

Cytokines in nasal fluids from school children with seasonal allergic rhinitis

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Allergic rhinitis is a particularly good model for studies of cytokine production *in vivo*. In this study the occurrence of the cytokines IL-4, IL-5, IL-10 and IFN- γ as well as the soluble receptor for IL-4 in nasal lavage fluids were assayed in 38 school children, with seasonal allergic rhinitis, and 19 healthy age-matched, non-atopic controls, using highly sensitive enzyme immunoassays.

IL-4 levels in patients with seasonal allergic rhinitis were markedly increased in comparison with those in non-atopic controls or in atopic patients before the start of the pollen season. In controls, but not in the atopic patients, levels of IFN- γ and IL-5 were significantly higher in specimens obtained during the pollen season than in those obtained outside the season. The IL-4/IFN- γ ratios were significantly higher in atopic than in non-atopic subjects and further increased in atopic patients during the season. In addition to IL-4, elevated levels of IL-10 were observed in association with seasonal rhinitis. Following treatment with a topical steroid (budesonide) there was a statistically significant increase of the levels of soluble IL-4 receptor.

These findings indicate that nonatopic and atopic individuals react to pollen exposure with distinct cytokine patterns in agreement with the Th1/Th2 concept. Topical steroids may possibly decrease inflammation by increasing the formation of soluble IL-4 receptor.

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Introduction

Allergic inflammation is caused by a T-cell-dependent activation of a complex cellular network, including B cells, eosinophils, mastcells and basophils. From early observations that atopy was associated with an abnormality of T cells (1), subsequent research showed that this abnormality corresponded to a shift in the balance between two lymphocyte subsets i.e. from a Th1 to a Th2 cell preponderance (2). These two subsets of lymphocytes can be identified by different profiles regarding cytokine secretion. Secretion of interferon- γ (IFN- γ) is often used as a marker of Th1 cell activity and interleukin-4 (IL-4) as a marker of Th2 cells although these two cytokines can also be produced by several other types of cells. Since IFN- γ is produced by and stimulates Th1 cells and inhibits Th2 cell activity and the opposite is true for IL-4 (3) these cytokines are particularly well suited as markers for the determination of the Th1/Th2 balance. Although the general

validity of the Th1/Th2 concept has been questioned (4, 5) it still seems justifiable to characterize immune responses as being Th1- or Th2-like (6).

The clinical relevance of the Th1/Th2 concept in atopic allergy is supported by many studies. Thus, increased serum levels of IL-4 in allergic patients have been observed (7–9) and stimulation of peripheral blood mononuclear cells from atopic individuals with appropriate allergens results in higher levels of IL-4 and lower levels of IFN- γ than those observed after stimulation of cells from non-atopic individuals (10, 11). However, it should be stressed that Th-1 and Th-2 cells as well as IL-4 and IFN- γ are parts of complex networks that involve many other cells and cytokines and also soluble receptors which modulate the effects of the cytokines. For example, the effects of IL-4 may be inhibited by the soluble IL-4 receptor (IL-4sR). The clinical relevance of this inhibition as well as the importance of IL-4 is shown by the results of phase I and II trials in which IL-4sR is used as an antiasthmatic drug (12).

Allergic rhinitis is a particularly good model for studies of the Th1/Th2 concept *in vivo*, since the cytokines can be determined locally, in nasal lavage fluid. Important aspects that can be studied are the cytokine response before and during the pollen season in both atopic and healthy individuals, as well as the effects of treatment with, for example, topical steroids.

Recently developed ultrasensitive immunoassays for the determination of cytokines in body fluids allow accurate measurement of cytokines, including those that occur in very low concentrations. Using such assays the aims of our study were to investigate the occurrence of IL-4, IL-4sR, IL-5, IL-10 and IFN- γ in nasal fluid from patients with seasonal allergic rhinitis before and during symptoms, as well as after treatment with topical steroids (budesonide) and in healthy controls before and during the pollen season.

