Expression of RANTES, eotaxin-2, ICAM-1, LFA-1 and CCR-3 in chronic rhinosinusitis patients with nasal polyposis[1]

Expressão de RANTES, eotaxina-2, ICAM-1, LFA-1 e CCR-3 em pacientes com rinossinusite crônica associada à polipose nasossinusal

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ABSTRACT

PURPOSE: To compare gene expression of the chemokines RANTES and eotaxin-2, its receptor, CCR-3, adhesion molecule ICAM-1 and its receptor LFA-1 in eosinophilic polyps and in control normal nasal mucosa.

METHODS: Gene expression was quantified by Real Time PCR in polyps (n=35) and in healthy nasal mucosa (n=15).

RESULTS: Eosinophilic polyps showed a higher expression of eotaxin-2 and RANTES, but not of CCR-3, ICAM-1 or LFA-1 compared to control nasal mucosa.

CONCLUSION: Eosinophilic polyps present greater expression of eotaxin-2 and RANTES, but not of CCR-3, ICAM-1 or LFA-1 compared to control nasal mucosa.

Key words: Nasal Polyps. Chemokines. Intercellular Adhesion Molecule-1. Sinusitis.

RESUMO

OBJETIVO: Comparar a expressão gênica das quimiocinas RANTES e eotaxina-2, do seu receptor CCR-3, da molécula de adesão ICAM-1 e do seu receptor LFA-1 entre pólipos nasais eosinofílicos (PE) (n=35) e mucosa nasal controle (n=15).

MÉTODOS: Quantificou-se a expressão gênica dos mediadores citados pela técnica de PCR em tempo real em PEs e em mucosas de concha média de pacientes sem doenças nasais ou alteração endoscópica.

RESULTADOS: Pólipos eosinofílicos apresentam maior expressão de eotaxina-2 e RANTES, mas não de CCR-3, ICAM-1 e LFA-1, quando comparados as mucosas nasais controles.

CONCLUSÃO: Pólipos eosinofícios apresentaram maior expressão de eotaxin-2 and RANTES, mas não de CCR-3, ICAM-1 ou LFA-1,comparada à mucosa nasal controle.

Descritores: Pólipos Nasais. Quimiocinas. Molécula 1 de Adesão Intercelular. Sinusite

Introduction

Nasosinusal polyposis (NSP) is a disease with a variety of clinical manifestations which is frequently associated with other diseases such as asthma, cystic fibrosis, primary ciliary dyskinesia, and hypersensitivity to aspirin. It is considered a chronic inflammatory disease associated with rhinosinusitis (CRS with NSP) characterized by intense eosinophilia and tissue remodeling consisting of epithelial proliferation, hyperplasia of secretory cells, pseudocyst formation, thinning of basement membranes, localized fibrosis and edema[1,2].

Several hypothesis have been raised to elucidate the disease's mechanisms of action, but its etiopathogeny is not yet completely understood.

Some factors may influence the severity of the clinical symptoms, one of the main factors being related to predisposing genetic components. These may affect the expression of inflammatory cytokines and chemokines, with consequences for the severity of the inflammatory process[2,3]. This "genetic predisposition" may be added to external factors such as allergy, bacterial infection (especially in the presence of the formation of biofilms and/or superantigens) or even fungal infection to form the phenogenotype of CRS with NSP[4-7].

Some proinflammatory factors have been proven to be involved in the process of formation of CRS with NSP, such as interleukins 4, 5, 6 and 8, transforming growth factor (TGF), some chemokines (eotaxins and RANTES), metalloproteinases (MMPs), and adhesion molecules (VCAM-1)[1-3].

The chemokines RANTES (Regulated on Activation Normal T Expressed and Secreted) and eotaxin-2 are important recruiters and activators of eosinophils and have been postulated to be responsible for eosinophilia in the tissue stroma of the polyp[8-18]. About this matter, the interaction of chemokines with their receptors in CRS with NSP is important, with CCR-5 and CCR-3 being the receptors more extensively studied[19]. Both are present in the normal nasal mucosa and both have been widely studied in allergy, where obvious tissue eosinophilia has also been observed. They are expressed both in basophils and in eosinophils. However, the quantification of their expression and consequent correlation with chemokine expression have not been properly studied in CRS with NSP.

Another class of inflammatory mediators whose role in CRS with NSP needs to be better studied is the adhesion molecules (such as VCAM-1 and ICAM-1) and their receptors (such as LFA-1). The adhesion molecule ICAM-1 plays an important role in the intercellular inflammatory cascade and is responsible for the migration of inflammatory cells to the affected tissue. Its role in CRS with NSP has not been clarified despite its high levels of expression[20].

Thus, the objective of the present study was to investigate the expressions of the chemokines RANTES and eotaxin-2, of their receptor CCR-3, of the adhesion molecule ICAM-1 and its receptor LFA-1 in CRS with NSP and compare them to the expression of control nasal mucosa.

