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Increased eotaxin-mRNA expression in non-atopic and atopic nasal polyps: Comparison to RANTES and MCP-3 expression*

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SUMMARY

Eosinophilic tissue infiltration of nasal mucosa typical for allergic rhinitis and chronic polypous sinusitis may be due to chemotactic activity of chemokines specific for eosinophils. The CC-chemokines eotaxin, RANTES and MCP-3 have been postulated to be involved in the recruitment of eosinophils to certain inflamed tissues. To explore their possible role in chronic polypous sinusitis we examined eotaxin-, RANTES- and MCP-3-gene expression in human nasal polyps and normal human nasal mucosa of patients undergoing endonasal surgery for treatment of chronic polypous sinusitis. Using gene-specific primers in semi-quantitative reverse-transcriptase polymerase-chain-reaction experiments we found elevated expression of eotaxin- and RANTES-mRNA but no MCP-3-mRNA in non-atopic and atopic nasal polyps when compared to normal nasal mucosa.

Key words: eotaxin, chemokines, chronic polypous sinusitis, chemotaxins, allergic rhinitis

INTRODUCTION

By unknown reasons activated eosinophils are present in nasal mucosa of patients suffering from allergic rhinitis and chronic polypous sinusitis (Horn et al., 1975; Jankowski et al., 1989; Gleich, 1990; Terada et al., 1992; Moneret Vautrin et al., 1992; Ford et al., 1992). Their number in nasal polyps has been reported to be strongly elevated when compared to non-affected nasal tissue (Stoop et al., 1993), indicating an important role for eosinophils in the pathogenesis of nasal polyposis. Eosinophils are known to cause tissue damage through the release of reactive oxygen species and granular highly-basic constituents (Meurer et al., 1993; Kapp, 1993; Baggiolini and Dahinden, 1994; Clark Lewis et al., 1995). The mechanisms determining selective eosinophilic tissue infiltration into diseased nasal mucosa as yet are speculative. Pan-leukotactic factors known to be present in nasal polyps, such as PAF or complement C5a-derived factors, cannot explain the type-selective tissue infiltration in eosinophilic- or neutrophilic-featured diseases (McLean, 1994). Chemokines are known to have leukocyte subtype-selective chemotactic properties *in vitro* and thus are candidates explaining leukocyte-characteristic tissue infiltration. In particular the ß-chemokines RANTES and MCP-3 and, to a lesser degree, macrophage inflammatory protein (MIP)-1a and monocyte chemoattractant protein (MCP)-2 have been reported to attract among monocytes also eosinophils *in vitro* (Ebisawa et al., 1994; Dahinden et al., 1994; Weber et al., 1995). Recently, another ß-chemokine, eotaxin, has been cloned. Recombinant eotaxin was identified to induce chemotaxis of eosinophils but not of neutrophils, lymphocytes or monocytes (Burke-Gaffney and Hellewell, 1996; Bartels et al., 1996; Ponath et al., 1996; Garcia-Zepeda et al., 1996), indicating a selectivity for eosinophils. Immunohistochemistry on human nasal polyps with anti-eotaxin monoclonal antibodies showed that certain leukocytes as well as respiratory epithelium were intensely immunoreactive, and eosinophil infiltration occurred at sites of eotaxin up-regulation (Ponath et al., 1996). Immunostaining of nasal polyp tissue with anti-RANTES was largely localized to airway and glandular epithelium (Beck et al., 1996). In order to further explore the molecular mechanisms playing a role in chronic polypous sinusitis, we assessed relative eotaxin-, RANTES- and MCP-3-mRNA expression in human nasal polyps.

PATIENTS AND METHODS

Patients

The aim of this study was to investigate eotaxin-, MCP-3- and RANTES-mRNA expression in eosinophilic nasal polyps derived

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from patients with and without atopic disposition. Therefore, RNA was isolated from nasal polyps of 6 atopic patients (4 males; median age: 39 years; range: 26-44 years). Atopic disposition was defined by a >3 mm skin wheal response to *Dermatophagoides pteronyssinus* combined with a positive intranasal provocation test with this allergen. The other nasal polyps derived from 11 patients (7 males; median age: 41 years; range: 27-46 years) without allergic symptoms in their medical histories and without a response of common allergens (*Dermatophagoides pteronyssinus*, mixed grass pollen, dog, feathers and cat dander). Specimens of non-eosinophilic nasal turbinates were used as control group from 6 patients (3 males; median age: 32 years; range: 24-38 years) who were operated for nasal blockage.

