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Elevated expression of CC Chemokine ligand 23 in eosinophilic chronic rhinosinusitis with nasal polyps

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Abstract

Background—Chronic rhinosinusitis (CRS) is a heterogeneous chronic disease characterized by local inflammation of the sinonasal tissues. The pathogenesis of CRS remains controversial but it has been associated with the accumulation of various immune and inflammatory cells in sinus tissue.

Objectives—The objective of this study was to investigate the expression of chemokine CCL23, known to bind to CCR1 and recruit monocytes, macrophages, and dendritic cells, in patients with CRS.

Methods—We collected nasal tissue from patients with CRS and control subjects. We assayed mRNA for CCL23 by using real-time PCR and measured CCL23 protein by ELISA, immunohistochemistry and immunofluorescence.

Results—CCL23 mRNA was significantly elevated in nasal polyps from patients with polypoid CRS (CRSwNP) ($p<0.05$) compared to inferior turbinate and uncinate tissue from patients with CRS or control subjects. CCL23 protein was also elevated in nasal polyps, although these levels were not statistically significant. Immunohistochemical analysis revealed CCL23 expression in mucosal epithelial cells and inflammatory cells, but accumulation of CCL23 positive inflammatory cells occurred only in nasal polyps. Immunofluorescence data showed CCL23 co-localization with ECP positive eosinophils. The concentration of CCL23 in nasal polyps positively correlated with the concentration of ECP, suggesting that eosinophils are major CCL23 producing

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Clinical implications: Over expression of CCL23 in nasal polyps may have a pathogenic role in eosinophilic CRSwNP.

Capsule summary

CCL23, which is a ligand for CCR1 and can recruit monocytes, macrophages and dendritic cells, is elevated in eosinophilic nasal polyps. It may have an unrecognized role, contributing to the inflammation in eosinophilic CRSwNP.

cells in nasal polyps. Finally, we found that CCL23 protein was significantly elevated in nasal polyps from patients with CRSwNP with aspirin sensitivity.

Conclusion—Overproduction of CCL23 in nasal polyps may contribute to the pathogenesis of eosinophilic CRSwNP via the recruitment of CCR1 positive inflammatory cells including monocytes and macrophages, and the amplification of local inflammation.

Keywords

Chronic rhinosinusitis; Nasal polyps; CCL23; CCR1; Eosinophils; Aspirin sensitivity

INTRODUCTION

Chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by local inflammation of the upper airways and sinuses that is unresponsive to antibiotic therapy and which persists for at least 12 weeks. CRS is one of the most common chronic diseases in adults in the United States, affecting over 30 million Americans, and is responsible for over 250,000 surgical interventions annually.[1-3] The etiology and pathogenesis of CRS remain controversial, but both fungi and bacteria have been implicated as key infectious agents in inciting the intense host inflammatory responses.[4] Primarily on the basis of physical examination, histology, and clinical course, CRS is frequently divided into 2 types: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Histologic studies have demonstrated significant tissue eosinophilia in CRSwNP, most prominently in western countries. T cells in the mucosa are elevated in both forms of CRS and are skewed to Th2 cytokine expression in CRSwNP.[2, 3, 5] In addition, we and other groups have found that B cells, plasma cells, macrophages and neutrophils are also accumulated in the nasal mucosa of patients with CRSwNP.[5-9] Therefore it is now recognized that accumulation of inflammatory cells and up-regulation of the factors that recruit them are important in the pathogenesis of CRS, especially in CRSwNP.

Chemokines mainly participate in the selective recruitment of inflammatory cells into tissue sites. Chemokines are a large superfamily of low molecular mass, secreted and heparin-binding molecules that can be classified into several groups based on their molecular structures.[10, 11] They are known to be involved in many inflammatory diseases including asthma, atopic dermatitis, rheumatoid arthritis, Crohn's disease and inflammatory bowel disease.[11, 12] Several chemokines have also been reported to be involved in CRS. The regulation and production of chemokines involved in the recruitment of $CCR3^+$ cells, (e.g. eosinophils) such as RANTES (CCL5) and eotaxins (CCL11 (eotaxin-1), CCL24 (eotaxin-2) and CCL26 (eotaxin-3)) in patients with CRSwNP have been studied by many groups.[5, 13-17] In addition, several other chemokines, including CCL2 (MCP-1), CCL20 (LARC), CXCL8 (IL-8), CXCL12 (SDF-1α) and CXCL13 (BCA-1) have also been reported to be elevated in patients with CRS.[8, 18-22]

CCL23 (also known as myeloid progenitor inhibitory factor 1 (MPIF-1), macrophage inflammatory protein 3 (MIP-3) and CKβ8) was originally identified from a human aortic endothelial cell library and from the human monocytic cell line THP-1.[23, 24] Because a homolog of CCL23 in rodents has not been discovered, function and regulation of CCL23 is not well described. CCL23 has chemotactic activity for monocytes, dendritic cells and lymphocytes via the chemokine receptor CCR1.[23-25] In addition, it has been reported that CCL23 can induce endothelial cell migration, tube formation and angiogenesis via CCR1.[26] A protease cleaved product of CCL23 has chemotactic activity for monocytes and neutrophils via formyl peptide receptor like-1 (FPRL1).[27] In contrast, CCL23 has been shown to suppress progenitor cells of granulocyte and monocyte lineages.[23, 24] CCL23 has

been reported to be expressed in activated monocytes and dendritic cells and is a biomarker for some inflammatory diseases including atopic dermatitis, rheumatoid arthritis and systemic sclerosis.[25, 28-30] Expression of CCL23 in monocytes has been shown to be regulated by the Th2 cytokines IL-4 and IL-13.[28] Although CRSwNP is classically a Th2 related sinus disease, and inflammatory cells are recruited to nasal polyps, expression of CCL23 in CRS has not been studied. In this study, we examined expression of CCL23 in CRS and found that CCL23 was elevated in patients with eosinophilic CRSwNP.

