

Cytokine pattern in allergic and non-allergic chronic rhinosinusitis in asthmatic children

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Summary

Background Rhinosinusitis represents one of the most common chronic diseases. The association of rhinosinusitis with asthma has been frequently reported. Eosinophils and Th2 cells play a pathogenic mechanism in asthma.

Objective The aims of the study were to evaluate the cytokine pattern in chronic rhinosinusitis in asthmatic children and to compare the findings in allergic vs. non-allergic asthmatics.

Methods Thirty-five asthmatic children were evaluated, 19 males and 16 females, with an average age of 8.7 years. All children were asthmatic and suffered from chronic rhinosinusitis. Twenty were allergic and 15 were non-allergic. Ten healthy children were studied as normal controls. Evaluated parameters were the levels of the following cytokines: IL-1 β , IL-4, IL-6, IL-8, IL-12, IFN- γ and TNF- α . Cytokines were recovered from rhinosinusal lavage and measured by immunoassays. Nasal cytology was also performed in all subjects and inflammatory cells were counted by conventional staining.

Results Allergic subjects showed a significant increase of IL-4 ($P < 0.01$) and TNF- α ($P < 0.05$) and a significant decrease of IL-12 ($P < 0.05$) and of IFN- γ ($P < 0.0001$), whereas IL-1 β , IL-6 and IL-8 were not significantly increased. Non-allergic children showed a significant increase of IL-4 ($P < 0.05$) and a significant decrease of IFN- γ ($P < 0.0001$), IL-12 was not significantly decreased, and IL-1 β , IL-6 and IL-8 were not significantly increased. A significant inflammatory infiltrate was present in all asthmatic children. Significant correlations were demonstrated between IL-4 and IL-12 ($P < 0.001$), IL-12 and IFN- γ ($P < 0.001$), IL-8 and neutrophils ($P < 0.01$), and TNF- α and monocytes/macrophages ($P < 0.05$), in allergic asthmatics. IL-4 and IL-12 were significantly correlated ($P < 0.05$) as well as IL-8 and neutrophils ($P < 0.01$) in non-allergic asthmatics.

Conclusion This study shows that allergic asthmatic children with chronic rhinosinusitis have a typical Th2 cytokine pattern, but also non-allergic asthmatic children share a similar pattern. These findings would suggest the existence of a common pathophysiological mechanism shared by upper and lower airways and are consistent with the concept of united airways disease.

Keywords allergy, asthmatic children, chronic rhinosinusitis, cytokines, inflammation, united airways disease

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Introduction

Sinusitis represents one of the most common chronic diseases, affecting more than 14% of the general population in the United States of America (USA), but at paediatric age it is probably underestimated [1]. The definition of rhinosinusitis seems to be more appropriate from a pathophysiological point of view, as coexistence of rhinitis and sinusitis has been broadly reported [2].

Rhinosinusitis has been considered by several authors as a significant pathogenic factor in asthmatic patients [3–7]. We previously reported that rhinosinusitis was diagnosed in the

44% of 128 asthmatic children evaluated by nasal endoscopy [8]. Rhinosinusitis is mainly characterized by nasal congestion, rhinorrhea and facial pain; if symptoms persist longer than 12 weeks, chronic rhinosinusitis is considered [9]. Chronic rhinosinusitis is sustained by mucosal oedema, subepithelial fibrosis, basement membrane thickening, goblet cell hyperplasia and inflammatory cell infiltrate [10,11].

The pathophysiological features of chronic rhinosinusitis have been related to the effects of Th2 cytokines in allergic patients [10]. Hamilos et al. reported increased levels of GM-CSF, IL-3, IL-4 and IL-5 correlating with the density of tissue eosinophils in allergic rhinosinusitis [12]. Moreover, IFN- γ , IL-3 and GM-CSF are produced in non-allergic rhinosinusitis, even though the mechanisms remain unknown [12]. Al-Ghamdi et al. found high levels of IL-4, IL-5 and IL-13 in allergic chronic sinusitis, whereas IL-4 was not increased in non-allergic subjects [13]. A reduced expression of IL-12 has

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been reported in both allergic and non-allergic patients with chronic rhinosinusitis [14]. IL-6 has been colocalized to eosinophils, macrophages, T cells and mast cells within the sinus mucosa of allergic subjects [15], IL-1 β and TNF- α have been reported increased in rhinosinusitis [16], and IL-8 levels were significantly higher in patients with chronic sinusitis than in controls [17].

