

Mechanism of neutrophil recruitment induced by IL-8 in chronic sinusitis

Hideaki Suzuki, MD,^a Yuichi Takahashi, MD,^b Hideya Wataya, MD,^{a,c}
Katsuhisa Ikeda, MD,^a Seiichiro Nakabayashi, MD,^a
Akira Shimomura, MD,^a and Tomonori Takasaka, MD^a Sendai, Japan

Background: The mechanism of neutrophil recruitment in patients with chronic sinusitis is unclear.

Objective: This study aims to elucidate the role of IL-8 in inducing neutrophil accumulation in the nasal discharge of patients with chronic sinusitis.

Methods: Nasal discharge and mucosal specimens were obtained from two groups of patients, those with chronic sinusitis and those with allergic rhinitis. The samples were subjected to immunohistochemical examination and in situ hybridization. The IL-8 level in the nasal discharge was measured by enzyme immunoassay.

Results: Immunoreactivity to IL-8 was observed in polymorphonuclear cells of nasal smear, in nasal gland duct cells, and in epithelial cells of the chronic sinusitis group; whereas those of the allergic rhinitis group mostly showed little or no reaction. Similar patterns of localization were shown by in situ hybridization for IL-8 messenger RNA. The IL-8 level in nasal discharge was significantly higher in the chronic sinusitis group than in the allergic rhinitis group.

Conclusion: These results suggest that chemotactic factors in sinus effusion, including IL-8 derived from nasal gland duct cells and epithelial cells, attract neutrophils out of mucosa, and the neutrophils that have emigrated into the sinus effusion secrete IL-8. This induces further neutrophil accumulation in the sinus effusion of patients with chronic sinusitis. (*J Allergy Clin Immunol* 1996;98:659-70.)

Key words: IL-8, neutrophil, chemotaxis, chronic sinusitis, nasal discharge

Chronic sinusitis is a common inflammatory paranasal sinus disease, which is clinically characterized by edematous hypertrophy of the paranasal mucosa and persistent purulent nasal discharge and paranasal sinus effusion with selective and vigorous recruitment of neutrophils.¹⁻³ Despite the high incidence of the disease and many investigations into chronic sinusitis, its cause and pathogenesis are not clearly understood. Some patients are

Abbreviation used

PBS: Phosphate-buffered saline

known to have allergic predisposing factors,⁴ but in many patients upper respiratory infection precedes the onset of the disease.⁵ Because differentiation between allergic and infective sinusitis is sometimes difficult,⁶ careful clinical and laboratory examinations should be performed for accurate diagnosis. In bacterial culture, indigenous bacteria such as *Staphylococcus aureus* and *Haemophilus influenzae* are frequently isolated from the secretion in the middle nasal meatus where paranasal sinus ostia are located.⁷ However, remissions achieved in response to antibiotic therapy are only partial and temporary, and the course of the disease tends to be protracted and repeatedly aggravated. In recent years, it has been reported that administration of erythromycin is effective in

From ^aDepartment of Otolaryngology, Tohoku University School of Medicine, Sendai; ^bDepartment of Clinical and Laboratory Medicine, Tohoku University School of Medicine, Sendai; and ^cDepartment of Otolaryngology, Tohoku Rosai Hospital, Sendai.

Supported by Grant-In-Aid 05807156 to K. Ikeda from the Ministry of Education, Science and Culture of Japan.

Received for publication Aug. 1, 1995; revised Feb. 21, 1996; accepted for publication Feb. 23, 1996.

