

Local IgE and inflammation in chronic rhinosinusitis of asthmatics and non-asthmatics

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Abstract The aim of the study was to show the difference in the pattern of inflammation, and Th1/Th2 polarization between asthmatic and non-asthmatic patients with CRS, specifically eosinophil activation, local IgE levels in the sinus fluid and tissue, and the severity of inflammation were measured. The maxillary sinus lavages, mucosal biopsies and bacteriological swabs were taken in 17 asthmatic and 36 non-asthmatic adult patients with CRS. The concentrations of IgE, eosinophil cationic protein (ECP), myeloperoxidase (MPO), and tryptase were analyzed and IgE+ cells, eosinophils, lymphocytes and plasma cells were counted. The granulocyte activation markers and IgE in sinus lavages, and the inflammatory and IgE+ cells counts were significantly higher in the asthmatics with the greatest difference in ECP and IgE concentrations. The tryptase concentrations did not differ, but only in the asthmatics they correlated significantly with the IgE concentrations and IgE+ cells count. Asthmatic patients present a distinct subgroup among the patients with chronic rhinosinusitis (CRS). The levels of the cellular markers and IgE in the sinus fluid differ from those of non-asthmatic patients with CRS. The activation of granulocytes (especially eosinoph-

ils), local IgE concentrations and the inflammatory cells infiltration are significantly higher in the asthmatics.

Keywords Sinus lavage · Histomorphometry · Immunohistochemistry · ECP · MPO · Tryptase · Eosinophil · Th-2 · Bacteria · Superantigen

Introduction

Chronic rhinosinusitis (CRS) is a common clinical syndrome; however, its complexity arises from the interaction of many individual risk factors, which have been associated with this disease [1]. Sinus mucosal biopsies and sinus fluid analyses have revealed different patterns of inflammatory and regulatory cytokines, inflammatory cells activation and Th1/Th2 polarization comparing CRS and nasal polyposis (NP) [2, 3]. Furthermore, distinct subgroups, related to certain risk factors, within CRS and NP, in terms of pathogenesis and inflammatory response, have been recognized [1]. However, most studies, on the basis of sinus sample analysis, such as in this study, have an important selectional bias, as they include surgical cases, which might differ from those patients with CRS who respond to medical treatment. The sensitization to aeroallergens was more common in the patients with CRS [1]; however, the role of allergy in the pathogenesis of CRS remains unclear. Increased local IgE was found more often in Th-2 polarized immune response, but not necessarily linked to atopy [4]. Expression of IgE heavy chain transcripts in the sinus mucosa of the atopics was significantly higher than in non-atopics with CRS, but the latter had a higher expression than in the control group [5]. Recent data indicate that increased local IgE production in the tissue might be related to the response to bacterial superantigens, presumably *Staphylococcus aureus* exotoxins

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[6]. Previous studies on CRS in asthmatics mostly included patients with NP. In contrast, we were interested in analyzing the pattern of the inflammation in asthmatics without NP.

The aim of the study was to test the hypothesis that sinus fluid and mucosa in the asthmatics with CRS would show Th-2-like inflammation (as a part of the united airways disease), even without NP. As markers of inflammation, concentrations of eosinophil cationic protein (ECP), myeloperoxidase (MPO), and tryptase in the maxillary sinus fluid were used to assess the degree of activation of eosinophils, neutrophils, and mast cells within the study, respectively. IgE concentrations were measured in the sinus fluids, and IgE+ cells, eosinophils, lymphocytes and plasma cells were counted in maxillary sinus biopsies of the asthmatic and non-asthmatic patients with CRS.

Patients and methods

The study was conducted in a group of 17 asthmatic (18–65 years, 8 females, 9 males and mean age 36) and 36 non-asthmatic patients (18–65 years, 19 females, 17 males and mean age 38) with CRS scheduled for endosinus treatment [7], due to persisting CRS symptoms, despite long-term medical treatment. All patients gave their informed consent and the study was approved by the Ethics Committee of University Hospital “Sestre milosrdnice”, Zagreb School of Medicine. Sensitization to common aeroallergens was tested using skin prick testing (SPT), and specific IgE in the serum supported the reactivity to relevant allergens.