Materials and methods

Subjects

The subjects included 38 patients with seasonal allergic rhinitis aged 12 to 20 years and 19 healthy nonatopic controls aged 13 to 19 years. Eighteen of the patients and eight of the controls were girls. Allergic rhinitis was defined by a positive history of seasonal symptoms and a positive skin prick test to either birch pollen or timothy pollen or both. Skin prick testing was performed on the volar side of one arm according to the "Nordic Guidelines". Soluprick 10 Hep (ALK, Hørsholm, Denmark) was used and normal saline was used as a negative control and histamine dihydrochloride 10 mg/mL served as positive control. The test was considered positive when the wheal was at least 50% of the area of the wheal induced by histamine dihydrochloride 10 mg/mL (13). Eleven subjects had a positive reaction against birch pollen, six against timothy and 21 against both. Two patients with positive history but negative skin tests and three, who refused skin tests, were excluded from the study.

The control subjects had no history whatsoever of seasonal or perennial rhinitis, asthma, atopic dermatitis or urticaria and they were all symptom-free and received no medication. The controls were not skin prick-tested. However, the lack of history of atopy together with the total absence of eosinophils in nasal lavage fluid from these children (see below) was considered to indicate that they were truly nonatopic.

Twenty of the patients were on medications as part of their regular treatment; 13 were treated with antihistamines immediately prior to and during the pollen season, one with topical sodium cromoglycate only and six with both drugs. For ethical rea-

sons these patients were not asked to discontinue their medications, to comply with study purposes. Patients continuously treated with topical or systemic steroids were not included in the study, however, since evaluation of the cytokine response in relation to steroids was one of the aims of the study. Skin tests were performed outside the pollen season and none of the subjects were then treated with antihistamines at the time of the testing.

Local pollen counts in the air were determined daily by the Department of Botany at Göteborg University during the whole birch and grass pollen seasons.

Study design

Patients and healthy control subjects were prospectively enrolled from two schools in Göteborg, Sweden during the Spring 1996. Informed consent was obtained from both the subjects and the parents. The study was approved by the Ethics Committee of Göteborg University.

Before the season nasal lavage samples were obtained from 19 healthy controls and from 22 symptom-free patients. The patients were asked to return as soon as possible when rhinitis occurred to give new nasal lavage fluids. In addition 16 new patients with rhinitis were recruited during the pollen season. The median (range) duration of symptoms before start of steroid therapy was 1.5 (1–14) days. Treatment with budesonide nasal spray 100 μ g in each nostril b.i.d. and if needed levocabastin eye-drops 0.5 μ g/mL 1–2 drops in each eye b.i.d. was then initiated. Thirty-three of these patients subsequently gave a final nasal lavage sample within a median (range) of 7 (1–21) days).

All subjects were asked to mark their symptoms (sneezing, rhinorrhoea, congestion and itching) on a visual analogue scale of 10 cm. These values were measured and added to a total symptom score. The maximum obtainable score was 40.

Collection of nasal lavage fluid

The subjects were asked to exhale forcefully through the nose to clear excess mucous. Nasal lavage fluid was then obtained using a disposable metered-dose pump filled with 6 mL of sterile normal saline solution at room temperature. Each activation delivered 50 μ L of the solution. The saline solution was aerosolized into each nostril, while alternately occluding the other. The nasal washings were then allowed to return passively and were collected in a specimen cup. The aim was to recover 3 mL in each patient but this was not possible in all cases. The median volume of introduced fluid was 4 (3.5–6) mL. The median recovered fluid was 3 (1–4.5) mL. The study was based on recovered rather than intro-

duced volumes (14). The samples were immediately placed in iced water. Within three hours the samples were centrifuged at 2500 rpm at -4°C , for 10 minutes. The supernatant was separated from the pellet and stored at -70°C . The pellet was resuspended in 50 μL of Ringer's solution. Slides were prepared and stained according to the Giemsa method. The percentage of eosinophils out of total of 100 cells/slide was then computed.

Cytokine assays

All cytokines, except IFN- γ , were assayed by means of immunoassay kits obtained from R&D Systems. Regular ELISA kits (Quantikine) for IL-4sR and IL-5 and assays with improved sensitivity (Quantikine HS) for IL-4 and IL-10 were used (R & D Systems, Minneapolis, MN, USA).

