

Low levels of interferon- γ in nasal fluid accompany raised levels of T-helper 2 cytokines in children with ongoing allergic rhinitis

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The T-helper 2 (Th2) cytokines interleukin-(IL-) 4, IL-5, IL-6, IL-10 and the Th1 cytokine IFN- γ and their associations with eosinophil, eosinophil cationic protein (ECP) and immunoglobulin (Ig) E were studied in nasal lavage fluid from 60 school children with allergic seasonal rhinitis and 36 nonatopic healthy controls, before and during the pollen season. Eosinophil differential counts and IgE increased significantly in the patients during the pollen season. The eosinophil differential counts, ECP and IgE were all significantly higher during the season than in specimens simultaneously obtained from the nonatopic controls. Before season, the levels of ECP and IgE, but not eosinophils, were significantly higher in the patients than in the controls. During the season the nasal lavage fluid levels of IFN- γ were significantly lower and the IL-4/IFN- γ quotients significantly higher in the allergic than in the control children. In the allergic children, but not in the controls, the nasal fluid levels of the Th2 cytokines IL-4, IL-5 and IL-10 increased during the season, and together with IL-6, were correlated with the differential counts of eosinophils, and with the levels of ECP and IgE. These findings are compatible with the hypothesis that a deficient release of the Th1 cytokine IFN- γ plays an important role in the pathogenesis of allergic inflammation. Regardless of whether the defective IFN- γ secretion is primary or a consequence of suppression by other cytokines, it will in the atopic subjects enhance the release of Th2 cytokines, which in turn will facilitate the development of allergic inflammation.

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Atopic diseases are considered to be associated with a shift in the balance between type 1 and type 2 T-helper cells (Th1 and Th2 cells) towards a Th2 predominance (1–3). Th1 and Th2 cells are characterized by different profiles of cytokine production. Thus, although there may be a considerable overlap *in vivo*, Th1 cells produce interferon- γ (IFN- γ) and Th2 cells interleukin-(IL-) 4, IL-5, IL-6, IL-10 and IL-13. Th1 and Th2 cells antagonize each other (4), and the importance of Th2 cytokines in allergic inflammation is evidenced by their role in inducing IgE and eosinophilia (2,3).

Interferon- γ down-regulates cytokine production of Th2 cells but not Th1 cells in a relatively specific manner because the former but not the latter express the β -chain of the IFN- γ receptor (5). This means that the Th1/Th2 imbalance in atopic diseases could be a consequence of two principal types of events: either a hyperproduction or increased reactivity of Th2-type cytokines; or a subnormal production or function of Th1-type cytokines. We have previously found increased levels of certain Th2 cytokines and decreased levels of IFN- γ in nasal fluids from children with allergic rhinitis, compared with

healthy controls (6). The decreased levels of IFN- γ in atopic individuals were only observed in nasal fluids collected during the pollen season, and the levels in specimens obtained outside the season in patients and controls were in fact similar. These findings suggested that a basic abnormality in allergic rhinitis may be an inability to mount an adequate IFN- γ response, which in turn secondarily might lead to an excessive production of Th2 cytokines. In agreement with this suggestion several recent studies have suggested that a defective production of IFN- γ underlies the increased tendency to produce IL-4 and other Th2 cytokines and may therefore be of fundamental importance for the development of allergic rhinitis and other atopic diseases (7–9). In the present study we describe extended investigations on the intranasal inflammatory response in allergic rhinitis to further test this hypothesis.

Patients and methods

Patients

The material has been described in detail previously (10). In summary, it consists of 60 school children with seasonal allergic rhinitis, and 36 nonatopic school children. Allergic rhinitis was defined by a positive seasonal history and a positive skin prick test to either birch pollen or timothy pollen or both. Twenty-seven patients had a positive reaction against birch pollen, eight against timothy and 25 against both. Local pollen counts in the air were determined daily by the Department of Botany at Göteborg University, Sweden during the whole pollen season.

