Seasonal variations of interleukin-4 and interferon-γ release by peripheral blood mononuclear cells from atopic subjects stimulated by polyclonal activators

Béatrice Lagier,[a] Nathalie Pons,[a] Agnès Rivier,[a] Isabelle Chanal, MD,[b] Pascal Chanez, MD,[a, b] Jean Bousquet, MD, PhD,[a, b] and Jérôme Pène, PhD[a]
Montpellier, France

IgE synthesis is controlled by interleukin (IL)-4 and interferon (IFN)-γ, but there is heterogeneity in the IL-4 response depending on the sensitization of patients and natural allergen exposure. In patients sensitized to various allergens, we studied the synthesis of IL-4, IFN-γ, and IgE to determine to what extent their in vitro immune response may be influenced by pollen season, depending on their sensitization. We studied 12 nonallergic individuals, seven patients sensitized to cypress pollen, 12 sensitized to grass pollen, 14 sensitized to several pollens, and 42 patients with polysensitization. The release of IL-4 and IFN-γ from peripheral blood mononuclear cells stimulated by polyclonal agents (calcium ionophore A23187 and phorbol myristate acetate) was measured by ELISA. The spontaneous and IL-4–induced release of IgE was measured by ELISA. In patients with cypress pollen allergy, IL-4 and IgE release were significantly lower than in patients with other allergies. In the pollen-sensitized group, IL-4 and IgE release were significantly higher during the pollen season than out of it. No variation in IL-4 or IgE release was observed in the polysensitized group. IFN-γ production was not affected by the pollen season. These data show that the seasonal variations of IL-4 and IgE synthesis differ according to the sensitization of patients.
(J ALLERGY CLIN IMMUNOL 1995;96:932-40.)

Key words: *Interleukin-4, IgE, atopy, pollen, season*

The immune response to environmental allergens depends mainly on genetic and environmental factors.[1] The regulation of IgE synthesis is controlled by several factors, among which cytokines play a central role. Interleukin (IL)-4 induces IgE and IgG_4 secretion by peripheral blood mononuclear cells (PBMCs) of atopic and nonatopic donors[2-5] by promoting isotype switching from IgM to IgE on resting B cells.[6, 7] In addition, other cytokines can modulate IgE synthesis. IL-13 appears to share similar effects with IL-4 on the IgE isotype switching and IgG_4 regulation.[8, 9] Several other cytokines, such as IL-5, IL-6, IL-9, and tumor necrosis factor-α, were reported to act synergistically with suboptimal amounts of IL-4.[10, 11] On the other hand, interferon (IFN)-γ has suppressive effects on both IL-4–induced and spontaneous IgE synthesis.[2, 3, 12] The inhibitory roles of IFN-α,[3] transforming growth factor-β, IL-10,[13] and IL-12 have also been observed.[10, 11] It has been recently

Abbreviations used

IFN-γ:	Interferon-γ
IL:	Interleukin
mAb:	Monoclonal antibody
PBMCs:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
PHA:	Phytohemagglutinin
PMA:	Phorbol myristate acetate
SPT:	Skin prick test

From the [a]Institut National de Santé et de la Recherche Médicale Contrat Jeune Formation 92-10; and [b]the Clinique des Maladies Respiratoires, Centre Hospitalo-Universitaire, Montpellier.
Supported by an educational grant from the Laboratoires des Stallergènes, Fresnes, France. Béatrice Lagier is a recipient of a grant from the Fondation Mérieux, Lyon, France.
Received for publication Aug. 5, 1994; revised Jan. 12, 1995; accepted for publication Feb. 22, 1995.
Reprint requests: Jérôme Pène, PhD, INSERM CJF 92.10, Hôpital Arnaud de Villeneuve, 34295 Montpellier, Cedex 5, France.

0091-6749/95 $5.00 + 0 **1/1/64683**

shown that IL-4 genes are linked with the IgE immune response.[14] IL-4 and IFN-γ are produced by distinct subsets of $CD4^+$ helper/inducer T lymphocytes, which are separated into a T_{H1} phenotype (IFN-γ) or T_{H2} phenotype (IL-4, IL-5, IL-6).[15, 16]

However, IgE and IL-4 responses are heterogeneous in atopic individuals.[17-19] Patients allergic to a single allergen (monosensitized) differ from those allergic to many allergen species (polysensitized). In comparison with polysensitized patients, monosensitized patients have lower total serum IgE levels,[17, 20] and PBMCs produce lower levels of IL-4 and IgE in vitro.[19] Moreover, patients who are only allergic to cypress pollen may have an IgE-immune response close to that of nonallergic individuals.[18, 21]

