Up-regulation of Chemokine Ligand 20 in Chronic Rhinosinusitis

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Objectives: To investigate the up-regulation of chemokine ligand 20 (CCL20) in chronic rhinosinusitis mucosa and to localize the distribution of CCL20 in the human paranasal sinus mucosa.

Design: Prospective study.

Setting: Tertiary academic institution.

Patients: Ten patients who underwent functional endoscopic sinus surgery for chronic rhinosinusitis without nasal polyps and 10 normal control subjects.

Interventions: Messenger RNA was extracted from the sinus mucosa, and semiquantitative reverse transcriptase–polymerase chain reaction was performed.

Immunohistochemical staining was used to localize the CCL20 protein.

Results: The expression levels of CCL20 messenger RNA level in chronic rhinosinusitis without nasal polyps were significantly increased compared with those in normal sinus mucosa. The expression of CCL20 protein was greater in chronic rhinosinusitis without nasal polyps mucosa and was localized to the epithelial and submucosal glandular cells.

Conclusion: CCL20 is an inducible product of human paranasal sinus epithelium that may play a role in modulating mucosal immunity of the sinus mucosa.

Arch Otolaryngol Head Neck Surg. 2006;132:537-541

Methods

Subjects

Ten patients (5 men and 5 women; age range, 28-40 years) who underwent surgery for chronic rhinosinusitis and 10 normal control subjects (5 men and 5 women; age range, 35-50 years) were recruited for this study. Clinical diagnosis of chronic rhinosinusitis followed the guidelines proposed by the American Academy of Otolaryngology–Head and Neck Surgery. In the chronic rhinosinusitis group, pa-
tients experienced typical symptoms, such as headache, nasal obstruction, and discolored nasal drainage arising from the nasal passages, that persisted for 12 weeks or longer and showed edema or erythema of the middle meatus, as identified by nasal endoscopy. Inflamed maxillary sinus mucosal tissues were removed during functional endoscopic sinus surgery through extended middle meatal antrostomy. For the controls, normal maxillary sinus mucosa was obtained from 10 patients with blowout fractures who underwent endoscopic endonasal reconstruction of the medial orbital walls. The patients in the control group had no sinonasal symptoms or signs and had no evidence of inflammation of paranasal sinus mucosa on preoperative computed tomography of the paranasal sinuses. The patients and controls had no personal or family history of allergy and had negative results on skin prick tests to 20 common airborne allergens and on multiple simultaneous allergen tests. Patients with nasal polyposis or immunodeficiency and individuals who had undergone prior sinus surgery were excluded from the study. No patient had been receiving any medication, including antibiotics, topical or oral corticosteroids, antihistamines, and mucokinetic agents, for at least 4 weeks before the study. Informed consent was obtained from all patients and control subjects, and the study protocols were approved by the Committee of Ethics, College of Medicine, Korea University, Seoul, South Korea.

PREPARATION OF SINUS MUCOSA

Tissues were cut into 2 portions. One portion was immediately flash frozen in liquid nitrogen and stored at −70°C for subsequent RNA studies. Another portion was fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline (pH, 7.4) overnight at 4°C and then embedded in paraffin for immunohistochemical staining.

RNA EXTRACTION AND RT-PCR

Total RNA was extracted from maxillary sinus mucosal specimens using a commercially available reagent (TRIzol; Gibco BRL, Grand Island, NY) according to the manufacturer’s recommendations. Total RNA from each sample was reverse transcribed in 20 µL of reaction mixture containing 2.5 U of Maloney murine leukemia virus RT (Gibco BRL, Grand Island, NY) according to the manufacturer’s recommendation, using 20 µL of reaction mixture containing 2.5 U of Maloney murine leukemia virus RT (Gibco BRL) and 50pM random hexanucleotide primer. The cDNA products were used as templates for the PCR reactions. The cDNA products from both normal controls and patients with chronic rhinosinusitis were used in experiments. The cDNA products were considered statistically significant at P<.05.

IMMUNOHISTOCHEMICAL STAINING

Before immunohistochemical staining, the sections were deparaffinized and rehydrated in xylene and a graduated dilution of ethanol. Immunohistochemical staining was performed by the avidin-biotin-peroxidase method (Vectastain ABC-Elite kit; Vector Laboratories, Burlingame, Calif). For CCL20 immunostaining, monoclonal anti–mouse CCL20 antibody (R&D Systems, Inc, Minneapolis, Minn) was used. Distribution of peroxidase was revealed by incubating the sections in a solution containing 3,3′-diaminobezidine tetrahydrochloride (Sigma-Aldrich, St Louis, Mo). The antibody concentrations, incubation time, and other conditions were determined according to the instructions of the supplier and the results of preliminary experiments carried out by the authors. For negative control, non-immune serum IgG or phosphate-buffered saline, instead of the primary antibody, was used.

STATISTICAL ANALYSIS

The Mann-Whitney U test was used to test for differences in each of CCL20/GAPDH ratios between the 2 groups. The difference in CCL20/GAPDH ratio between the 2 groups was considered statistically significant at P<.05.

