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SHORT COMMUNICATION

Increased gene expression of inflammatory markers in nasal turbinate of patients with persistent allergic rhinitis and chronic obstruction

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Abstract

Purpose The pathogenesis of persistent allergic rhinitis with chronic and refractory nasal obstruction is still unknown. Inflammation and tissue remodeling are known to play a role, but this has not been studied thoroughly. The purpose of this study is to identify the profile of gene expression of inflammatory and remodeling markers in nasal mucosa of patients with PAR and chronic obstruction.

Methods After informed consent, we obtained nasal mucosa tissue from five aeroallergen-sensitized PAR patients undergoing anterior turbinectomy, and control non-sensitized individuals undergoing cerebrospinal fluid fistula repair or rhinoplasty. We assessed the expression of 34 genes related to inflammation and tissue remodeling using the real-time polymerase chain reaction (qPCR) to quantify each mRNA.

Results IL-4 mRNA was upregulated in nasal mucosa of all five patients; CCR3, CCR8 and Eotaxin-2 were upregulated in four out of five patient samples; while IL-5 and IL-13 were upregulated in two of them. TGF-β1 was not upregulated in PAR samples. mRNA from metalloproteinases MMP-7, MMP13 and MMP15 were upregulated in three out of five samples. Our results indicate a typical mRNA expression profile of the infiltrating inflammatory Th2 cells and eosinophils, combined with altered gene expression of remodeling-related proteins in stromal cells from the mucosa.

Conclusion Prolonged allergen challenge can lead to persistent upregulation of genes for inflammatory mediators such as IL-4 Th2/eosinophil cytokines, chemokines and receptors, which may play an important role in maintaining PAR with chronic nasal obstruction. Our findings may have therapeutic implications, including the use of anti-IL4, -CCR3 or -MMP therapy to ameliorate the condition.

Keywords Allergic rhinitis · Gene expression · Nasal mucosa · Cytokines · IL-4 · Remodeling

Persistent allergic rhinitis (PAR) with chronic and refractory nasal obstruction is a clinically significant problem, but its causes remain unknown. The pathophysiology of PAR has been studied in recent years, and remodeling is not well understood. Remodeling occurs in all inflammatory responses although the mechanism and severity vary depending on the disease [1]. There are very few studies that investigate upper airway structural remodeling in the nasal mucosa [2, 3] and the results are conflicting. Inflammatory mediators and infiltration of inflammatory cells in nasal tissue may be responsible for increased collagen deposition and matrix metalloproteinase (MMP) expression in the interstitial matrix resulting in fibrosis and tissue remodeling [2] but this hypothesis has not been proven yet. The study of gene expression profile for inflammatory mediators in the hypertrophied nasal turbinate can be the key to better understand pathophysiology of chronic allergic rhinitis and to identify new treatment targets. The purpose of this

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Table 1 Relative expression (fold increase values over control samples) of the 34 genes by qPCR in five patients with perennial AR