Seasonal exposure to allergens induces variations in the levels of serum allergen-specific IgE,[22] T-cell responses,[23] and $Fc\epsilon R^+$ of peripheral blood lymphocytes,[24] as well as in the in vitro synthesis of IgE and cytokines.[25, 26] The de novo IgE synthesis by PBMCs of pollen-sensitized donors appears to be confined to the peak of the pollen season.[25] The IL-4–induced IgE synthesis by PBMCs of allergic patients is increased during the pollen season and is higher than that of nonatopic donors, whereas no difference was observed between the two groups out of the pollen season.[26] Finally, the synthesis of IL-4 induced by *Lolium perene* pollen (Lol p 1) was only observed during the pollen season, whereas out of the season it was not detectable.[23]

The in vitro synthesis of IL-4 and IFN-γ by PBMCs activated by polyclonal agents and the in vitro synthesis of IgE by PBMCs spontaneously and after IL-4 activation were studied in patients allergic to cypress, grass, or multiple pollen species and in polysensitized patients during the pollen season and out of it. The study was carried out in order to determine whether: (1) the release of these cytokines and IgE is influenced by allergen exposure during pollen season, (2) the different groups of subjects have a similar pattern of response during the pollen season, and (3) the cytokine pattern and IgE synthesis of patients with cypress pollen allergy differs from that of patients with other allergies.

METHODS

Patients

Eighty-three allergic patients (42 men), ranging in age from 9 to 69 years (mean ± SD, 32.0 ± 17 year), volunteered to enter the study after they gave informed consent. The study was approved by the ethical committee of Montpellier University. All patients were allergic and selected according to the criteria of a previous study[27]: (1) a history suggestive of seasonal or perennial allergic diseases with seasonal exacerbations (asthma, rhinitis, or both), (2) a positive skin prick test (SPT) response to at least one of the aeroallergens of the Montpellier area when available standardized extracts (Laboratoires des Stallergènes, Fresnes, France) were used, and (3) the presence of specific IgE (Phadebas CAP System; Pharmacia Diagnostics, Uppsala, Sweden). None of the patients had received any form of specific immunotherapy because this treatment might have modified the cytokine pattern of T cells.[28] None of the patients had been treated with any form of corticosteroids for the previous 3 months because these drugs might have immunomodulatory effects. Patients were placed in the pollen-sensitized or the polysensitized group according to their sensitivity, as previously described.[19, 27] Seven patients were sensitized to cypress pollen only, 13 to grass pollen only, and 21 to several pollen species; 42 were sensitized to pollens and house dust mites of the species *Dermatophagoides pteronyssinus* and *D. farinae* and/or various allergens including animal danders and molds.

Twelve nonallergic subjects (7 men and 5 women), ranging in age from 24 to 52 years (mean ± SD, 32.6 ± 8.8 years) were included as control subjects.

Reagents and cytokines

Purified recombinant human IL-4 (1×10^7 U/mg) was a gift of Dr. Françoise Rousset (Schering-Plough-France, Dardilly, France). Recombinant human IFN-γ (1×10^6 IU/mg) and recombinant human IL-2 were kindly provided by Pr. Gianni Garotta (Hoffmann-Laroche and Co. AG, Basel, Switzerland). The anti-human IL-4 monoclonal antibody (mAb) 11B4, the polyclonal goat anti-IL-4 antiserum, the two anti-IFN-γ mAbs A35 and B27, and the anti-IgE mAb I-27 were gifts of Dr. Rousset (Schering-Plough-France). For mAbs A35 and B27, the immunoglobulin fraction was purified by absorption onto a G column (Pierce, Oud-Beijerland, The Netherlands) according to the instructions of the manufacturer. The mAb B27 was biotinylated by coupling long-chain biotin (Pierce) according to a method previously described.[29] Standardized extracts of allergens were prepared according to the proposals of the Allergen Subcommittee of the International Unions of Immunological Societies by Laboratoires des Stallergènes (Fresnes, France) and have been described previously.[20, 30]

Preparation of PBMCs

Fifty milliliters of peripheral venous blood from each patient was drawn into a tube containing heparin. PBMCs were isolated from whole blood as previously described.[29] In brief, anticoagulated blood was diluted with phosphate-buffered saline (PBS) and layered over Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala,

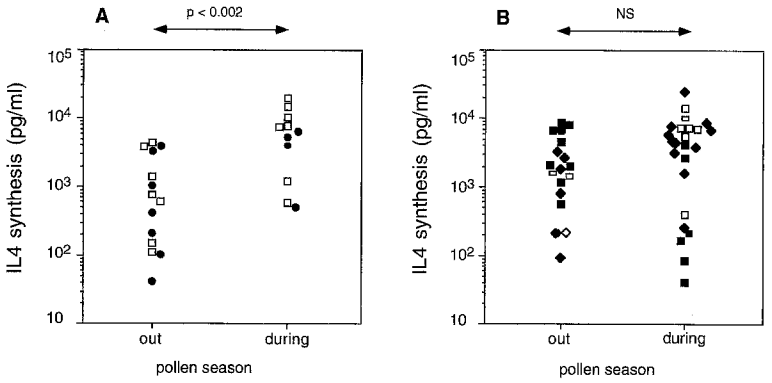

FIG. 1. Comparison of IL-4 synthesis by PBMCs of atopic subjects activated by PMA + calcium ionophore A23187 during and out of the pollen season. **A,** Pollen-sensitized patients: ● sensitized to grass pollen only; ❑ sensitized to various pollens. **B,** Polysensitized patients: ◆ sensitized to pollens and house dust mite; ■ sensitized to pollens, house dust mite, and other allergens; ❑ sensitized to pollens and other allergens but not to house dust mite. Statistical analysis is according to the Mann-Whitney U test. *NS,* Not significant.

