

Sinus and adenoid inflammation in children with chronic rhinosinusitis and asthma



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ABSTRACT

Background: Chronic rhinosinusitis (CRS) and asthma frequently coexist in children and adults. However, the precise pathophysiologic mechanism of this interaction is still poorly understood, especially in children, owing to the lack of direct measurements of mucosal inflammation in the upper airways.

Objective: To determine the pathophysiologic mechanism by analyzing the expression of a large array of inflammatory cytokines and chemokines in the sinus and adenoid tissues surgically removed from pediatric patients with CRS refractory to medical management.

Methods: Twenty-eight children 2 to 12 years old diagnosed with CRS with or without asthma and 10 controls were included in this prospective, nonrandomized study. Mucosal expression of 40 inflammatory cytokines was measured with a multiplex assay and was normalized to total tissue protein.

Results: Compared with children with CRS and without asthma, children with CRS and asthma had significantly higher sinus levels of tumor necrosis factor- α and adenoid levels of epidermal growth factor, eotaxin, fibroblast growth factor-2, growth-related oncogene, and platelet-derived growth factor-AA.

Conclusion: The inflammatory response in the upper airway mucosa of children with asthma and CRS was similar, but more severe, compared with children with CRS without asthma. This observation is consistent with the hypothesis that asthma in these patients is caused or exacerbated by severe upper airway disease and supports the concept that treating sinus disease is paramount in the management of chronic asthma in children using, for the first time, direct measurements of airway inflammation in children.

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Introduction

Chronic rhinosinusitis (CRS) and asthma frequently coexist in children and adults. In a series of pediatric patients admitted with status asthmaticus, 27% had radiologic evidence of sinusitis,^{1,2} and in another study, 61 of 128 children with asthma had evidence of rhinosinusitis on endoscopic examination.³ Fewer studies have investigated whether asthma affects the upper airways. The

authors previously reported that children with asthma have worse surgical outcomes after sinus surgery and/or adenoidectomy compared with children without asthma.⁴ Similarly, adults with asthma undergoing endoscopic sinus surgery for CRS have worse surgical outcomes compared with controls without asthma.^{5,6}

The epidemiologic link between CRS and asthma has been confirmed by pathophysiologic and therapeutic observations. Histologic studies have shown mast cells and eosinophils in the nasal mucosa of individuals with allergic rhinitis and in the bronchial mucosa of patients with asthma^{7–9} and the exposure of patients with rhinitis to specific allergens triggers eosinophilic infiltration into nasal and bronchial mucosa.^{2,10} Furthermore, several studies have shown that medical management of CRS alleviates asthma symptoms and improves lung function,^{2,11–14} and that surgical management alleviates asthma symptoms and decreases emergency visits in children with these 2 diseases.¹⁵

Despite this body of work, the precise interaction between asthma and CRS remains poorly understood, especially in children.

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This is due primarily to the lack of direct measurements of mucosal inflammation in the upper airways of children with vs without asthma, because most patients are managed medically and do not require surgery. Thus, the primary purpose of the present study was to fill this void by analyzing the expression of a large array of inflammatory cytokines and chemokines in the sinus and adenoid tissues surgically removed from pediatric patients with CRS refractory to medical management compared with control patients without upper or lower airway disease.

Furthermore, by defining quantitative and qualitative differences in cytokine expression associated to the coexistence of asthma and CRS in children, the authors sought to gain a better understanding of the clinical relation between these highly common pathologic conditions and possibly help in the interpretation of clinical trials and in the choice of more targeted therapeutic strategies.

Methods

Thirty-eight children 2 to 12 years of age were included in this prospective, nonrandomized study. Twenty-eight children had CRS resistant to maximal medical management and underwent endoscopic sinus surgery at West Virginia University from March 2010 to September 2011. Surgery involved maxillary sinus lavage, balloon maxillary antrostomy, maxillary antrostomy, or maxillary antrostomy with anterior ethmoidectomy. When indicated, endoscopic sinus surgery was combined with adenoidectomy. An adenoidectomy was performed in children with CRS based on evidence that adenoids act as a bacterial reservoir in these children and removing the adenoids improves outcomes.⁴ All patients with CRS underwent computed tomography of the sinuses to confirm the diagnosis of sinusitis and received maximal medical therapy, including a 3-week course of antibiotics, topical nasal saline sprays, and topical nasal steroid sprays. Exclusion criteria included a diagnosis of cystic fibrosis, congenital syndromes, ciliary dyskinesia, invasive fungal sinusitis, immunodeficiency, and trauma to the affected sinuses. Parental consent and child assent were required to enter this study and the West Virginia University institutional review board approved the experimental protocol.

The control group included 10 children whose parents signed informed consent for tonsillectomy, adenoidectomy, and biopsy examination of sinus tissue for pathologies other than CRS, including recurrent streptococcal pharyngitis and adenotonsillar hypertrophy. These patients did not have computed tomograms to document the absence of CRS, because it would have been unethical to expose them to x-rays. Instead, all parents completed a preoperative sinonasal symptoms score questionnaire (SN-5) and patients were excluded from the control group if their SN-5 score was higher than 3.5. The SN-5 is a validated symptom score questionnaire for the evaluation of CRS in children that consists of 5 domains: infection symptoms, nasal obstruction, allergy symptoms, emotional distress, and activity limitations.¹⁶ The SN-5 score was found to correlate strongly with a computed tomographic diagnosis of sinusitis in children, with a score higher than 3.5 being strongly indicative of true sinus disease.¹⁶

Demographic and historical data recorded from all patients included age, sex, previous adenoidectomy, and nasal steroid use. Diagnosis of asthma was based on guidelines published by the Global Initiative for Asthma using medical history and spirometry data interpreted by a board-certified pediatric pulmonologist. Specific serum IgE levels were measured by the ImmunoCAP Fluorezyme Immunoassay (>0.35 kU/L was considered positive; Phadia AB, Uppsala, Sweden) or a standard prick skin test for aeroallergens was used to confirm a diagnosis of allergic rhinitis.