Methods

Thirty-five patients with CRS and NSP were selected and submitted to endoscopic surgery at the Rhinology unit of a university hospital after failure to obtain beneficial effects with an optimized clinical treatment. The diagnosis was confirmed in all patients by nasofibroscopy and computed tomography according to the Brazilian directives for rhinosinusitis[21]. Nasal polyp samples were obtained during the surgical procedure and immediately stored in Trizol® at -70ºC. Samples of the meatal surface of the middle turbinate were obtained from 15 patients submitted to aesthetic rhinoplasty who had no nasal complaints, no history of nasal diseases, or abnormal findings in the physical examination of the nasal cavity.

The study was approved by the Ethics Committee of the hospital (protocol no. 8897/09) and all patients gave written informed consent to participate. Patients younger than 13 years, with unilateral nasal disease, cystic fibrosis, inverted papilloma or ciliary dyskinesia were excluded from the study.

mRNA was extracted from all samples by the Trizol® technique (Invitrogen) according to manufacturer specifications. The extracted mRNA was quantified with a ThermoScientific® spectrophotometer model Nanodrop 1000. After confirmation of its integrity, cDNA was determined in each sample using the Improm-II Promega kit according to manufacturer instructions.

For real-time PCR, the cDNAs of the samples were added to the primers obtained from Invitrogen of the genes RANTES, eotaxin-2, CCR-3, ICAM-1 and LFA-1, in addition to the housekeeping gene beta-actin. The reaction was performed using the SYBR Green® marker in the ABI 7000 Biosystems® Detection System (SDS ABI 7000),and quantification was carried out by delta-delta CT analysis.

Data were analyzed statistically and graphs were constructed using the GraphPad-Prism 5.0 software. All reactions were analyzed and compared by the nonparametric Mann-Whitney test, with the level of significance set at $p < 0.05$.

Results

Comparison of RANTES expression between nasal polyps and control nasal mucosa samples revealed a significantly higher expression of the genes RANTES (4.07±1.18 for polyps vs. 0.75±0.69 for control mucosa, $p<0.05$) and eotaxin-2 (17.97±8.13 for polyps vs. 2.03±1.32 for control mucosa, $p<0.05$) (Figures 1 and 2).

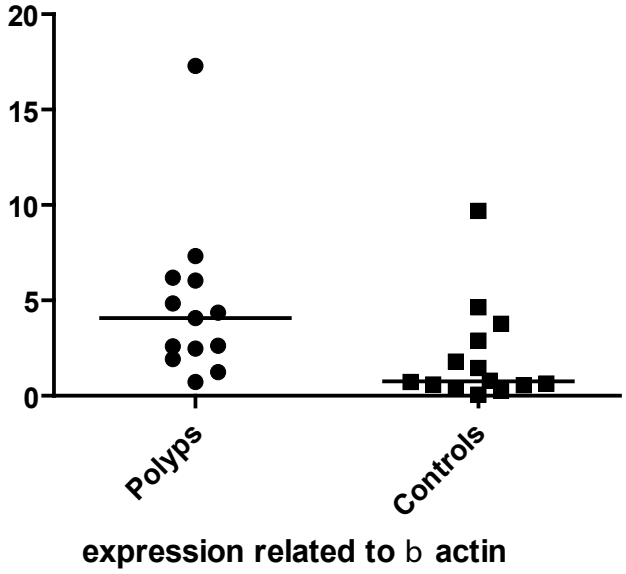

FIGURE 1 - Comparison of the expression of RANTES in nasal polyps vs. controls.

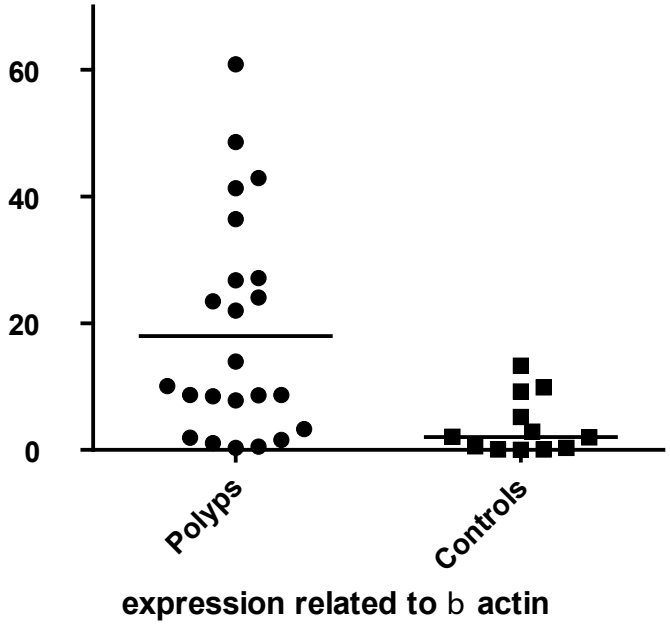

FIGURE 2 - Comparison of the expression of eotaxin-2 in nasal polyps vs. controls.