Sweden), and the PBMCs were collected after centrifugation. Then the PBMCs were washed three times in PBS and resuspended in Iscove's culture medium (Gibco, Paisley, Scotland) supplemented with 2 mmol/L L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco). For the determination of IL-4 and IFN-γ synthesis, culture medium was supplemented with 10% autologous serum, whereas for the production of IgE, it was supplemented with 10% fetal calf serum (Gibco).[19]

Cytokine production

PBMCs were stimulated with polyclonal agents according to a method previously described.[19] The cells were plated at 2×10^6 cells/ml in duplicate, in 48-well cluster plates (Costar, Cambridge, Mass.), in a volume of 1 ml/well. Cells were stimulated at 37° C in a humidified atmosphere containing 5% CO_2 in the presence or absence of 10 ng/ml of phorbol myristate acetate (PMA), (Sigma Chemicals, St. Louis, Mo.) plus 100 ng/ml of calcium ionophore A23187 (Sigma Chemicals). After a 48-hour incubation, the culture supernatants were harvested, centrifuged at 4° C and stored at −20° C for quantification of IFN-γ synthesis. The cells received fresh culture medium and were incubated for an additional period of 6 days in the presence of IL-2, 20 IU/ml, and purified phytohemagglutinin (PHA) (Murex Diagnostics Ltd., Dartford, U.K.), 0.05 μg/ml; the cells were fed on day 3 by replacing 0.5 ml of the culture supernatant with fresh medium containing IL-2 and PHA. On day 6, the culture supernatants were aspirated and replaced with fresh medium, and the PBMCs were restimulated with PHA plus PMA or PMA plus calcium ionophore A23187 at the concentrations used initially. The culture supernatants were collected at 48 hours and treated as described previously for IL-4 determination.

In vitro IgE production

The PBMCs were resuspended, as previously described,[19, 35] at a concentration of 5×10^6 cells/ml in Iscove's culture medium supplemented with 10% fetal calf serum. IgE production was measured in a two-step culture procedure. To eliminate passive transfer of serum IgE, PBMCs were preincubated for 48 hours in a culture flask (Costar, Cambridge, Mass.) in the presence of IL-4, 200 U/ml. After this preincubation period, the nonadherent cells were collected, washed three times in culture medium, resuspended at a concentration of 1×10^6 cells/ml in the absence or presence of various amounts of IL-4 (0, 20, 200, and 400 U/ml). After an incubation period of 9 days, culture supernatants were harvested, centrifuged, and stored at −20° C until IgE titration was carried out by ELISA as described below. To determine de novo IgE production, cycloheximide (Sigma Chemicals), an inhibitor of protein synthesis, was included in some experiments.

ELISA for IL-4 and IFN-γ

ELISAs were performed with 96-well, flat-bottom microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark) as previously described in detail.[19] The ELISA for IL-4 was modified as follows. Microplates were coated overnight for 18 hours at + 4° C with a purified immunoglobulin fraction of the rat monoclonal anti-IL-4 antibody 11B4 diluted at 1 μg/ml in a carbonate buffer (pH 8.6). Calibration curves, which consisted of serial dilutions of purified recombinant IL-4, were made in parallel. The second antibody was a purified immunoglobulin fraction of a polyclonal goat anti-IL-4 antiserum, added at a concentration of 2 μg/ml. Subsequently, a peroxidase-conjugated swine anti-goat immunoglobulin (Tago Inc., Burlingame, Calif.) diluted 1:10,000 was used for labeling. The sensitivity of the assay was 20 pg IL-4/ml.

TABLE I. Production of IL-4 and IFN-γ in vitro by PBMCs of allergic and nonallergic individuals

Groups of subjects	Season	n	IL-4 (pg/ml) Median	IL-4 (pg/ml) Range	IFN-γ (ng/ml) Median	IFN-γ (ng/ml) Range
Nonatopic		12	281	0-538	173	4.2-312
Cypress-sensitized		7	310	0-3410	3.4	0.8-40.5
Grass pollen–sensitized	Out	7	402	0-3724*	11.0	0.1-31.4
	In	5	5052	541-9221*	30.0	1.4-151
Pollen-sensitized	Out	7	681	105-4264†	16.5	2.6-38.6
	In	7	7380	570-17899†	8.3	0.9-207
Polysensitized	Out	18	1894	89-8357	4.3	0.1-30.0
	In	24	4769	0-23330	5.1	0.9-191

Results are expressed as median and range for each group of study subjects.
*$p < 0.035$ (Mann-Whitney U test).
†$p < 0.02$ (Mann-Whitney U test).