Sinus tissue samples were removed from the middle meatus region under endoscopic guidance using a biting or cutting forceps.

Table 1

Comparison of demographic and clinical data of control patients (no CRS, asthma, or allergic rhinitis) vs patients with CRS only (without allergic rhinitis or asthma)

	Control (n = 10)	CRS only (n = 10)	P value
Girls, n (%)	7 (70)	5 (50)	
Age (y), mean	4.5	6.5	.08
Nasal steroid use, n (%)	4 (40)	3 (30)	.65
Revision adenoidectomy, n (%)	0 (0)	2 (20)	.14
SN-5 score, mean	2.02	4.33	<.0001
Allergic rhinitis, n (%)	0 (0)	0 (0)	0
Asthma, n (%)	0 (0)	0 (0)	0

Abbreviations: CRS, chronic rhinosinusitis; SN-5, sinonasal symptoms score questionnaire.

Adenoid tissue was removed through the oropharynx with a biting or cutting forceps before adenoidectomy. All specimens were immediately snap-frozen with liquid nitrogen and then stored at -80°C . Each sample was homogenized in $1\times$ protein lysis buffer, homogenized twice for 30 seconds each, and centrifuged to remove debris.

Mucosal expression of inflammatory cytokines and chemokines was measured using the Luminex 100 System (Luminex, Austin, Texas) and a Milliplex kit (Millipore, Billerica, Massachusetts) according to the manufacturers' specifications. Each mediator was normalized to total protein tissue content measured with the Precision Red Advanced Protein Assay (Cytoskeleton, Denver, Colorado). The 40 cytokines and chemokines tested included epidermal growth factor (EGF), eotaxin, fibroblast growth factor-2 (FGF-2), Flt-3 ligand, fractalkine, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, growth-related oncogene (GRO), interleukin (IL)-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IL-1 α , IL-1 β , IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, interferon (INF)- α 2, INF- γ , INF- γ -induced protein-10, monocyte chemoattractant protein-1 (MCP-1/CCL2), MCP-3, macrophage-derived chemokine/CCL22, macrophage inflammatory protein-1 α , macrophage inflammatory protein-1 β , platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB, regulated on activation normal T cell expressed and secreted (RANTES), soluble CD40 ligand, soluble IL-2 receptor antagonist, transforming growth factor- α , tumor necrosis factor (TNF)- α , TNF- β , and vascular endothelial growth factor.

Data were analyzed using the Student *t* test for continuous variables or the χ^2 test for nominal variables. Differences with a *P* value less than .05 were considered statistically significant.

Results

The 10 control patients did not have CRS (based on clinical score), asthma, or allergic rhinitis. Their average age was 4.5 years and 7 were girls (Table 1). Four were being treated with nasal steroids at the time of surgery in an attempt to decrease swelling of the adenoids. There was no statistically significant difference in demographic and clinical data between the control patients and the 10 patients diagnosed with CRS only (ie, without asthma or

Table 2

Comparison of demographic and clinical data of patients with chronic rhinosinusitis with vs without asthma

	Asthma (n = 15)	No asthma (n = 13)	P value
Girls, n (%)	5 (33)	6 (46)	
Age (y), mean	6.87	6.38	.67
Nasal steroid use, n (%)	12 (80)	6 (46)	.06
Revision adenoidectomy, n (%)	7 (47)	4 (31)	.39
SN-5 score, mean	4.77	4.34	.23
Allergic rhinitis, n (%)	5 (33)	3 (23)	.59

Abbreviation: SN-5, sinonasal symptoms score questionnaire.