The diagnosis of sinus disease was based on history, nasal endoscopy and radiological criteria according to EP³OS guidelines [8]. The diagnosis and the stage of asthma stage were based on Global Initiative for Asthma (GINA) guidelines criteria [9]. Only mild to moderate asthma patients were included. The patients receiving systemic steroid or antibiotic treatment during the past month, those who had a recent upper respiratory infection, suspected odontogenic pathology, increased CRP, NP, aspirin sensitivity or any systemic disease that may influence the study protocol, were excluded. Patients with severe asthma were excluded as they would make the asthma group even more heterogeneous, which was already shown by the distribution of the markers data when only mild and moderate were included. All patients stopped topical nasal steroid use 1 week before the procedure, but continued nasal saline washings.

After disinfection, inferior maxillary anastomostomy was performed in local anesthesia with a sterile trocar, bacteriological swabs and sinus lavage with 5 ccm of saline were taken. Lavage samples were stored at room temperature for 2 h, centrifuged at 1,000×g for 10 min and placed in a

refrigerator at −20°C. The concentrations of ECP, IgE and tryptase in the lavages were determined by fluoroenzyme-immunoassay (UniCAP, Pharmacia and Upjohn, Uppsala, Sweden), and MPO by radioimmunoassay (Pharmacia, Sweden). Bacterial swabs and aspirates were cultured according with standard typing and identification techniques [7].

Biopsy specimens were formalin-fixed, routinely processed, paraplast-embedded, cut at 3 µm, and hemalaun-eosine stained. Additional sections were prepared for immunohistochemical IgE staining (polyclonal rabbit anti-human IgE, DAKO, Glostrup, Denmark).

Each cell type (eosinophils, lymphocytes, plasma cells, and IgE+ cells, respectively) was microscopically assessed by a blinded pathologist, counting the cells in eight randomly selected high power fields (400×), expressing the final number for each specimen and cell type as a sum of two highest counts divided by 2 [4].

The distribution of the data was calculated using the Kolmogorov–Smirnov test. Statistical analysis was performed by the Kruskal–Wallis and Mann–Whitney *U* two-tailed tests for unpaired comparisons. The Mann–Whitney *U* test was then used for between-group comparison and the correlations with Spearman rank correlation test. All conclusions were based on a significance level of $P < 0.05$.

Results

Although the calculated distribution was normal, the distribution curves indicated that some parameters were not distributed normally (ECP in the asthmatics demonstrated trimodal curve, and MPO bimodal, most of the other parameters have shown skewed curves). The statistician indicated non-parametric tests. Sensitization to common aeroallergens was found in 12/17 in the asthmatic group and in 19/36 in the CRS group. Bacterial growth was present in 24 of the 34 specimens in the asthma group, 4 commensals and 24 pathogens, 19 aerobes and 5 anaerobes, as mixed flora was found in three samples. In the CRS group, bacterial growth was present in 47/60 samples (8 patients had unilateral anastomostomy), 15 commensal and 32 pathogens (24 aerobes, 8 anaerobes). *Staphylococcus aureus* was the most common in both groups; gram-negative rods were more common in the asthma group (25 vs. 15%, respectively). Most common gram-negative rods in CRS were *Haemophilus influenzae* followed by *Klebsiella*, which were not found in the asthma group, where *Pseudomonas aeruginosa* was the most common. The granulocyte activation markers as well as the cell counts were significantly higher in the asthma group (Table 1). Correlations between the parameters were similar in both groups (Table 2); however, tryptase correlated significantly with ECP, IgE,

Table 1 Presenting mean and median values (extremes in the brackets), and significance of difference (*P* value) between cellular markers concentrations in maxillary sinus lavages, IgE and eosinophils, lymphocytes, IgE+ and plasma cells count per high power field (HPF) in the maxillary sinus mucosa of the asthmatic and non-asthmatic patients with CRS

