

Short Report

Medical treatment reverses cytokine pattern in allergic and nonallergic chronic rhinosinusitis in asthmatic children

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A Th2 cytokine pattern has recently been reported both in allergic and nonallergic chronic rhinosinusitis in asthmatic children. The aim of the study was to evaluate the cytokine pattern in chronic rhinosinusitis in allergic and nonallergic asthmatic children before and after medical treatment. Thirty asthmatic children were evaluated, 18 males and 12 females (mean age 9.1 years). Sixteen were allergic and 14 were nonallergic. All children were asthmatic and suffered from chronic rhinosinusitis, whose diagnosis was confirmed by endoscopy. All of them were treated with amoxicilline-clavulanate (20 mg/kg b.i.d.) and fluticasone propionate aqueous nasal spray (100 µg daily) for 14 days; a short course of oral corticosteroid was also prescribed (deflazacort 1 mg/kg daily for 2 days, 0.5 mg/kg daily for 4 days and 0.25 mg/kg daily for 4 days). Rhinosinusal lavage and nasal cytology were performed in all subjects before and after medical treatment. IL4 and IFN γ were measured by immunoassay and inflammatory cells were counted by conventional staining. Thirteen allergic children and 12 nonallergic children showed a negative endoscopy after the treatment. Allergic subjects showed a significant decrease of IL4 ($p = 0.0002$) and a significant increase of IFN γ ($p = 0.03$) after the treatment. Nonallergic children showed a significant decrease of IL4 ($p = 0.0007$) and a non-significant increase of IFN γ . A significant reduction of the inflammatory infiltrate was detected in all asthmatic children ($p < 0.05$). This study confirms a Th2 polarization in chronic rhinosinusitis both in allergic and nonallergic asthmatic children. Moreover, the medical treatment of chronic rhinosinusitis reversed the cytokine pattern from a Th2 towards a Th1 profile both in allergic and nonallergic children.

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Rhinosinusitis has been considered by several authors as a significant pathogenic factor in asthmatic patients (1). We previously reported that rhinosinusitis was diagnosed in the 44% of 128 asthmatic children evaluated by nasal endoscopy (2).

Chronic rhinosinusitis is considered when symptoms persist longer than 12 weeks (3). The pathophysiological features of chronic rhinosinusitis have been related to the effects of

Th2-type cytokines in allergic patients (4). Increased levels of GM-CSF, IL3, IL4, IL5 and IL13 were reported in allergic rhinosinusitis, whereas IL4 was not increased in nonallergic subjects (5). Moreover, IFN γ , IL3 and GM-CSF were produced in nonallergic rhinosinusitis (5).

We recently reported that allergic asthmatic children with chronic rhinosinusitis had a typical Th2 cytokine pattern, but also that nonallergic asthmatic children shared a similar pattern

(6). These findings would suggest the existence of a common immunopathological mechanism shared by upper and lower airways, and are consistent with the concept of united airways disease (7).

The aims of our study were: to evaluate the cytokine pattern in chronic rhinosinusitis in asthmatic children before and after medical treatment and to compare the findings in allergic and nonallergic patients.

Materials and methods

Subjects

30 asthmatic children were evaluated consecutively, 18 males and 12 females, with an average age of 9.1 years (range 5–12). A detailed clinical history and a complete physical examination, including allergy evaluation, were carried out by a pediatrician and an ENT specialist for each patient.

The asthma diagnosis and severity were established, according to GINA Criteria (8), and by performing spirometry in all children.

The diagnosis of chronic rhinosinusitis was based on nasal symptoms (mucopurulent rhinorrhea and nasal congestion), facial pain, postnasal drip and hyposmia, occurring for more than 3 months, according with validated criteria (3). All patients had evidence of endoscopic signs of rhinosinusitis, according with validated criteria (2, 3).

The diagnosis of associated allergy was made on the basis of a skin prick test for a panel of common allergens described elsewhere (2, 6).