The patients and controls were prospectively enrolled from a school in Göteborg during the spring term of 1997. Before the season, nasal lavage samples were obtained from 40 symptom-free patients with seasonal allergic rhinitis and from 36 healthy controls. The samples from the patients were taken from February 18–March 7, and from the controls from February 18–21. The patients were asked to return when symptoms of rhinitis occurred to give nasal lavage samples (April 10–June 6). In case of concurrent infection both patients and controls were asked to return at a later date. In addition, 20 new patients with allergic rhinitis were recruited during the pollen season. The controls were asked to return during the season, and samples were taken from May 16–21. All subjects were asked to mark their symptoms (sneezing, rhinorrhea, congestion and itching) on a visual analog scale of 10 cm. These values were measured and added to a total

symptom score. The maximum obtainable score was 40. The mean $\pm$ SEM symptom scores before and during the pollen season were 0.2 ± 0.1 and 16 ± 0.9 , respectively. None of the controls had any symptoms of rhinitis.

Collection of nasal lavage fluids

Nasal lavage fluids were obtained according to a previously described method (11). After clearing excess mucous by forceful exsufflation, sterile normal saline solution of normal room temperature was aerosolized into each nostril, while alternately clearing the other. The nasal washings were allowed to return passively and collected in a graded test tube until 3 ml were recovered. The samples were immediately placed in iced water. Within 3 h the samples were centrifuged at 1377 *g* at 4°C, for 10 min. The supernatant was separated from the pellet and stored at –70°C. Slides were prepared and stained according to the May–Grünwald–Giemsa method for morphological assessment of cells in the fluid (12). The percentages of epithelial cells, eosinophils, neutrophils and mononuclear cells out of a total of 100 cells/slide were then computed.

Mediator assays

All cytokines were measured using specific ELISA kits with improved sensitivity (Quantikine HS) obtained from R & D Systems (Abingdon, Oxon, UK), according to the manufacturer's instructions. The assays have undergone extensive testing with a multitude of different cytokines to ensure absence of cross-reactivity with other molecules. Cytokine concentrations in nasal fluids were quantified by comparison with a standard curve generated using the appropriate recombinant human cytokine. Validation experiments in our laboratory showed that all assays had a very high sensitivity, and inter- and intra-assay variations were generally low except when low cytokine concentrations were measured. To avoid errors due to this fact the sensitivity limits were set at levels that in some cases were higher than those recommended by the manufacturer. Thus the sensitivity limits were set at 7.5, 0.25, 3, 0.16 and 0.1 pg/ml for IFN- γ , IL-4, IL-5, IL-6 and IL-10, respectively. For calculation of ratios, concentrations of IL-4 less than 0.25 pg/ml were assigned a value of 0.25 pg/ml and IFN- γ concentrations below 7.5 pg/ml a value of 7.5 pg/ml.

The ECP concentrations were determined by means of a competitive radioimmunoassay, performed as described by the manufacturer (Pharmacia ECP RIA kit, Pharmacia & Upjohn

Diagnostics AB, Uppsala, Sweden). The assay for ECP has shown no cross-reactivity with other eosinophil proteins or neutrophil proteins. The IgE concentrations were determined using a radioimmunoassay kit obtained from the same manufacturer. The detection limit for this assay was set at 0.5 kU/l.

Protein assay

The protein concentration in all supernatants were determined using a modified Lowry method (Protein Assay, BIO-RAD, Hercules, CA, USA). The mean ($\pm$ SEM) protein content of samples obtained from the controls before and during the season were 0.49 ± 0.04 and 0.48 ± 0.05 mg/ml, respectively, and the protein contents in samples from allergic subjects before and during the season were 0.31 ± 0.03 and 0.33 ± 0.02 mg/ml, respectively.