The aims of our study were: to evaluate the cytokine pattern in chronic rhinosinusitis in asthmatic children; and to compare the findings in allergic vs. non-allergic patients.

Materials and methods

Subjects

Thirty-five asthmatic children were evaluated consecutively, 19 males and 16 females, with an average age of 8.7 years (range 5 to 12 years). A detailed clinical history and a complete physical examination, including allergy evaluation, were carried out by a paediatrician and an ENT specialist for each patient.

The asthma diagnosis and severity were established, according to GINA criteria [18] and after performing spirometry in all children. The diagnosis of chronic rhinosinusitis was based on nasal symptoms (mucopurulent rhinorrhea, nasal congestion), facial pain, post-nasal drip and hyposmia, occurring for more than 3 months. All patients had evidence of endoscopic signs of rhinosinusitis, in accordance with validated criteria [8,9,19]. The diagnosis of associated allergy was made on the basis of skin prick test for a panel of common allergens described elsewhere [8].

Ten healthy children with headache and submitted to nasal endoscopy to rule out rhinosinusal disease were enrolled as normal controls.

Study design

To evaluate the cytokine pattern in a group of asthmatic children with chronic rhinosinusitis, we excluded all the children who met the following exclusion criterion: use of antibiotics, nasal corticosteroids or oral corticosteroids within the previous 4 weeks. The study was approved by the Institutional Review Board and an informed oral consent was obtained from their parents.

ENT examination consisted of nasal endoscopy with optical fibres, and otoscopy.

Nasal endoscopy

The endoscopy was performed as previously published in detail [8]. 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 [20,21]. 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 220 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 cytopsin slides were prepared by using standard techniques.

Smears were stained with Diff Quik stain to differentiate between eosinophils, neutrophils, lymphocytes, monocytes/macrophages and epithelial cells and were analysed by optic microscope (Olympus U-SPT). The number of inflammatory cells was expressed as a percentage of cells. Samples are examined in a blinded fashion.

Rhinosinusal lavage

A lavage of the rhinosinusal cavity was done using 5 mL of physiological saline, according to standard methods described elsewhere [22]. This was performed with the patient's head bent backwards during closure of the soft palate. The recovered fluid was comparable in all groups and was collected to dose cytokine levels and stored at -20° .

Cytokine assessment

The cytokines were measured with ELISA method (R & D Systems, Minneapolis, MN, USA). This assay employed the quantitative sandwich enzyme immunoassay technique. The minimum detectable dose was typically less than 10 pg/mL for IL-4, 8 pg/mL for IFN- γ , 5 pg/mL for IL-12, 4.4 pg/mL for TNF- α , 1 pg/mL for IL-1 β , 0.7 pg/mL for IL-6 and 10 pg/mL for IL-8, respectively. A monoclonal antibody specific for each cytokine was pre-coated 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 for 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 ANOVA. Correlations were analysed by the parametric paired *t*-test. All results with *P*-values less than 0.05 were considered statistically significant.

Results

Subjects

Thirty-five patients with asthma occurring in the previous year were examined: 23 with mild intermittent asthma, 11 with mild persistent asthma and one with moderate asthma (according to the GINA criteria). A reduced FEV₁ was detectable in 12 children, all of them showed a reversible bronchoconstriction.

Twenty patients were allergic to the more common aeroallergens: seven were sensitized to *Dermatophagoides farinae* and *Pteronyssinus*, three to pollens, and 10 were polysensitized to mites and pollens.

Nasal endoscopy was successfully performed on all patients.