METHODS

Patients

CRS patients were recruited from the Allergy-Immunology clinic and the Otolaryngology clinic of the Northwestern Medical Faculty Foundation (NMFF), the group practice for physician faculty members of Northwestern University and the Northwestern Sinus Center at NMFF. Sinonasal and nasal polyp tissues were obtained from routine Functional Endoscopic Sinus Surgery in patients with CRS. All subjects met the criteria for CRS as defined by the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force.[1, 31] Patients with an established immunodeficiency, pregnancy, coagulation disorder, diagnosis of classic allergic fungal sinusitis, Churg-Strauss syndrome or cystic fibrosis did not participate in the study. Details of subjects' characteristics are included in Table I and in the Online Repository. All subjects signed informed consent forms and the protocol governing procedures for this study has been approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine.

Real-time PCR

Total RNA from sinus tissue was extracted using QIAzol (Qiagen, Valencia, CA) and the quality of total RNA from sinus tissue was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Semi-quantitative real-time RT-PCR was performed using the TaqMan method as described previously.[32] The mRNA expression levels were normalized to the median expression of the housekeeping gene, β-glucuronidase (GUSB). Details are in the Online Repository.

ELISA and eosinophil culture

The methods are described in the Online Repository.

Immunohistochemistry

Immunohistochemistry was performed as described previously.[7] Briefly, tissue sections were incubated with 1.5 μg/ml biotinylated goat anti-human CCL23 antibody (R&D systems) at 4°C overnight. After washing, sections were incubated in ABC reagent (Vector Laboratories) for 1 hour. Sections were rinsed and incubated in DAB reagent (Invitrogen, Carlsbad, CA), and then counterstained with hematoxylin. Slides were blinded and ten pictures were randomly taken from each slide. The number of CCL23 positive cells in nasal mucosa was counted by two independent observers. Details of the methods for immunofluorescence and immunohistochemistry are described in the Online Repository.

Statistics

All data are reported as the mean ± SEM unless otherwise noted. Differences between groups were analyzed using the Mann-Whitney U-test. Correlations were assessed by using the Spearman's rank correlation. A p value of less than 0.05 was considered significant.

RESULTS

CCL23 expression in CRS

To determine the presence of CCL23 expression in chronic rhinosinusitis, sinonasal and polyp tissues were collected from 49 subjects with CRSsNP, 95 subjects with CRSwNP and 42 control subjects. Subject characteristics are shown in Table I.

We assessed the expression of CCL23 in inferior turbinate (IT) and uncinate tissue (UT) from patients with CRSsNP, CRSwNP and controls, as well as in nasal polyp (NP) tissue from patients with CRSwNP. Expression of the housekeeping gene GUSB was not significantly different among the 7 groups (data not shown). CCL23 mRNA was only significantly increased in NP tissue from patients with CRSwNP ($p<0.001$) in comparison to IT and UT from either patients with CRS or control subjects (Fig 1, *A*). To confirm this observation at the protein level, we made detergent extracts from homogenates of IT and NP tissues and then measured the concentration of CCL23 using ELISA. Although not statistically significant, CCL23 protein was elevated in NP tissue from CRSwNP (n=44; 218.0 ± 48.4 pg/mg; range 10.9-1899 pg/mg) compared to IT tissue from CRSsNP (n=20; 80.9 ± 6.5 pg/mg; 27.0-139.3 pg/mg), CRSwNP (n=21; 175.5 ± 39.4 pg/mg; 16.3-636.4 pg/mg) and control subjects (n=12; 84.8 ± 12.8 pg/mg; 15.3-173.8 pg/mg) (Fig 1, *B*). We thought the large variation of concentration of CCL23 in NP tissue might be due to asthmatic or atopic status in patients. However this did not turn out to be the case, as mean levels of CCL23 were not affected by asthmatic status or atopic status (Fig 1, *C*).

Localization of CCL23 positive cells in nasal mucosa of CRS

To examine the CCL23 producing cells in nasal mucosa, we used immunohistochemistry to detect $CCL23^+$ cells in UT and NP tissue. As shown in Fig 2, CCL23 staining was observed mainly in submucosal inflammatory cells, although light CCL23 staining was also observed in some mucosal and glandular epithelium. In addition, we did not observe a significant difference in the number of $CCL23^+$ cells in the epithelium and glands among the 3 groups of subjects (Fig 2 and data not shown). We therefore focused on inflammatory cells in this study. We found that $CCL23^+$ inflammatory cells were highly elevated in NP tissue from patients with CRSwNP (Fig 2, *D*). We counted the number of $CCL23^+$ inflammatory cells using a semi-quantitative method and found that $CCL23^+$ inflammatory cells were significantly elevated in the submucosal region of NP tissues from CRSwNP (n=10; 10.6 ± 2.5 $CCL23^+$ cells/high power field (HPF)) compared to UT from CRSsNP (n=12; 1.4 ± 1.7 $CCL23^+$ cells/HPF, $p<0.05$), CRSwNP (n=10; 3.2 ± 2.1 $CCL23^+$ cells/HPF, $p<0.05$) and control subjects (n=11; 1.3 ± 1.8 $CCL23^+$ cells/HPF, $p<0.05$) (Fig 2, *F*).