	P2	P4	P12	P9	P15
Inflammation/infiltrate					
CCR7	0.4	**6.0**	**16.3**	0.5	**2.1**
CCL19/ECL	0.2	1.0	**6.5**	0.3	0.8
CCL21/SLC	1.0	1.7	**12.9**	0.5	1.1
CCR5	1.0	**5.1**	**11.3**	1.3	**2.7**
CCL5/RANTES	0.9	**2.5**	**3.5**	0.7	0.9
IL-15	1.3	1.3	**2.5**	0.4	**2.0**
IL2	0.6	**3.5**	**2.3**	1.1	1.6
IL-1β	0.2	0.3	**4.4**	0.1	1.5
IL-8	0.2	1.9	0.6	0.1	0.1
Th2 Profile					
CCR8	1.2	**5.1**	**20.4**	**3.2**	**4.4**
CCL1/I-309	0.5	0.4	1.9	indetectable	0.5
CCR4	0.4	**13.9**	**13.8**	0.8	1.4
CCL17/TARC	**3.0**	1.7	0.8	1.3	0.5
CCL22/MDC	0.3	0.9	**6.7**	0.1	0.1
IL-4	**8.6**	**3.1**	**31.3**	**15.3**	**14.9**
IL-4 Rα	1.9	**6.7**	**4.5**	0.2	0.4
IL-5	indetectable	**0.54**	**24.32**	0.736	**5.73**
IL-13	0.6	0.2	**3.0**	0.2	**15.0**
IL-13 Rα2	1.1	1.1	**4.2**	0.4	1.4
Eotaxin	1.3	0.2	**2.2**	0.1	0.1
Eotaxin-2	**2.1**	**2.5**	**14.5**	0.9	**4.1**
CCR-3	**3.2**	**2.7**	**5.1**	0.4	**27.0**
Th1 profile					
IFNγ	0.5	0.8	**9.2**	0.9	**3.6**
CXCR3	**5.03**	**2.87**	**3.36**	**3.46**	1.09
CXCL9/MIG	1.24	1.24	1.74	**13.75**	1.39
CXCL10/IP10	0.89	0.07	0.49	**9.12**	0.31
IL-18	**4.3**	**4.7**	0.6	0.7	0.2
Anti-inflammatory					
IL10	0.0	0.0	0.0	0.0	0.0
TGF-β1	0.1	0.6	1.7	0.2	0.7
FoxP3	**20.32**	**40.49**	0.76	**13.33**	1.48
Fibrosis/remodeling					
MMP-7	**5.8**	**3.1**	**2.6**	0.2	0.4
MMP-13	**6.2**	**8.6**	**2.6**	0.4	0.6
MMP-15	**5.8**	3.1	**2.6**	0.2	0.4
MMP-9	0.1	0.6	1.7	0.2	0.7
TGF-b RI	**2.1**	**3.3**	**2.5**	0.3	0.5

Relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Values are normalized in comparison with the average expression of each gene in control samples (here set to 1). Bold values indicate, genes with significantly increased expression (fold increase > 2). Oligonucleotide primer sequences are available upon request.

study is to identify gene expression of multiple inflammatory markers in nasal turbinate of patients with perennial AR and chronic obstruction compared with a control group.

After signed informed consent, participants were divided into two groups. Five patients were in the studied group with PAR, symptomatic chronic nasal obstruction for at least 3 years, absence of nasal polyposis, not using nasal corticosteroids for at least 2 weeks, and positive prick test for at least one aeroallergen who underwent surgery for anterior turbinectomy (age avg 33 years; 40% male). Four non-allergic patients were in the control group with negative prick test, undergoing cerebrospinal fluid fistula repair or rhinoplasty (age avg 46 years; 70% male). After surgical excision of the turbinate, fragments were immediately frozen in liquid nitrogen. To evaluate the gene expression "ex vivo", without nasal challenge, we used the real-time polymerase chain reaction (quantitative PCR; qPCR) using the SYBR green© protocol to quantify mRNA encoding the inflammatory mediators. We analyzed the expression of 34 genes associated with Th1, Th2, regulatory T cell (Treg) responses and remodeling as normalized by the expression of GAPDH, using control samples as calibrators. Oligonucleotide primer sequences are available upon request. We used the ΔΔCt quantification method [4]. Relative expression values (fold expression over controls) greater than two were considered indicative of upregulated expression.

The expression of the 34 genes in each patient sample is summarized in Table 1. Among the genes of the Th2 phenotype, we found that IL-4 was upregulated in nasal mucosa of all five patients; CCR3, CCR8 and Eotaxin-2 were upregulated in four out of five patient samples; IL-5 and IL-13 were upregulated in only two of them. Chemokine receptors CCR5 and CCR7 were upregulated in three out of five patient samples. Paradoxically, upregulation of expression of CXCR3, a marker of Th1 cells, was observed in four out of five patient samples. Metalloproteinases MMP-7, MMP13 and MMP-15 but not MMP-9 were upregulated in three out of five samples as was TGF-β receptor 1. The regulatory cytokines IL-10 and TGF-β1 were not found to be upregulated in chronic rhinitis patient's samples.