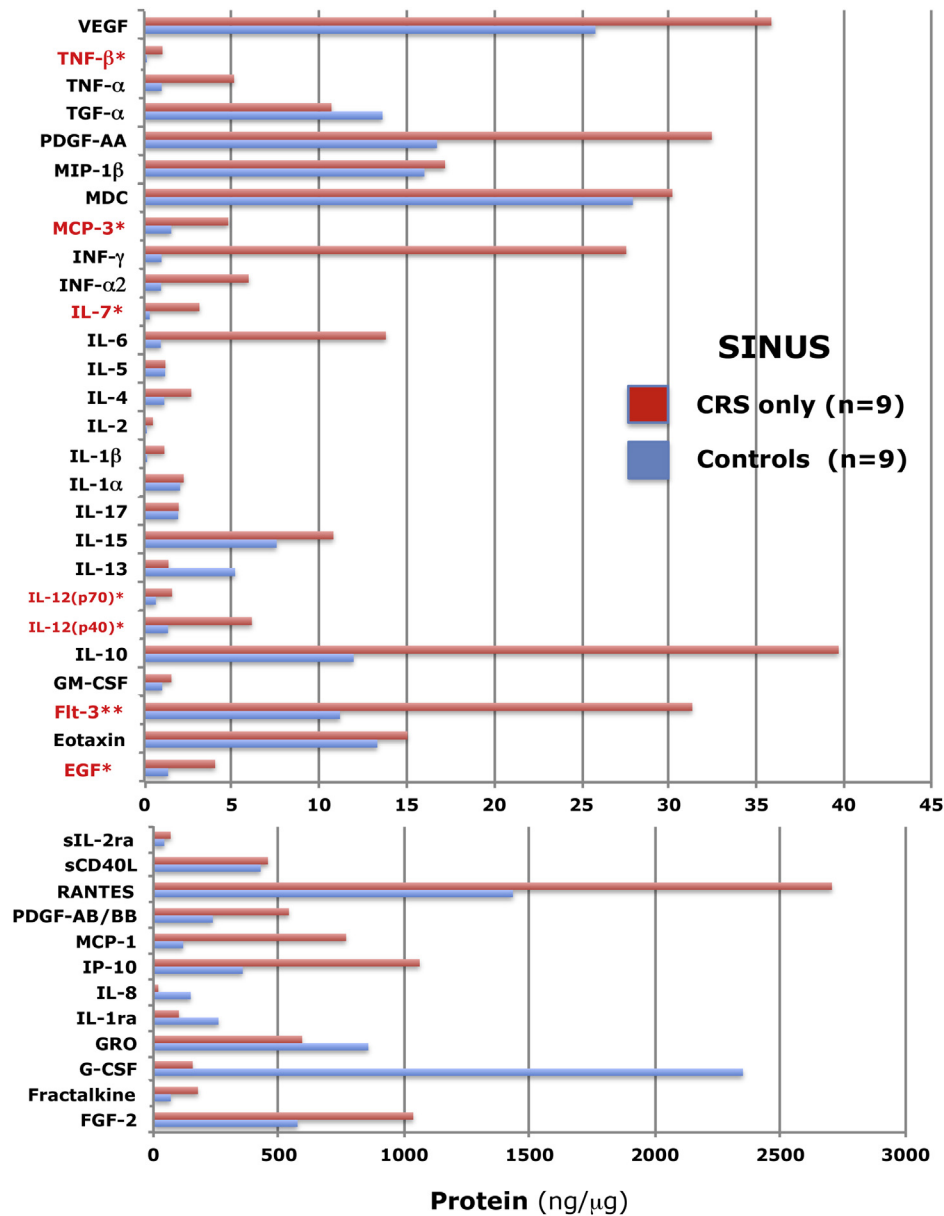


Figure 1. Expression of inflammatory cytokines and chemokines in sinus tissue of children with CRS only (ie, without asthma or allergic rhinitis) vs control children without CRS, asthma, or allergic rhinitis. Seven of the cytokines tested were significantly increased in the CRS group (red). Data are presented as mean concentrations in nanograms per microgram of total tissue protein. * $P < .05$; ** $P < .01$, significantly different from control. CRS, chronic rhinosinusitis; EGF, endothelial growth factor; FGF-2, fibroblast growth factor-2; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; INF, interferon; IP-10, interferon- γ -induced protein-10; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine/CCL22; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; sCD40L, soluble CD40 ligand; sIL-2ra, soluble interleukin-2 receptor antagonist; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

allergic rhinitis), except for the lower average SN-5 clinical score ($P < .0001$).

Twenty-three of the 28 children with CRS resistant to maximal medical management also underwent adenoidectomy, and 11 of them (48%) had a previous adenoidectomy (Table 2). Fifteen patients (54%) had asthma and 8 (29%) had allergic rhinitis. Eleven patients (39%) were girls and the average age at the time of surgery was 6.6 years. Eighteen patients (64%) were taking a nasal steroid at the time of surgery for an average duration of 3 months. The average SN-5 score was 4.59. There was no significant difference in sex, age, history of adenoidectomy, SN-5 score, or diagnosis of allergic rhinitis between the asthmatic and nonasthmatic groups. However, twice as many patients with CRS and asthma were treated with nasal steroids

compared with patients with CRS without asthma, and this difference was close to statistical significance ($P = .06$).

When sinus tissue of control patients was compared with sinus tissue of children with CRS, but no asthma or allergic rhinitis, 34 of the 40 inflammatory cytokines tested were increased in the CRS group (Fig 1), and the difference was significantly different for 7 cytokines, including TNF- β ($P = .046$), MCP-3 ($P = .042$), IL-7 ($P = .023$), IL-12(p70) ($P = .015$), IL-12(p40) ($P = .044$), Flt-3 ligand ($P = .007$), and EGF ($P = .033$). Similarly, when adenoid tissue of control patients was compared with adenoid tissue of children with CRS, but no asthma or allergic rhinitis, 36 of the 40 cytokines tested were increased in the CRS group (Fig 2), and the difference was statistically significant for 16 of these cytokines, including vascular

