

Seasonal enhancement of IL-4 induced IgE synthesis by peripheral blood mononuclear cells of atopic patients

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Summary

The role of IL-4 on IgE synthesis has been well established. IL-4 has been shown to promote IgE production by B cells from atopic and non-atopic donors. In this study, the effects of natural exposure to pollens on IL-4-induced IgE synthesis by peripheral blood mononuclear cells of atopic and non-atopic donors were examined. The results confirm production of IgE in an IL-4 dose-dependent manner by PBMC cultures of these two groups. When cultures were performed out of the pollen season, following stimulation by IL-4, no significant difference was observed between the levels of IgE produced by PBMC of atopic and non-atopic donors. In contrast, upon natural exposure to pollens, significant higher levels of IgE were measured in the atopic group than in the non-atopic one. These results show that the pollen season influences the IL-4-induced IgE synthesis by PBMC of allergic patients and are in keeping with seasonal rise of specific IgE antibodies.

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Introduction

In the last few years, it has become clear that the T cell-derived interleukin-4 plays a crucial role in the regulation of IgE synthesis. Indeed, in mice, in the presence of IL-4, a polyclonal *in vitro* IgE and IgG1 production by liposaccharide-stimulated B cell blasts was reported [1–4]. This IgE response was inhibited by *in vivo* administration of an anti-IL-4 Ab [5,6]. In humans, recombinant IL-4 and supernatants from PHA-stimulated T-cell clones containing IL-4 have been shown to induce preferentially the synthesis of IgE [7–10] and IgG4 [11,12] isotypes by peripheral blood mononuclear cells. However, IL-4 alone failed to induce IgE production by highly purified B-cells and a second signal is necessary. This second signal to activate B cells could be provided by cognate or non-cognate T–B cell interaction [10,13], by Epstein–Barr virus [14,15], by hydrocortisone [16] or after activation with a MoAb directed to a 50 kD surface glycoprotein expressed on all human B lymphocytes (CD40) [17–19].

Because of the effects of IL-4 on IgE synthesis, it has been hypothesized that a higher proportion of circulating T cells able to produce IL-4 could be responsible for enhanced IgE synthesis in atopic patients [9].

A number of studies have shown that *in vitro* spontaneous human IgE synthesis by B cells is greater in allergic than in non-allergic subjects [20–26]. In contrast, it has recently been reported that *in vitro* IgE production by PBMC in the presence of IL-4 was not significantly different between atopic and non-atopic donors [27]. However, the effects of natural exposure to pollens on IL-4-induced *in vitro* IgE synthesis have not been clarified. In this report, the effects of IL-4 on IgE synthesis by PBMC of grass pollen sensitive subjects and non-atopic donors were studied during and out of the pollen season. We report that, during the pollen season, upon stimulation by IL-4, higher levels of IgE were produced by PBMC of atopic patients compared to non-atopic donors while out of the season, no difference was observed between the two groups.

Materials and methods

Cell donors

Twenty-nine adult atopic donors (Table 1) were selected from the Allergy and Immunology Clinic of Centre

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Table 1. Study population

Subjects	RAST to grass pollens	Sex (F/M)	Serum IgE (ng/ml)	
			Mean	Range
Atopic	+	12/17	1840	60–10030
Non-atopic	–	5/8	307	10–920

Hospitalier de l'Université Laval (CHUL) on the basis of a history of seasonal allergic rhinitis, positive skin-prick test (Omega Laboratories, Montréal, Canada) and positive RAST (Pharmacia, Piscataway, NJ, U.S.A.) to *Lolium perenne*, and with no previous immunotherapy. None was suffering from atopic dermatitis. Eight atopic donors were studied during the pollen season alone, seven during and out of the season and seven subjects out of the season alone. Thirteen non-atopic subjects (five studied during the pollen season and eight out of the season) without any symptoms of seasonal allergic rhinitis, negative skin-prick test and RAST to a panel of common inhalants were also included in this study.