Reprint requests: Hideaki Suzuki, MD, Department of Otolaryngology, Tohoku University School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-77, Japan.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/1/73133

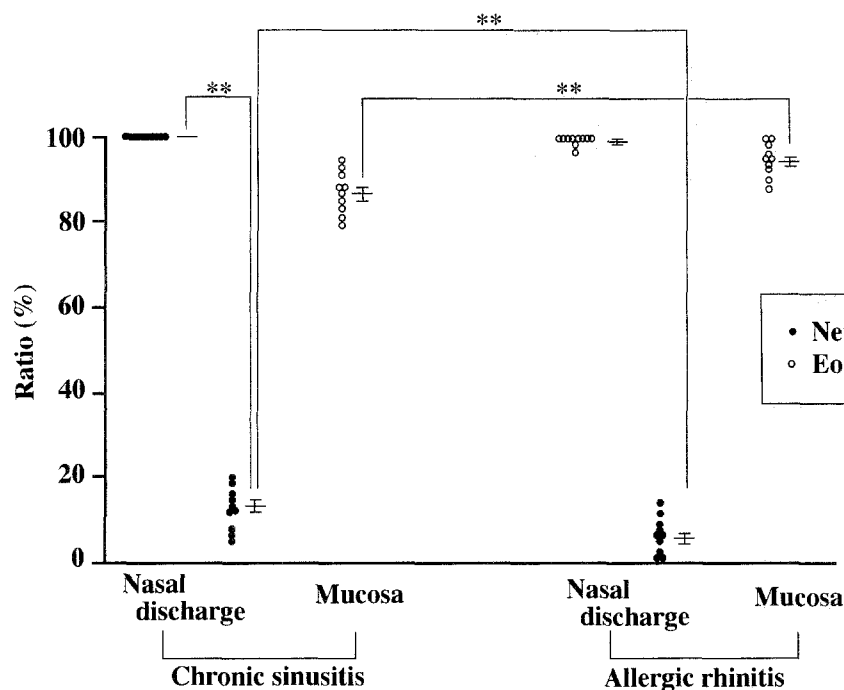


FIG. 1. Profile of infiltrated polymorphonuclear cells in patients with chronic sinusitis and patients with allergic rhinitis. Each value represents the ratio of neutrophils and eosinophils to the total number of polymorphonuclear cells ($n = 10$). Bars indicate mean $\pm$ SEM. $**p < 0.001$.

treating sinobronchial syndrome, even if the dose of the drug is below the level that exerts bactericidal action.⁸⁻¹⁰ These observations suggest that chronic sinusitis is not simply a chronic bacterial infection and that some other mechanism underlies the prolongation of this disease.

So far, the mechanism of neutrophil chemotaxis and the function of the neutrophils in the inflamed site of chronic sinusitis are not well documented. It has been observed that activation of recruited neutrophils brings about tissue destruction, as well as antibacterial defense by the host. In addition, neutrophils are known to be not merely terminal effector cells; they can also affect other cells by releasing various cytokines.¹¹⁻¹⁷ More recently, IL-8 has been discovered to be an inflammatory cytokine that is a potent activating and chemotactic factor of neutrophils.^{18, 19} It has been revealed that IL-8 participates in the pathogenesis of a variety of neutrophil-infiltrating chronic inflammatory diseases such as chronic bronchitis,^{20, 21} bronchiectasis,²⁰ cystic fibrosis,²⁰ rheumatoid arthritis,²²⁻²⁴ psoriasis,²⁵⁻²⁸ ulcerative colitis,²⁹⁻³¹ and Crohn's disease.^{30, 31} These results suggest an important reciprocal relationship between neutrophils and IL-8 in chronic sinusitis. This study was performed in an attempt to elucidate the role of IL-8 in

inducing neutrophil accumulation in nasal discharge of patients with chronic sinusitis.

METHODS

Reagents

Anti-human IL-8 polyclonal antibody (made in rabbit) was purchased from Upstate Biotechnology, Inc. (Lake Placid, N.Y.). Biotinylated anti-rabbit IgG antibody (made in goat), normal goat serum, normal rabbit serum, and avidin-biotinylated peroxidase complexes were obtained from Vector Laboratory, Inc. (Burlingame, Calif.). Diaminobenzidine tetrahydrochloride was purchased from Sigma Chemical Co. (St. Louis, Mo.), and Hansel solution (Eosinostain) was purchased from Torii Co. (Tokyo, Japan). DIG Oligonucleotide Tailing Kit and DIG Nucleic Acid Detection Kit were purchased from Boehringer Mannheim (Mannheim, Germany).