RESULTS

CCL20 mRNA was expressed in the paranasal sinus mucosa from both normal controls and patients with chronic rhinosinusitis. The PCR products extracted from the chronic rhinosinusitis mucosa had the size (156 bp) that was expected from the selected primers. There were no significant differences in the degree of CCL20 mRNA expression among the 10 chronic rhinosinusitis mucosa samples examined (P>.05). The same-size product was expressed in the positive control. For negative control, RT-PCR was performed without the CCL20 primer or RT (Figure 1). As a control for RNA integrity and RT-PCR procedure, the same RNA samples were also analyzed for GAPDH gene expression. The GAPDH primer produced as the ratio of the intensity of CCL20 to the intensity of GAPDH. The PCR products obtained from the sinus mucosa were cut from the agarose gel, subcloned with a TA cloning kit (Invitrogen, Carlsbad, Calif), which is used to clone Taq polymerase-generated PCR products, and sequenced. To establish the specificity of responses, we used negative controls in which input RNA was omitted or in which RNA was used but RT was omitted. Messenger RNA extracted from lung tissues known to express CCL20 was used as the positive control.
a PCR product (248 bp) from each of the tissue samples. All samples were standardized to their corresponding GAPDH control. The expression of CCL20 mRNA in chronic rhinosinusitis was increased significantly compared with that in normal sinus mucosa (P < .05) (Figure 2). The PCR products obtained from lung and sinus mucosa were isolated and sequenced. In both tissues, the sequences obtained were identical to the corresponding sequences published for human CCL20, confirming that the correct transcript was being amplified in the PCR. These data indicate that CCL20 mRNA expression in the human sinus mucosa is up-regulated in the presence of inflammation.

**IMMUNOHISTOCHEMICAL STAINING OF CCL20 PROTEIN**

To localize the distribution of the CCL20 protein, the paranasal sinus mucosal samples from the controls and the patients with chronic rhinosinusitis were immunostained for CCL20. Immunohistochemical staining of the chronic rhinosinusitis mucosa showed strong staining for the CCL20 protein in the epithelial and submucosal glandular cells (Figure 3). In contrast, the CCL20 protein was weakly expressed in the normal sinus mucosa. These results demonstrate that the CCL20 protein is localized in epithelial and submucosal glandular cells and is up-regulated in chronic rhinosinusitis.

**COMMENT**

The present study demonstrated expression of CCL20 mRNA in normal and chronically inflamed human paranasal sinus mucosa and showed its up-regulation in chronic rhinosinusitis using RT-PCR. Furthermore, we localized the distribution of the CCL20 protein in the human sinus mucosa to the epithelial cells and submucosal glandular cells and demonstrated its up-regulation in rhinosinusitis mucosa through immunohistochemistry.

Chemokines are small-molecular-weight proteins that regulate leukocyte migration via the activation of 7 transmembrane-spanning G protein–coupled receptors. CCL20 has been demonstrated in human tonsillar crypts, inflamed intestinal epithelial cells, keratinocytes, lungs, appendix, and liver.7–9 It is released upon stimulation of cells with various proinflammatory cytokines, such as interleukin 1, tumor necrosis factor α, interleukin 17, and interferon gamma, as well as by other stimuli, including lipopolysaccharides, 12-O-tetradecanoylphorbol-13-acetate, and viral infection.11,12

CCL20 is a unique functional ligand for the chemokine receptor CCR6. The receptor is selectively expressed on immature dendritic cells, such as Langerhans cell precursors, a subpopulation of dendritic cells that reside at mucosal surfaces.3 It is also expressed on distinct subpopulation of antigen-activated CD4+ T lymphocytes and B cells.13,14 Therefore, it is possible that CCL20 functions as an important chemokine for the recruitment of a distinct population of CCR6-expressing immature dendritic cells to the airway for subsequent antigen presentation.

In this study, RT-PCR analyses of human sinus mucosal cells indicated that CCL20 mRNA is constitutively expressed in the normal paranasal sinus mucosa and is
up-regulated in chronic rhinosinusitis mucosa. Also, immunohistochemical staining demonstrated predominant localization of CCL20 protein in the epithelium and submucosal glands in chronic rhinosinusitis mucosa. The localization pattern of CCL20 protein suggests that the expression of CCL20 may be induced by external stimuli, because the sinus mucosa first contacts the external milieu in the epithelium and submucosal glands. The constitutive and up-regulated expression of CCL20 by the human sinus mucosa demonstrated in the present study is consistent with a role for epithelial cell–produced CCL20 in modulating mucosal adaptive immune responses.

The up-regulation of CCL20 mRNA and protein in chronic rhinosinusitis epithelium is in agreement with previous reports of its up-regulation in other organs in response to inflammatory stimuli. This up-regulation of CCL20 in chronic rhinosinusitis suggests that human paranasal sinus epithelial cells regulate the expression of CCL20, which is an important chemokine for the recruitment of immature dendritic cells that are essential for subsequent antigen presentation and the development of host adaptive immune response. Thus, in response to inflammatory stimuli, the paranasal sinus mucosal epithelial cells may develop the ability to alter the cytokine microenvironment to influence the recruitment of immature dendritic cells that reside in close proximity to epithelial cells. It is also possible that constitutive low levels of epithelial expression of CCL20 in normal human sinus mucosa may serve to keep immature dendritic cells and memory T cells close to the epithelial surface, the first site of contact with antigen.

In conclusion, the expression of CCL20 protein and mRNA is up-regulated in chronic rhinosinusitis mucosa, and the CCL20 protein is localized in the epithelial and submucosal glandular cells. These findings support the current view that CCL20 is an inducible product of human airway epithelium that plays a role in modulating mucosal immunity. Further studies regarding the exact signaling pathways that induce the regulation of the CCL20 gene and protein are warranted.

Submitted for Publication: September 28, 2005; final revision received February 2, 2006; accepted February 12, 2006.

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Author Contributions: Dr H.-M. Lee had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: None.

Funding/Support: This study was supported by the Brain Korea 21 Project of the Ministry of Education and Human Resources Development, Seoul, Korea, 2005.
REFERENCES