CCL23 is expressed by eosinophils in nasal polyps

CCL23 is known to be produced by monocytes, macrophages and dendritic cells.[25, 28, 33] We therefore first examined whether macrophages and dendritic cells were major CCL23 producing cells in NP tissue. Immunofluorescence data showed that less than 5% of $CCL23^+$ cells were co-stained with the macrophage marker CD68, and the majority of $CD68^+$ cells lacked CCL23 staining in NP tissue (Fig 3, *A* and Fig E1). In addition, we could not find CCL23 staining in $CD1c^+$ dendritic cells in NP tissue (Fig E1). To identify the major CCL23 producing cells in submucosal tissue of nasal polyps, we performed immunofluorescence analysis using anti-CCL23 and antibodies against several other cell specific markers including CD3 (T cells), CD20 (B cells), CD138 (plasma cells), ECP (eosinophils) and tryptase (mast cells). Interestingly, we found CCL23 co-localization in ECP^+ eosinophils but not in T cells, B cells, plasma cells or mast cells (Fig 3, *B* and E1).

Nasal polyps from patients with CRSwNP have long been known to be characterized by eosinophilic inflammation.[15] Immunofluorescence staining showed that more than 90% of $CCL23^+$ cells in NP tissue were eosinophils (Fig 3). However, to our knowledge, no one has reported production of CCL23 in eosinophils. We first examined the expression of CCL23 in eosinophils isolated from peripheral blood from control subjects. We found that CCL23 mRNA was constitutively expressed in isolated eosinophils and that levels were significantly higher than in peripheral blood mononuclear cells (PBMC) or neutrophils (n=3-4, Fig E2, *A*). In order to investigate the regulation of CCL23 in eosinophils, purified blood eosinophils were stimulated with common eosinophil activators, IL-5, GM-CSF, PMA/A23187 and TNF, and the known CCL23 inducers[28, 33], IL-4, IL-13 and IFN-γ. However, the concentration of CCL23 was below the lowest standard in the ELISA (15.6 pg/ml) in most eosinophil supernatants (Fig E2, *C*). We also measured the expression of CCL23 mRNA in eosinophils 24 hours after stimulation. Interestingly, CCL23 mRNA was decreased 24 hours following culture (n=3, 0.02-fold, Fig E2, *A* and *B*). Decreased expression of CCL23 mRNA was rescued by GM-CSF, IL-5 and TNF (Fig E2, *B*). Since the levels of CCL23 protein were at the limit of the detectability in cultured eosinophils, we then assessed whether CCL23 was detected in eosinophils that were freshly isolated from nasal lavage in patients with CRSwNP. We found that CCL23 was expressed in in ECP^+ eosinophils from nasal lavage but not in $elastase^+$ neutrophils (Fig 4).

We next examined whether levels of CCL23 correlated with eosinophilic inflammation in nasal polyps. We assayed the levels of ECP as a marker for the presence of eosinophils in NP tissue. We found that the concentration of CCL23 in NP tissue significantly correlated with the concentration of ECP (r=0.4952, p=0.004, n=32; Fig 5, *A*). Nasal polyps in aspirin sensitive (AS) patients are typically more eosinophilic than in aspirin tolerant patients.[15] Although we initially excluded patients who had aspirin sensitivity in this study, we decided to determine levels of CCL23 in nasal polyps from patients with CRSwNP/AS. We found that CCL23 protein was significantly elevated in NP tissue from CRSwNP/AS (n=12; 499.1 ± 142.7 pg/mg; range 25.7-1640 pg/mg) compared to IT tissue from control subjects (n=12, 84.8 ± 12.8 pg/mg, p=0.040), CRSsNP (n=20, 80.9 ± 6.5 pg/mg, p=0.023) and CRSwNP (n=21, 175.5 ± 39.4 pg/mg, p=0.070) (Fig 1, *B* and 5, *B*).

Correlation of CCL23 and CCR1 in nasal polyps

CCL23 is a ligand of chemokine receptor CCR1 which is expressed on monocytes, macrophages and dendritic cells. We therefore measured the expression of CCR1 in sinus tissue. CCR1 mRNA was significantly upregulated in NP tissue from patients with CRSwNP ($p<0.05$) in comparison to UT from either patients with CRS or control subjects (Fig 6, *A*). We next examined whether the expression of CCL23 correlated with the levels of CCR1 and markers of macrophages and dendritic cells in NP tissue. We found that CCL23 mRNA significantly correlated with mRNA levels for CCR1 (r=0.5591, p=0.0045), macrophage mannose receptor (MMR; r=0.5739, p=0.0034) and CD1c (r= 0.4304, p= 0.0358) in NP tissue (n=24, Fig 6).

DISCUSSION

This study provides the first demonstration that CCL23 is increased in polypoid tissue from patients with CRSwNP (Fig 1 and 2). Significant levels of CCL23 protein were found in polypoid tissue obtained from patients with CRSwNP, especially in patients with aspirin sensitivity (Fig 1 and 5). CCL23 positive cells were elevated in the submucosal tissue of nasal polyps from patients with CRSwNP (Fig 2 and 3), and CCL23 was found to be co-localized predominantly with ECP, indicating that eosinophils are major CCL23 producing cells in nasal polyps (Fig 3 and 4). We also demonstrate that levels of CCL23 significantly correlated with ECP, MMR and CCR1 in nasal polyps (Fig 5 and 6).