Histological examination, RNA isolation and cDNA synthesis

Human nasal tissue (500 mg) obtained from nasal polyps or inferior turbinates was dissected and divided into two parts upon surgical removal and immediately processed as follows: one part was fixed in formalin solution and stained with haematoxylin-eosin to detect and quantify eosinophils. The polyp was defined as eosinophilic when >100 eosinophils per high-power field were present. The specimens from turbinates were classified as non-eosinophilic when <10 eosinophils per high-power field were detected.

The other part was frozen in liquid nitrogen for subsequent RNA isolation. The frozen tissue was grounded in a pre-cooled mortar together with frozen lysis-buffer containing 2 M guanidinium isothiocyanate, 50% phenol (pH 4), 0.25% sarkosyl, 50 mM mercaptoethanol and 140 mM sodium acetate (pH 4). Total RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction essentially as described by Chomczynski and Sacchi (1987). One microgram of total RNA was reverse transcribed using an oligo-dT_{18} primer, Superscript^{TM} II RNase H-reverse transcriptase (Gibco/BRL; Eggenstein, Germany) and standard reagents according to the recommendations of the manufacturer (Gibco/BRL; Eggenstein, Germany).

Semi-quantitative duplex RT-PCR (SQRT-PCR)

Intron-spanning sets of primers specific for GAPDH (Bartels et al., 1996), MCP-3 (forward primer: 5'-ACCAAACCAGAAACCTCCAATTC-3'; reverse primer: 5'-AGGTAGAGAAGGGAGGAGCAT-3'), RANTES (forward primer: 5'-CATCCTCATTGCTACTGCCCTCTG-3'; reverse primer: 5'-CGGGTTCACGCCATTCTCCT-3) and eotaxin (Bartels et al., 1996) were used to differentiate between genomic and cDNA templates. cDNA corresponding to 50 ng RNA served as template in a duplex-PCR-reaction containing 0.8 μM of primers specific for MCP-3, RANTES or eotaxin and (as internal control for equal amounts of cDNA before PCR) 0.1 μM of a GAPDH-specific primer pair (Aschoff et al., 1994; Bartels et al., 1996). Amplification and analysis of the PCR products were done as described by Bartels et al. (1996).

Statistical analysis

Densitometric levels of mRNA expression were analyzed using the chi-square test. A p-value of <0.05 was regarded as significant.

RESULTS

Chemokine-specific cDNA amplification relative to GAPDH-cDNA amplification was used to monitor disease-dependent changes in RANTES- and MCP-3-mRNA expression, assuming that mRNA expression of the housekeeping gene GAPDH is not affected by the disease (Aschoff et al., 1994). The results from individual patients are shown in Figures 1A-C and summarized in Figures 1D-E.

Tissue derived from non-eosinophilic inferior turbinates of non-atopic patients (n=6; patients 1-6) served as control and showed only low basic eotaxin- and RANTES- and no MCP-3-mRNA expression (Figures 1A-C).

In nasal polyps of non-atopic patients (n=11; patients 7-17) average eotaxin-mRNA expression was approximately 3 times higher relative to control as judged by densitometric evaluation (Figure 1D) of semi-quantitative RT-PCR results (Figure 1A). RANTES-mRNA seemed not to be significantly increased in this group of patients (Figure 1E).

Atopic patients (n=6, patients 18-23) showed an average increase of both chemokine-mRNA species by a factor of approximately two (RANTES; cf. Figure 1E) or better (eotaxin; cf. Figure 1D). No MCP-3-mRNA expression could be observed in nasal polyps (patients 7-23; cf. Figure 1C).

DISCUSSION

Nasal epithelium forms the initial barrier between the environment and the respiratory system and is a potential source of pro-inflammatory cytokines, which are believed to contribute to the pathophysiology of allergic rhinitis and chronic polypous sinusitis. Nasal polyps contain elevated numbers of activated eosinophils (Stoop et al., 1993). This was also found in the polyps but not in inferior turbinates of non-atopic patients used for this study. We studied the expression of candidate genes, which may be involved in the production of eosinophilic chemotaxins using semi-quantitative assessment of mRNA expression relevant to local production of eosinophil chemotactic chemokines in human nasal polyps. We examined MCP-3-, RANTES- and eotaxin-mRNA production in normal nasal mucosa and nasal polyps from patients with or without atopic diathesis. These three chemokines have been shown to be potential mediators of eosinophil recruitment at allergic inflammatory sites (for a review, see Teran and Davies, 1996). In contrast to MCP-3, we found elevated mRNA expression for eotaxin and RANTES in human nasal polyps indicating a role for these CC-chemokine genes in chronic polypous sinusitis. Densitometric evaluation (Figures 1D-E) of chemokine-mRNA expression in nasal polyps (patients 7-23; Figures 1A-B) relative to normal nasal mucosa (patients 1-6; Figures 1A-B) suggests that the increase in eotaxin-mRNA expression in nasal polyps is more pronounced than the increase in RANTES-mRNA expression. While eotaxin expression has been reported to be associated with a type-II allergic immune response (Moshizuki et al., 1997), RANTES has been associated with a type-I immune response (Schrum et al., 1996).