All the above assays had, according to the manufacturer, undergone extensive testing with a multitude of different cytokines to ensure absence of crossreactivity with other molecules. The assay for IL-4sR has been tested by the manufacturer for interference from IL-4. At IL-4 concentrations < 15 ng/mL no interference was found. Similarly the assay for IL-4 was tested for interference from IL-4sR. At IL-4sR concentrations < 50 ng/mL no interference was found.

IFN- γ was measured by an ELISA test using monoclonal antibodies, purchased from MABTECH AB, Stockholm, Sweden. The assay was performed as described by the manufacturer (15). The specificity of this test was ensured after cross-testing with different cytokines.

Validation experiments in our laboratory showed that all assays employed had a very high sensitivity and inter and intra assay variations were generally low except when very low cytokine concentrations were measured. To avoid errors due to this fact the sensitivity limits of the tests were set at levels that in some cases were higher than those given by the manufacturer. Thus, the sensitivity limits were set at 7.5, 0.5, 0.5, 0.25 and 0.1 pg/mL for IFN- γ , IL-4sR, IL-5, IL-4 and IL-10, respectively. For calculation of ratios all concentrations of IL-4 less than 0.25 pg/mL were assigned a value of 0.25 and all IFN- γ concentrations below 7.5 pg/mL a value of 7.5. By choosing comparatively high sensitivity limits, the confounding effects of high variability at low cytokine concentration on statistical calculations could thus be minimized.

Correlations between cytokine levels, symptom scores and pollen counts

In the birch and grass pollen allergic patients cytokine levels and symptom scores were correlated to

the birch and grass pollen counts, respectively. In patients who had both birch and grass pollen sensitivity, cytokine levels and symptom scores were correlated to the counts of the particular pollen that was the highest one on the day when the sample was collected. In the part of Sweden where the study was conducted, the birch pollen season occurs from late April through the first half of May, while the grass pollen season starts in late May/early June. In the controls, who all lacked specific allergy, correlations were calculated between cytokine levels and the total as well as the predominant pollen counts.

Statistics

Results are given as the median (range) of the cytokine values in pg/mL. The Kruskal-Wallis test was used to test differences between the groups and the Spearman rank correlation test to determine the correlations. p -values < 0.05 were considered statistically significant.

Results

Pollen counts

The median (range) birch pollen (*Betula*) counts were 23 (0–1338) counts/ m^3 air and grass pollen (*Poaceae*) counts were 0.5 (0–11) counts/ m^3 air during the study period.

Eosinophils and symptom scores

No eosinophils were found in nasal lavage fluid from healthy controls before or during the pollen season. In allergic patients the median percentage of eosinophils was significantly higher in symptomatic than in asymptomatic patients (14 (0–60) and 0 (0–10), respectively; $p < 0.001$). After treatment with budesonide the eosinophils decreased to 0 (0–28) percent ($p < 0.001$ for difference between treated and untreated patients).

All controls were asymptomatic. The median (range) symptom scores of the allergic patients before the pollen season, during the season and after budesonide treatment were 0 (0–2), 20 (4–36) and 4.5 (0–36.5), respectively.

Cytokine levels in nasal fluids of control children

Some of the cytokines studied appeared to be produced constitutively since they occurred in detectable quantities in individuals who were healthy and had no signs of rhinitis. Thus, IFN- γ , IL-4sR and IL-5 occurred in median concentrations of about 8, 3 and 1, pg/mL respectively in non-atopic children (Table 1).

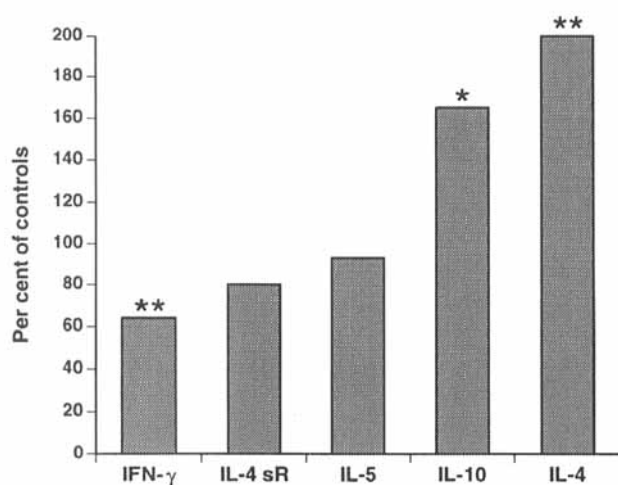


Fig. 1. Cytokine levels in nasal fluids of children with seasonal rhinitis in relation to controls. Bars indicate percentage value of cytokine level in nasal fluid from patients with rhinitis in relation to levels found in control children sampled during the pollen season (* = $p < 0.05$ and ** = $p < 0.01$, Kruskal-Wallis test).