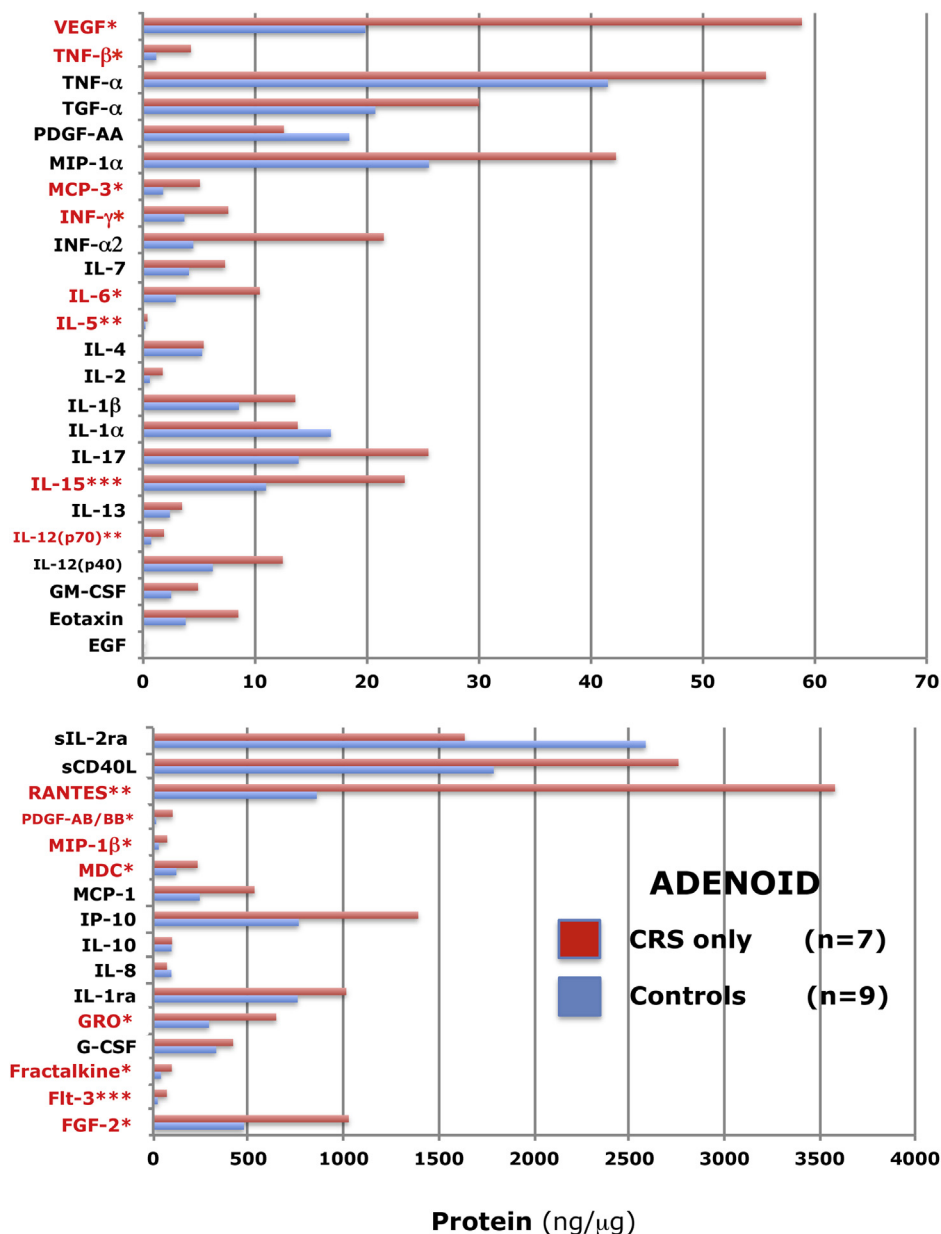


Figure 2. Expression of inflammatory cytokines and chemokines in adenoid tissue of children with CRS only (ie, without asthma or allergic rhinitis) vs controls without CRS, asthma, or allergic rhinitis. Sixteen of the cytokines tested were significantly increased in the CRS group (red). Data are presented as mean cytokine concentrations in nanograms per microgram of total tissue protein. * $P < .05$; ** $P < .01$; *** $P < .001$, significantly different from control. CRS, chronic rhinosinusitis; EGF, endothelial growth factor; FGF-2, fibroblast growth factor-2; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; INF, interferon; IP-10, interferon- γ -induced protein-10; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine/CCL22; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; sCD40L, soluble CD40 ligand; sIL-2ra, soluble interleukin-2 receptor antagonist; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

endothelial growth factor ($P = .018$), TNF- β ($P = .028$), MCP-3 ($P = .027$), INF- γ ($P = .041$), IL-6 ($P = .024$), IL-5 ($P = .006$), IL-15 ($P = .0007$), IL-12(p70) ($P = .003$), RANTES ($P = .008$), PDGF-AB/BB ($P = .036$), macrophage inflammatory protein-1 β ($P = .028$), macrophage-derived chemokine/CCL22 ($P = .033$), GRO ($P = .023$), fractalkine ($P = .014$), Flt-3 ligand ($P = .0007$), and FGF-2 ($P = .012$).

Compared with children with CRS without asthma, the sinus tissue of children with asthma and CRS showed increased levels for 27 of the 40 inflammatory cytokines tested (Fig 3), but the increase was statistically significant only for TNF- β ($P = .009$). In the adenoid tissue, 39 of the 40 cytokines tested were higher in children with than in those without asthma (Fig 4), and the increase was

statistically significant for 5 of these, including EGF ($P = .018$), eotaxin ($P = .037$), FGF-2 ($P = .013$), GRO ($P = .037$), and PDGF-AA ($P = .049$).

Table 3 presents the statistical significance of the differences of cytokine expression in sinus tissue measured between the control and CRS-only groups (ie, no asthma or allergic rhinitis) and between the asthmatic and nonasthmatic CRS groups. All cytokines that were significantly increased in the CRS-only group compared with the control group also were increased in the asthmatic CRS group compared with the nonasthmatic CRS group. TNF- β was the only cytokine that was significantly increased in the asthmatic CRS group compared with the nonasthmatic CRS group and was significantly increased in the CRS-only group compared with the control group.