It has been reported that lymphocytes, macrophages, dendritic cells and neutrophils also accumulate in the nasal mucosa of patients with CRSwNP.[5-9] In the current study we demonstrated that mRNA encoding CCL23, which is a known ligand of CCR1 and is able to recruit monocytes, dendritic cells, lymphocytes and endothelial cells, was significantly increased in NP tissue (Fig 1, *A*). Although elevated CCL23 mRNA was detected in 70 to 80% of NP tissue, elevated CCL23 protein was detected in only 50% of NP extracts (Fig 1, *B*). It has been reported that CCL23 can be cleaved by proteases including mast cell chymase, neutrophil elastase and cathepsin G,[27, 34] suggesting that the stability of CCL23 protein in sinus mucosa may be compromised by the presence of activated immune cells. Alternatively, it may be that the extraction procedure that we use does not adequately free the CCL23 for detection in our ELISA assay. Further studies will be required to generate an assay system for the cleaved product of CCL23 and test these possibilities. Importantly, the known CCL23 cleaved product does not activate CCR1 but has activity for another receptor, FPRL1, that is known to be expressed on monocytes and neutrophils.[27] Although neutrophils generally accumulate in NP tissue, the precise mechanisms for their recruitment in CRSwNP are not well understood. Nasal polyps from patients with cystic fibrosis are characterized by neutrophilia and IL-8 is known to be chemotactic for neutrophils in this disease.[5, 35] In contrast, the effect of IL-8 in the recruitment of neutrophils in CRSwNP is still unclear. The relationship between the production and degradation of CCL23 and the recruitment of neutrophils in CRSwNP will hence be worthy of investigation.

It has long been known that nasal polyps from patients with CRSwNP are characterized by eosinophilic inflammation. Our immunofluorescence data clearly shows that CCL23 was detected in ECP positive eosinophils in the submucosa of nasal polyps (Fig 3). However, the production of CCL23 in eosinophils has not been reported. In addition, our understanding of the regulation of CCL23 is limited. Forssmann et al showed that IL-1β and IFN-γ had the ability to induce CCL23 expression at low levels in monocytes.[33] Novak et al showed that Th2 cytokines, IL-4 and IL-13 were potent inducers of CCL23 in monocytes and that the activity was dependent on STAT6.[28] We therefore tested whether purified eosinophils from the peripheral blood of healthy subjects were able to produce CCL23 when stimulated by known CCL23 inducers and the common eosinophil activators. Although CCL23 mRNA was constitutively expressed in eosinophils and levels were 100 fold higher than in PBMC or neutrophils, we could not obtain significant evidence indicating that either resting or stimulated eosinophils purified from peripheral blood release CCL23 (Fig, E2). Interestingly, levels of mRNA for CCL23 fell to 2% of basal expression in blood eosinophils following 24 hours culture and this down-regulation was somewhat rescued by eosinophil activators. We also assessed the concentration of CCL23 in serum and found that levels of CCL23 in patients with CRSwNP (1.10 ± 0.12 ng/ml, n=15) were not different compared to patients with CRSsNP (0.83 ± 0.19 ng/ml, n=15) or control subjects (0.94 ± 0.22 ng/ml, n=9). This suggests that overproduction of CCL23 in eosinophils occurs only locally in nasal polyps. Previous studies have shown that eosinophils isolated from bronchoalveolar lavage are phenotypically distinct from peripheral blood eosinophils and *in vitro* primed eosinophils.[36-38] We therefore examined whether eosinophils that were freshly isolated from nasal lavage in patients with CRSwNP expressed CCL23. We found that CCL23 was detected in nasal lavage eosinophils but not in neutrophils (Fig 4). Future studies will be required to identify the regulation and production of CCL23 in nasal polyp eosinophils.

Initially, we did not find a statistically significant elevation of CCL23 protein in NP tissue. Nasal polyps are usually characterized by eosinophilic inflammation but this is not observed in all patients. In fact, 20-30% of nasal polyps do not have eosinophilia (i.e., less than 5 eosinophils/HPF) in our clinic. In the current study, we found that CCL23 was detected in eosinophils in NP tissue (Fig 3) and that CCL23 was significantly elevated in NP tissue from patients with CRSwNP with aspirin sensitivity who usually had more eosinophilic

inflammation (Fig 5).[15] Although we found that the concentration of CCL23 was significantly correlated with ECP in NP tissue (Fig 5), there were some patient samples in which ECP was high and CCL23 was low, suggesting that our ability to detect CCL23 may be compromised in some patient samples due to the protease activity referred to above or another factor. On balance, these data suggest that CCL23 is produced by eosinophils in affected tissue and may contribute to the pathogenesis of eosinophilic CRSwNP.

We detected ECP^+ eosinophils using an EG2 antibody. $EG2^+$ cells are classically defined as activated eosinophils. Importantly, it has been shown that $EG2^+$ activated eosinophils are found in about 80% of NP tissue from patients with CRSwNP.[39] In contrast, no $EG2^+$ eosinophils are detected in turbinate tissue from patients with CRSsNP and from control subjects, although some resting eosinophils are found.[39] In this study, we found CCL23 was detected in $EG2^+$ cells (Fig 3). This suggests the possibility that activated eosinophils but not resting eosinophils produce CCL23 in NP tissue.