A comparison between cytokine levels in nasal fluid before and during the pollen season showed that there was a statistically significant rise of the levels of IFN- γ and IL-5 during the pollen season (Table 1). IFN- γ concentrations showed a good correlation to total pollen counts in the air ($r = 0.6$; $p < 0.001$). Although the correlation between IL-5 and total pollen count was non-significant, IFN- γ as well as IL-5 levels correlated significantly to the birch pollen count ($p < 0.05$ and $p < 0.01$, respectively; both r -values = 0.5).

Cytokine levels in nasal fluids of children with seasonal rhinitis

Children with ongoing rhinitis had significantly increased levels of IL-4 ($p < 0.01$) and IL-10 ($p < 0.05$) as compared to control children tested during the pollen season (Fig. 1).

The median levels of IFN- γ were significantly lower in children with rhinitis than those in non-at-

opic children sampled during the pollen season (9 vs. 15 pg/mL, $p < 0.01$) (Tables 1, 2 and Fig. 1).

A comparison between cytokine levels in nasal samples obtained from the atopic children before and during the pollen season revealed a significant rise of the IL-4 level during the season (Table 2). IL-4 correlated to eosinophil counts in nasal lavage fluid ($r = 0.4$, $p < 0.001$), to specific pollen counts ($r = 0.3$, $p < 0.05$) and to symptom scores ($r = 0.4$, $p < 0.001$). As expected IL-5 levels correlated to eosinophil counts in nasal lavage fluid ($r = 0.4$, $p < 0.01$). There were no significant differences in IL-4, IFN- γ and IL-4sR levels between untreated patients or patients treated with antihistamines or cromolyn.

Cytokine levels in steroid-treated children with seasonal rhinitis

Generally, differences in cytokine levels between treated and non-treated children with rhinitis were modest. However, as seen in Table 2, IL-4sR levels were significantly higher in the treated patients than in the nontreated children.

IL-4/IFN- γ ratios in nasal fluids of healthy controls and patients

The geometric mean values of the IL-4/IFN- γ quotients were higher in all groups of atopic patients than in the controls. Strongly elevated values were observed in both untreated and steroid-treated patients with rhinitis, but were also found in some patients outside the pollen season when asymptomatic (Fig. 2). The highest quotient value observed among the controls was 70×10^{-3} . Values exceeding this were found in 18% of the patients pre-seasonally, in 54% during the season and in 31% of those treated with budesonide. The differences between patients in season and controls as well as between patients during and outside the season were statistically significant ($p < 0.001$ and $p < 0.01$, respectively). By contrast, the difference between untreated and budesonide-treated patients was not statistically significant and, likewise, the lower mean ratio found in controls during the pollen season was not significantly different from that found in these subjects before the pollen season.