The ELISA for IFN-γ used the two anti-IFN-γ mAbs that detect different epitopes on the molecule as catcher (mAb A35) and tracer antibodies (mAb B27), respectively. A35 was coated to the plates at 2 μg/ml. Calibration curves were made with serial dilutions of purified recombinant IFN-γ. Biotinylated mAb B27 was added at 1 μg/ml. The sensitivity of the assay was 20 pg IFN-γ/ml.

IgE titration

Total serum IgE concentrations and IgE released in the culture supernatants were determined by using an ELISA previously described in detail.[3, 19] Briefly, ELISA plates were coated with a rabbit anti-IgE (Dakopatts, Glostrup, Denmark) diluted 1:2000. A standard IgE serum (Phadexact serum, Pharmacia Diagnostics AB) was used for calibration. The catcher antibody was made of the anti-human IgE mAb I-27 (in the form of ascite, 1:40,000), and a peroxidase-conjugated goat anti-mouse immunoglobulin (1:5000, Tago Inc.) was used for labeling. The limit of sensitivity of the assay was 75 pg IgE/ml. We tested the correlation of this assay with the Phadebas PRIST (Pharmacia Diagnostics AB) in 30 serum samples and obtained a highly significant correlation ($p < 0.0001$, Spearman's rank correlation).[19]

Pollen counts

Pollen counts were obtained by the method of Cour.[31] The sampler was placed outdoors, 2 m above the ground, and counts were available throughout the study.

Design of the study and statistical analysis

All subjects had the same tests done, including a titration of total and allergen-specific serum IgE and lymphocyte cultures. Thirty-six of the patients were studied during the peak of the pollen seasons, and 32 were studied out of the pollen season (i.e., between 3 months after the end of the season and 1 month before the onset of the next season). In addition, eight patients (1 sensitized only to grass pollen, 7 sensitized to several pollen species) were studied during and out of the season. The results for seven patients allergic to cypress pollens only were averaged because of the low number of these subjects and the similar levels of cytokine and IgE production during and out of the pollen season. The production of IgE in vitro was not carried out in all subjects because of the amount of supernatant. The study was carried out over 3 years (1991 to 1993).

Statistical analysis was performed by means of nonparametric tests. The overall interpatient analysis was performed with the Kruskall-Wallis test. The Mann-Whitney U test was used for intergroup analysis, and the Wilcoxon signed-rank test was used for intragroup analysis of the subjects studied during and out of the season. Results are expressed as median and range.

RESULTS

IL-4 production

PBMCs of 14 allergic patients spontaneously produced low amounts of IL-4 (20 to 200 pg/ml). None of the nonallergic individuals demonstrated any spontaneous IL-4 release.

In the presence of PMA plus calcium ionophore A23187, and regardless of the pollen season, IL-4 was released by PBMCs of 81 of 83 allergic patients and 10 of 12 nonallergic individuals (Table I). Cypress pollen–sensitized patients produced low amounts of IL-4 ($p < 0.01$, Mann Whitney U test) as compared with the three other groups of allergic patients. Out of the pollen season, IL-4 production was low in patients allergic to grass pollen, increased in patients allergic to multiple pollens, and highest in polysensitized patients ($p < 0.04$, Kruskal-Wallis test) (Fig. 1). During the pollen season, there was no significant difference among different groups of allergic patients. There was a significant increase in IL-4 release in patients allergic to grass and multiple pollens who were

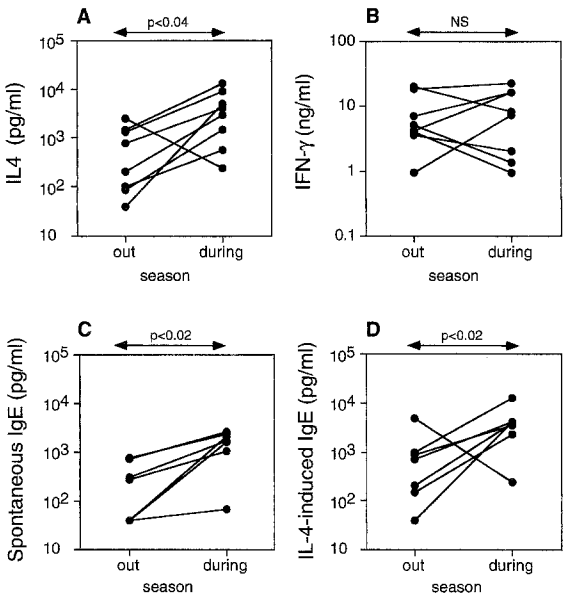

FIG. 2. Comparison of IL-4 and IFN-γ synthesis, spontaneous IgE and IL-4–induced IgE release by PBMCs of eight atopic subjects during and out of the pollen season. PBMCs were isolated from the same eight patients, during and out of the pollen season, and activated by PMA + calcium ionophore A23187 for cytokine production or in the presence of IL-4 for IgE measurement. **A,** IL-4 synthesis. **B,** IFN-γ synthesis. **C,** Spontaneous IgE. **D,** IL-4–induced IgE. Statistical analysis is according to the Wilcoxon signed-rank test. *NS,* Not significant.