CCL23 is known to recruit monocytes and macrophages via CCR1,[24, 25] and we found that both CCL23 and CCR1 were significantly upregulated in NP tissue. In contrast to eosinophils, the role of monocytes and macrophages in the pathogenesis of CRS is poorly understood. $CD68^+$ macrophages were reported to be elevated in CRSwNP but this did not achieve statistical significance in some small studies.[5, 40] Macrophages can be polarized by their microenvironment, especially by Th cytokines and pathogens. Alternatively activated macrophages (also known as M2 macrophages) are primed by Th2 cytokines IL-4 and IL-13.[41, 42] Importantly, eosinophilic nasal polyps contain an environment enriched for Th2 cytokines.[5] Interestingly, Claeys et al showed that mRNA for macrophage mannose receptor (MMR, also known as mannose receptor C type 1 and CD206) and MMR^+ macrophages were significantly elevated in NP tissue from patients with CRSwNP.[6, 35] MMR is now widely accepted to be a marker of M2 macrophages.[41, 42] We therefore examined the correlation of CCL23 and MMR in NP tissue and found that MMR was significantly elevated in NP tissue and positively correlated with expression of CCL23 (Fig 6 and data not shown). Very recently Krysko et al showed that CD68 positive macrophages and M2 macrophages (MMR^+, $HLADR^+$, $CD14^+$, $CD11c^+$, $CD20^-$) were increased in NP tissue.[43] One possibility is that CCL23 may be involved in the recruitment of monocytes and macrophages into nasal polyps followed by polarization of the recruited cells to the M2 phenotype by Th2 cytokines. In addition, eotaxin is elevated in nasal polyps and is released from nasal epithelial cells and macrophages in nasal mucosa of patients with CRS and allergic rhinitis.[44] M2 macrophages are now known to be a rich source of eotaxin/CCL11 in mice.[45] In humans, high levels of CCL11 and eotaxin-3/CCL26 are expressed in *in vitro* polarized M2 macrophages.[41, 42, 46, 47] Thus the production of CCL23 from eosinophils and the subsequent recruitment of macrophages which in turn release eotaxins that are chemotactic for eosinophils, may represent a cycle through which inflammation in polyp tissue is perpetuated. This suggests that production of chemokines, CCL23, CCL11 and CCL26, recruitment of macrophages and eosinophils, and the presence of Th2 cytokines in NP may be linked to each other and may be involved in the pathogenesis of eosinophilic CRSwNP. Future study will be required to determine the relationships of these factors in eosinophilic nasal polyps. It has been widely accepted that other allergic airway diseases including asthma and allergic rhinitis are characterized by Th2 inflammation and eosinophilia. Thus future studies may be directed towards investigating the presence of CCL23 in these patients and may help explain their disease pathology.

Angiogenesis and microvascular remodeling are features of tissue remodeling in chronic inflammatory diseases. Recently a number of studies have reported a role for angiogenesis, based on up-regulation of proangiogenic factors including vascular endothelial growth factor angiopoietin and metalloproteinase 33, in nasal polyps.[48-51] CCL23 has been reported to

induce the migration of endothelial cells as well as tube formation[26] and is known to induce the production of matrix metalloproteinase-2 in endothelial cells.[52] In addition, increased expression of matrix metalloproteinase-2 in nasal polyps has been reported.[53] These data suggest that there should be consideration of the possibility that CCL23 plays a role in angiogenesis in CRSwNP.

In summary, we report here that eosinophils produce the chemokine CCL23, and that patients with eosinophilic CRS have elevated levels of CCL23 in polypoid tissue. Our findings indicate that CCL23 is a novel marker of patients with eosinophilic CRSwNP and the overproduction of CCL23 in NP may contribute the pathogenesis of eosinophilic CRSwNP.

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Abbreviations

CRS	Chronic rhinosinusitis
CRSwNP	CRS with nasal polyps
CRSsNP	CRS without nasal polyps
CCL23	CC Chemokine Ligand 23
ECP	eosinophil cationic protein
MMR	macrophage mannose receptor
GUSB	β-glucuronidase
IT	inferior turbinate
UT	uncinate tissue
NP	nasal polyp

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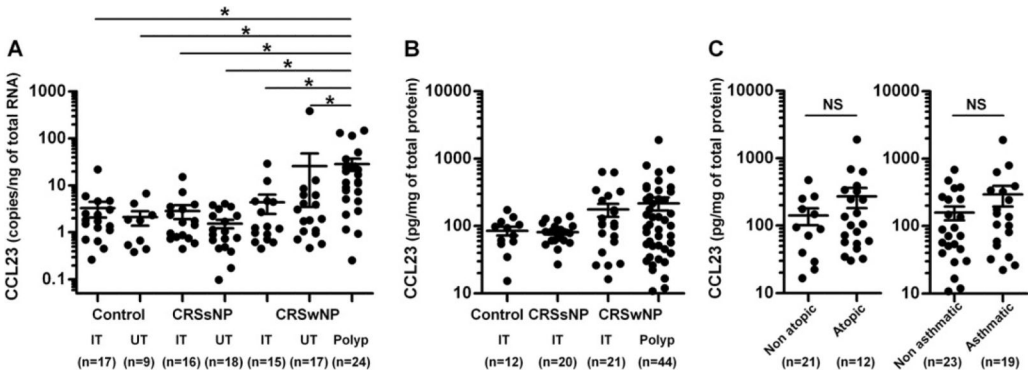


Figure 1.

Increased expression of CCL23 in nasal polyp tissue. (A) Total RNA was extracted from inferior turbinate (IT), uncinate tissue (UT) and nasal polyps (Polyp) and expression of CCL23 was analyzed using real-time PCR. The concentration of CCL23 in tissue homogenates of IT (B) and polyp (B and C) was measured using ELISA. CCL23 concentration was normalized to the concentration of total protein. * $p < 0.05$. NS; not significant.