Study design

To evaluate the cytokine pattern in a group of asthmatic children with chronic rhinosinusitis, we excluded all children who met the following exclusion criteria: use of antibiotics, and nasal or oral corticosteroids within the previous 4 weeks.

A placebo group was not considered, since the main scope of this study was the evaluation of the immunopathological phenomena appearing after the treatment and it was not considered ethical by our Ethics Committee.

All children were treated with amoxicilline-clavulanate (20 mg/kg bid) and fluticasone propionate aqueous nasal spray (100 µg/daily: 50 µg/nostril in the morning) for 14 days; a short course of oral corticosteroid was also prescribed (deflazacort 1 mg/kg daily for 2 days, 0.5 mg/kg daily for 4 days, and 0.25 mg/kg daily for 4 days).

Rhinosinusal lavage and nasal cytology were performed in all subjects before and after medical treatment.

The study was approved by the Institutional Review Board and informed oral consent was obtained from the parents of the children.

ENT examination consisted of nasal endoscopy with optical fibers and otoscopy and was performed before and after treatment.

Nasal endoscopy

The endoscopy was performed as previously published (2,6). After each ENT examination and rhinosinusal endoscopy, all clinical data were registered on a proper form.

Nasal cytology

Nasal scrapings were obtained by scraping the head of the inferior turbinate with a cotton swab, as described in previous reports (2,6). Briefly, after the nasal scraping, the cotton tip of the swab was immersed in a plastic tray with phosphate-buffered saline (PBS) and transferred to a 10-ml polypropylene tube. The recovered fluids were centrifuged at 1500 g for 10 min, and each pellet was re-suspended in PBS (2 ml). Cell suspensions were filtered to reduce the quantity of mucus, and cytospin slides were prepared by using standard techniques.

Smears were stained with Diff Quik stain (Sigma, Milan, Italy) and were analyzed by optic microscope (Olympus U-SPT; Olympus, Italia, Rome, Italy).

Rhinosinusal lavage

A lavage of the rhinosinusal cavity was performed using 5 ml of physiologic saline, according to standard methods described elsewhere (2,6). This was performed with the patient's head bent backwards during closure of the soft palate. The recovered fluid was comparable in all groups; it was collected to dose cytokine levels and stored at -20°C (6).

Cytokine assessment

The cytokines were measured with ELISA method (R & D Systems, CO, USA). This assay employed the quantitative sandwich enzyme immunoassay technique. The minimum detectable dose was typically less than 10 pg/ml for IL4 and 8 pg/ml for IFN γ , respectively. A monoclonal antibody specific to each cytokine was precoated onto a microplate. Standards and

samples were pipetted into the wells and each cytokine present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific to cytokine was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of cytokine bound in the initial step. The colour development was stopped and the intensity of the colour measured.

Statistical analysis

The data collected were subjected to statistical analysis by M-ANOVA and post hoc comparison by Duncan's test. All results with p-values of less than 0.05 obtained were considered statistically significant.

Results

Subjects

Thirty children with asthma occurring in the previous year were examined: 11 with mild intermittent asthma; 18 with mild persistent asthma; and one with moderate asthma (according to the GINA criteria). A reduced FEV1 was detectable in four children, all of whom showed a reversible bronchoconstriction.

Sixteen patients were allergic to the more common aeroallergens: six were sensitized to *Dermatophagoides farinae* and *pteronysinus*, two to pollens and eight were poly sensitized to mites and pollens.

All children assumed the prescribed therapy without significant side-effects.

Nasal endoscopy

Nasal endoscopy was successfully performed on all patients. Signs of rhinosinusitis (i.e. mucopurulent drainage) were detected in all children.

Thirteen allergic children and 12 nonallergic children showed a negative endoscopy after the treatment.

Nasal cytology

Inflammatory cells were significantly decreased both in allergic children ($p < 0.05$) and in nonallergic subjects ($p < 0.05$) after the treatment. In particular, eosinophils ($p < 0.05$) and neutrophils ($p < 0.05$) significantly decreased both in allergic and in nonallergic children.