Statistics

Results are expressed as means ($\pm$ SEM). The Kruskal–Wallis test was used to compare differences between patients and controls before and during season. When a large proportion of the variables were below the detection limit (IFN- γ , IL-4, IL-5 and IL-10), the χ^2 test was also used to analyze differences in the proportions of patients and controls with cytokine responses above the detection limits. In the 39 patients that were seen consecutively before and during the pollen season, the proportions of patients with either increased or decreased cytokine levels during season were analyzed with the Sign test. Patients with unchanged levels were excluded from the analysis. The Spearman–Rank correlation test was used to determine correlations between the cytokines and eosinophils, ECP and IgE, and p-values were calculated with permutation tests owing to the dependency between the patient data before and during pollen season. P-values < 0.05 were considered statistically significant. The analyses were performed with the SAS statistical analysis system (SAS Institute Inc., Cary, NC, USA).

Results

Eosinophils, ECP and IgE in patients with allergic rhinitis and controls

Eosinophil differential counts, ECP and IgE levels were all significantly higher in the allergic patients than in the controls during the season ($p < 0.0001$, < 0.05 , and < 0.0001 , respectively). In specimens obtained before the pollen season

there were higher values of IgE ($p < 0.05$) and ECP ($p < 0.01$), but not eosinophils, in the patients than in controls (Table 1). In the patients the eosinophil differential counts in nasal fluids increased from 0 before the pollen season to $11.0 \pm 2.0\%$ during the pollen season, IgE levels increased from 0.4 ± 0.2 to 2.7 ± 0.4 kU/l, and ECP levels from 32.5 ± 6.4 to 37.9 ± 5.9 μ g/ml.

Changes of eosinophils, ECP and IgE in individual patients during the pollen season

In the patients the differences in eosinophil differential counts, ECP and IgE levels were analyzed statistically in the paired samples obtained from the 39 patients seen both before and during season. This was done with the Sign test, which compares the number of patients with increasing levels of the analyzed variable against the number of patients with decreasing levels. Patients with unchanged levels are excluded from the analysis. Increased eosinophil differential counts were found in 27 of these patients during, as compared to before, the season, whereas none of the patients had decreased levels ($p < 0.0001$). The corresponding figures for ECP were 24 of 39 patients (n.s), and for IgE 25 of 31 patients ($p < 0.001$).

Levels of IFN- γ , IL-4, IL-5, IL-6, IL-10 and IL-4/IFN- γ ratios in patients and controls

The proportion of subjects with detectable IFN- γ levels was significantly lower in patients than in controls sampled during the pollen season: 55% vs. 82% ($p < 0.01$) (Table 2). In contrast, the proportion of subjects with detectable IL-10 was higher in patients than in controls during pollen season: 46% vs. 16% ($p < 0.01$). Before the season no significant difference in the proportions of patients and controls with detectable IFN- γ levels was found, but the proportion of patients with detectable IL-10 was higher: 32% vs. 11% ($p < 0.05$). The percentages of patients having detectable nasal levels of IL-4, IL-5 and IL-10 increased during the pollen season compared with before the season, from 15% to 27%, 10% to 27%, and 32% to 46%, respectively (Fig. 2, Table 2). All patients displayed detectable levels of IL-6, before as well as during the pollen season. In contrast, the percentages of patients with detectable IFN- γ decreased from 74% before season to 55% during season (Fig. 2, Table 2). In the controls, the corresponding proportions for IL-4, IL-5 and IL-10 were 9% and 12%, 26% and 30%, and 11% and 16%, respectively. The percentages of controls with detectable IFN- γ

Table 1. IgE, ECP and eosinophils in nasal fluids from patients and controls

	IgE (kU/l)	ECP (μ g/ml)	Eosinophils (%)
Controls			
<i>before pollen season</i>			
mean $\pm$ SEM	0.1 $\pm$ 0.1	13.4 $\pm$ 3.3	0.1 $\pm$ 0.0
median	0	7.2	0
range	0–3.5	0–84	0–1
n	32	32	35
<i>during pollen season</i>			
mean $\pm$ SEM	0	18.0 $\pm$ 3.2	0.1 $\pm$ 0.1
median	0	12.9	0
range	0–0	0–72	0–3
n	29	30	33
Allergic children			
<i>before pollen season</i>			
mean $\pm$ SEM	0.4 $\pm$ 0.2**†	32.5 $\pm$ 6.4***†	0
median	0	14	0
range	0–4.3	0–140	0
n	39	39	39
<i>during pollen season</i>			
mean $\pm$ SEM	2.7 $\pm$ 0.4****‡	37.9 $\pm$ 5.9*‡	11.0 $\pm$ 2.0****‡
median	2.5	19.5	3
range	0–12.6	0–200	0–55
n	60	60	59

* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ † Compared with controls before season, Kruskal–Wallis test. ‡ Compared with controls during season, Kruskal–Wallis test.

increased from 66% before season to 82% during season (Fig. 1, Table 2).

In agreement with the results obtained when proportions of positive samples were computed, the absolute levels of the different Th2 cytokines in the patients tended to increase during the pollen season, whereas there was an opposite trend regarding IFN- γ . The absolute nasal levels of IL-6, expressed as mean $\pm$ SEM pg/ml, were 4.9 ± 0.7 before and 14.8 ± 4.8 during season. The corresponding levels of IL-10 were 0.23 ± 0.10 and 0.43 ± 0.10 , and of IFN- γ 16.7 ± 3.3 and 15.5 ± 3.3 (Table 2). As reported elsewhere (10) the levels of IL-5 increased from 0.44 ± 0.21 to 1.70 ± 0.50 . The IL-4 levels were 0.16 ± 0.10 before season and 0.17 ± 0.10 during season. In the controls the mean levels were essentially unchanged (Table 2), although the proportion of patients with detectable cytokines tended to increase (Fig. 2).

The IL-4/IFN- γ ratios were significantly higher in patients than in controls during, but not before, the pollen season ($p < 0.01$), and there was also a trend towards a rise of these ratios during the pollen season (Fig. 1, Table 2).

Changes of cytokine levels in individual patients during the pollen season

The differences in cytokine responses in the patients before and during season were analyzed statistically in the paired samples obtained from

the 39 patients seen both before and during season. Fifteen patients had detectable IL-4 levels either before or during the season. Twelve of these 15 patients showed a demonstrable increase of IL-4 during the season ($p < 0.05$). The corresponding proportions for IL-5 were 10 of 13 ($p < 0.05$); for IL-6, 16 of 37 (n.s); and for IL-10, 12 of 16 patients ($p < 0.05$). Twenty of 32 patients with detectable IFN- γ showed a decrease in IFN- γ levels (n.s).

Correlations between IFN- γ , IL-4, IL-5, IL-6, and IL-10 and eosinophils, ECP or IgE in the allergic patients, before and during the pollen season

As shown in Table 3, IFN- γ correlated with ECP ($r = 0.27$, $p < 0.05$) but not with IgE or eosinophils. Interleukin-4 correlated with eosinophils ($r = 0.28$, $p < 0.05$), ECP ($r = 0.39$, $p < 0.001$) and IgE ($r = 0.23$, $p < 0.05$). Interleukin-5 correlated with ECP ($r = 0.36$, $p < 0.001$), IgE ($r = 0.29$, $p < 0.05$) and, as previously reported (10), with eosinophils ($r = 0.44$, $p < 0.001$). Interleukin-6 correlated with IgE ($r = 0.23$, $p < 0.05$), ECP ($r = 0.48$, $p < 0.001$) and eosinophils ($r = 0.21$, $p < 0.05$). Interleukin-10 correlated with IgE ($r = 0.28$, $p < 0.05$), ECP ($r = 0.28$, $p < 0.05$) and eosinophils ($r = 0.26$, $p < 0.05$).

Discussion

In a previous study we found higher levels of the type 2 cytokines IL-4 and IL-10 but lower nasal fluid levels of IFN- γ in children with allergic rhinitis than in controls (6). In the present study these findings were extended; and further evidence was obtained that nonatopic individuals tend to respond by mounting an inflammatory response that includes a rise in the intranasal levels of IFN- γ , whereas atopic individuals respond by producing Th2-type cytokines, but not IFN- γ , upon natural allergen stimulation (i.e. during the pollen season). In fact, in the present study there was a clear tendency towards declining rather than increasing levels of IFN- γ in the atopic patients during the pollen season. Thus, there seems to be an important difference between the Th1 cytokine IFN- γ on one hand and the Th2 cytokines on the other, in that the latter, but not the former, show a rise in response to allergen stimulation in atopic subjects.