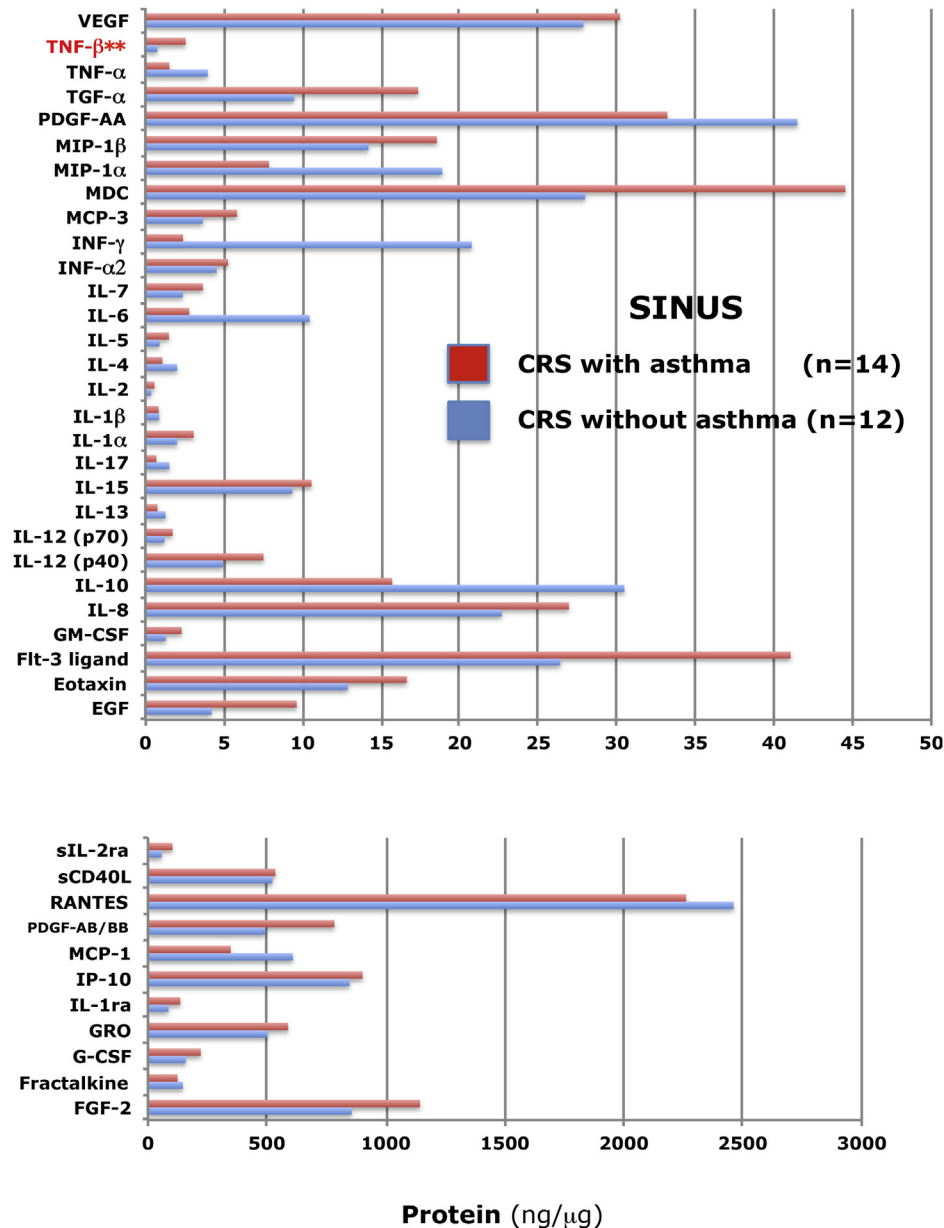


Figure 3. Expression of inflammatory cytokines and chemokines in sinus tissue of children with CRS and asthma vs children with CRS without asthma. Only TNF-β was significantly increased in children with asthma (red). Data are presented as mean cytokine concentrations in nanograms per microgram of total tissue protein. ** $P < .01$, significantly different from children with CRS without asthma. CRS, chronic rhinosinusitis; EGF, endothelial growth factor; FGF-2, fibroblast growth factor-2; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; INF, interferon; IP-10, interferon-γ-induced protein-10; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine/CCL22; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; sCD40L, soluble CD40 ligand; sIL-2ra, soluble interleukin-2 receptor antagonist; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Table 4 presents the statistical significance of the differences of cytokine expression in adenoid tissue measured between the control group and the CRS-only group (ie, no asthma or allergic rhinitis) and between the asthmatic and nonasthmatic CRS groups. Fifteen of 16 cytokines that were significantly increased in the CRS-only group compared with the control group were increased in the asthmatic CRS group compared with the nonasthmatic CRS group. FGF-2 and GRO were significantly increased in the CRS-only group compared with the control group and in the asthmatic CRS group compared with the nonasthmatic CRS group. Fractalkine and IL-12(p70) were significantly increased in the CRS-only group compared with the control group and approached significance in the asthmatic CRS group compared with the nonasthmatic CRS group.

Discussion

This study shows that the inflammatory response in the sinus and adenoid tissues of children with CRS is qualitatively similar but quantitatively amplified in the setting of a concomitant diagnosis of asthma. Specifically, the cytokines and chemokines that were significantly increased in the CRS-only (ie, no asthma or allergic rhinitis) group compared with the control group were almost universally expressed at higher levels in the asthmatic CRS group compared with the nonasthmatic CRS group. Although this evidence is consistent with previous epidemiologic and clinical observations,^{17,18} the present study is the first confirming this pathophysiologic model with direct measurement of mucosal inflammation in children.