Nasal cytology

Total cells were significantly increased both in allergic children ($P < 0.001$) and in non-allergic subjects ($P < 0.05$). Neutrophils and monocytes/macrophages were significantly increased in allergic children (respectively $P < 0.001$ and $P < 0.05$) in

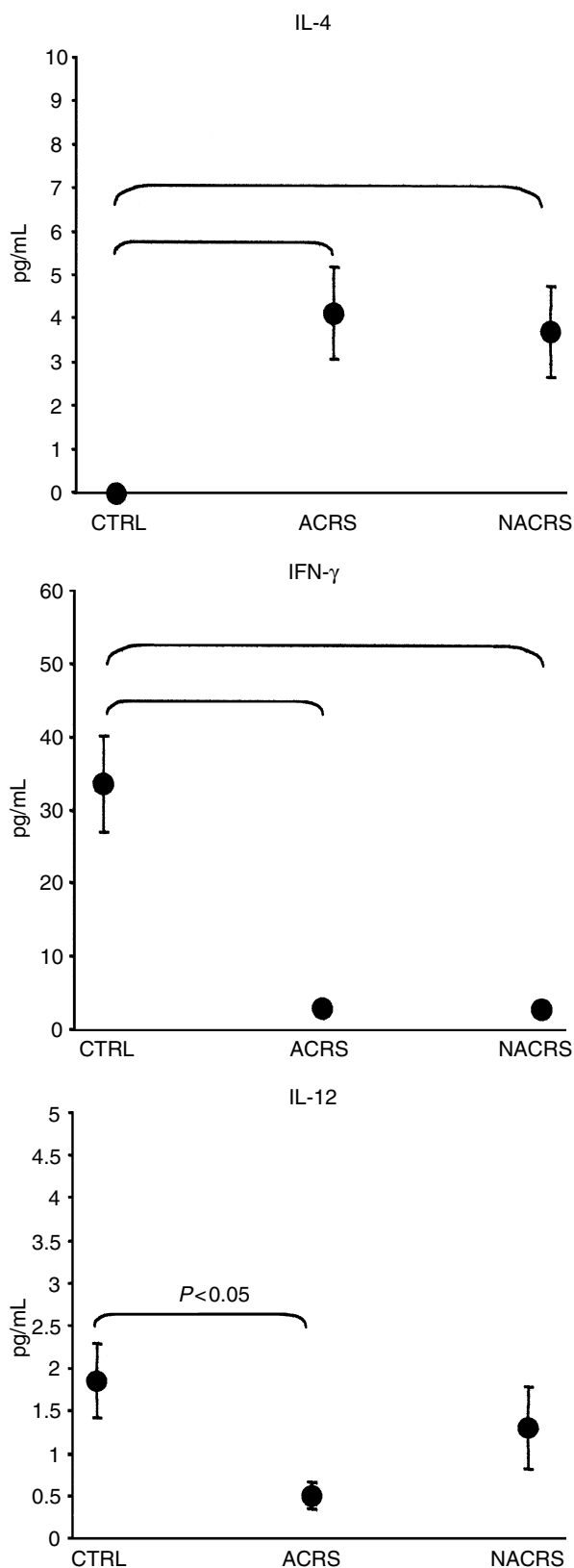


Fig. 1. IL-4, IFN- γ and IL-12 levels in healthy children (CTRL) and allergic (ACRS) and non-allergic asthmatic children with chronic rhinosinusitis (NACRS).

comparison with normal subjects. Normal children did not show a cellular infiltrate.

Cytokine dosage

Cytokine levels are singly reported in Figs 1 and 2.

IL4 was significantly increased both in allergic patients ($P < 0.01$) and in non-allergic children ($P < 0.05$) in comparison with normal subjects (Fig. 1). IFN- γ was significantly decreased both in allergic children ($P < 0.0001$) and in non-allergic patients ($P < 0.0001$) in comparison with normal children (Fig. 1). IL-12 was significantly reduced only in allergic asthmatics ($P < 0.05$) in comparison with normal controls (Fig. 1). TNF- α was significantly reduced only in allergic asthmatics in comparison with both non-allergic children ($P < 0.05$) and normal controls ($P < 0.05$) (Fig. 2). IL-1 β , IL-6 and IL-8 were increased both in allergic and non-allergic asthmatics (Fig. 2). There were significant correlations between IL-4 and IL-12 ($P < 0.001$), IL-12 and IFN- γ ($P < 0.001$), IL-8 and neutrophils ($P < 0.01$), and TNF- α and monocytes/macrophages ($P < 0.05$), in allergic asthmatics. IL-4 and IL-12 were significantly correlated ($P < 0.05$), as well as IL-8 and neutrophils ($P < 0.01$), in non-allergic asthmatics.