Despite the small number of analyzed samples, we observed that there is a significant inter-individual variation in gene expression in the turbinate of patients with PAR and chronic obstruction, with a clear predominance of Th2 profile. IL-4 showed increased expression in nasal mucosa samples of all five patients, and other genes related to Th2/eosinophil phenotype showed increased expression in four patients, such as CCR8, CCR3 and its chemokine ligand Eotaxin-2. This shift to a chronic Th2 pro-inflammatory phenotype was described to cause eosinophil infiltration, epithelial damage, cell hyperplasia and extracellular matrix deposition with the development of airways remodeling in asthmatic epithelium [5, 6] and the overexpression of those genes in nasal mucosa supports the hypothesis that airway

remodeling can also be a feature of chronic AR [5–7]. Accordingly, three out of these samples also presented increased gene expression of MMP such as MMP7, MMP13, MMP15 and TGF-β receptor 1. MMPs include a wide spectrum of zinc-dependent collagenases and elastases, which are expressed in tissues during inflammation. MMPs play a central role in the tissue remodeling underlying several systemic and local inflammatory diseases and MMP-7 and MMP-9 are believed to cleave major collagen components of extracellular matrix and basement membrane [8]. Lung gene expression microarray studies have consistently identified MMP7 to be one of the most upregulated genes in interstitial lung diseases such as idiopathic pulmonary fibrosis [8]. The overexpression of MMP7 and other MMPs support the hypothesis of tissue remodeling in the nasal mucosa of chronic obstructed AR.

We found no upregulation of expression of regulatory cytokines such as IL-10 and TGF-β, and this may be related to the chronicity of inflammatory process [9]. However, the transcriptional factor FoxP3, marker of regulatory T cells or recently activated T cells, was present in three samples, which may suggest a role for CD4 + CD25 + FoxP3 + regulatory T cells in PAR with chronic obstruction [9, 10]. To our knowledge, this is the first study of local gene expression in obstructive PAR and it establishes a typical Th2 expression profile where IL-4 is dominant. The identification of this local expression profile also suggests genes that can be used as peripheral markers to monitor patients. Finally, the set of 34 primers for qPCR used in this study may be a suitable tool for investigation of other diseases of allergic background. One limitation of this study was that we measured only mRNA, but not protein levels. However, given the comparison with inflammation-free control samples, results in nasal PAR mucosa mirror the typical mRNA expression profile of the infiltrating inflammatory Th2 cells and eosinophils, combined with altered gene expression in stromal cells from the mucosa. Protein detection studies will allow definitive confirmation of the expression of immune molecules in PAR nasal mucosa.

Prolonged allergen challenge can lead to upregulation of genes for inflammatory mediators such as IL-4 Th2/eosinophil cytokines, chemokines and receptors, which may play an important role in PAR with chronic nasal obstruction. The elucidation of the pathophysiology of this condition may have important therapeutic implications as it might indicate the applicability of selective inhibitors of IL-4, CCR3, CXCR3 and MMP to treat the disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the University of São Paulo School of Medicine ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Eloy P, Poirrier AL (2011) Actual concepts in rhinosinusitis: a review of clinical presentations, inflammatory pathways, cytokine profiles, remodeling, and management. Curr Allergy Asthma Rep 11(2):146–162
2. Montero Mora P, Blanco E, Matta Campos JJ et al (2003) Nasal remodeling in patient with perennial allergic rhinitis. Rev Alerg Mex 50(3):79–82
3. Lei F, Zhu D, Sun J et al (2010) Effects of minimal persistent inflammation on nasal mucosa of experimental allergic rhinitis. J Rhinol Allergy 24(1):23–28
4. Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3(6):1101–1108
5. Bousquet J, Jeffery PK, Busse WW et al (2000) From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 161(5):1720–1745
6. Francis JN, Lloyd CM, Sabroe I, Durham SR, Till SJ (2007) T lymphocytes expressing CCR6 are increased in allergic rhinitis compared with non-allergic controls and following allergen immunotherapy. Allergy 62(1):59–65
7. Pawankar RU, Okuda M, Hasegawa S et al (1995) Interleukin-13 expression in the nasal mucosa of perennial allergic rhinitis. Am J Respir Crit Care Med 152(1):2059–2067
8. Richards TJ, Park C, Chen Y et al (2012) Allele-specific transactivation of matrix metalloproteinase 7 by FOXA2 and correlation with plasma levels in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 302:L746–L754
9. Bellinghausen I, König B, Böttcher I et al (2005) Regulatory activity of human CD4+ CD25+ T cells depends on allergen concentration, type of allergen and atopy status of the donor. Immunology 116(1):103–111
10. Sanjabi S, Zenewicz LA, Kamanaka M et al (2009) Anti-inflammatory and pro-inflammatory roles of TGF-β, IL-10, and IL-22 in immunity and autoimmunity. Curr Opin Pharmacol 9(4):477–553