Discussion

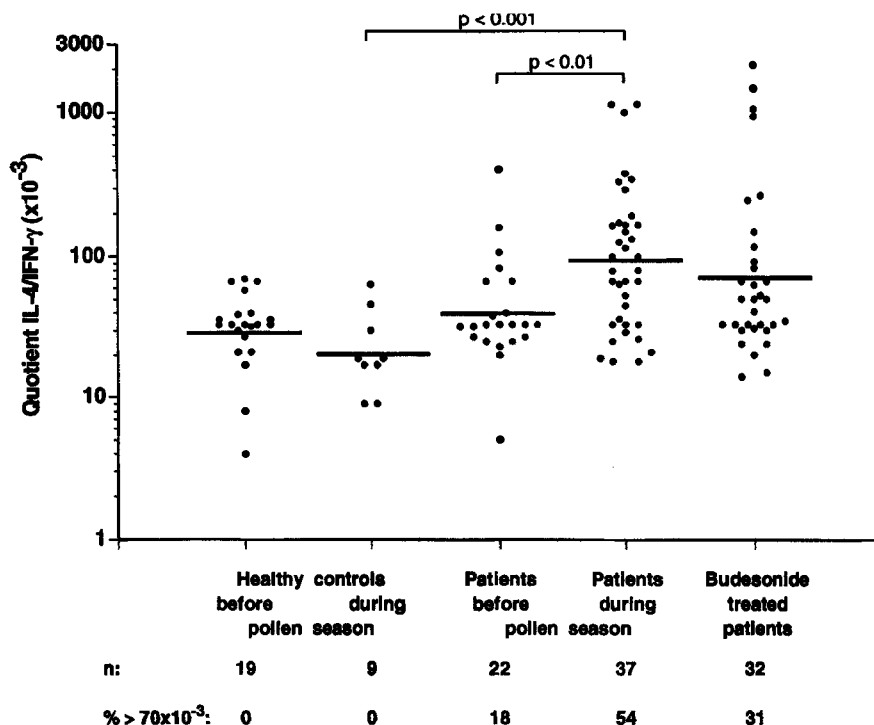
Cytokines in allergic rhinitis have been studied in investigations based on both natural and provoked rhinitis using a variety of methods (16). Using enzyme immunoassays, increases in the concentrations in nasal fluid of cytokines like IL-5, IL-6, TNF- α and GM-CSF have been described, whereas IL-4, IL-10 and IFN- γ have escaped detection in these studies (17–20). This may be due to low sensitivities of the assays used. With the aid of other methods such as immunofluorescence or measure-

Table 1. Nasal lavage fluid cytokines in healthy controls, before and during pollen season

	IL-4	IFN- γ	IL-4sR	IL-5	IL-10
<i>Before season (N = 19)</i>					
median (pg/mL)	0.3	7.5	3.0	1.3	<0.1
range	<0.25–1.4	<7.5–60	<0.5–140	<0.5–17.5	<0.1–1.1
<i>Season (N = 9)</i>					
median (pg/mL)	<0.25	15.0*	11.0	6.5*	<0.1
range	<0.25–0.6	7.5–28.0	<0.5–225	1.0–24.0	<0.1–1.8

* $p < 0.05$, Kruskal-Wallis test.

Fig. 2. IL-4/IFN- γ ratios in nasal fluids from allergic and non-allergic individuals. The IL-4/IFN- γ quotients in healthy controls before and during pollen season and in patients before and during the pollen season and on budesonide are indicated. Statistically significant differences are indicated (Kruskal-Wallis test). Horizontal bars show geometric means. Figures below panel indicate number of subjects in each group and percentages of subjects displaying a quotient of 70×10^{-3} (the highest value recorded among the controls).



ment of mRNA transcripts following reverse transcription polymerase chain reaction, it has been possible to measure the production of both IL-4 and IFN- γ from nasal cells (21, 22). Although such methods do not allow any accurate quantification of cytokines, important information has been obtained following their use in allergic rhinitis. Thus, increased production of IL-4, but little or no IFN- γ from mucosal lymphocytes has been observed in allergic rhinitis.

In the present study the most prominent findings were the increased IL-4 and IL-10 levels in patients

with seasonal allergic rhinitis. IL-4 levels correlated to pollen counts, eosinophils in nasal fluids and to symptom scores. In contrast to elevation of IL-5, which was also observed in non-atopic individuals during the pollen season, the increase of IL-4 was specific to atopics. Elevated IL-4 in allergic rhinitis has also been found by others using immunofluorescence or determination of cytokine mRNA (21, 23, 24, 25). The present study allowed, because of the ultrasensitive methodology, very accurate determination of IL-4 concentrations and thereby also of the relationships between the levels of IL-4 and other cytokines. It should be noted that overexpression of IL-4 in transgenic mice results in inflammation and tissue damage (26). Therefore, it seems possible that IL-4 may evoke pathogenic effects, that result in rhinitis, but are independent of the ability of the cytokine to stimulate IgE synthesis.