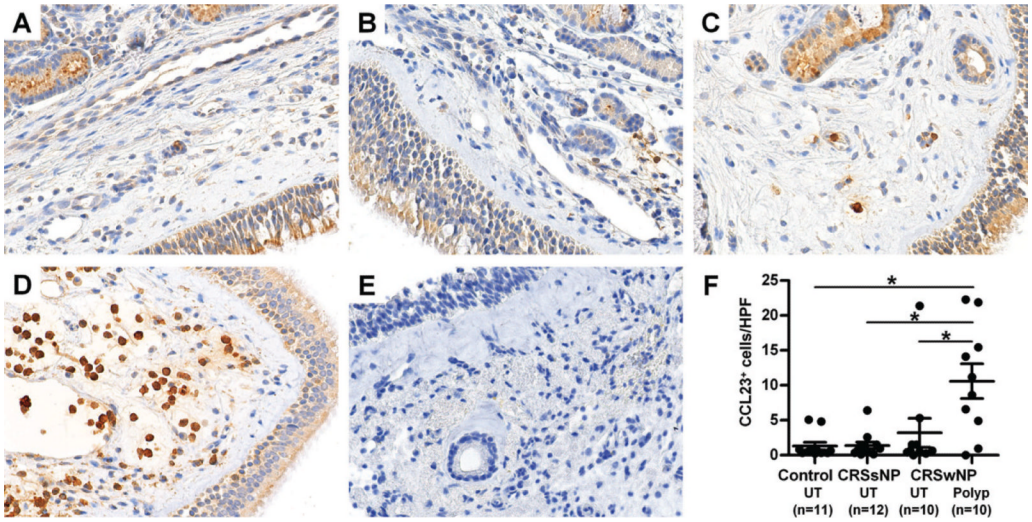


Figure 2.

Immunohistochemistry of CCL23 was performed with an anti-human CCL23 antibody. Representative immunostaining for CCL23 in uncinate tissue (UT) from a control subject (A), a patient with CRSsNP (B), a patient with CRSwNP (C), and in nasal polyp tissue (D). Negative control antibody staining in nasal polyp tissue from a patient with CRSwNP (E). The number of CCL23 positive cells in UT from control (n=11), CRSsNP (n=12) and CRSwNP (n=10) and in nasal polyps (n=10) was counted using the NIH-issued Image J software (F). Magnification; ×400. * $p < 0.05$.

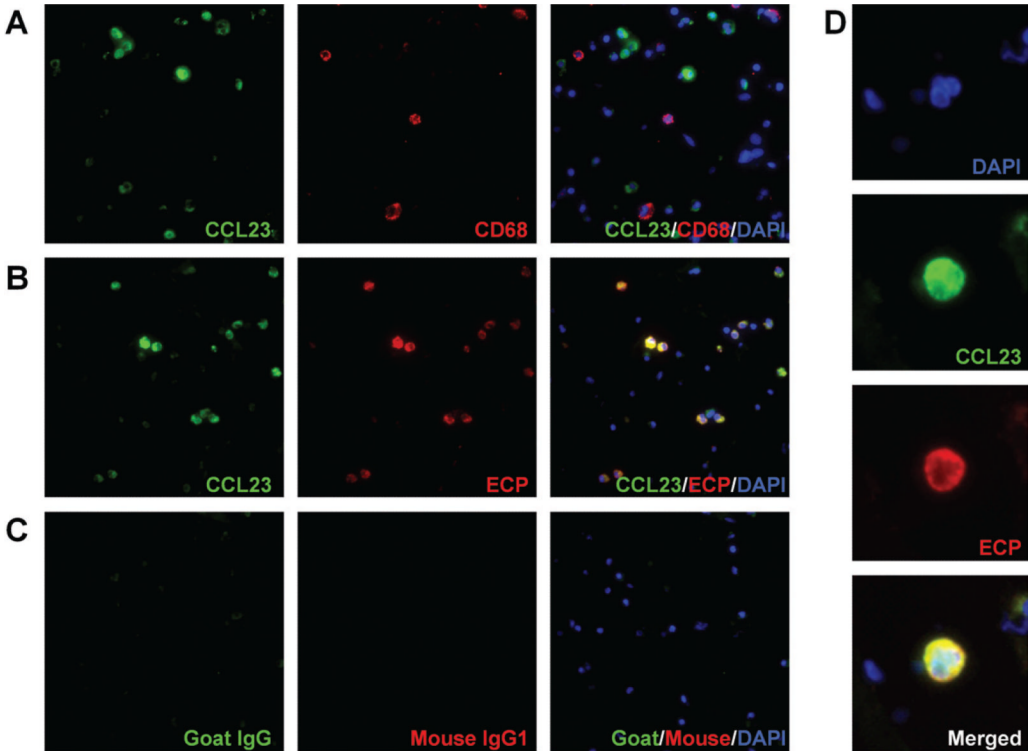


Figure 3.
Immunofluorescence of CCL23 in nasal polyp tissue. Immunofluorescence assay was performed using anti-CCL23 (green fluorescence), anti-CD68 mAb (red fluorescence) for macrophages (A), anti-ECP mAb (red fluorescence) for eosinophils (B and D) and control IgG (C). Nuclei were counterstained with 4’,6-diamidino-2-phenylindole (DAPI) (blue fluorescence). The results are representative of 3 to 6 separate patients.