Reagents

Human recombinant IL-4 (Sp.Act. = 2×10^7 U/mg) was purchased from Amersham Corporation (Arlington Heights, IL, U.S.A.). The goat anti-human IgE antiserum (Atlantic Antibodies, Scarborough, ME, U.S.A.) was purified as previously described [28]. The anti-human IgE MoAbs (7H8; 3D10) were prepared in our laboratory as reported in other studies [29,30]. These MoAbs were purified from mouse ascitic fluids or culture supernatants by passage through a protein A-Sepharose CL4B column (Pharmacia, Piscataway, NJ, U.S.A.). The MoAbs were then eluted, dialysed against PBS, and stored at -80°C until used. The culture medium was c-IMDM described by Claassen *et al.* [31] which consisted in Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY, U.S.A.) enriched with 0.5% BSA (Sigma, St Louis, MO, U.S.A.), 50 $\mu\text{g}/\text{ml}$ human transferrin (Sigma, St Louis, MO, U.S.A.), 5 $\mu\text{g}/\text{ml}$ bovine insulin (Sigma, St Louis, MO, U.S.A.), 1 $\mu\text{g}/\text{ml}$ each of oleic, linoleic and palmitic acids (Sigma, St Louis, MO, U.S.A.) and 10% heat-inactivated pooled FCS (Hyclone Laboratories, Inc., Logan, Utah, U.S.A.).

Radioiodination

Purified Ab (30 μg) were iodinated with 200 μCi of ^{125}I (Amersham, Arlington Heights, IL, U.S.A.) by using

Iodo-Gen (Pierce Chemical Co., Rockford, IL, U.S.A.) and free iodine was separated from ^{125}I -labelled Ab by passage through an Econo-Pac 10DG column (Bio-Rad, Richmond, CA, U.S.A.). Specific activity was estimated in accordance with the instructions of the manufacturer.

Culture conditions for in vitro IgE production

PBMC were isolated from heparinized venous blood of the subjects by density-gradient centrifugation as previously described [32]. The cells were washed three times in Hank's balanced salt solution (Gibco, Grand Island, NY, U.S.A.) and resuspended in the culture medium. Unfractionated PBMC (2×10^6 cells/ml) were cultured at 37°C in a humidified 5% CO_2 atmosphere for 18 days alone or in the presence of different concentrations of IL-4 (25 to 100 U/ml). The cultures were done in triplicate 17×100 mm polystyrene tubes. At the end of the culture, cells were harvested and supernatants lyophilized and reconstituted in 220 μl of Tris-HCl-buffered saline (TBS; pH 7.4; 50 mM)–0.1% Tween-20 containing horse serum (HS). Control cultures for the evaluation of preformed IgE were set up in the presence of cycloheximide (Sigma, St Louis, MO, U.S.A.; 100 $\mu\text{g}/\text{ml}$). Net IgE synthesis was evaluated by subtracting the IgE values detected in cycloheximide-treated cultures from the values found in untreated cultures.

IgE assay

IgE values were determined in duplicate for each triplicate culture by a RIA performed in polystyrene removable strips (Dynatech Laboratories, Alexandria, VA, U.S.A.). The wells were coated with 0.1 ml of 2:1 mixture of two anti-IgE MoAb (7H8 at 2 $\mu\text{g}/\text{ml}$ and 3D10 at 1 $\mu\text{g}/\text{ml}$) in bicarbonate buffer, pH 9.6. After an overnight incubation, the wells were washed, saturated with TBS-10%HS for 2 hr and subsequently washed with TBS-1%HS. The culture supernatants or different concentrations of standard IgE were added to the wells and incubated in a humidified chamber. After an overnight period of incubation, they were washed and incubated with ^{125}I -labelled goat anti-human IgE (10^5 c.p.m./well/100 μl) for 6 hr. After extensive washes, bound radioactivity was determined in a gamma counter (LKB, Bromma, Sweden). The results were expressed in count per minute (c.p.m.). The sensitivity of this assay is 200 pg/ml.

Statistical analysis

Results were expressed in means of IgE (pg/ml) $\pm$ s.e.m. and compared with the unpaired Student's *t*-test. The comparison of the response obtained using the same patients during and out of the pollen season was performed with the paired Student's *t*-test.