We found no significant increase of IFN- γ in symptomatic allergic patients and the levels of this cytokine were in fact similar to those found in healthy controls outside the pollen season. During the pollen season, however, the controls reacted with increased IFN- γ and the levels of this cytokine correlated to pollen counts. *In vitro* studies have shown higher production of IFN- γ from peripheral blood mononuclear cells from non-atopic than atopic subjects upon stimulation with allergen (10, 11). In studies of nasal provocation challenge of atopic individuals with ragweed allergen and diesel exhaust particles Diaz-Sanchez and Saxon (27) found decreased accumulation of IFN- γ mRNA, whereas transcripts of IL-4 and other Th2 cytokines were in-

Table 2: Nasal lavage fluid cytokines in atopic patients before and during pollen season and after treatment with budesonide

	IL-4	IFN- γ	IL-4sR	IL-5	IL-10
<i>Before the pollen season</i>					
median (pg/mL)	0.3	7.5	3.0	4.0	<0.1
range	<0.25–3.0	<7.5–55.0	<0.5–23.0	0.7–11.9	<0.1–2.0
n	22	22	12	12	13
<i>During the pollen season</i>					
median (pg/mL)	1.0***	8.9	3.6	5.0	0.5
range	<0.25–16.0	<7.5–56.5	<0.5–30.0	<0.5–16.5	<0.1–5.1
n	37	38	36	36	37
<i>Budesonide treated</i>					
median (pg/mL)	0.6	12.0	9.5**	7.8	0.4
range	<0.25–16.0	<7.5–47.0	<0.5–50.0	<0.5–19.0	<0.1–2.3
n	33	32	33	33	33

*** $p < 0.001$, Kruskal-Wallis test, symptomatic compared to asymptomatic patients.

** $p < 0.01$, Kruskal-Wallis test, budesonide treated compared to symptomatic patients.

creased following challenge. Together with the present findings, these results suggest that IFN- γ production is less easily induced in atopic than in nonatopic individuals following allergen stimulation.

In addition to increased IFN- γ concentrations, the controls showed significantly increased IL-5 levels during the birch pollen season, and both IL-5 and IFN- γ showed significant correlation to the birch pollen counts. This raises the question whether pollen may have a non-specific stimulatory effect on cytokine release. In this context it is interesting to note that birch pollen in early studies was found to have an adjuvant effect on antibody formation (28).

The balance between IL-4 and IFN- γ may be crucial for the development of allergic inflammation. In our symptomatic patients the increase in IL-4 and essentially unchanged levels of IFN- γ certainly resulted in increased IL-4/IFN- γ ratios. In fact, 54% of the patients during the pollen season and 18% out of season had ratios of IL-4/IFN- γ that exceeded the highest value observed in any of the healthy control children. The increased IL-4/IFN- γ ratio that we have observed in nasal fluid of atopic individuals is clearly an analogue of what has been obtained in cell cultures using stimulation with a variety of allergens (10, 11). Furthermore, the results are also in agreement with those obtained using semiquantitative measurements of cytokines in nasal cells (21, 22, 27).

It thus appears that the increased IL-4/IFN- γ ratio in nasal lavage is a hallmark of allergic rhinitis and certainly in agreement with findings in atopy in general. Although present in the atopic individuals when asymptomatic, it increases in association with the development of allergic symptoms. It is not clear whether the IL-4/IFN- γ imbalance in atopy is due to an increased ability to produce IL-4 or to a decreased ability to produce IFN- γ . In this context it seems important, however, that a reduced ability of blood cells of newborn babies to produce IFN- γ is predictive of later development of atopic sensitization (29, 30). On the other hand, infants, who later develop atopy may have elevated serum levels of IL-4 at a very young age (9). IFN- γ and IL-4 are mutually inhibitory and it is possible that the low IFN- γ and high IL-4 in allergic patients may be secondary to some underlying disturbance in the balance or regulation of T cell subsets, rather than to an inherent decreased or increased capacity to produce either cytokine.

In addition to a rise in IL-4 we also found significantly increased levels of IL-10 in symptomatic patients. To our knowledge increased IL-10 levels have not been described before in allergic rhinitis. In apparent contrast to our results, Borish and co-

workers found high levels of IL-10 in bronchoalveolar lavage fluid of normal, nonasthmatic subjects and considerably lower levels of this cytokine in asthmatic subjects (31). After allergen challenge, however, there was an increase in IL-10 transcripts in BAL cells from the asthmatic patients.