studied during and out of the pollen season ($p < 0.02$ and $p < 0.035$, respectively; Mann-Whitney U test) but not in the polysensitized group.

In the eight allergic patients who were tested twice, there was a significant increase in IL-4 production during the pollen season ($p < 0.04$, Wilcoxon W test) (Fig. 2).

IFN-γ production

PBMCs of 12 patients and of all nonallergic individuals spontaneously produced low amounts of IFN-γ (20 to 600 pg/ml).

In the presence of PMA plus calcium ionophore A23187, and regardless of the pollen season, IFN-γ was released by PBMCs of all patients and nonallergic individuals. Nonallergic individuals produced significantly higher amounts of IFN-γ than allergic patients ($p < 0.001$, Mann-Whitney U test) (Table I). The four groups of allergic patients produced similar amounts of IFN-γ during and out of the pollen season (Fig. 3).

In the eight allergic patients who were tested twice, there was no significant increase in IFN-γ production during the pollen season (Fig. 2).

Total serum IgE

The levels of total serum IgE measured out of the pollen season were increased in patients with cypress pollen allergy compared with polysensitized patients ($p < 0.005$, Kruskall Wallis test). Total serum IgE titers were not significantly modified by the pollen season (Table II). In addition, in the eight patients who were tested during and out of the pollen season, total serum IgE titers were similar out of the pollen season (median and range: 240 ng/ml, 160 to 1738 ng/ml) and during the pollen season (median and range: 256 ng/ml, 50 to 1649 ng/ml).

Spontaneous and IL-4–induced IgE production in vitro by PBMC

Regardless of the pollen season, the spontaneous IgE synthesis in vitro was the lowest in the cypress pollen–sensitized group and the highest in the polysensitized group; grass pollen–sensitized patients had intermediate values (Table II). In the entire group of patients, spontaneous IgE production was not significantly affected by the pollen season (Table II). In the eight patients who were tested during and out of the pollen season, spontaneous IgE synthesis was significantly increased ($p < 0.02$, Wilcoxon signed-rank test) during the pollen season (during pollen season, median and range: 1675 pg/ml, 70 to 2648 pg/ml; out of pollen season, median and range: 275 pg/ml, 0 to 749 pg/ml) (Fig. 2).

Regardless of the pollen season, the IL-4–induced IgE synthesis in vitro was the lowest in the cypress pollen–sensitized group and was the highest in the polysensitized individuals; grass pollen–sensitized patients had intermediate values (Table II). Patients sensitized to multiple pollens had variable results, but the number of subjects tested in this group was low. The IL-4–induced IgE production was not significantly affected by the pollen season (Table II). In the eight patients who were tested during and out of the pollen season, the IL-4–induced IgE synthesis was significantly ($p < 0.02$, Wilcoxon signed-rank test) increased during the pollen season (during pollen season, median and range: 3943 pg/ml, 235 to 12755 pg/ml; out of pollen season, median and range: 680 pg/ml, 0 to 4751 pg/ml).

DISCUSSION

In this study we have shown that the release of IL-4, a major cytokine that regulates IgE synthesis, is expressed at a higher level during the pollen season in patients allergic to pollen. In contrast,

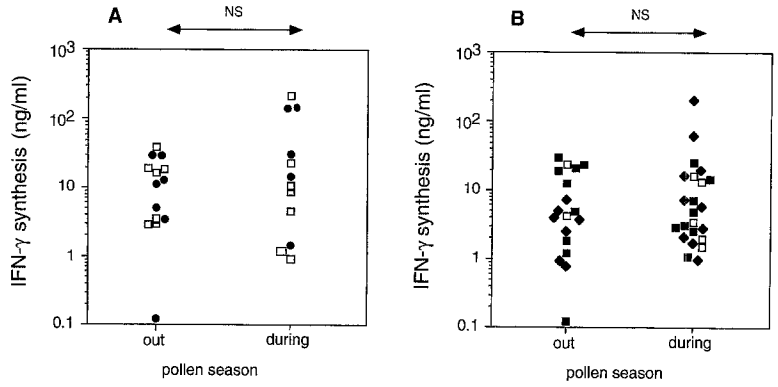

FIG. 3. Comparison of IFN-γ synthesis by PBMCs of atopic subjects activated by PMA + calcium ionophore A23187 during and out of the pollen season. **A,** Pollen-sensitized patients: ● sensitized to grass pollen only; ❑ sensitized to various pollens. **B,** Polysensitized patients: ◆ sensitized to pollens and house dust mite; ■ sensitized to pollens, house dust mite, and other allergens; ❑ sensitized to pollens and other allergens but not to house dust mite. Statistical analysis is according to the Mann-Whitney U test. *NS,* Not significant.