In contrast, the expression of the other inflammatory mediators studied did not differ significantly between nasal polyps and normal control mucosa (CCR-3: 0.47±18.09 for polyps vs. 1.05±0.57 for control mucosa; ICAM-1: 1.00±0.26 for polyps vs. 0.79±1.21 for control mucosa; LFA-1: 3.43±1.06 for polyps vs. 3.29±0.72 for control mucosa, with a non significant p value for all of them).

Discussion

Cytokines, chemokines, adhesion molecules, metalloproteinases and inducers of cell apoptosis are becoming increasingly important targets in the area of rhinosinusology[1-3,8-24] for a better understanding of the mechanism of onset and maintenance of the inflammatory cascade in CRS with NSP and the consequent determination of therapeutic targets for the inhibition of these inflammatory mediators.

RANTES is an important chemokine for the recruitment and activation of eosinophils in different types of tissues and its relation to CRS with NSP has been studied in different manners. Using semi-quantitative RT-PCR, Lane *et al.*[17]detected higher expression of RANTES in patients with chronic rhinosinusitis (with and without polyposis) compared to patients with a normal mucosa. Marcella *et al.*[16] compared nasosinusal polyps of allergic and non-allergic patients to normal mucosa by semi-quantitative RT-PCR and detected higher expression of RANTES in both polyp groups than in normal nasal mucosa. Using the same methodology, Meyer *et al.*[12] observed higher expression of RANTES in eosinophilic than non-eosinophilic polyps. These studies demonstrated the importance of RANTES in the eosinophilia of nasal polyps regardless of their allergic or non-allergic origin.

Eotaxin-2 is another chemokine related to eosinophilia which has been strongly related to CRS with NSP, and associated with RANTES on many occasions. In a study with a methodology similar to that used in the present investigation, Valera *et al.*[24] evaluated the expression of various inflammatory mediators, including eotaxin-2. Comparing polyp and control nasal mucosa, they noted higher expression of eotaxin-2 in the polyps. Shin *et al*.[13]studied the expression of eotaxin-2 and RANTES in normal tissues, eosinophilic and non-eosinophilic polyps and observed that the eosinophilic polyps expressed more eotaxin than the other two groups. However, these authors did not observe a difference in the expression of RANTES, in contrast to previous literature data and to the data obtained in the present study. Using ELISA, Olze *et al.*[14]observed a higher concentration of proteins of eotaxins-1, 2 and 3 in eosinophilic polyps than in normal nasal concha mucosa.

The CCR-3 receptor is related to both RANTES and eotaxin-2, but with greater specificity for the latter. It is widely related to the type Th2 (eosinophilic) inflammatory response. However, its study in CRS with NSP is not so extensive regarding the chemokines. In one of the rare relevant studies involving CCR-3 and CRS with NSP, Kim *et al*.[19] showed higher CCR-3 expression in eosinophilic than non-eosinophilic polyps using immunohistochemistry. There are no reports thus far of studies comparing the expression of CCR-3 between samples of CRS with NSP and control nasal mucosa.

The adhesion molecule ICAM-1 and its receptor LFA-1 are highly related to the proinflammatory infiltrate although they are not specific for the lymphocyte response mainly of the Th2 type. We did not observe a difference in the expression of these two genes between polyps and controls, but this finding may be explained exactly by the type of lymphocyte response to which these mediators are related. Among studies of adhesion molecules in NSP, Olejniczak *et al.*[25] compared the protein expression of ICAM-1 between allergic and non-allergic patients with NSP by immunohistochemistry and observed higher expression in the group of allergic patients. In a report previously cited in the present paper because it also involved the study of eotaxin-2, Valera *et al*.[24] compared nasal polyp and normal nasal mucosa regarding the expression of ICAM-1 and found no significant difference between groups. Corsi *et al.*[20] evaluated by microarray the expression of adhesion molecules in individuals with CRS with NSP compared to individuals without nasal disease and detected increased expression of selectins and VCAM-1, but not of ICAM-1, in nasal polyps.

The present study demonstrates the close relation between eosinophilic polyps and the expression of chemokines with a pattern of Th2 inflammation, confirming the effect of the inflammatory process in this direction, with a clear and continuous induction of eosinophilia in tissue. The possible determination of medications that would inhibit the expression of these chemokines may be of help in reducing the inflammatory process present in CRS with NSP.

Conclusion

Eosinophilic polyps present greater expression of eotaxin-2 and RANTES, but not of CCR-3, ICAM-1 or LFA-1 compared to control nasal mucosa.

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