2

Presence of IgE antibodies against timothy pollen in serum and in MNC cultures in early spring before the pollen season and in the summer. The values are expressed in Pharmacia paper RAST units (PRU) per ml

Patient IgE, kU/I	Spring			Summer		
	Serum	Spontaneous	<i>de novo</i> synthesis	Serum	Spontaneous	Synthesis
1. 90	7.6	<0.1	<0.1	12.8	0.1	0.1
2. 628	53.0	<0.1	<0.1	28.4	<0.1	0.6
3. 306	9.3	<0.1	<0.1	23.6	0.1	0.1
4. 685	40.0	<0.1	<0.1	29.3	0.1	0.1
5. 404	52.0	0.1	<0.1	28.8	0.2	0.1
6. 270	n.d.*	<0.1	<0.1	29.7	0.1	0.2
7. 119	14.4	<0.1	<0.1	23.5	0.2	<0.1
8. 230	14.7	<0.1	<0.1	20.1	0.1	<0.1

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