Nasal lavage	Group	Mean	Median (range)	<i>P</i>	Cells/HPF 400×	Mean	Median (range)	<i>P</i>
ECP (mcg/l)	Asthma	64.61*	36.05 (2–273)	0.0001	IgE+	4.75*	3.25 (1–18)	0.00001
	CRS	19.48	2.65 (2–231)			1.50	1 (0–9.5)	
MPO (mcg/l)	Asthma	456.67*	211.8 (2.7–1,879)	0.027	Eosinophils	33.61*	10.5 (0–121)	0.004
	CRS	342.68	31.05 (2.4–3,800)			11.04	2.75 (0–103.5)	
Tryptase (mcg/l)	Asthma	15.64	5.76 (1–166)	0.714	Lymphocytes	25.26*	19.5 (2–68)	0.0001
	CRS	9.89	3.28 (1–112)			9.06	0 (41.5)	
IgE (kU/l)	Asthma	44.25*	19.4 (2.1–420)	0.0001	Plasma cells	20.97*	14.25 (3.5–75)	0.0001
	CRS	7.34	1.6 (0.6–66.9)			7.57	4.5 (0–47.5)	

**P* < 0.05 between asthmatic and non-asthmatic group

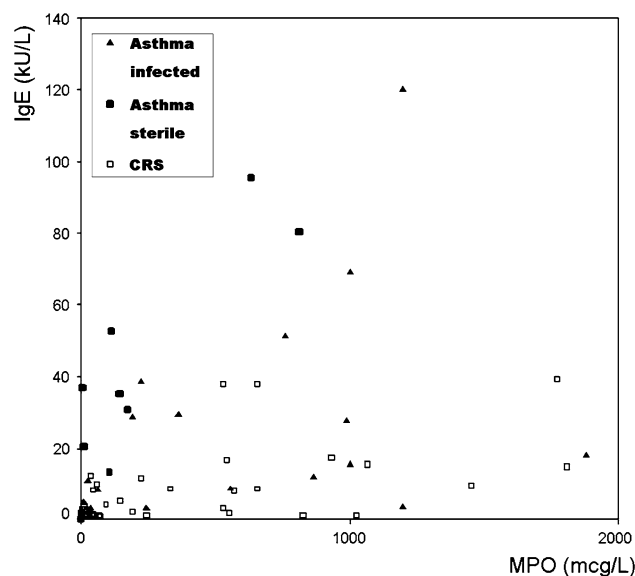
Table 2 Presenting correlation coefficients (Spearman ρ) between cellular markers and IgE in the sinus lavages, and between IgE and cells/high power field count in asthmatic and non-asthmatic patients with CRS

Parameters	Asthma Spearman ρ	<i>P</i>	CRS Spearman ρ	<i>P</i>
ECP–MPO	0.607*	0.0001	0.644*	0.0001
ECP–tryptase	0.418*	0.014	−0.22	0.88
ECP–IgE	0.699*	0.0001	0.49*	0.0001
ECP–eosinophils	0.358*	0.038	0.506*	0.0001
ECP–IgE+	0.502*	0.02	0.276*	0.05
MPO–tryptase	0.173	0.329	−0.027	0.854
MPO–IgE	0.293	0.093	0.581*	0.0001
MPO–IgE+	0.293	0.093	0.358*	0.011
Tryptase–IgE	0.451*	0.007	−0.037	0.802
Tryptase–IgE+	0.537*	0.001	0.151	0.664
IgE–IgE+	0.771*	0.0001	0.664*	0.0001
IgE–eosinophil	0.557*	0.001	0.447*	0.0001
IgE–lymphocyte	0.448*	0.008	0.644*	0.0001
IgE–plasma	0.447*	0.008	0.604*	0.001

Correlations between inflammatory cells count—eosinophils, lymphocytes and plasma cells (not shown in the table) are highly significant for both groups

*Significant correlation at significant level *P* < 0.05

eosinophils and IgE+ cells count in the asthma group only. Interestingly, MPO correlated significantly with IgE only in the CRS group. However, IgE correlated significantly with MPO in the asthma group in the patients where the bacterial growth was shown (Fig. 1). The bacterial growth had no impact on the markers and IgE in the CRS group. In the asthma group, only the MPO was significantly higher in the samples where bacteria were detected (Table 3). The IgE in the sinus fluid and the IgE+ cells count significantly correlated, and both significantly correlated with the count of all inflammatory cells observed. Although sensitization to aeroallergens was more common in the asthmatics (70% vs.

