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Cysteinyl leukotriene 1 receptor expression in nasal polyps

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ABSTRACT: Cysteinyl leukotrienes (CysLTs) have been implicated in the pathogenesis of chronic rhinosinusitis (CRS). This study was undertaken to better understand the role of CysLTs in the development of CRS through 1). assessment of the pattern of expression of CysLT1 receptor in nasal polyps from patients with CRS and 2). correlation of expression of CysLT1 receptor with clinical features. Expression of CysLT1 receptor was evaluated immunohistochemically in nasal polyps from 20 patients with CRS and nasal mucosa from 10 control subject undergoing plastic surgery of the nose. Patients with CRS showed significantly higher expression of CysLT1 receptor as compared with the control group ($p<0.05$). The expression of CysLT1 receptor in sub-epithelial inflammatory infiltrates tended to be higher in patients with CRS and allergy as compared with patients with CRS but without allergy ($p=0.07$). In particular, the expression of CysLT1 receptor in sub-epithelial inflammatory infiltrates was significantly greater in 3 patients with CRS and drug allergy as compared with patients with CRS but without drug allergy ($p=0.03$). Increased expression of CysLT1 receptor in inflamed mucosa of nasal polyps in patients with allergy might suggest particular role of CysLTs in the pathogenesis of nasal polyps in this group of patients.

KEYWORDS: nasal polyps, rhinosinusitis, leukotrienes

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease characterized by the accumulation of eosinophils, fibroblasts, mast cells and globlet cells in the affected mucosa of the upper airways. A common feature of this disease is the presence of nasal polyposis (NP) [1]. With a prevalence of up to 10% of the adults, CRS is considered as one of the most common laryngological diseases in the world [2]. Untreated CRS leads to nasal obstruction, discharge, and facial pain which in turn cause significant impairment of the quality of life, diminished productivity and economic burden.

Management of CRS with nasal polyps includes endoscopic surgery and usage of anti-inflammatory therapies. However, the results of these treatments are often considered insufficient or transient only and the disease often relapse [3]. Searching of new therapies which could improve effectiveness of treating CRS is therefore of great interest.

The etiology of CRS is unclear and is considered to include different factors including systemic diseases [4]. Some forms of the disease are driven by allergy, often in association with asthma. Leukotrienes (LTs) are inflammatory mediators synthesized from arachidonic acid by a number of cell types including mast cells, eosinophils, basophils, macrophages and monocytes. The family of LTs consist of leukotriene B (LTB4) and a group of cysteinyl leukotrienes (cysLTs) including leukotriene C4 (LTC4), D4 (LTD4) and E4 (LTE4). CysLTs, also known as slow-reacting substance of anaphylaxis or SRS-A, has been implicated in the pathogenesis od asthma and asthma-related conditions, including allergic rhinitis [5].

CysLTs exert their biological effects by binding and activating specific receptors [6]. In consistency with the potential role of CysLTs in the pathogenesis of allergy-driven disease, selective antagonists of the cysteinyl leukotriene 1 (CysLT1) receptor have been introduced for treatment of asthma and allergic rhinitis [7, 8, 9, 10, 11].

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Although published studies indicate that CysLT1 receptor antagonists improve clinical status in certain groups of patients with CRS, their results appear somehow inconsistent with regard to the target population of patients who could most benefit from this kind of therapy. Considering pathogenic and clinical heterogeneity of CRS we undertook this study to better identify the group of patients who can particularly benefit form treatment with selective antagonists of CysLT1 receptor.

To address this question expression of CysLT1 was assessed by immunohistochemistry in nasal polyps from patients with CRS and correlated with clinical features with particular attention to the presence of the different forms of allergy.

PATIENTS AND METHODS

Expression of CysLT1 was evaluated in nasal polyps removed by means of endoscopy from patients with CRS and in unchanged mucosa taken during plastic surgery of the nose.