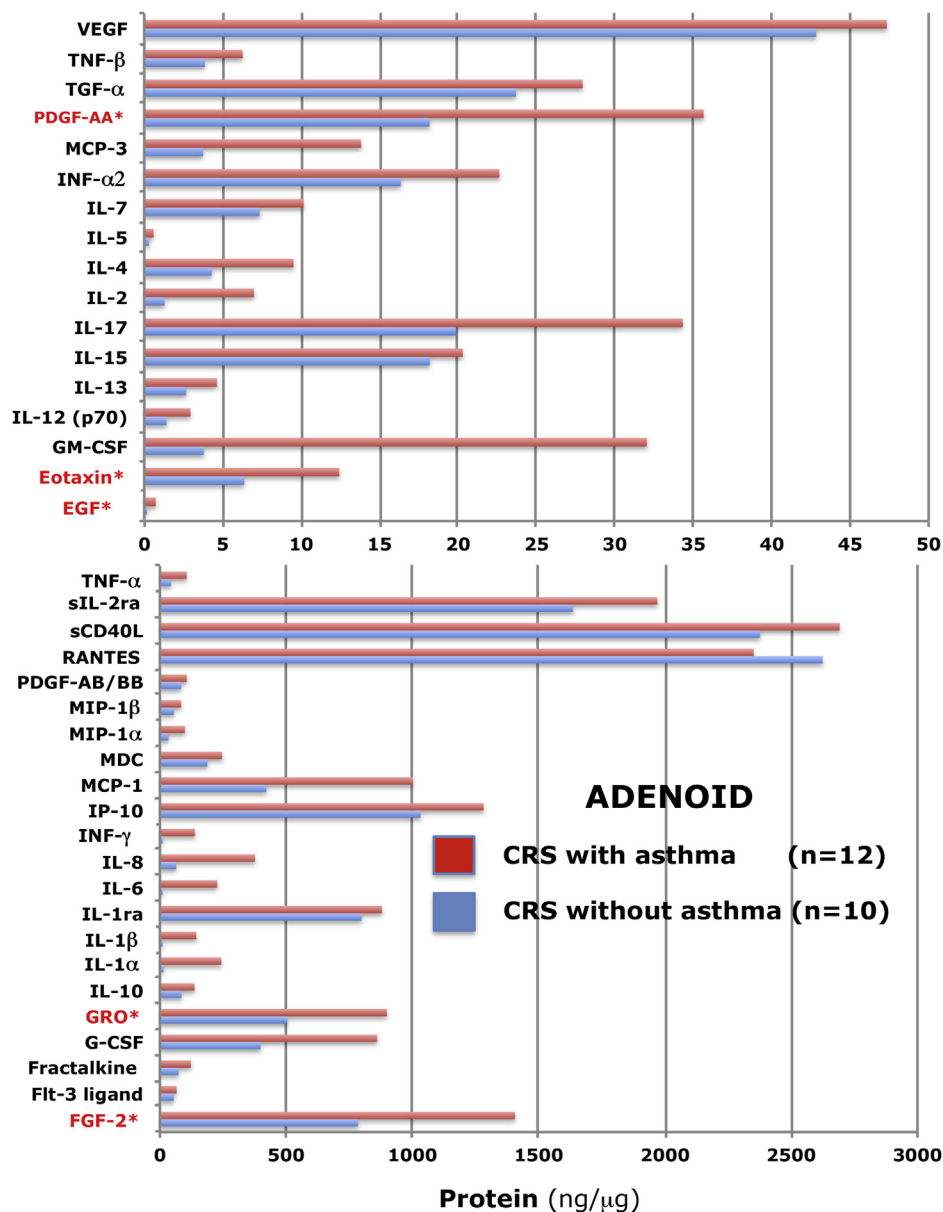


Figure 4. Expression of inflammatory cytokines and chemokines in adenoid tissue of children with CRS and asthma vs children with CRS without asthma. Five of the cytokines tested were significantly increased in children with asthma (red). Data are presented as mean cytokine concentrations in nanograms per microgram of total tissue protein. * $P < .05$, significantly different from children with CRS without asthma. CRS, chronic rhinosinusitis; EGF, endothelial growth factor; FGF-2, fibroblast growth factor-2; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; GRO, growth-related oncogene; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; INF, interferon; IP-10, interferon- γ -induced protein-10; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine/CCL22; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; sCD40L, soluble CD40 ligand; sIL-2ra, soluble interleukin-2 receptor antagonist; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

In the sinus tissue, TNF- β was the only cytokine whose increase reached statistical significance in the asthmatic CRS group compared with the nonasthmatic CRS group. This cytokine also was significantly increased in the CRS-only group compared with the control group. In the adenoid tissue, FGF-2 and GRO were significantly increased in the CRS-only group compared with the control group and in the asthmatic CRS group compared with the nonasthmatic CRS group. Fractalkine and IL-12(p70) were significantly increased in the CRS-only group compared with the control group and approached significance in the asthmatic CRS group compared with the nonasthmatic CRS group.

These inflammatory patterns can inform the future use of specific cytokines as biomarkers and identify specific targets for biological therapies using humanized anticytokine antibodies. Indeed,

the results of this study suggest that new therapeutic strategies are necessary, considering the strong inflammatory response found in the tissues of patients with CRS and asthma despite the use of nasal steroids by most of them. Because the bioavailability of nasal sprays is not dependent on effort or maneuver, the sinus and adenoid tissues examined in this study might have been exposed to significant concentrations of potent topical corticosteroids, raising some concern about the actual anti-inflammatory potency of these drugs in vivo. Because the children included in this study had failed medical management, they could represent a distinct subpopulation with particularly severe inflammation or steroid resistance or perhaps were less compliant with therapy.

Furthermore, these data provide insight about the relation between CRS and asthma. Specifically, these data support the

Table 3

Summary of statistical significance of differences in cytokine and chemokine expressions in sinus tissue

	P value for CRS only vs controls	P value for CRS with vs without asthma
EGF	.0325 ^a	.1466
Flt-3 ligand	.0072 ^a	.1212
IL-12(p40)	.0440 ^a	.2547
IL-12(p70)	.0151 ^a	.2546
IL-7	.0233 ^a	.2210
MCP-3	.0417 ^a	.3128
TNF- β	.0455 ^a	.0095 ^a

Abbreviations: CRS, chronic rhinosinusitis; EGF, endothelial growth factor; IL, interleukin; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor.

^aP < .05, statistically significant differences.

hypothesis that children with asthma and CRS have a similar, but more severe, form of the same inflammatory process as children with CRS but without asthma. This pattern of cytokine expression could be a manifestation of the “united airways” hypothesis, that is, that the inflammatory process associated with atopy is homogeneous across contiguous upper and lower segments of the respiratory tract. Conversely, lower airway disease in patients with CRS and asthma could be driven, at least in part, by a more severe inflammatory process in the upper airway. This could be the result of postnasal secretions seeding the lower respiratory tract or could derive from the systemic absorption of inflammatory mediators synthesized in the upper airways. More likely, a neurogenic link based on cholinergic-mediated naso-sino-bronchial reflexes and/or non-cholinergic, non-adrenergic axon reflexes best fits the pathophysiology models developed in animals and other experimental systems. In all these scenarios, the present data strongly support the practice of aggressively treating sinus disease in children with asthma to achieve better or full control of asthma symptoms.