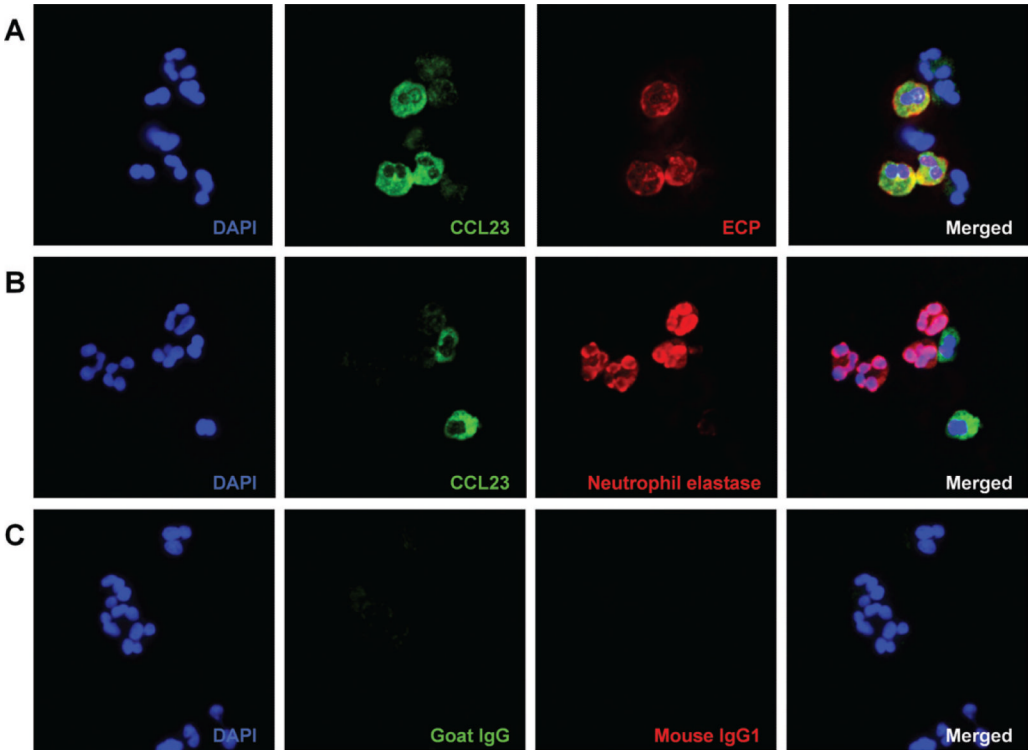


Figure 4.
Detection of CCL23 in nasal lavage eosinophils from patients with CRSwNP. Immunofluorescence assay was performed using anti-CCL23 (green fluorescence), anti-ECP mAb (red fluorescence) for eosinophils (A), anti-neutrophil elastase (red fluorescence) for neutrophils (B), and control IgG (C). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue fluorescence). The results are representative of four separate patients.

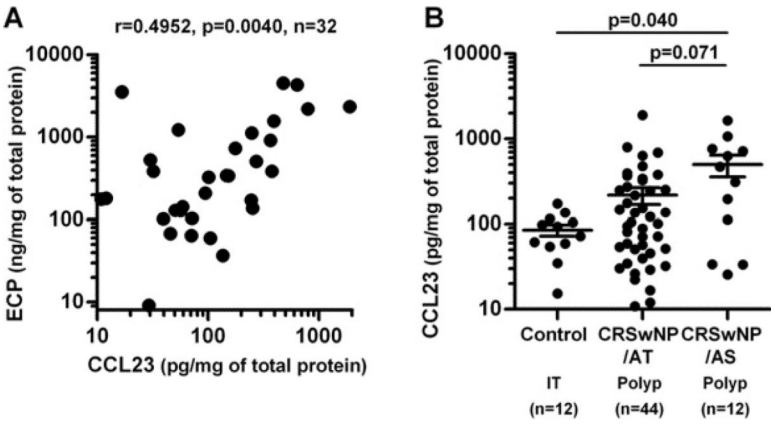


Figure 5.

Elevated expression of CCL23 in eosinophilic nasal polyps. Relationship of CCL23 and ECP in nasal polyp tissue was evaluated by using ELISA. The correlations were assessed using the Spearman rank correlation (A).The concentration of CCL23 in nasal polyp tissue homogenates from aspirin sensitive patients (CRSwNP/AS) and aspirin tolerant patients (CRSwNP/AT) (B). The concentration of CCL23 in IT from control subjects and in nasal polyps from CRSwNP/AT was used from Fig. 1B.