Twenty consecutive patients who underwent endoscopic surgery due to CRS with nasal polyps in the Department of Otolaryngology at the Medical University of Bialystok were included. There were 6 females and 14 males, aged 25 to 77 years (mean (+/- SD) age: 51 +/- 16 years). Four patients had history of bronchial asthma, another 4 were diagnosed with pollen allergy, 3 with mites allergy, and another 3 with drug allergy (all three patients were hypersensitive to antibiotics, none had aspirin hypersensitivity). In total, 7 patients had pollen, mite and/or drug allergy. Six patients had underwent polypectomy before.

All but one patients complained about difficulty with breathing through nose and/or nasal obstruction, 10 patients had headaches, and 4 had smell disturbances.

The following pattern of sinus involvement were noted: right and/or left maxillary sinus were involved in 13 patients, sphenoidal sinus in 10, ethmoid sinus (anterior and/or posterior) in 20, and right and/or left frontal sinus in 6. In 3 patients endoscopy of the nose was proceeded by septoplasty.

Control group consisted of 10 patients undergoing plastic surgery of the nose. The mean age of patients from control group (mean +/- SD: 39 +/- 14 years) was slightly lower that the mean age of the patients with CRS but the difference was not significant ($p>0.05$). None of the patients in the control group had CRS, bronchial asthma, pollen, mites or drug allergy. Clinical characteristics of the patients with CRS and the control group is given in table I.

The project of this study was approved by the local ethics committee and written informed consent was obtained from all patients included in the study.

IMMUNOHISTOCHEMISTRY AND HISTOLOGICAL ASSESSMENT

We retrospectively waived the 10% buffered formalin fixed, paraffin embedded sections. Evaluation of CysLT1 receptor expression was done using immunohistochemical method. Following the deparaffinision and rehydration, epitope retrieval was carried out in the EnVision Flex Target Retrieval Solution (DAKO) in low pH. Endogenous peroxidases were blocked by incubating the sections in methanol and 3% hydrogen peroxidase for 40 minutes. Next slides were incubated with rabbit polyclonal antibody against CysLT1 receptor (CysLT1 Receptor Antibody (H-60):sc-25448) in 1:100 dilution 1 hour in room temperature. Visualization reagent EnVision (DAKO) was applied for 30 minutes followed by DAB solution for 10 minutes. The slides were than counterstained with hematoxylin and examined under the light microscope. The intensity of immunostaining was evaluated in random 10 fields under 20xmagnification. The results were expressed as semi-quantitative score calculated based on the percentage of cells with a strong positive staining as follows: <or = 10% positive cells - negative (0), between 11–50% (1), and > or = 51% positive cells (2). Appropriate positive and negative controls were performed.

STATISTICS

The between-group comparisons were performed using the U Mann Whitney teat or Fisher test, as appropriate. The Spearman's rank test was used to evaluate correlations between continues values. $P<0.05$ was considered as significant. Values are given as mean and standard deviations (SD) unless stated otherwise.

RESULTS

In the samples taken from patients with CRS expression of CysLT1 receptor was seen in the epithelial cells (mean score: 1.50 +/- 0.51), sub-epithelial inflammatory infiltrates (mean score: 1.20 +/- 0.41), and in leukocytes within small blood vessels (Fig. 1, 2). In contrast, in the samples taken from the control group expression of CysLT1 was found only in single cells within sub-epithelial layer and in leukocytes seen within small blood vessels (mean score: 0.40 +/- 0.52, $p<0.05$ versus expression of CysLT1 receptor in

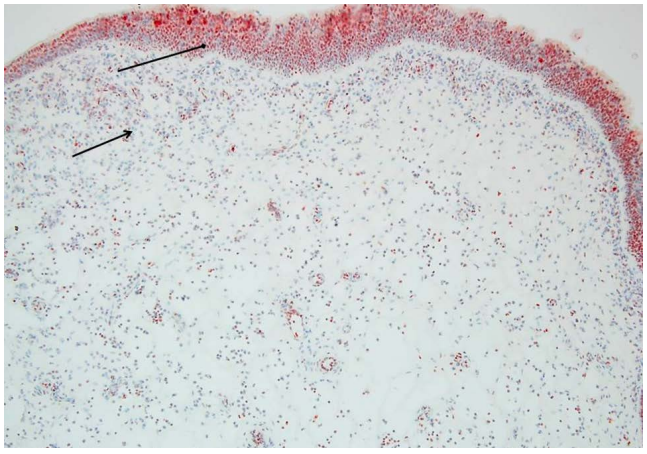

Fig. 1. Expression of CysLT1 receptor in the covering epithelium of the nasal inflammatory polyp. The arrows show the significant expression within the epithelium and underlying lymphocyte infiltration. Magn. 40X.