Glucocorticoids are generally considered to downregulate the cytokine response (32, 33). In this study the decrease in IL-4 levels in nasal fluid following treatment with a topical steroid did not reach statistical significance. However, there was a concomitant decrease of the symptom scores from a median of 20 to 4.5 and a significant decrease in eosinophil counts in nasal lavage indicating compliance with therapy. Decreasing IL-4 was observed in another study of allergic rhinitis, where topical application of fluticasone suppressed the number of IL-4 reactive cells (23).

The IL-4 assay used in our study would be assumed to measure both free IL-4 and IL-4 bound to IL-4sR. It is therefore possible that, even if we failed to detect any significant decrease of total IL-4 levels, the budesonide treatment might still have caused a decrease in the levels of free, biologically active IL-4.

Cytokine receptors like IL-4sR are important parts of cytokine signalling. In our study, median levels of IL-4sR showed a statistically significant threefold increase in budesonide treated patients. Since IL-4sR may inhibit allergic inflammation (34), steroid-induced expression of this receptor might be one of several possible explanations for the beneficial clinical effects of steroids on allergy. Glucocorticoids have been reported to enhance the expression of one of the soluble IL-1 receptors which inhibits IL-1 (35). The balance between cytokines and their receptors may be yet another important field to explore when assessing the biological effects of cytokines or antiinflammatory drugs.

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References

1. STRANNEGÅRD Ö, STRANNEGÅRD I-L. T lymphocyte numbers and function in IgE-mediated allergy. *Immunol Rev* 1978; 41: 148-70.