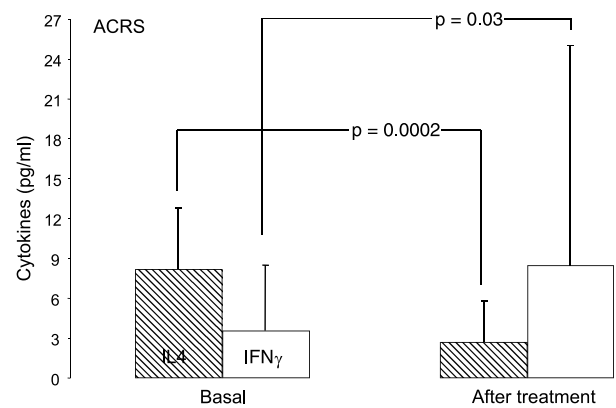


Fig. 1. IL4 and IFN γ levels in allergic asthmatic children with chronic rhinosinusitis (ACRS) before and after treatment. Data are expressed as median $\pm$ SE.

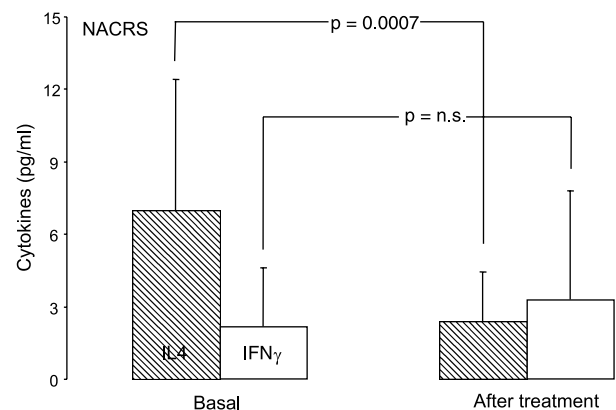


Fig. 2. IL4 and IFN γ levels in nonallergic asthmatic children with chronic rhinosinusitis (NACRS) before and after treatment. Data are expressed as median $\pm$ SE.

Cytokine dosage

IL4 was significantly decreased ($p = 0.0002$) and IFN γ was significantly increased ($p = 0.03$) in allergic subjects after the treatment (Fig. 1).

IL4 was significantly decreased ($p = 0.0007$) and IFN γ was not significantly increased ($p = \text{n.s.}$) in nonallergic patients after the treatment (Fig. 2).

The intergroup analysis did not reveal any difference between allergic and nonallergic children both before and after the treatment ($p = \text{n.s.}$).

Discussion

We recently reported that allergic asthmatic children with chronic rhinosinusitis had a typical Th2 cytokine pattern and there was a correlation between high levels of IL4 and low levels of IFN γ

(6). On the other hand, high levels of IL4 and low levels of IFN γ were reported also in nonallergic asthmatic children with chronic rhinosinusitis (6).

Therefore, the present study was established to evaluate the effects of medical treatment of chronic rhinosinusitis on the cytokine pattern in allergic and nonallergic asthmatic children.

A standard pharmacological protocol was chosen according to the literature (9). This protocol consisted of the association of oral amoxicilline-clavulanate plus fluticasone propionate aqueous nasal spray administered for 2 weeks and a short course of oral deflazacort.

Amoxicilline-clavulanate is considered a reasonable initial antibiotic choice in patients with uncomplicated rhinosinusitis. It is generally effective, it is relatively inexpensive and side-effects are rare. The combination amoxicilline-clavulanate is typically effective against most β -lactamase-producing germs (such as *Moraxella catarrhalis*, *Haemophilus influenzae*, *Staphylococcus aureus*) and many other anaerobic bacteria (9).

Fluticasone propionate aqueous nasal spray is a topically active corticosteroid and has been demonstrated to be effective in the treatment of chronic rhinosinusitis, reducing both clinical and inflammatory parameters (10).