Patients

Samples were obtained from two groups of patients, those with nonallergic chronic sinusitis (chronic sinusitis group) and those with allergic rhinitis but without sinusitis (allergic rhinitis group). Chronic sinusitis was diagnosed by clinical history, rhinoscopic examination, paranasal sinus x-rays studies, dominant neutrophils without eosinophils in nasal smear, and negative RAST for specific serum IgE. Allergic rhinitis was diagnosed by clinical history, rhinoscopic examination, paranasal sinus x-ray studies, dominant eosinophils with a negligible

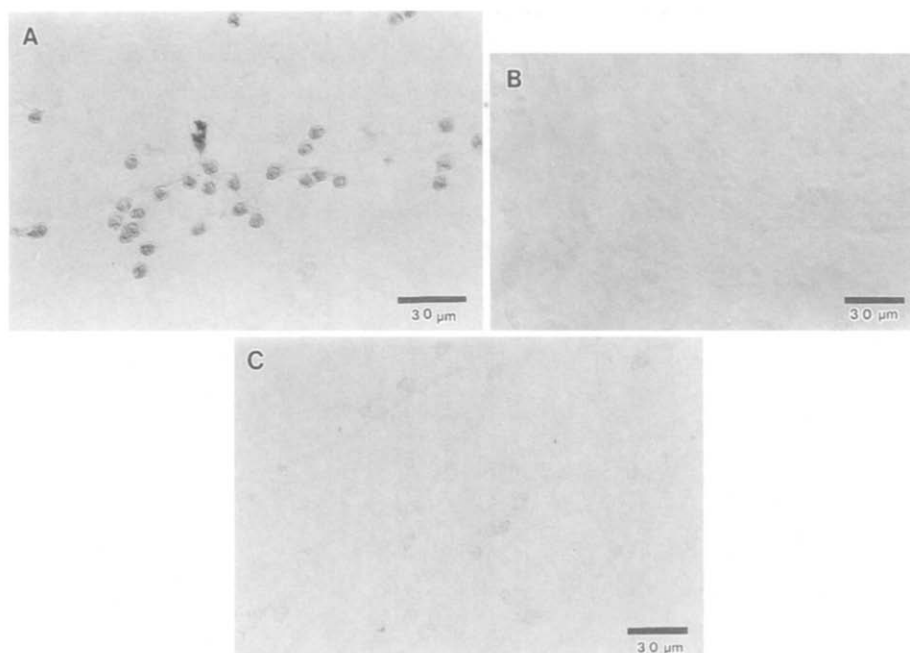


FIG. 2. Immunohistochemical staining for IL-8 of the nasal smear. **A**, Chronic sinusitis. Positive staining is observed in the cytoplasm of polymorphonuclear cells. **B**, Allergic rhinitis. **C**, Chronic sinusitis. Negative control.

number of neutrophils in nasal smear, and positive RAST. House dust mite, Japanese cedar pollen, orchard grass pollen, and short ragweed pollen were examined as allergic antigens in RAST, which were evaluated to be positive when at least one item was present. The patients with allergic rhinitis were those with perennial allergy or seasonal allergy in their allergy season. The chronic sinusitis group consisted of 46 patients, 30 male and 16 female subjects, ranging in age from 5 to 84 years, with an average age of 43.9 years. The allergic rhinitis group consisted of 41 patients, 17 male and 24 female subjects, ranging in age from 7 to 48 years, with an average age of 24.1 years.