It should, however, be emphasized that although the Th2 cytokines IL-4, IL-5 and IL-10 increased significantly in the patients during season compared with before season, most

Table 2. IFN- γ , IL-4, IL-6, IL-10 and IL-4/IFN- γ quotients in nasal fluids from patients and controls

	IFN- γ (pg/ml)	IL-4 (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	IL-4/IFN- γ ($\times 10^{-3}$)
Controls					
<i>before pollen season</i>					
mean $\pm$ SEM	41.8 $\pm$ 10.5	0.03 $\pm$ 0.02	8.2 $\pm$ 2.2	0.18 $\pm$ 0.10	18.2 $\pm$ 2.3
median	17.5	0	4.3	0	16.7
range	0–235	0–0.63	0.4–55.5	0–2.43	1.1–36
% > detection limit	66	9	100	11	100
n	35	35	35	35	35
<i>during pollen season</i>					
mean $\pm$ SEM	47.3 $\pm$ 18.1	0.07 $\pm$ 0.04	7.4 $\pm$ 1.6	0.10 $\pm$ 0.05	18.5 $\pm$ 2.1
median	16.5	0	3.9	0	15.6
range	0–510	0–1	0.4–40.5	0–1.18	0.5–40
% > detection limit	82	12	100	16	100
n	33	33	33	32	33
Allergic children					
<i>before pollen season</i>					
mean $\pm$ SEM	16.7 $\pm$ 3.3	0.16 $\pm$ 0.10	4.9 $\pm$ 0.7	0.23 $\pm$ 0.10	25.8 $\pm$ 2.5
median	11	0	3.7	0	27.8
range	0–100	0–3.6	0.7–25.5	0–2.5	3.7–76.6
% > detection limit	74	15	100	32*†	100
n	39	39	39	38	39
<i>during pollen season</i>					
mean $\pm$ SEM	15.5 $\pm$ 3.3**‡	0.17 $\pm$ 0.10	14.8 $\pm$ 4.8	0.43 $\pm$ 0.10**‡	33.9 $\pm$ 5.5**‡
median	8	0	3.9	0	33.3
range	0–116	0–2.5	0.5–183	0–2.4	2.8–312.5
% > detection limit	55**§	27	100	46**§	100
n	60	59	60	41	59