**Fig. 1** Scatter plot showing relationship between IgE and MPO in non-asthmatic, and infected and non-infected samples in asthmatic patients with CRS, respectively. Two patients (one in each group) with the extreme IgE and MPO values were excluded from the figure. This correlation was significant in CRS (*P* = 0.011), and in the subgroup of infected samples of the asthmatics (*P* = 0.006), but not in whole asthma group (*P* = 0.093). MPO/IgE correlation in the sterile samples of the asthmatic patients was borderline significant (*P* = 0.057)

53%, respectively), it may not explain higher local IgE and ECP concentrations in the asthmatics, as no difference was observed in the cellular markers and IgE concentrations in the lavages of sensitized and non-sensitized patients (Table 3).

Discussion

A number of recent studies indicated distinct patterns of inflammation and Th1/Th2 polarization in patients with NP and CRS without NP [2, 3]. Significantly higher local

Table 3 The cellular markers and IgE in the lavage in the subgroups of infected and sterile samples and sensitized and non-sensitized patients

Subgroup	ECP (mcg/l) median (range)	MPO (mcg/l) median (range)	Tryptase (mcg/l) median (range)	IgE (kU/l) median (range)
Asthma infected	38.4 (2–273)	460.1 (2.7–1,879)*	5.48 (1–62.9)	13.85 (2.1–420.7)
Asthma sterile	32.7 (3.5–254.1)	134.3 (6.8–812.4)	8.55 (1–166)	35.95 (7.8–95.4)
Asthma SPT+	21.5 (2–254.1)	129.2 (6.8–1,200)	4.03 (1–166)	14.5 (2.1–420.7)
Asthma SPT-	42.2 (3.46–273)	392.3 (2.7–1,879)	7.39 (1–62.9)	24.05 (6.8–180)
CRS infected	2.8 (2–231)	36.4 (1.1–3,800)	3.28 (1–68.9)	2.1 (0.9–66.9)
CRS sterile	3.7 (2–207.2)	41.7 (4.4–931)	3.34 (1–112)	3.3 (0.6–52.5)
CRS SPT+	3.3 (2–231)	36.4 (7.7–1,308)	3.9 (1–23.4)	1.3 (0.8–66.9)
CRS SPT-	2.4 (2–214)	24.5 (8–3,800)	4.48 (1–112)	1.4 (0.6–52.5)

SPT+ sensitized to common aeroallergen, SPT– non-sensitized to common aeroallergen

* $P < 0.05$ compared within subgroup (infected vs. non-infected or sensitized vs. non-sensitized)

expression of interleukin-5 (IL-5) ECP, IgE and eosinophils, was found in nasal polyp tissue, as compared to CRS mucosa and CF polyps [2]. Even the early stage nasal polyps have shown significantly higher tissue eosinophilia and inflammatory cells infiltration, but no difference was found between number of IgE+ cells and mastocytes when compared to CRS without NP [10]. Cellular activation markers in the sinus fluid and histomorphometry of the maxillary sinus mucosa in our study indicated that CRS in the asthmatics have a distinct, Th-2-like, pattern of inflammation. The differences in the number of eosinophils, lymphocytes, plasma and IgE+ cells in sinus mucosal biopsies, and the IgE and ECP concentrations in the sinus fluid between the asthmatics and non-asthmatics were highly significant. Although these results may be interpreted as a higher degree of inflammation in the asthmatics, significant correlations between the concentrations of the tryptase and IgE, and tryptase and ECP, respectively, in the sinus fluid, which were not significant in the CRS samples, may suggest a different type of inflammatory process, at least in terms of mast cells activation. Although proportion of patients sensitized to common aeroallergens was higher in the asthmatics, which is expected to influence the results, no difference between sensitized and non-sensitized patients was observed in either of the groups (Table 3). The highly significant correlation between ECP and MPO, which was found in the sinus lavages of both groups, may be explained by the upregulation of chemokines, IL-8 or granulocyte macrophage colony stimulating factor (GM-CSF). Similarly, IL-8 significantly correlated with ECP and MPO in bronchoalveolar lavages of the steroid-untreated asthmatics [11]. The activation of neutrophils and MPO release may be IgE dependent, as they possess high- and low-affinity IgE receptors. Furthermore, IgE-dependent activation and IL-8 release from neutrophils was demonstrated in vitro [12]. Interestingly, there was a significant correlation between IgE and MPO only in the samples of