Deflazacort is a systemic glucocorticoid with very low systemic side-effects.

The present study shows that a standard pharmacological treatment is capable, as expected, to improve the endoscopic and cytological features in asthmatic children with chronic rhinosinusitis.

Moreover, this pharmacological treatment induces a total reversal of the ratio IL4/IFN γ in allergic children and a partial reversal in nonallergic subjects. This is the first evidence that a pharmacological treatment shifts the Th2 polarization towards a Th1 profile in asthmatic children with chronic rhinosinusitis. The mechanisms of action of the single drugs might explain that effect. Amoxicilline-clavulanate has been reported to reduce cytokine production both in *in vitro* and *in vivo* studies (11). Fluticasone reduced IL4 and IL13 in allergic subjects with chronic rhinosinusitis as well as the numbers of cells expressing receptors for IL4, IL5 and GM-CSF (10). Aside from directly reducing the synthesis of Th2-type cytokines, corticosteroids may also increase the level of Th1-type cytokines, particularly IFN γ and IL12, which can suppress the transcription of IL4 (12).

Our findings are consistent with these previous studies and confirm the capacity of corticosteroids and/or antibiotics to reverse the Th2 response. It is noteworthy that this effect is demonstrable also in nonallergic children.

Thus, this study confirms our previous findings concerning a Th2 polarization both in allergic and nonallergic asthmatics with chronic rhinosinusitis (6), and demonstrates the reversibility of IL4/IFN γ ratio after adequate treatment.

Further studies should established the correlation of clinical, functional and endoscopic parameters with immunopathological data in asthmatic children with chronic rhinosinusitis.

Acknowledgments

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References

1. KALINER MA, OSGUTHORPE JD, FIREMAN P, et al. Sinusitis: Bench to Bedside. *J Allergy Clin Immunol* 1997; 99: S829-48.
2. TOSCA A, RICCIO AM, MARSEGLIA GL, et al. Nasal endoscopy in asthmatic children: assessment of rhinosinusitis and adenoiditis incidence, correlations with cytology and microbiology. *Clin Exp Allergy* 2001; 31: 609-15.
3. ANON JB (ed). Report of the Task Force on Rhinosinusitis. *Otolaryngol Head Neck Surg* 1997; 117: 7-14.
4. CHRISTODOUPOULOS P, CAMERON L, DURHAM S, HAMID Q. Molecular pathology of allergic disease II. upper airway disease. *J Allergy Clin Immunol* 2000; 105: 211-23.
5. HAMILLOS DL, LEUNG DYM, WOOD R, et al. Evidence for distinct cytokine expression in allergic versus non-allergic chronic sinusitis *J Allergy Clin Immunol* 1995; 96: 537-44.
6. RICCIO AM, TOSCA MA, COSENTINO C, et al. Cytokine pattern in allergic and non-allergic chronic rhinosinusitis in asthmatic children. *Clin Exp Allergy* 2002; 32: 422-6.
7. PASSALACQUA g, CIPRANDI g, CANONICA gw. United airways disease: therapeutical implications. *Thorax* 2000; 55: S26-7.
8. Guidelines For the Diagnosis and Management of Asthma. NIH Publication No. 97-4051, April 1997.
9. WALD ER. Chronic sinusitis in children. *J Pediatrics* 1995; 127: 339-47.
10. KONDO H, NACHTIGAL D, FRENKIEL S, et al. Effect of steroids on nasal inflammatory cells and cytokine profile. *Laryngoscope* 1999; 109: 91-7.
11. REATO G, CUFFINI AM, TULLIO V, et al. Co-amoxiclav affects cytokine production by human polymorphonuclear cells. *J Antimicrob Chemother* 1999; 43: 715-8.
12. SORNASSE T, LARENAS PV, DAVIS KA, et al. Differentiation and stability of T helper 1 and 2 cells derived from naive human neonatal CD4⁺ T cells, analysed at the single-cell level. *J Exp Med* 1996; 184: 473-83.