TABLE II. Serum total IgE and spontaneous and IL-4–induced IgE in vitro by PBMCs of allergic and nonallergic individuals

			Serum IgE (ng/ml)			Spontaneous IgE (pg/ml)		IL-4–induced IgE (pg/ml)	
Groups of subjects	**Season**	***n***	**Median**	**Range**	***n***	**Median**	**Range**	**Median**	**Range**
Nonatopic		7	60	6-117		ND	ND	ND	ND
Cypress-sensitized		5	98	15-226	3	195	126-393	398	299-812
Grass pollen–sensitized	Out	6	89	31-310	3	300	0-961	847	150-2794
	In	4	272	49-823	3	1260	597-1675	2318	1517-3391
Pollen-sensitized	Out	7	158	82-1738	5	1175	0-2334	3501	0-4751
	In	7	166	50-1780	3	110	70-411	826	235-1541
Polysensitized	Out	15	525	87-4546	8	445	0-794	837	150-3822
	In	21	238	69-2864	11	2080	116-4427	3367	183-12755

Results are expressed as median and range for each group of study subjects.
ND, Not done.

there was no increase in IL-4 production by PBMCs in polysensitized individuals. IFN-γ synthesis was not affected by the pollen season. Levels of IgE produced in vitro by PBMCs were parallel to those of IL-4. Patients allergic to cypress pollen have values for IL-4 and IgE in vitro synthesis between those of normal subjects and patients allergic to other pollen species.

The aim of this study was to examine the production of two major cytokines that regulate IgE production in vitro (IL-4 and IFN-γ) in order to determine whether the natural exposure to allergens may modify the release of these cytokines, depending on the allergenic sensitization of the patients.

Although other cytokines display inducing (especially IL-13) and suppressive effects on IgE synthesis,[8-13] we did not test these cytokines because: (1) amounts of cell supernatants would not have allowed us to study many other cytokines, (2) we had previously found that IL-10 is not released in the model studied (unpublished observation), and (3) the assay for IL-13 was not available when the study was started.

Methods used

One of the critical problems of this study was the characterization of the allergenic sensitivities of the patients. For this purpose, we used standardized allergen extracts, which were not available for all allergens. In particular, allergen extracts of cypress pollen and some molds are not in standardized form. The characterization of the sensitivity of the patients was defined according to previous

studies by our group on the history of allergic symptoms, SPTs, and serum-specific IgE.[18-20] We did not use intradermal skin tests because the clinical relevance of the positivity of this test in patients with negative SPT responses is still unclear.[32, 33]

A second clinical problem is differentiation between "during" and "out of" season. The pollen season was determined by pollen counts performed with a very precise technique.[31] However, pollens are often present in the atmosphere in low concentrations before any symptom occurs, and they can induce a biologic effect; therefore, we did not include patients sensitized to a given pollen species 1 month before the onset of that pollen season. On the other hand, patients allergic to pollen sometimes have an increase in bronchial reactivity that lasts for some weeks after the pollen season ends,[34] indicating that biologic effects of pollen exposure may last longer than the pollen season. Thus we did not include patients sensitized to a given pollen species between the pollen season and 3 months after its end.

Another problem with this study is that we did not test the same polysensitized patients in and out of the pollen season. However, the number of patients in the polysensitized group was large, and the results appear to be clear enough. For these reasons, we did not believe it was necessary to test the same patients during and out of the pollen season.

As previously shown,[35, 36] PBMCs did not release IL-4 or IFN-γ spontaneously in vitro. We therefore devised an in vitro system with polyclonal activators in which a consistent and relatively high IL-4 and IFN-γ production could be easily measured in both normal individuals and allergic patients.[19] Although this stimulation does not represent a physiologic condition for T-cell activation, we did not use an allergen-specific cytokine production as it was used previously[23, 26] because we wanted to compare several groups of subjects including normal individuals and patients allergic to different allergen species. However, the polyclonal stimulation reflects the optimal IL-4– or IFN-γ–producing capacity of the PBMCs. Alternatively, we may have used more physiologic systems, such as activation with anti-CD2 or anti-CD3 and anti-CD28 mAbs,[37, 38] but the study has been carried out over the past 4 years, these systems have been more recently validated, and we have compared the three methods and have not found any significant difference in the release of cytokines. The IgE production by PBMCs of atopic and nonatopic subjects is usually spontaneously low and increases when PBMCs are stimulated with IL-4.[2, 3, 26, 35] IgE was measured in supernatants in fewer patients because the number of cells recovered did not always make it possible to carry out all assays.