Using *in situ* hybridization increased MCP-3- and RANTES-mRNA expression has been demonstrated in bronchial mucosa

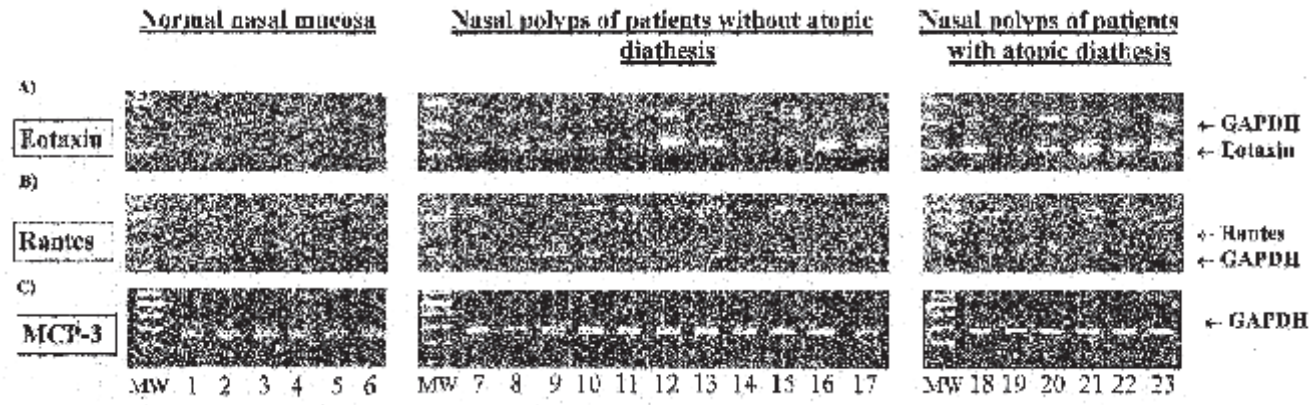

Figure 1A-C. Chemokine- and GAPDH-mRNA expression in individual samples of normal nasal mucosa and nasal polyps detected by semi-quantitative duplex RT-PCR (SQRT-PCR). The amplification products are shown after separation by gel electrophoresis. GAPDH- (318 base pairs), eotaxin- (207 base pairs; Figure 1A) and RANTES-specific amplification products (618 base pairs; Figure 1B) are indicated. A 100-bp ladder was used as molecular-weight size marker (MW).