Collecting samples

The patients did not receive antibiotics, antihistamines, antiallergic medicine, or corticosteroids for at least 1 week before sample collection. Three kinds of samples were collected from patients. (1) Nasal discharge was collected with a cotton-tipped swab, and spread on a poly-L-lysine-coated slide (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) for immunohistochemical examination or on an aminopropyltriethoxysilane-coated slide (Dako, Tokyo, Japan) for in situ hybridization. In the case of thick discharge, a small volume of saline solution was added before spreading. The smear preparations were dried in air and stored at -80°C until use. (2) Ethmoidal mucosa of patients with chronic sinusitis and inferior turbinate mucosa of patients with allergic rhinitis were obtained during sinus surgery and turbinectomy, respectively. The operation

TABLE I. Immunohistochemical reactivity for IL-8 of the nasal smear

	0	1+	2+	3+	Total
Chronic sinusitis	3	2	4	5	14
Allergic rhinitis	9	2	1	0	12

The number of positively stained cells was scored on a 4-point scale: 0 (no positive cells), 1+ (a few positive cells), 2+ (positive cells scattered throughout the field), and 3+ (positive cells forming clusters). Each digit represents the number of cases. The ratio of positive cases (1+ or more) of chronic sinusitis ($11/14 = 79\%$) was significantly higher than that of allergic rhinitis ($3/12 = 25\%$). (Chi square test, $p < 0.02$).

was performed while patients were under general anesthesia without local administration of anesthetic agents or vasoconstrictor drugs. The surgical specimens were fixed with 4% paraformaldehyde in 10 mmol/L phosphate-buffered saline (PBS) at pH 7.4 for 4 to 6 hours. (3) Nasal discharge was collected by suction and diluted 5 to 10 times with saline solution. After vortexing, the sample was centrifuged at 1000 g for 5 minutes, and the supernatant was stored at -20°C until use.

Cell counting

The nasal smears were stained with Hansel solution. The mucosal specimens were dehydrated in ethanol, sectioned at $4\text{ }\mu\text{m}$ while frozen, and stained with hematoxylin-eosin. The slides were examined under a light microscope, and about 100 polymorphonuclear cells

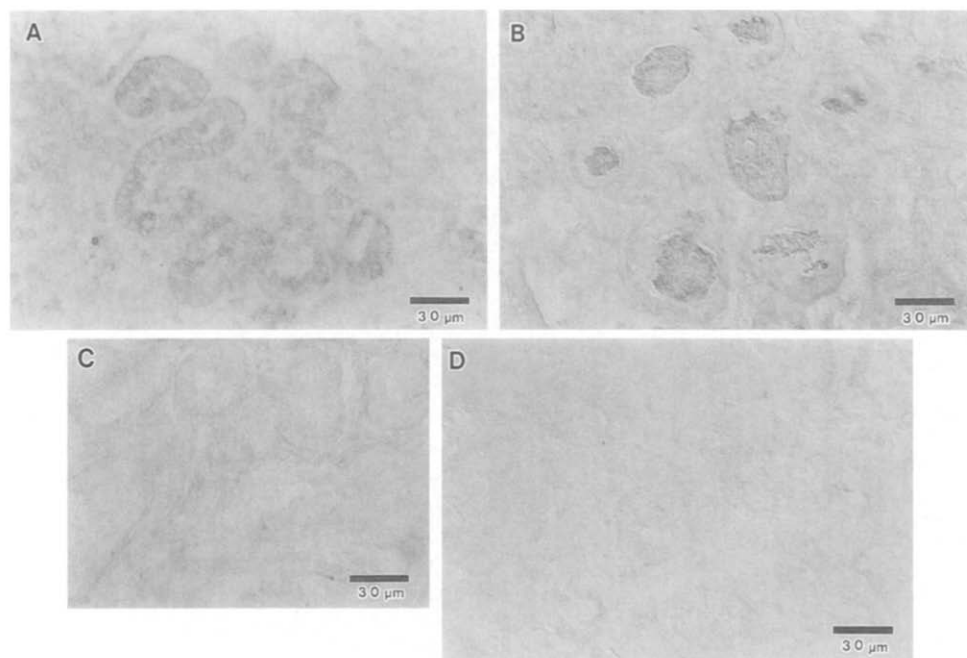


FIG. 3. Immunohistochemical staining for IL-8 of mucosal sections. **A**, Chronic sinusitis. Nasal gland duct cells are positively stained. **B**, Chronic sinusitis. Mucus in the nasal gland shows strong reactivity. **C**, Allergic rhinitis. **D**, Chronic sinusitis. Negative control.

were counted in each slide. In mucosal specimens infiltrated polymorphonuclear cells in the epithelium and the lamina propria were counted.