Importance of IL-4 in atopic patients

The results of this study are consistent with previous findings by our group.[3, 19] Levels of IL-4 released by PBMCs stimulated by polyclonal agonists were low in nonatopic individuals and increased with the sensitization of the patients, whereas IFN-γ production was high in nonatopic individuals and low in the four groups of allergic patients. However, this study shows that IL-4 production differs according to the pollen season and that the pollen season differently affects PBMCs according to the sensitization of the patients. In patients who are allergic to pollens only, a significant increase in IL-4 production occurred during the pollen season, reflecting the increase in allergen-specific IgE in serum during the pollen season.[22, 39] Our results are in agreement with those of Gagnon et al.[23] who studied the Lol p 1–induced IL-4 and IFN-γ production by PBMCs of patients allergic to grass pollen. However, we extend these data by showing that only a subset of allergic patients can have a seasonal variation because this increase in IL-4 production was not observed in patients sensitized to many allergen species who are continuously exposed to allergens throughout the year. IgE production followed a similar pattern, but the differences were less well characterized, possibly because the number of patients tested was lower. In the eight patients studied during and out of the pollen season, both IL-4 production and spontaneous IgE production were significantly increased during the season, and these results are in accord with those of previous studies.[23, 25] The results of this study confirm the importance of IL-4 in differentiating atopic subjects of variable allergenic sensitivity but show that IL-4 and IgE production can be modulated by allergen exposure. Thus both genetic[14] and environmental influences appear to govern the overall IgE production.

Patients allergic to cypress pollen

Patients allergic to cypress pollen are heterogeneous in terms of immune reactivity. In comparison with polysensitized patients, those only allergic to cypress pollen had normal or subnormal total serum IgE levels and lower serum-specific IgE; they also had symptoms during the cypress pollen

season at an older age (between 25 and 65 years).[18] This contrasts with the natural history of patients allergic to pollen of the Northern Mediterranean area in which most patients with grass pollen allergy have had symptoms by 20 years of age. The differences between patients allergic to grass pollen and those allergic to cypress pollen may be related to the very high amounts of cypress pollens released in the atmosphere, and, it may be considered that patients allergic to cypress pollen are not congenitally atopic but were sensitized as a result of the very high concentration of allergens in the atmosphere.[18, 21, 40] Although the number of subjects tested was low because these patients are rather infrequent, the results of this study confirm that patients allergic to cypress pollen only fall between nonatopic and atopic individuals in terms of IL-4 and IgE release by PBMCs, whereas IFN-γ production is similar to that of atopic individuals.

We thank Drs. Françoise Rousset, Jacques Banchereau, Jan E. De Vries, and Gianni Garotta for providing the mAbs and recombinant cytokines. We also thank Dr. Mike Campbell for revising the manuscript.