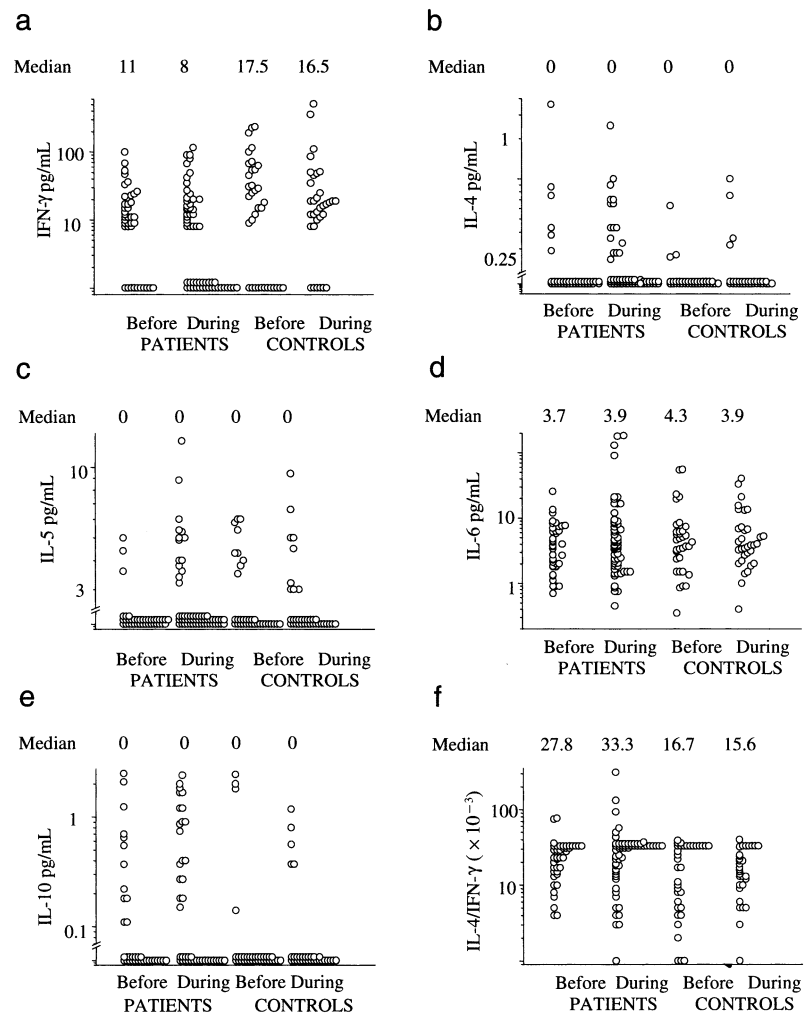
*p < 0.05, **p < 0.01. † Compared with controls before season, χ^2 test. ‡ Compared with controls during season, Kruskal–Wallis test. § Compared with controls during season, χ^2 test.

patients did not have detectable levels of these cytokines, either before or during the season. Furthermore, only IL-10 was higher in the patients than in the controls during season. Similarly, while IFN- γ levels tended to decrease in the patients during season, these differences were not significant. However, during season IFN- γ levels were significantly lower in the patients than in the controls. The lack of clear-cut differences between patients before and during season, or between patients and controls, could be explained by other mechanisms than that implied by the Th1/Th2 paradigm (13). An alternative explanation could, for instance, be that cytokine expression could vary at different time points of allergic inflammation. Indeed, studies of bronchial lavage (BAL) fluids from patients with asthma, and skin biopsies from patients with acute and chronic atopic dermatitis, indicate that increased IL-4 expression may be more pronounced during the early stages of allergic inflammation, and that Th1-like or mixed Th1 and Th2 responses occur during the later stages (14,15). Because our patients had an average duration of symptoms of one week before giving their nasal fluids, it is likely that they would be in the later stages, which could explain why they had cytokine patterns that were only partially consistent with the Th1/Th2

paradigm. Furthermore, cytokine expression could vary with the degree of pollen exposure. This possibility is supported by a comparison with a study performed in 1996 (6). Both studies were of similar design and the same cytokine assays were used. Air pollen levels were twice as high as during the former season, and the differential counts of eosinophils and IL-4 levels were three to four times higher compared with this study.

However, our finding of a possibly defective secretion of IFN- γ in atopic patients following pollen stimulation *in vivo* is concordant with several reports showing that the ability of peripheral mononuclear cells to produce IFN- γ upon stimulation with mitogens *in vitro* is impaired in patients with atopic dermatitis or asthma (7–9, 16–21). By contrast, the release of Th2 cytokines has been described as either unchanged or increased (7, 20–22). Because IL-4 and IFN- γ are mutually cross-regulatory (4,23), it can be surmised that the ratio of IL-4 and IFN- γ release in each individual, rather than the absolute levels of either cytokine, is decisive for the type of inflammatory reaction evolving after allergenic stimulation. Indeed, this is supported by reports of increased IL-4/IFN- γ ratios in allergic patients in this and other studies (6,16,20).

Fig. 1. (a)–(f). Scattergrams of the levels of IFN- γ , IL-4, IL-5, IL-6, IL-10 and the IL-4/IFN- γ quotients in the patients and controls, before and during the pollen season. The median levels are indicated above the scattergrams.