Among the limitations of this study, the most important is the relatively small sample. However, the collection of samples in a pediatric population is always problematic, especially when the

samples need to be obtained from asymptomatic controls. Although it is likely that a larger sample would increase the number of differences reaching statistical significance, power analysis suggests that this would be prohibitive in time and costs. One limitation directly related to the small sample is the inability to determine the potential influence of allergen sensitization and exposure on cytokine levels. The same is true for the use of topical corticosteroids, which have been shown to significantly decrease adenoid tissue^{19,20} and treat rhinosinusitis.²⁰ The authors could have observed significant statistical differences had they obtained the samples after a period of washout of topical steroids. However, this would have been unethical. Randomization would have contributed to the quality of the study but also would have prevented access of some patients with severe symptoms to therapies proven to be effective. The SN-5 score used to exclude CRS in control patients is not as accurate as a computed tomogram of the sinuses, but it would have been unethical to irradiate asymptomatic children.

Compared with children with CRS and without asthma, children with CRS and asthma had significantly higher sinus levels of TNF- β and adenoid levels of EGF, eotaxin, FGF-2, GRO, and PDGF-AA. Although, to authors' knowledge, TNF- β expression has not been studied in sinus or adenoid tissues in CRS or asthma, there have been reports demonstrating a high prevalence of TNF- β polymorphisms in chronic sinusitis²¹ and in asthma.²² A recent study found increased T-cell activation markers in the adenoids of children with CRS. This study also showed that CRS severity was associated with levels of activated cells and cytokines in adenoid tissues.²³ Therefore, the present findings suggest that TNF- β could be a major player in the mechanism of CRS and asthma. However, more studies are needed to explore this possibility.

The present investigation did not permit the precise identification of the cellular origin or role in pathogenesis of these cytokines. Such investigations would be complicated by the well-known cytokine characteristics of redundancy and pleiotropy (multiple cell sources and an ability to exert >1 action often on multiple cell types). Therefore, confirmation of a causal role for any of these cytokines in pathophysiology will require the conduct of a controlled clinical trial using a specific blocking agent. Not surprisingly, these cytokines are involved in the stimulation of cell growth, proliferation, and differentiation and several key processes linked with allergy and asthma, such as chemoattraction of inflammatory cells (eg, eosinophils) and tissue remodeling.^{24,25} Also, more differences were observed in the adenoids compared with the sinuses, perhaps reflecting the major role of the adenoids in regulating immune function.

The present research focused on sinus and adenoid tissue in children, but other investigators have conducted studies in children with otitis media.^{26–29} A recent study found higher IL-5 and TNF- α production in stimulated cells from hypertrophic adenoids of pediatric patients with vs without otitis media with effusion.²⁹ Sobol et al²⁶ assessed middle ear fluids for cells by immunocytochemistry and for expression of cytokines by in situ hybridization in 26 patients with otitis media with effusion undergoing myringotomy and ventilation tube placement. Compared with children without atopy, there was a predominance of eosinophils, T lymphocytes, and T-helper cell type 2 mediators (IL-4 and IL-5) in the middle ear effusions of children with atopy. In a follow-up study using middle ear biopsy specimens from 7 children with persistent otitis media with effusion and 7 controls,²⁷ there were statistically significant increases in the expression of CD3, a major basic protein, and IL-5 in the atopic population. The present study also assessed IL-5 and there were significant differences between the CRS-only and control groups in adenoid tissue (Table 4) but not between the asthmatic CRS group and the nonasthmatic CRS group and not in sinus tissue.

In summary, the present data show that the inflammatory response in the upper airway mucosa of children with asthma and

Table 4

Summary of statistical significance of differences in cytokine and chemokine expressions in adenoid tissue

	P value for CRS only vs controls	P value for CRS with vs without asthma
FGF-2	.0121 ^a	.0133 ^a
Flt-3 ligand	.0007 ^a	.4566
Fractalkine	.0141 ^a	.0581
GRO	.0227 ^a	.0368 ^a
IL-12(p70)	.0029 ^a	.0676
IL-15	.0007 ^a	.5948
INF- γ	.0409 ^a	.3711
MCP-3	.0266 ^a	.1770
MDC	.0328 ^a	.2602
MIP-1 β	.0275 ^a	.2318
PDGF-AB/BB	.0356 ^a	.6515
RANTES	.0082 ^a	.7540
TNF- β	.0282 ^a	.2674
VEGF	.0175 ^a	.7050
IL-5	.0063 ^a	.4207
IL-6	.0243 ^a	.3437
EGF	.0000	.0183 ^a
Eotaxin	.0891	.0368 ^a
PDGF-AA	.4355	.0493 ^a

Abbreviations: CRS, chronic rhinosinusitis; EGF, endothelial growth factor; FGF-2, fibroblast growth factor-2; GRO, growth-related oncogene; IL, interleukin; INF- γ , interferon- γ ; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine/CCL22; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

^aP < .05, statistically significant differences.

CRS is similar to, but more severe than, that of children with CRS without asthma. This observation is consistent with the hypothesis that asthma in these patients is caused or exacerbated by severe upper airway disease and could provide a basis for the observation that children with asthma are at risk for worse surgical outcomes after sinus surgery and/or adenoidectomy compared with children without asthma.⁴ The present findings support the concept that treating sinus disease is paramount in the management of chronic asthma in children. In addition, this study provides specific and direct information on inflammatory profiles critical for the future validation of biomarkers and identification of targets for biological therapies of pediatric CRS and asthma.