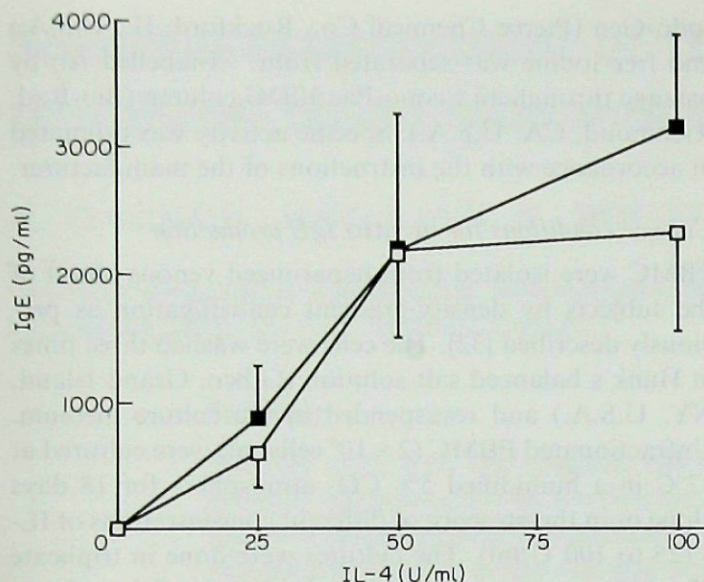


Fig. 1. Dose-response curve of IL-4-induced IgE synthesis by unfractionated PBMC from atopic ($n=7$; opened squares) and non-atopic donors ($n=5$; closed squares). Data are expressed in means $\pm$ s.e.m. The levels of IgE induced by IL-4 was significantly different than the levels measured in unstimulated cultures for the two groups ($P<0.05$) while no significant difference was observed between the two groups.

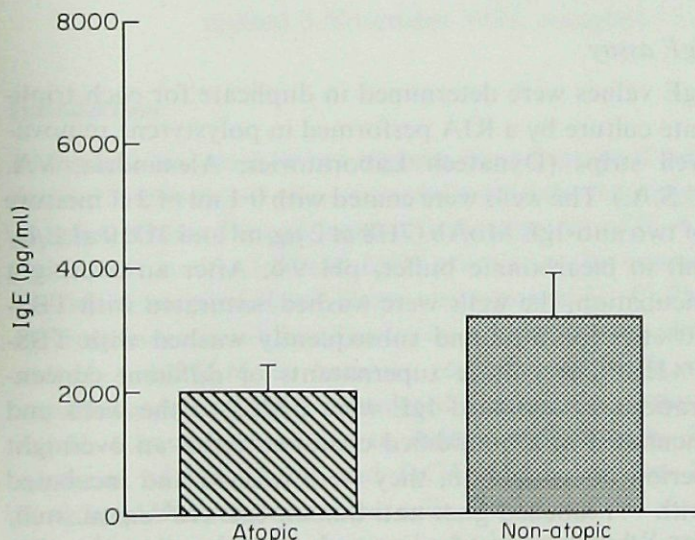


Fig. 2. IL-4-induced IgE synthesis by PBMC of atopic ($n=15$) and non-atopic ($n=5$) donors: out of the pollen season. Data are expressed in means $\pm$ s.e.m. No significant difference ($P>0.1$) was observed between the two groups.

Results

Dose-response curve of IL-4-induced IgE synthesis

In order to study the effects of natural exposure to pollens on IL-4-induced IgE synthesis, it was of prime importance to determine in a dose-response curve the optimal

concentration of IL-4 required. First, our study confirmed that, after 18 days of culture in the presence of IL-4, unfractionated PBMC of both atopic ($n=7$) and non-atopic ($n=5$) donors could produce significant amounts of IgE in a dose-dependent fashion (Fig. 1). For the two groups, this IgE antibody response was significant ($P<0.05$) with IL-4 concentrations ranging from 25 to 100 U/ml. This dose-response assay was performed out of the pollen season, e.g. at least 4 months after the grass pollens season. No significant difference was seen between the amounts of IgE antibody produced by atopic and non-atopic groups at each concentration of IL-4 used in this assay.

Effects of the pollen season on IL-4-induced IgE synthesis

In subsequent studies, optimal concentration of cytokine (100 U/ml) was used. Out of the pollen season, as seen in Fig. 2, the level of IgE produced in the presence of IL-4 was not significantly different ($P>0.1$) from PBMC of atopic ($n=15$) than from PBMC of non-atopic donors ($n=5$). The mean IgE concentrations were respectively 1976 ± 478 pg/ml and 3166 ± 730 pg/ml. To assess the effects of the natural exposure to the allergen on this IL-4-induced IgE production, results obtained during and out of the pollen season were compared. As shown in Fig. 3, atopic subjects ($n=14$) produced significantly more ($P<0.05$) IgE than non-atopic controls ($n=8$) when the assay was performed during the pollen season. The mean IgE concentrations were respectively 5480 ± 1650 pg/ml and 1450 ± 830 pg/ml. Furthermore, the levels of IL-4-induced IgE production by PBMC of atopic donors were also higher during the pollen season than out of the season ($P<0.05$) whereas, for the non-atopic donors, no significant difference ($P>0.1$) was observed between the two periods of assay (not shown). During and out of the season, no spontaneous IgE synthesis was observed for atopic and non-atopic subjects (not shown). Values of preformed IgE were not significantly different between the two groups neither during nor out of the season. During the season, the mean of preformed IgE was 499 ± 131 pg/ml while out of the season it was 449 ± 71 pg/ml.