REFERENCES

1. Marsh DG. Genetic factors determining asthma in childhood. N Engl J Med 1981;9:60-1.
2. Del-Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 1988;140:4193-8.
3. Pène J, Rousset F, Brière F, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci U S A 1988;85:6880-4.
4. Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. J Exp Med 1989;169:1295-307.
5. Ishizaka A, Sakiyama Y, Nakanishi M, et al. The inductive effect of interleukin-4 on IgG4 and IgE synthesis in human peripheral blood lymphocytes. Clin Exp Immunol 1990;79: 392-6.
6. Lundgren M, Persson U, Larsson P, et al. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. Eur J Immunol 1989;19:1311-5.
7. Gascan H, Gauchat JF, Aversa G, Van-Vlasselaer P, de-Vries JE. Anti-CD40 monoclonal antibodies or CD4+ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. J Immunol 1991;147:8-13.
8. Defrance T, Carayon P, Bilian G, et al. Interleukin 13 is a B cell stimulating factor. J Exp Med 1994;179:135-43.
9. Punnonen J, de Vries JE. IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells. J Immunol 1994;152:1094-102.
10. Yssel H, Aversa G, Punnonen J, Cocks B, de Vries JE. Regulation of IgE synthesis by T cells and cytokines. Ann Fr Anesth Reanim 1993;12:109-13.
11. Wilhelm D, Kirchner H. T cells and their role in allergies. Exp Clin Immunogenet 1994;10:208-18.
12. Pène J, Rousset F, Brière F, et al. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. J Immunol 1988;141:1218-24.
13. Punnonen J, de Waal Malefyt R, van Vlasselaer P, Gauchat JF, de Vries JE. IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. J Immunol 1993;151:1280-9.
14. Marsh D, Neely J, Breazeale D, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 1994;264: 1152-6.
15. Mosmann TR, Schumacher JH, Street NF, et al. Diversity of cytokine synthesis and function of mouse CD4+ T cells. Immunol Rev 1991;123:209-29.
16. Romagnani S. Human TH1 and TH2 subsets: doubt no more. Immunol Today 1991;12:256-7.
17. Bousquet J, Becker WM, Hejjaoui A, et al. Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple-pollen species. II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts. J ALLERGY CLIN IMMUNOL 1991;88:43-53.
18. Bousquet J, Knani J, Hejjaoui A, et al. Heterogeneity of atopy. I. Clinical and immunologic characteristics of patients allergic to cypress pollen. Allergy 1993;48:183-8.
19. Pène J, Rivier A, Lagier B, Becker WM, Michel FB, Bousquet J. Differences in IL-4 release by PBMC are related with heterogeneity of atopy. Immunology 1994;81: 58-64.
20. Bousquet J, Hejjaoui A, Becker WM, et al. Clinical and immunologic reactivity of patients allergic to grass pollens and to multiple pollen species. I. Clinical and immunologic characteristics. J ALLERGY CLIN IMMUNOL 1991;87:737-46.
21. Ramirez DA. The natural history of mountain cedar pollinosis. J ALLERGY CLIN IMMUNOL 1984;73:88-93.
22. Weyer A, David B, Laurent M, Henocq E. Cellular histamine release, specific and total serum IgE levels in hay fever patients and controls. Clin Allergy 1978;8:187-94.
23. Gagnon R, Akoum A, Hebert J. Lol p I-induced IL-4 and IFN-gamma production by peripheral blood mononuclear cells of atopic and nonatopic subjects during and out of the pollen season. J ALLERGY CLIN IMMUNOL 1993;91:950-6.
24. Spiegelberg HL, Simon RA. Increase of lymphocytes with Fc receptors for IgE in patients with allergic rhinitis during the grass pollen season. J Clin Invest 1981;68:845-52.
25. Björksten B, Gamkrelidze A, Vanto T, Kjellman M. Seasonal variation of IgE synthesis in vitro by human peripheral blood mononuclear cells. Allergy 1990;45:572-6.
26. Gagnon R, Lian J, Boutin Y, Hebert J. Seasonal enhancement of IL-4 induced IgE synthesis by peripheral blood mononuclear cells of atopic patients. Clin Exp Allergy 1993;23:498-503.
27. Bousquet J, Hejjaoui A, Becker WM, et al. Clinical and immunologic reactivity of patients allergic to grass pollens and to multiple pollen species. I. Clinical and immunologic characteristics. J ALLERGY CLIN IMMUNOL 1991;87:737-46.
28. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. J Exp Med 1993;178:2123-30.
29. Chrétien I, Pène J, Brière F, De-Waal-Malefijt R, Rousset

F, De-Vries JE. Regulation of human IgE synthesis. I. Human IgE synthesis in vitro is determined by the reciprocal antagonistic effects of interleukin 4 and interferon-gamma. Eur J Immunol 1990;20:243-51.
30. Bousquet J, Djoukadar F, Hewitt B, Guérin B, Michel FB. Comparison of the stability of a mite and a pollen extract stored in normal conditions of use. Clin Allergy 1985;15: 29-35.
31. Cour P. Nouvelles techniques de détection des flux et retombées polliniques. Pollens et Spores 1973;16:103-62.
32. Bousquet J, Michel F. In vivo methods for study of allergy. Skin tests, techniques and interpretation. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. Allergy: principles and practice. 4th ed. St. Louis: Mosby, 1993:573-94.
33. Malling H. Position Paper of the EAACI: Allergen standardization and skin tests. Methods of skin testing. Allergy 1993;48(suppl 14):55-6.
34. Prieto L, Berto JM, Lopez M, Peris A. Modifications of PC20 and maximal degree of airway narrowing to methacholine after pollen season in pollen sensitive asthmatic patients. Clin Exp Allergy 1993;23:172-8.
35. Rousset F, Robert J, Andary M, et al. Shifts in interleukin-4 and interferon-gamma production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. J ALLERGY CLIN IMMUNOL 1991;87:58-69.
36. Rocken M, Muller KM, Saurat JH, Hauser C. Lectin-mediated induction of IL-4-producing CD4+ T cells. J Immunol 1991;146:577-84.
37. Van-der-Pouw-Kraan T, Van-Kooten C, Rensink I, Aarden L. Interleukin (IL)-4 production by human T cells: differential regulation of IL-4 vs. IL-2 production. Eur J Immunol 1992;22:1237-41.
38. Van-der-Pouw-Kraan T, de-Jong R, Aarden L. Development of human Th1 and Th2 cytokine responses: the cytokine production profile of T cells is dictated by the primary in vitro stimulus. Eur J Immunol 1993;23:1-5.
39. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J ALLERGY CLIN IMMUNOL 1982;70: 261-71.
40. Reid M, Schwietz L, Whisman B, Moss R. Mountain cedar pollinosis: can it occur in non-atopics? N Engl Regional Allergy Proc 1989;9:225-32.