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References

- [1] Fuller CG, Schoettler JJ, Gilsanz V, Nelson MD Jr, Church JA, Richards W. Sinusitis in status asthmaticus. *Clin Pediatr*. 1994;33:712–719.
- [2] Smart BA, Slavin RG. Rhinitis and pediatric asthma. *Immunol Allergy Clin North Am*. 2005;25:67–82.
- [3] Tosca MA, Riccio AM, Marseglia GL, Caligo G, Pallestrini E, Ameli F. Nasal endoscopy in asthmatic children: assessment of rhinosinusitis and adenoiditis incidence, correlations with cytology and microbiology. *Clin Exp Allergy*. 2001;31:609–615.
- [4] Ramadan HH. Surgical management of chronic sinusitis. *Laryngoscope*. 2004;114:2103–2109.
- [5] Dejiima K, Hama T, Miyazaki M, et al. A clinical study of endoscopic sinus surgery for sinusitis in patients with bronchial asthma. *Int Arch Allergy Immunol*. 2005;138:97–104.
- [6] Pawankar R, Zernotti ME. Rhinosinusitis in children and asthma severity. *Curr Opin Allergy Clin Immunol*. 2009;9:151–153.
- [7] Togias A. Rhinitis and asthma: evidence for respiratory system integration. *J Allergy Clin Immunol*. 2003;111:1171–1183.
- [8] Pipkorn U, Karlsson G, Enerback L. The cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol*. 1988;82:1046–1054.
- [9] Ollerenshaw S, Woolcock A. Characteristics of the inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. *Am Rev Respir Dis*. 1992;145:922–927.
- [10] Braunstahl GJ, Overbeek SE, Kleinjan A, Prins JB, Hoogsteden HC, Fokkens WJ. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol*. 2001;107:469–476.
- [11] Rachelefsky G, Katz R, Siegel SC. Chronic sinus disease with associated reactive airway disease in children. *Pediatrics*. 1984;73:526–529.
- [12] Tosca MA, Cosentino C, Pallestrini E, Caligo G, Milanese M, Ciprandi G. Improvement of clinical and immunopathologic parameters in asthmatic children treated for concomitant chronic rhinosinusitis. *Ann Allergy Asthma Immunol*. 2003;91:71–78.
- [13] Businco L, Fiore L, Frediani T, Artuso A, Di Fazio A, Bellioni P. Clinical and therapeutic aspects of sinusitis in children with bronchial asthma. *Int J Pediatr Otolaryngol*. 1981;3:287–294.
- [14] Oliveira C, Sole D. Improvement of bronchial hyperresponsiveness in asthmatic children treated for concomitant sinusitis. *Ann Allergy Asthma Immunol*. 1997;79:70–74.
- [15] Parsons D, Phillips S. Functional endoscopic surgery in children. *Laryngoscope*. 1993;103:899–903.
- [16] Terrell AM, Ramadan HH. Correlation between SN-5 and computed tomography in children with chronic rhinosinusitis. *Laryngoscope*. 2009;119:1394–1398.
- [17] Passalacqua G, Ciprandi G, Canonica GW. The nose-lung interaction: united airways disease. *Curr Opin Allergy Clin Immunol*. 2001;1:7–14.
- [18] Eriksson J, Bjerg A, Lotvall J, et al. Rhinitis phenotypes correlate with different symptom presentation and risk factor patterns of asthma. *Respir Med*. 2011;105:1611–1621.
- [19] Rezende RM, Silveira F, Barbosa AP, et al. Objective reduction in adenoid tissue after mometasone furoate treatment. *Int J Pediatr Otorhinolaryngol*. 2012;76:829–831.
- [20] Georgalas C, Thomas K, Owens C, et al. Medical treatment for rhinosinusitis associated with adenoidal hypertrophy in children: an evaluation of clinical response and changes on magnetic resonance imaging. *Ann Otol Rhinol Laryngol*. 2005;114:638–644.
- [21] Takeuchi K, Majima Y, Sakakura Y. Tumor necrosis factor gene polymorphism in chronic sinusitis. *Laryngoscope*. 2000;110(10 Pt 1):1711–1714.
- [22] Yang M, Fu X, Zhang Y, et al. The +252A/G polymorphism in the lymphotoxin-a gene increases the risk of asthma: a meta-analysis. *Respirology*. 2012;17:1229–1236.
- [23] Shin SY, Choi GS, Park HS, et al. Immunologic investigation in the adenoid tissues from children with chronic rhinosinusitis. *Otolaryngol Head Neck Surg*. 2009;141:91–96.
- [24] Kennedy JL, Borish L. Chronic sinusitis pathophysiology: the role of allergy. *Am J Rhinol Allergy*. 2013;27:367–371.
- [25] Borish L, Steinke JW. Cytokines and chemokines. *J Allergy Clin Immunol*. 2003;111:S460–S475.
- [26] Sobol SE, Taha R, Schloss MD, et al. T(H)2 cytokine expression in atopic children with otitis media with effusion. *J Allergy Clin Immunol*. 2002;110:125–130.
- [27] Wright ED, Hurst D, Miotto D, Giguere C, Hamid Q. Increased expression of major basic protein (MBP) and interleukin-5 (IL-5) in middle ear biopsy specimens from atopic patients with persistent otitis media with effusion. *Otolaryngol Head Neck Surg*. 2000;123:533–538.
- [28] Skoner AR, Skoner KR, Skoner DP. Allergic rhinitis, histamine, and otitis media. *Allergy Asthma Proc*. 2009;30:470–481.
- [29] Zelazowska-Rutkowska B, Ilendo E, Skotnicka B, Wysocka J, Kasprzycka E. Production of cytokines by mononuclear cells of hypertrophic adenoids in children with otitis media with effusion. *Folia Histochem Cytobiol*. 2012;50:586–589.