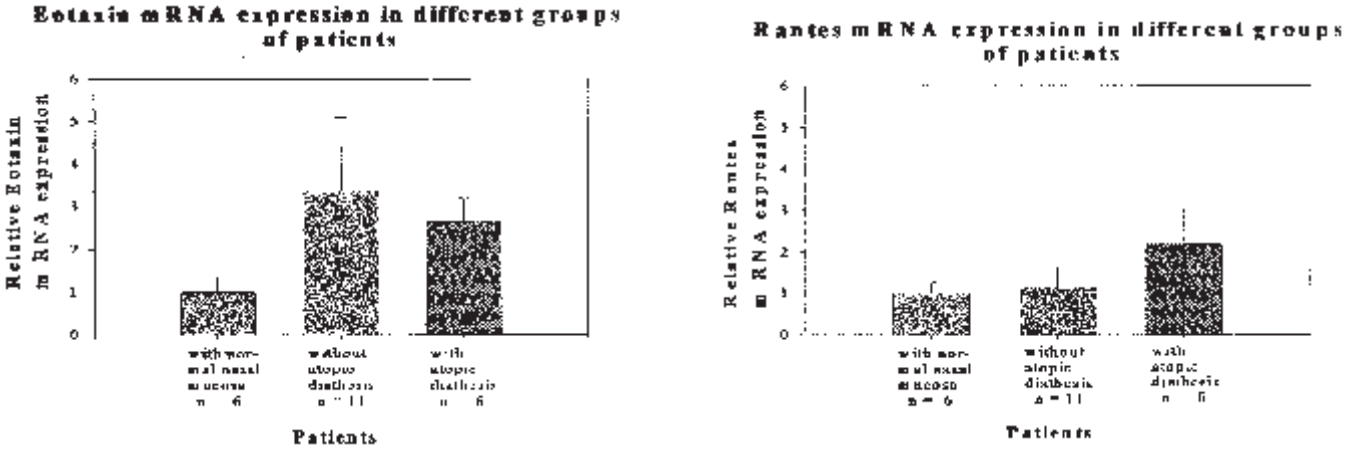

Figure 1D-E. Eotaxin- (Figure 1D) and RANTES-gene expression (Figure 1E) in nasal polyps of atopic and non-atopic patients relative to their expression in normal nasal tissue (chemokine-mRNA expression=1). Chemokine-mRNA expression relative to GAPDH expression was calculated for each sample (original data see Figure 1A-C) using the densitometric determined amounts of the corresponding amplification products. Average relative eotaxin- and RANTES-gene expression in normal nasal tissue and nasal polyps of atopic and non-atopic patients was determined based on these individual values and normalized by setting the average value of eotaxin/GAPDH-mRNA expression (Figure 1D) and RANTES/GAPDH-mRNA expression (Figure 1E) obtained for normal nasal tissue to 1.

of symptomatic atopic and non-atopic asthmatics (Humbert et al., 1997) as well as in human skin biopsies obtained from atopic subjects following allergen challenge (Ying et al., 1995). Using Northern-blot hybridization and RANTES-ELISA, RANTES-mRNA and RANTES-protein production could be shown in cultures of stimulated human nasal mucosa fibroblasts but not in cultured nasal epithelial cells, where only after PMA stimulation RANTES-mRNA but not protein could be detected (Maune et al., 1996a). While MCP-3-protein expression has only been reported from tumour cells (in trace amounts) but not from nasal polyps, RANTES protein has been detected in nasal polyps by immunohistochemical staining (Beck et al., 1996) and co-eluted with eosinophil chemotactic activity from extracts of nasal polyps (Maune et al., 1996b), although no significant correlation between counts of eosinophils and the intensity of epithelial RANTES staining in epithelial nasal tissues has been found (Beck et al., 1996). The ß-chemokine eotaxin, a strong and specific eosinophil chemoattractant *in vitro* and an effective eosinophil chemoattractant when injected into the skin of a rhesus monkey (Ponath et al., 1996), is regarded as a key element for the selective recruitment of eosinophils to certain inflamed tissues (Ponath et al., 1996). Immunohistochemistry on human nasal polyps with anti-eotaxin monoclonal antibodies showed that certain leukocytes as well as the respiratory epithelium were intensely immunoreactive, and that eosinophil infiltration occurred at sites of eotaxin up-regulation (Ponath et al., 1996). Histological studies demonstrated that polyp types appear to have a structural correlation with the specific infiltrate cell type, and no correlation between atopic status and polyp presence or polyp typology was found (Leprini et al., 1995). Therefore, it is possible that releasing factors, such as IL-4 and IL-5, derived from infiltrating cells (such as eosinophils) may influence the site of inflammation (Bachert et al., 1994), maybe more than the atopic disposition itself. IL-4 and IL-5 production by eosinophils may amplify local allergic inflammatory responses in local tissue survival (Kay et al., 1997). Atopic and non-atopic asthma, an eosinophilic inflammation in the upper respiratory tract, seems to be associated with combined bron-

chial mucosal expression of CC-chemokines (RANTES, MCP-3) together with eosinophil-active cytokines (IL-5, GM-CSF, IL-3; cf. Humbert et al., 1997). These cytokines may contribute either to the nasal or bronchial mucosal accumulation of eosinophils, both in atopic and non-atopic nasal polyps.

Our results indicate that eosinophilic tissue infiltration, a typical feature of allergic rhinitis and chronic polypous sinusitis (Jankowski et al., 1989; Linder et al., 1993), mainly correlates with increased eotaxin-gene expression and to some extent (especially in the case of patients with atopic diathesis) with RANTES-gene expression. The extent of eotaxin- and RANTES-gene expression may determine the course of nasal polyposis.

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