the CRS group. This correlation was significant in the subgroup of infected samples of the asthmatics ($P = 0.006$), but not in the whole asthma group ($P = 0.093$). On the other hand, MPO/IgE correlation in the sterile samples was borderline significant ($P = 0.057$). The difference in the IgE/MPO ratio between infected and non-infected samples, with tendency toward higher MPO and lower IgE values in the infected samples, might be the effect of the suppressive effect of Th-1 cytokines and presence of bacterial oligodeoxynucleotides [13], in the samples with bacterial growth. The role of bacterial antigens and superantigens in local IgE production was suggested in the literature [14]. It was demonstrated that most patients with NP and/or chronic sinusitis possess bacteria-specific IgE in their serum, whereas subjects with only allergic rhinitis do not [15]. Specific IgEs to *S. aureus* exotoxins were detected in nasal polyp tissue, and local IgE production was suggested, as the IgE/albumin ratios in the polyp tissue and serum were dissociated [16]. However, specific IgE antibodies to aeroallergens were also detected in the polyp samples, more often, not only in the atopic patients but also in SPT negative patients. Some earlier studies have suggested Th-2-like polarized inflammation in the sinus mucosa and fluid of the atopics with CRS [17–19]. However, the role of sensitization to aeroallergens and allergic rhinitis in the pathogenesis of CRS is not clear. It is unlikely that inhaled antigen-IgE antibody reaction occurs in the sinus mucosa, as aeroallergens cannot penetrate sinus cavities by inhalation. However, bilateral sinus mucosal thickening was demonstrated on CT scans following specific unilateral provocation in two-thirds of the patients with allergy to pollen [20]. Mechanisms of sinus mucosal changes in allergic rhinitis may be explained by congestion of nasal turbinates and ostiomeatal blockage induced by allergic response. Changes on the unprovoked side may be explained by reflex mechanisms (neurogenic inflammation) or by activation of the bone marrow cells after provocation. The IgE

levels in the sinus fluid were related neither to sensitization to aeroallergens nor to bacterial growth in our study. However, when individual data were analyzed in the allergic asthmatics, IgE levels in sinus fluid were higher in the patients with pollen allergy when sinus samples were collected soon after the end of the season, but the number of such patients was too low to show significant difference.

The IgE concentration in the sinus lavages significantly correlated with the number of IgE+ cells and with the inflammatory cells count in both groups. As the severity of inflammation assessed by histomorphometry was significantly correlated with the IgE concentrations in sinus fluid in the asthmatic and non-asthmatic patients with CRS, the IgE in the sinus fluid may serve as a good marker of the objective stage of inflammation in the respective sinus.

However, swabs taken from the sinuses may not necessarily reflect potential bacterial growth, as bacteria may escape detection in routine processing [21]. However, it is important to emphasize the possible impact of the selection criteria on the results in this study. The patients, who have been treated with long-term topical steroids and macrolides, and have not responded to such treatment, at least in terms of adequate subjective symptoms reduction, may have different immune responses than in previously untreated patients.

In summarizing the results of this study, it may be concluded that the asthmatic patients present a distinct subgroup among the patients with CRS. The concentrations of the granulocyte markers and IgE in the sinus fluid are significantly higher from those of non-asthmatic patients with CRS. The most prominent feature of the inflammation in the sinus mucosa of the asthmatics, as compared to non-asthmatics, which had a significantly more intense infiltration and activation of eosinophils and, according to the correlation between IgE and tryptase in the sinus fluid, mast cell activation seemed to be more likely IgE-dependent in asthmatic than in non-asthmatic patients with CRS, respectively. The results indicate that bacterial growth in CRS modifies local immune response in the asthmatics, whereas such a response was not confirmed for non-asthmatic patients with CRS.

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