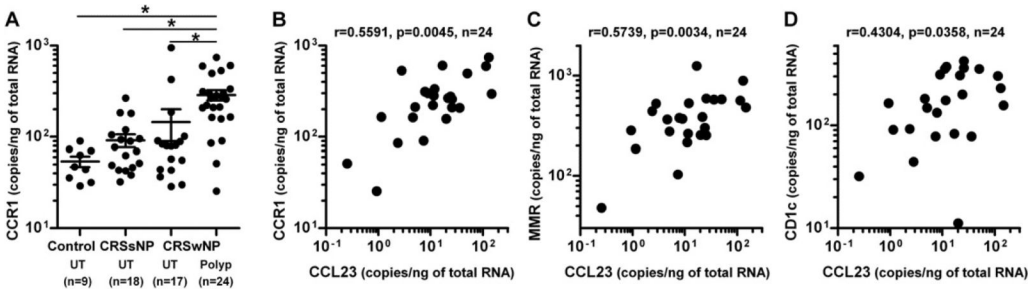
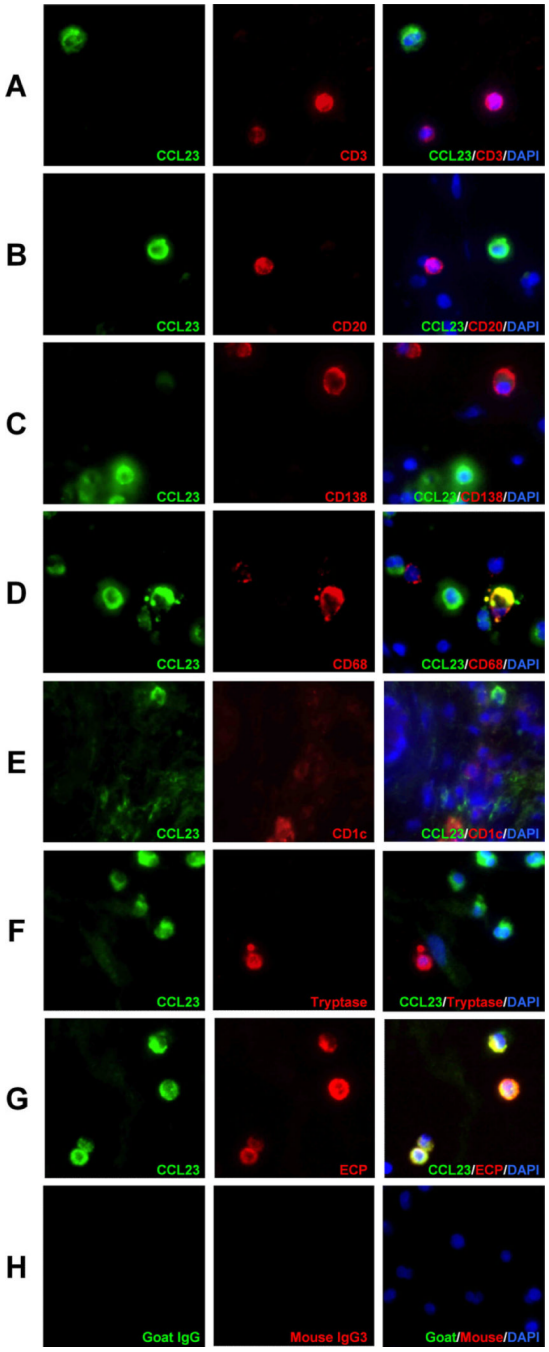


Figure 6.

Correlation of CCL23 with CCR1 in nasal polyps. Total RNA was extracted from UT and nasal polyp tissue and the expression of CCL23 (B-D), CCR1 (A, B) and cell specific markers, MMR (C) and CD1c (D) was analyzed by real-time PCR. The correlations were assessed by using the Spearman rank correlation. * $p < 0.05$.



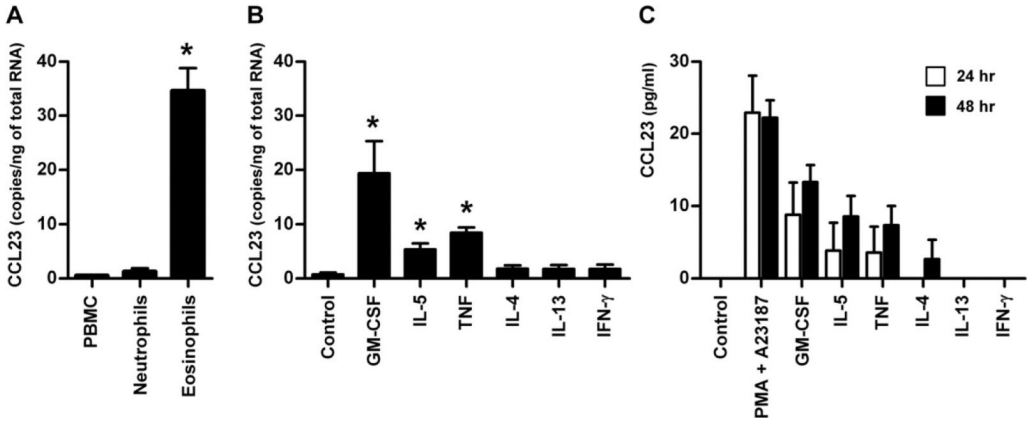


Table I

Subject characteristics

	Control			**CRSsNP**			**CRSwNP**			**CRSwNP with AS**		
Total no. of subjects (Male)	42 (27M)			49 (18M)			83 (61M)			12 (7M)		
Age (y), median (range)	37 (16-77)			36 (23-64)			42 (21-74)			50 (26-61)		
	Y	N	U	Y	N	U	Y	N	U	Y	N	U
Atopy	5	31	6	19	20	10	38	28	17	7	2	3
Asthma	0	38	4	5	40	4	35	45	3	10	2	0
Methodologies used												
PCR	IT		UT	IT		UT	IT	UT	NP			
	17 (14M)		9 (3M)	16 (7M)		18 (8M)	15 (8M)	17 (14M)	24 (18M)			
Age	36 (20-77)		41 (16-59)	36 (27-55)		36 (23-64)	40 (28-68)	43 (26-61)	46 (27-74)			
ELISA	IT			IT			IT		NP	NP		
	12 (7M)			20 (8M)			21 (14M)		44 (37M)	12 (7M)		
Age	40 (17-63)			35 (24-60)			41 (26-63)		41 (21-74)	50 (26-61)		
IHC	UT			UT			UT		NP			
	11 (4M)			12 (3M)			10 (8M)		10 (9M)			
	41 (16-77)			33 (26-55)			42 (26-67)		40 (26-70)			

M, male; Y; yes, N; no, U; unknown, AS; aspirin sensitive.