Previous results suggesting reduced activity of IFN- γ in atopic disease have relied on experiments utilizing mitogens or allergen stimulation in *in vitro* systems or demonstration of IFN- γ mRNA or protein in lymphocytes in the blood or in target organs. The present study suggests that the previously demonstrated relative inability of peripheral blood cells from atopics to produce IFN- γ upon allergen stimulation *in vitro* (24,25) is indeed reflected by reduced extracellular levels of the cytokine at the site of the allergic inflammation.

The above results suggest that a relative inability to produce IFN- γ may be associated

with all types of atopic allergy. As babies who later develop atopic disease have an impaired ability to produce IFN- γ (26,27), it seems possible that defective IFN- γ release may be a genetically determined trait that underlies all types of atopic disease. This possibility gets some support from the present study because low production of IFN- γ did not seem to be secondary to hyperproduction of IL-4. In some other studies, low production of IFN- γ , rather than high production of IL-4, has been found to be associated with high serum IgE levels (8,9) or with reduced lung function (8). In animal experiments it has been demonstrated that inhibition of allergen-induced

Table 3. The correlations, r , between the cytokines and IgE, ECP and eosinophils in the allergic patients, before and during pollen season

	IFN- γ	IL-4	IL-5	IL-6	IL-10
IgE	-0.01	0.23*	0.29*	0.23*	0.28*
ECP	0.27*	0.39***	0.36***	0.48***	0.28*
Eosinophils	-0.04	0.28*	0.44***	0.21*	0.26*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Spearman Rank correlation test.

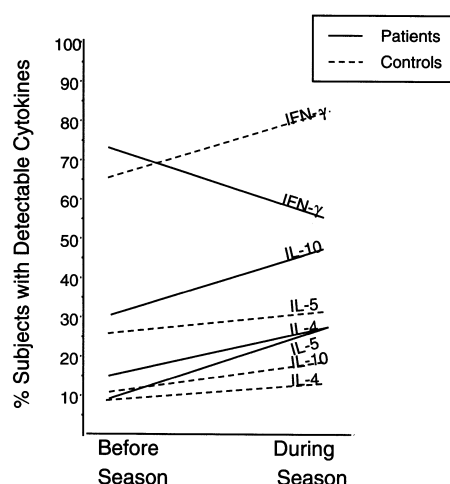


Fig. 2. The proportions of patients and controls with detectable cytokine levels in nasal fluids, before and during the pollen season. Patients are indicated by solid lines, and controls by dashed lines.

eosinophil influx in the airways is dependent on IFN- γ (28). It thus appears that a possible basic deficiency of IFN- γ production may have certain functional implications that are characteristic of atopic allergy.

Although the hypothesis that hypoproduction of IFN- γ is a primary, basic abnormality in atopic disease seems attractive, it is perhaps equally possible that the defective ability to mount IFN- γ responses is dependent on overproduction of factors, e.g. IL-4, that suppress IFN- γ responses. In this regard it is noteworthy that genetic abnormalities in atopic disease have been localized both to chromosome 5, which contains the IL-4 gene (29), and chromosome 12, to which the IFN- γ gene is localized (30). Genetic polymorphisms that might be associated with atopy have been found in the promoter of the IL-4 gene (31) but not in the IFN- γ gene (32).

It is also possible that an altered Th1/Th2 balance in atopic subjects might be the result of defects related to antigen presentation. Recently data have been presented indicating that dendritic cells, which process and present antigen to T cells, from atopic subjects may secrete cytokines that skew T cells towards a Th2-like phenotype (33).

In our previous study (6) there was a statistically significant rise in nasal IFN- γ in nonatopic controls during the pollen season, and the present study showed a tendency towards this. In the previous study pollen exposure during the season was twice as high as that occurring during the present study period. It is therefore possible that the lack of significant changes in this study could depend on lower pollen exposure. However, since only one sample was taken from each subject during the season, it is also possible that the results