2. ROMAGNANI S. Induction of Th1 and Th2 responses: a key role for the natural immune response. *Immunol Today* 1992; 13: 379–81.
3. MOSMANN TR, COFFMAN RL. Th1 and Th2 cells: differential pattern of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 1989; 7: 145–73.
4. KELSO A. Th1 and Th2 subsets: paradigm lost? *Immunol Today* 1995; 16: 374–9.
5. BORISH L, ROSENWASSER L. Th1/Th2 lymphocytes: Doubt some more. *J Allergy Clin Immunol* 1997; 99: 161–4.
6. MOSMANN TR, SAD S. The expanding universe of T-cell subsets. Th1, Th2 and more. *Immunol Today* 1996; 17: 138–46.
7. OSHIMA Y, KATAMURA K, MIURA M, MIKAWA H, MAYUMI M. Serum levels of interleukin 4 and soluble CD23 in children with allergic disorders. *Eur J Pediatr* 1995; 154: 723–8.
8. OHASHI Y, NAKAI Y, OKAMOTO H, et al. Serum level of interleukin-4 in patients with perennial allergic rhinitis during allergen-specific immunotherapy. *Scand J Immunol* 1996; 43: 680–6.
9. BORRES MP, EINARSSON R, BJÖRKSTÉN B. Serum levels of IL-4, soluble CD23 and IFN-gamma in relation to development of allergic disease during the first 18 months of life. *Clin Exp Allergy* 1995; 25: 543–8.
10. DORION BJ, WESLEY A, HARBECK R, et al. The production of interferon-gamma in response to a major peanut allergen, Ara h II, correlates with serum levels of IgE anti Ara h II. *J Allergy Clin Immunol* 1994; 93: 93–9.
11. IMADA M, ESTELLE F, SIMONS R, JAY FT, HAYGLASS KT. Allergen-stimulated interleukin-4 and interferon-gamma production in primary culture: responses of subjects with allergic rhinitis and normal controls. *Immunology* 1995; 85: 373–80.
12. LARKIN M. New asthma drugs target inflammatory cascade. *Lancet* 1997; 349: 1004.
13. DREBORG S. Allergy diagnosis. In: MYGIND N, NACLEIRO RM, eds. *Allergic and nonallergic rhinitis*. Copenhagen: Munksgaard, 1993, pp. 82–94.
14. KUNA P, LAZAROVICH M, KAPLAN AP. Chemokines in seasonal allergic rhinitis. *J Allergy Clin Immunol* 1996; 97: 104–12.
15. ANDERSSON G, EKRE HP, ALM G, PERLMANN P. Monoclonal antibody two-site ELISA for human IFN-gamma. *J Immunol Methods* 1989; 125: 89–96.
16. BOSQUET J, VIGNOLA AM, CAMPBELL AM, MICHEL F-M. Pathophysiology of allergic rhinitis. *Int Arch Allergy Immunol* 1996; 110: 207–18.
17. BACHERT C, WAGENMANN M, HAUSER U. Proinflammatory cytokines: measurements in nasal secretion and induction of adhesion receptor expression. *Int Arch All Immunol* 1995; 107: 106–8.
18. SIM TC, GRANT JA, HILSMEIER KA, FUKUDA Y, ALAM R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994; 149: 339–44.
19. LINDEN M, GREIFF L, ANDERSSON M, et al. Nasal cytokines in common cold and allergic rhinitis. *Clin Exp Allergy* 1995; 25: 166–72.
20. GARRELDI I, DE GRAFF T, NAHORI M, VARGAFTIG B, VAN WIJK R, ZILSTRA F. Interleukin-5 and eosinophilic cationic protein in nasal lavages of rhinitis patients. *Eur J Pharmacol* 1995; 275: 295–300.
21. PAWANKAR RU, OKUDA M, SUZUKI K, OKUMURA K, RA C. Phenotypic and molecular characteristics of nasal mucosal gamma delta T cells in allergic and infectious rhinitis. *Am J Respir Crit Care Med* 1996; 153: 1655–65.
22. DURHAM SR, YING S, VARNEY VA, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996; 97: 1356–65.
23. BRADDING P, FEATHER IH, WILSON S, HOLGATE ST, HOWARTH PH. Cytokine immunoreactivity in seasonal rhinitis: Regulation by a topical corticosteroid. *Am J Respir Crit Care Med* 1995; 151: 1900–6.
24. BRADDING P, FEATHER IH, WILSON S, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitis subjects. The mast cell as a source of IL-4, IL-5, IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; 151: 3853–65.
25. KARLSSON MG, DAVIDSSON Å, VIALE G, GRAZIANI D, HELLQUIST HB. Nasal messenger RNA expression of interleukins 2, 4 and 5 in patients with allergic rhinitis. *Diagn Mol Pathol* 1995; 4: 85–92.
26. DVORAK AM, TEPPER RI, WELLER PF, et al. Piecemeal degranulation of mast cells in the inflammatory eyelid lesions of interleukin-4 transgenic mice. Evidence of mast cell histamine release *in vivo* by diamine oxidase-gold enzyme-affinity ultrastructural cytochemistry. *Blood* 1994; 83: 3600–12.
27. DIAZ-SANCHEZ D, SAXON A. The effect of diesel exhaust particles on allergic disease. *Allergy Clin Immunol Int* 1996; 8: 57–9.
28. STRANNEGÅRD Ö. Adjuvant effect of birch pollen on antibody production. *Int Arch Allergy* 1974; 47: 14–22.
29. WARNER JA, MILES EA, JONES AC, QUINT DJ, COLWELL BM, WARNER JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24: 423–30.
30. TANG MLK, KEMP AS, THORBURN J, HILL DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994; 344: 983–5.
31. BORISH L, AARONS A, RUMBYRT J, CVIETUSA P, NEGRI J, WENZEL S. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 1996; 97: 1288–96.
32. FREW JA. Cytokines, chemokines, T cells and allergy. *Clin Exp Allergy* 1996; 26: 2–4.
33. SCHWIEBERT LA, BECK LA, STELLATO C, BICKEL CA, BOCHNER BS, SCHLEIMER RP. Glucocorticosteroid inhibition of cytokine production: Relevance to antiallergic actions. *J Allergy Clin Immunol* 1996; 97: 143–52.
34. RENZ H, ENSSLE L, LAUFFER R, KURRLE E, GELFAND W. Inhibition of allergen-induced IgE and IgG1 production by soluble IL-4 receptor. *Int Arch Allergy Immunol* 1995; 106: 46–54.
35. RE F, MUZIO M, DE ROSSI M, et al. The type II “receptor” as a decoy target for interleukin 1 in polymorphonuclear leukocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *J Exp Med* 1994; 179: 739–43.