Seven atopic subjects were studied both during and out of the grass pollen season. In Fig. 4, it is shown that a significant higher ($P<0.05$) production of IgE by IL-4-stimulated PBMC of the donors occurred when the cells were cultured during the pollen season. Mean IgE concentrations in the supernatants were 1310 ± 560 pg/ml out of the pollen season and 4960 ± 1560 pg/ml during the pollen season.

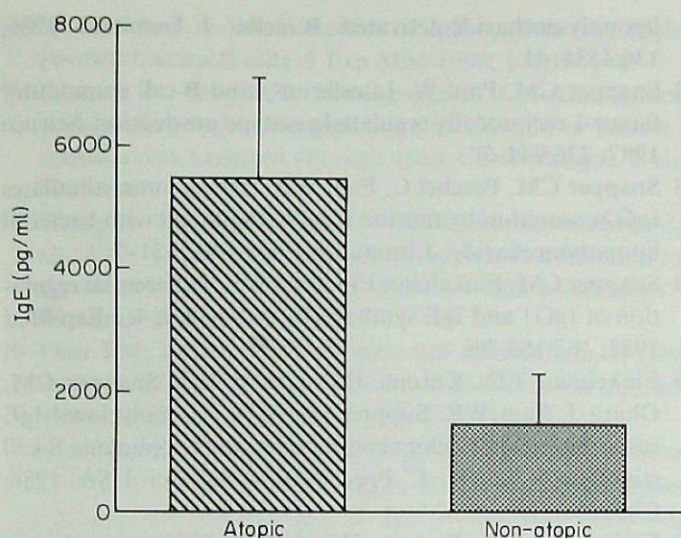


Fig. 3. IL-4-induced IgE synthesis by PBMC of atopic ($n=14$) and non-atopic ($n=8$) donors: during the pollen season. Data are expressed in means $\pm$ s.e.m. Significant higher level ($P<0.05$) was observed in the atopic group than in the non-atopic group.

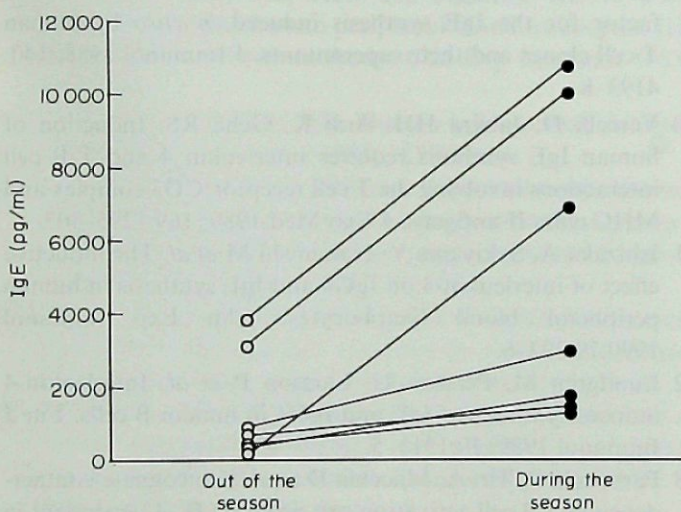


Fig. 4. IL-4-induced IgE synthesis by PBMC of atopic subjects studied during and out of the pollen season ($n=7$). Significant enhanced IgE production ($P<0.05$) was observed when the cultures were performed during the pollen season.

Discussion

This study was designed to look at the effects of the pollination period on IL-4-induced IgE antibodies *in vitro* synthesis by PBMC of atopic and non-atopic donors. Firstly, our results confirm the role of IL-4 on the induction of IgE synthesis. Indeed, the *in vitro* IgE synthesis by PBMC of atopic and non-atopic donors could be induced in a dose-dependent manner by IL-4.