Discussion

The relationship between the upper and lower airways has been deeply investigated over the past years. This strict link has been highlighted by epidemiological studies and clinical observations, but also by immunopathological investigations and outcomes of treatment. These concepts taken together led to the operative definition of rhinobronchitis [23] or, as we recently proposed, of united airways disease (UAD) [24,25].

Allergy should be considered as a disorder of the whole respiratory tract, and not limited to a specific target organ, with a broad spectrum of clinical features. The association of chronic rhinosinusitis with asthma has been reported in several studies showing that asthmatic symptoms, lung function and non-specific bronchial hyper-reactivity improve only after rhinosinusitis therapy [6,26–28]. Chronic rhinosinusitis is characterized by persistent inflammation. The inflammatory infiltrate has been reported to be similar to that observed in allergic rhinitis [29]. Like allergic rhinitis, the pathophysiological features of chronic rhinosinusitis have been broadly considered dependent on the effects of Th2 cytokines [10].

Increased levels of GM-CSF, IL-3, IL-4, IL-5 and IL-13 have been observed and they correlate with the eosinophil infiltrate [12,13]. Eosinophils, T cells, mast cells and macrophages, infiltrating the sinus mucosa of allergic patients with chronic rhinosinusitis, release IL-6, which stimulates fibroblast activation and collagen deposition [15]. On the other hand, a decreased expression of IL-12 has been observed in patients with allergy-associated and non-allergy-associated chronic rhinosinusitis, whereas IL-12 receptor is reduced only in allergic subjects, probably because of the inhibitory effect of IL-4 [14]. IL-12 is a Th1-associated cytokine, as it has been described to stimulate Th1 differentiation, promote T cell production of IFN- γ and inhibit IgE synthesis [30]. IL-12 has been suggested to play a role in the *in vivo* suppression of the allergic inflammatory sinonasal response; the control of this suppression may be exerted via the IL-12 receptor [14].

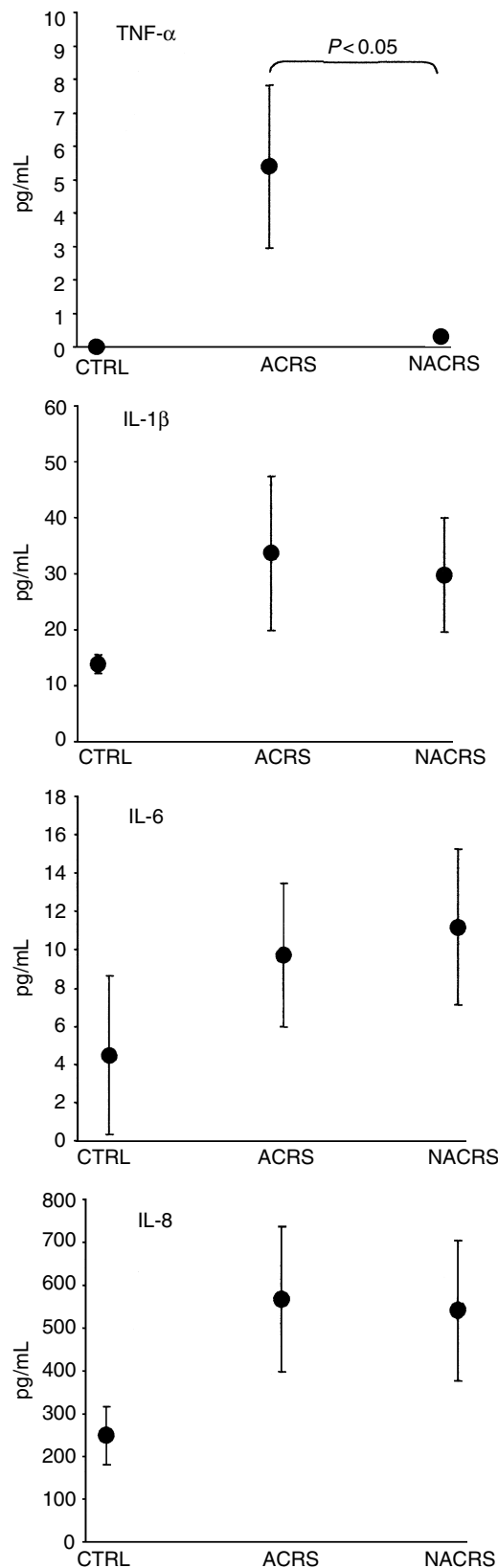


Fig. 2. TNF- α , IL-1 β , IL-6 and IL-8 levels in healthy children (CTRL) and allergic (ACRS) and non-allergic asthmatic children with chronic rhinosinusitis (NACRS).