could have been confounded by the kinetics of the IFN- γ response. In addition, exposure to environmental antigens such as air pollutants or viruses could confound the results (12,34). It should be noted, however, that although such exposure could affect IFN- γ levels, all controls were symptom-free. Interestingly, there was a tendency towards a rise in all other cytokines during the season, supporting an induction of an inflammatory response also in nonatopic individuals. This is reminiscent of the vigorous induction of a variety of proinflammatory cytokines in nasal and bronchial lavage fluids of workers exposed to swine dust (35). In a study of IgE and IgG immune responses in mice and rabbits it was found that birch pollen had adjuvant properties (36). Recently Tomee et al. (37) reported that pollen induced significant increases of IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) release from nasal epithelial cells in healthy subjects. Significantly higher levels of IFN- γ have been found in allergen-stimulated cell cultures from healthy than from atopic individuals (24,25). Thus it seems that an IFN- γ response to pollen may be a marker of a Th1-type immune response that could possibly have a protective role against atopic disease. Further studies are needed to elucidate whether Th1-like responses to pollen occur in healthy subjects.

We have previously found specific correlations between different cytokines and inflammatory cells in nasal fluids. For instance IL-5, but not IL-8, was found to correlate with eosinophils, and IL-8, but not IL-5, to correlate with neutrophils (10). This suggests that analysis of correlations between cytokines and other inflammatory markers in nasal fluids from patients with allergic rhinitis may yield information about the regulatory effects of Th1 and Th2 cytokines locally, *in vivo*. We therefore analyzed eosinophils, ECP and IgE in nasal fluids and their possible correlations with IFN- γ , IL-4, IL-5, IL-6 and IL-10. As expected, eosinophils as well as IgE increased significantly during the pollen season in the allergic patients. There was also a tendency towards a rise in ECP. By contrast, there were no changes regarding these parameters in the controls. The levels of all studied Th2 cytokines correlated with eosinophils, ECP and IgE. By contrast, IFN- γ levels correlated with neither eosinophils nor IgE, although there was a correlation to ECP levels. The correlation between ECP and IFN- γ was unexpected, in view of the supposedly opposing roles of these factors in allergic inflammation: ECP is associated with symptoms in allergic disorders whereas many reports, including the present

one, suggest that IFN- γ has an antiallergic effect. However, receptors for IFN- γ have been found on eosinophils, and if these cells are stimulated by IFN- γ they may release ECP (38). It is thus possible that the correlation between IFN- γ and ECP could reflect an involvement of IFN- γ in regulating release of ECP and that the actual intranasal levels of ECP could be the net result of many interacting cytokines.

The finding of a marked rise of intranasal IgE levels in conjunction with development of rhinitis contrasts with the results of an earlier study (39) but agrees with the notion that IgE plays a key role in allergic inflammation. The finding that all Th2 cytokines correlated with IgE concentrations is consistent with, for example, the notion that IL-4, IL-5, IL-6 and IL-10 regulate B-cell proliferation and IgE synthesis (22,40). In further agreement with this notion we did not find any correlation between the levels of IgE and IL-8, a chemokine with other types of biological activities (data not shown).

Interestingly, experimental data have shown that IL-10 may inhibit IgE synthesis in the early stages of allergic inflammation but act as an enhancer in later stages (40). Because our patients had an average symptom duration of one week before giving their nasal fluid samples, it is likely that they would be in the later stages of allergic inflammation. This emphasizes the importance of defining the effects of cytokines, not only with respect to their role in the cytokine network but also in relation to the kinetics of the inflammatory response. For instance, the lack of an inverse relationship between IFN- γ and IgE, which was somewhat unexpected in view of the recent results by Teramoto et al. (9), could be due to differing kinetics of the formation of the different factors involved in the allergic inflammation.

In summary, the principal finding in this study was the demonstration of discordant intranasal Th1 and Th2 cytokine responses in atopic children, resulting in lower levels of IFN- γ and higher levels of Th2 cytokines than those found in controls following stimulation with allergen *in vivo*. These results support the hypothesis that a deficient release of IFN- γ , which may be a basic trait in atopy, plays an important role in the pathogenesis of allergic rhinitis by permitting the Th2 cytokines to induce increased intranasal levels of IgE, eosinophils and ECP.

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