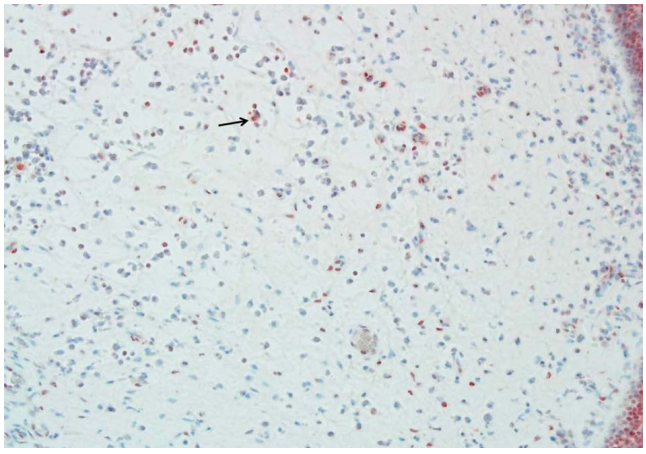

Fig. 2. Expression of CysLT1 receptor within the inflammatory cells in the stroma. Magn. 200X.

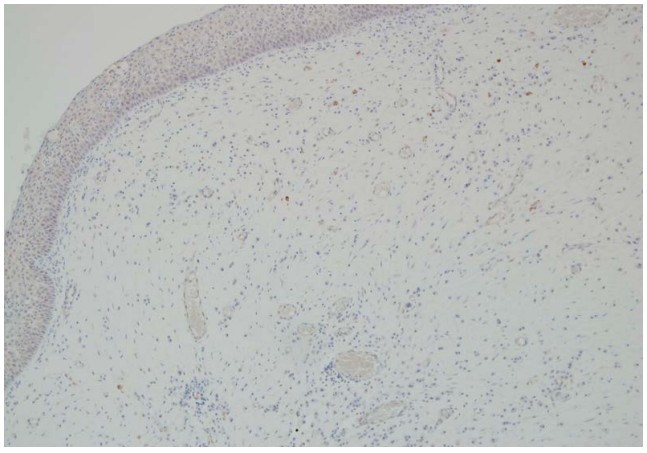

Fig. 3. Expression of CysLT1 receptor within the mucosa from controls. Magn. 40X.

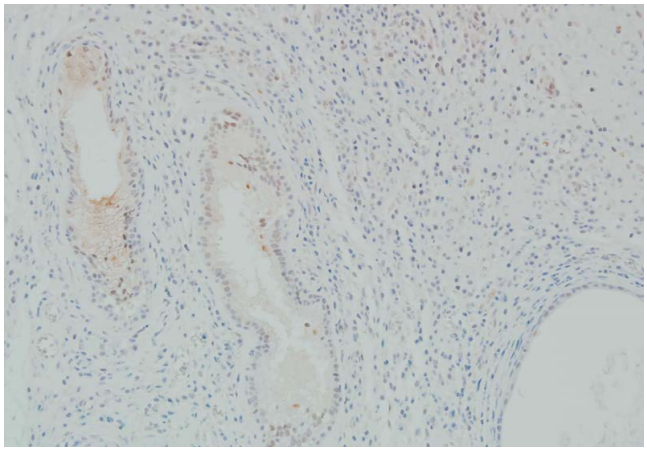

Fig. 4. Expression of CysLT1 receptor within the mucosa from controls. Magn. 200X: Expression of CysLT1 receptor is observed only in few inflammatory cells in the loose mucosal stroma and within the leukocytes of the small blood vessels.

sub-epithelial tissue from nasal polyps) (Fig. 3, 4). Accordingly, the total score of expression of CysLT1 receptor, calculated as a sum of scores for epithelial and sub-epithelial expression) was significantly higher in patients with CRS (2.7 +/- 0.80) as compared with the control group (0.40 +/- 0.52, $p<0.05$). There was significant, although rather weak, correlation between expression of CysLT1 receptor in epithelial cells and expression of CysLT1 receptor in sub-epithelial inflammatory infiltrates in patients with CRS ($R=0,5$, $p<0.5$) (Fig. 1, 2).