During the last few years, different concentrations of IL-4 have been reported to promote the IgE synthesis by human PBMC. In our study, IL-4 concentration of 25 U/ml was sufficient to obtain a significant IgE synthesis by PBMC of the donors. Del Prete *et al.* [9] showed that 400 U/ml of IL-4 was necessary for induction of IgE synthesis by B cells from normal donors while a lower concentration (100 U/ml) was apparently adequate with cells from atopic donors. A significant IgE synthesis was obtained with cells from atopic and non-atopic donors with 0.6 ng/ml (12.5 U/ml) in the report of Claassen *et al.* [27]. Although these results seem to be conflicting, it is not possible to compare the IL-4 concentrations used to stimulate a significant production of IgE since the biological activity of the different IL-4 preparations was not determined using the same bioassay.

In the present report, the dose-response curve was established out of the pollen season. At this time of study, for each concentration of IL-4 assayed, no difference was seen between the IgE levels that were produced by the two groups. These results are in agreement with another study [27] showing comparable IL-4-induced IgE production by atopic and non-atopic donors. To answer the question could natural exposure to the allergen influence the IL-4-induced IgE synthesis, this was assessed during and out of the pollen season. Interestingly, we report that significantly higher levels of IgE were produced *in vitro* by PBMC of atopic patients compared to the levels produced by non-atopic donors when the experiments were performed during the allergen season. This observation was confirmed when seven allergic subjects were studied both during and out of the pollen season. Different mechanisms to explain these findings could be postulated. First, in mice and humans, IL-4 has recently been shown to be secreted by a subset of T helper lymphocytes (TH-2) [33–37] and the presence of such lymphocytes has been observed in bronchoalveolar lavage from subjects with atopic asthma [38]. Also, an elevated number of TH-2 helper lymphocytes has been reported to be present at the site of allergen-induced late-phase cutaneous reaction in atopic patients [39]. These important observations support the possibility of an involvement of TH-2 cells in the pathophysiology of allergic diseases. Our results show that seasonal exposure to pollens led to a higher IL-4-induced *in vitro* IgE production in atopic patients than in non-atopic donors. These data could suggest that higher numbers of IgE-secreting B cells are circulating in peripheral blood of allergic subjects during the pollen season secondary to the increased *in vivo* production of IL-4 by activated TH-2 helper lymphocytes. It is also possible to speculate that such *in vivo* production of IL-4 could increase the level of IgE production per B cell. The use of ELISPOT methods [40] should determine if the

number of circulating IgE secreting B cells is enhanced during the pollen season in atopic patients.

Secondly, it is known that two signals are needed by B-cells to produce IgE: the first one is provided by IL-4 while the second one could be provided by different sources such as by cognate or non-cognate T-B cells contact, by Epstein-Barr virus, by hydrocortisone or by triggering of a B-cell surface glycoprotein (CD40). Therefore, we could not exclude the possibility that during the pollen season, in atopic patients, a specific stimulation of immunocompetent cells could lead to the production of one or several known second signals required to achieve an IgE production by IL-4-stimulated B cells. It could also be possible that the pollen season influences the IL-4 responsiveness by enhancing the number or affinity of IL-4 receptors or by enhancing the co-operative effects of other cytokines such as IL-5 and IL-6. In addition, the possible effects of pollutants on IgE production could not be ruled out [41].

In our experiments, IL-4 did not promote IgE synthesis in all atopic and non-atopic donors tested. However, such observation was reported by other groups as well [7,42]. It has been suggested by Zhang *et al.* [19] that this phenomenon could be related to non B-cell function. They reported that IL-4-induced IgE synthesis by PBMC from non-responder subjects could be restored by co-culture with CD40 MoAb which acts as second signal. In this study, no spontaneous IgE synthesis was observed. But the culture system used herein has not been optimized to measure spontaneous IgE synthesis and was somewhat different to those reported in other studies and designed to analyse spontaneous IgE synthesis [20–26].

In summary, the results reported herein confirm the IL-4-induced IgE synthesis by PBMC cultures from atopic and non-atopic donors. For the first time, the effects of the pollination period on IgE production in presence of IL-4 have been studied. We observed that, during the pollen season, IL-4-stimulated PBMC of atopic donors produced more IgE than IL-4-stimulated PBMC of non-atopic donors whereas out of the season no difference was observed between these two groups. These results suggest that natural exposure to the allergen influence the IL-4-induced IgE synthesis by PBMC of atopic subjects.

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