Immunohistochemical procedure

After fixation, the mucosal specimens were dehydrated in ethanol and sectioned at 4 μ m with a cryostat. The nasal smears were fixed with 4% paraformaldehyde in PBS for 5 minutes and subjected to the following process. The slides were soaked in 0.45% H_2O_2 /methanol for 30 minutes to block endogenous peroxidase, treated with 1% normal goat serum in PBS for 1 hour, and reacted with anti-human IL-8 antibody diluted $\times 2000$ in PBS containing 1% bovine serum albumin at 4° C overnight. The samples were treated with 10 μ g/ml biotinylated anti-rabbit IgG/PBS containing 1% bovine serum albumin for 1 hour, then incubated with avidin-biotinylated peroxidase complexes in PBS containing 1% bovine serum albumin for 1 hour and developed in 0.02% diaminobenzidine tetrahydrochloride and 0.006% H_2O_2 /0.05 mol/L Tris-HCl at pH 7.6 for 3 minutes. Between incubations the slides were washed with PBS. As a control, anti-human IL-8 antibody was replaced by normal rabbit serum.

In situ hybridization

In situ hybridization for IL-8 was performed as described by Miyamasu et al.³² A 5'-untranslated sequence of exon 1¹⁸ (Oncogene Science, Uniondale, N.Y.) was used for IL-8 antisense oligonucleotide probe and la-

beled with digoxigenin with a DIG Oligonucleotide Tailing Kit. Negative control was performed by substituting a sense probe for the antisense probe.

After fixation, ethmoidal mucosae from the chronic sinusitis group were dehydrated with ethanol and embedded in paraffin. They were then sectioned at a thickness of 2 μ m, placed on an aminopropyltriethoxysilane-coated slide, and deparaffined. The nasal smear was fixed with 4% paraformaldehyde in PBS and then subjected to the following process. The slides were treated with 0.2N HCl for 10 minutes, washed with PBS, and dehydrated with ethanol. They were then treated with predigested pronase (Calbiochem, San Diego, Calif.) at room temperature for 15 minutes for prehybridization. After washing with 0.2% glycine/PBS, the slides were acetylated in 0.25% acetic anhydride/0.1 mol/L triethanolamine, washed, dehydrated with ethanol, and dried in air. The hybridization mixture contained a digoxigenin-labeled IL-8 oligonucleotide probe (50 pmol/ml), salmon sperm DNA (80 mg/ml), yeast transfer RNA (550 μ g/ml), 50% formamide, 10 mmol/L Tris-HCl, 0.3 mol/L NaCl, 1 mmol/L ethylenediamine tetraacetic acid, 10% dextran sulfate, and 1 $\times$ Denhard's solution. The slides were incubated with the hybridization mixture for 18 hours at 45° C, washed, and treated in 3% blocking reagents (Boehringer Mannheim) for 30 minutes. The slides were then incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody for 2 hours and developed by nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate containing levamisole with a DIG Nucleic Acid Detection Kit.