The mean expression of CysLT1 receptor in sub-epithelial inflammatory infiltrates tended to be higher in patients with CRS and any allergy (mean score: 1.43 +/- 0.54) as compared with patients with CRS but without allergy (mean score: 1.08 +/- 0.28, $p=0,07$). In particular, the expression of CysLT1 receptor in sub--epithelial inflammatory infiltrates was significantly greater in 3 patients with CRS and drug allergy (mean score: 1,67 +/- 0,58) as compared with patients with CRS but without drug allergy (mean score: 1.12 +/- 0.33, $p=0,03$). The expression of CysLT1 receptor was not significantly different between patients with and those without bronchial asthma.

No relationships could be found between the expression of CysLT1 receptor and sex, age, clinical complains, location of nasal polyps or previous polypectomy, either.

DISCUSSION

We showed that expression of CysLT1 receptor is increased in nasal polyps from patients with CRS as compared with control

Tab. I. Clinical characteristics of the patients with CRS and the control group.

PARAMETER	PATIENTS WITH CRS (N=20)	CONTROL GROUP (N=10)	P VALUE
Sex (M/F)	14/6	4/6	NS
Age in years*	51 +/- 16	39 +/- 14	NS
Clinical features**			
Bronchial asthma	4	0	
Pollen allergy	4	0	
Mites allergy	3	0	
Drug allergy	3	0	
Any kind of allergy	7	0	
Patients with previous polypectomy	6	0	
Difficulty with breathing through nose	19	-	
Nasal obstruction,	19	-	
Headaches	10	-	
Smell disturbances	4	-	
Location of polyps			
Maxillary sinus (right and/or left)	13	-	
Sphenoidal sinus	10	-	
Ethmoid sinus (anterior and/or posterior)	20	-	
Frontal sinus (right and/or left)	6	-	

*mean and standard deviation, **number of patients with CRS and specific clinical feature, NS=not significant.

mucosa. Our results are in agreement with previous observations of other groups [12, 13, 14]. We found a trend toward higher expression of CysLT1 receptor in nasal polyps from patients with different types of allergy as compared with patients with CRS but without allergy. The most pronounced difference in expression of CysLT1 receptor was found in 3 patients with drug allergy compared with patients without drug hypersensitivity.

Interestingly, several groups have shown that expression of CysLT1 receptor in nasal polyps is higher in patients with aspirin hypersensitivity than in nasal polyps from aspirin-tolerated patients with CRS [16, 17, 18]. Authors conclude that the elevated number of nasal polyp cells expressing CysLT receptors and lack of cells expressing EP(2) receptor and COX-2 may be related to a more severe course of hyperplastic rhinosinusitis in aspirin hypersensitivity. There were however no patients with aspirin hypersensitivity among patients with CRS included in our study. Our observation linking increased expression of CysLT1 receptor in nasal polyps from patients with allergy is also supported by observation of others who have shown that allergen challenge increases expression of CysLT1 [19].

We have not found significant associations between expression of CysLT1 receptor and bronchial asthma or other than drug specific types of allergy, which could be due to relatively low number of patients with these conditions.

This observation of ours adds to the existing evidence regarding expression of CysLT1 receptor in patients with CRS and indicates that CysLTs, acting through the CysLT1 receptor, can contribute to the pathogenesis of nasal polyps and CRS, in particular in patients with allergy. In line with this our findings provide rationale for usage of CysLT1 blockers for treating nasal polyps in patients with different forms of allergy.

CONCLUSIONS

Increased expression of CysLT1 receptor in inflamed mucosa of nasal polyps in patients with allergy might suggest particular role of CysLTs in the pathogenesis of nasal polyps in this group of patients.

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