The up-regulation of VCAM-1 through TNF- α expression and the release of RANTES may be factors that contribute to the marked recruitment of inflammatory cells in non-allergic patients [31]. IL-8 and MPO levels are higher in patients with chronic rhinosinusitis than in normal controls: IL-8 attracts and activates neutrophils in sinus tissue [17]. IL-1, IL-6 and TNF- α are pro-inflammatory cytokines that act as unspecific alarm signals in infectious and inflammatory disorders [32]. They play a pathogenic role activating endothelial cells, releasing a cytokine cascade and activating T cells.

Atopic asthma is characterized by infiltration of the bronchial mucosa with eosinophils and Th2 cells [33]. Intrinsic asthma has a different clinical profile from allergic asthma, but it does not appear to be a distinct immunopathological entity. In fact, both variants of asthma are characterized by: infiltration of eosinophils and Th2 cells; presence of CC chemokines and Fc ϵ RI positive cells; and cells expressing mRNA for the ϵ germ-line transcript and the ϵ heavy chain of IgE [33]. The only difference seems to be a strong macrophage signal in biopsies from allergic asthmatic subjects compared with those from extrinsic asthmatics [33]. Thus, there appears to be more similarities than differences between allergic and intrinsic asthma. This evidence supports the concept that allergic and non-allergic asthma share many common immunopathological mechanisms, even if they show different clinical features.

The present study is, to our knowledge, the first investigation exploring the cytokine pattern in asthmatic children with chronic rhinosinusitis. The findings point to a Th2-dependent response, more evident in allergic children, at the level of the upper airways during a chronic inflammatory event (Fig. 9). The correlations between high level of IL-4 and low levels of both IFN- γ and IL-12 are consistent with this hypothesis in allergic asthmatics. Moreover, TNF- α and IL-8 correlate with monocyte/macrophage and neutrophil infiltrate, respectively, in allergic children.

The inflammatory events are sustained also by increased levels of IL-1, IL-6 and IL-8. On the other hand, high levels of IL-4 and low levels of IFN- γ in non-allergic asthmatic children with chronic rhinosinusitis are reported as first evidence in the literature. A possible explanation may be that the immunopathological mechanisms involved in the pathogenesis of intrinsic asthma should be superimposable to those leading upper airways inflammation. In this view, the concept of united airways disease may be further confirmed.

A polarization of Th2 response characterizes the inflammation of bronchi in both variants of asthma as well as upper airways inflammation may share a similar pathogenic mechanism, as provided by increased IL-4 levels. Anyway, allergic subjects show a more intense inflammation as provided by reduced IL-12 levels, increased TNF- α levels and more abundant infiltrate.

These data suggest the concept that allergy is not a disease confined to a specific target organ, but rather a systemic disorder of the respiratory tract with a broad spectrum of clinical manifestations, from rhinosinusitis to asthma. Moreover, the evidence of a Th2 response also at the upper airways level during chronic sinusitis in non-allergic asthmatics highlights the existence of pathophysiological mechanisms common to the entire respiratory tract. Thus, the definition of UAD is supported by epidemiological and clinical evidence and also by immunopathological data.

All these considerations prompted the WHO to publish an extensive position paper devoted to the relationship between rhinitis and asthma and its therapeutic implications. This position paper will be entitled 'ARIA: allergic rhinitis and its impact on asthma'.

In conclusion, this study points out the presence of a Th2-polarized response at the upper airways level in asthmatic children suffering from chronic rhinosinusitis, at least in part independently of atopy. Further studies should be addressed to evaluate the effects of adequate treatment of rhinosinusitis on the immune response.

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