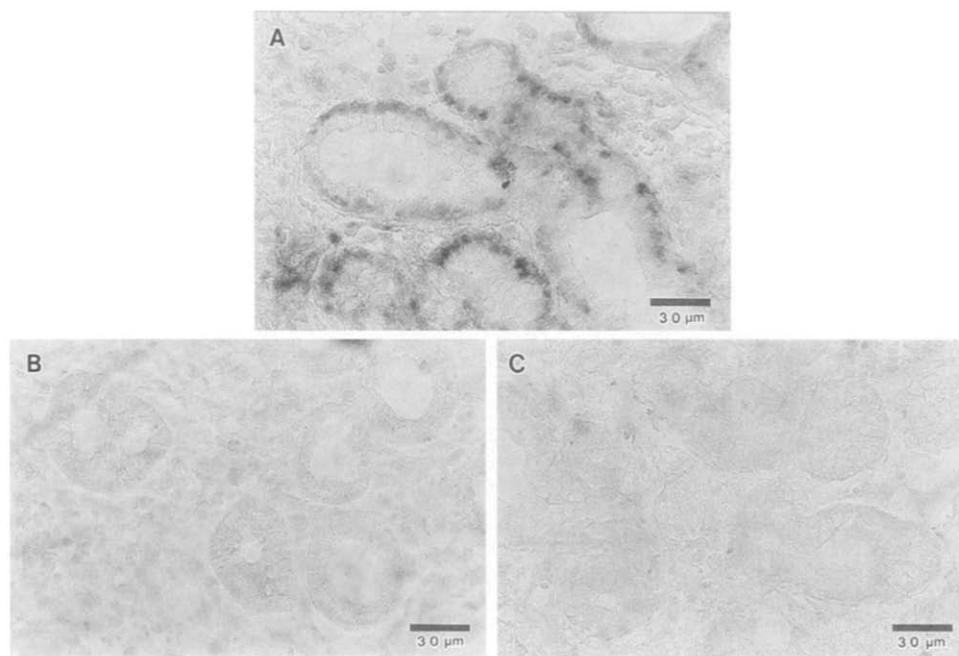


FIG. 4. In situ hybridization for IL-8 mRNA of mucosal sections. **A**, Chronic sinusitis. Expression of mRNA can be seen in nasal gland duct cells. **B**, Allergic rhinitis. **C**, Chronic sinusitis. Negative control.

Enzyme immunoassay

The supernatant of nasal discharge was subjected to enzyme immunoassay for IL-8 with the Biotrak IL-8 ELISA system (Amersham International plc., Little Chalfont, U.K.) according to the manufacturer's instructions. Assay range was 187.5 to 6000 pg/ml.

Statistics

Data values were expressed as means $\pm$ SEM, and statistical significance of differences was tested by using a two-tailed Student's *t* test, unless otherwise indicated. Differences were estimated to be significant when *p* was less than 0.05.

RESULTS

Profile of infiltrated polymorphonuclear cells

The ratios of neutrophils and eosinophils to the total number of polymorphonuclear cells are summarized in Fig. 1. Polymorphonuclear cells were all neutrophils in the nasal discharge of the chronic sinusitis group, whereas eosinophils were absolutely dominant in the allergic rhinitis group ($99.5\% \pm 0.3\%$, $n = 10$). In mucosal sections infiltrated polymorphonuclear cells were distributed mainly in the lamina propria, and eosinophils were predominant compared with neutrophils in both the chronic sinusitis group ($86.8\% \pm 1.6\%$, $n = 10$) and the allergic rhinitis group ($94.6\% \pm 1.1\%$, $n = 10$). The ratio of neutrophils in the mucosa of the chronic sinusitis group ($13.2\% \pm$

1.6% , $n = 10$) was significantly higher than that of the allergic rhinitis group ($5.4\% \pm 1.1\%$, $n = 10$; $p < 0.001$) but was still much lower than the ratio of neutrophils in the nasal discharge of the chronic sinusitis group ($p < 0.001$).

Immunohistochemical localization of IL-8

Two sets of nasal smear samples were obtained from one patient simultaneously, and immunostaining was performed on each of the samples. Most of the polymorphonuclear cells in the nasal smears of patients with chronic sinusitis stained positively for IL-8 (Fig. 2, *A*), whereas polymorphonuclear cells of patients with allergic rhinitis showed little or no reaction (Fig. 2, *B*). The number of positively stained cells was scored on a 4-point scale: 0 (no positive cells), 1+ (a few positive cells), 2+ (positive cells scattered throughout the field), and 3+ (positive cells forming clusters). The results are summarized in Table I. In the chronic sinusitis group, 11 of 14 patients (79%) showed positive staining (1+ or more), whereas in the allergic rhinitis group three of 12 patients (25%) showed positive staining. The positive ratios were significantly different between the two groups (chi square test, $p < 0.02$).

Eleven samples of ethmoidal mucosae in the chronic sinusitis group and five samples of inferior turbinate mucosae in the allergic rhinitis group

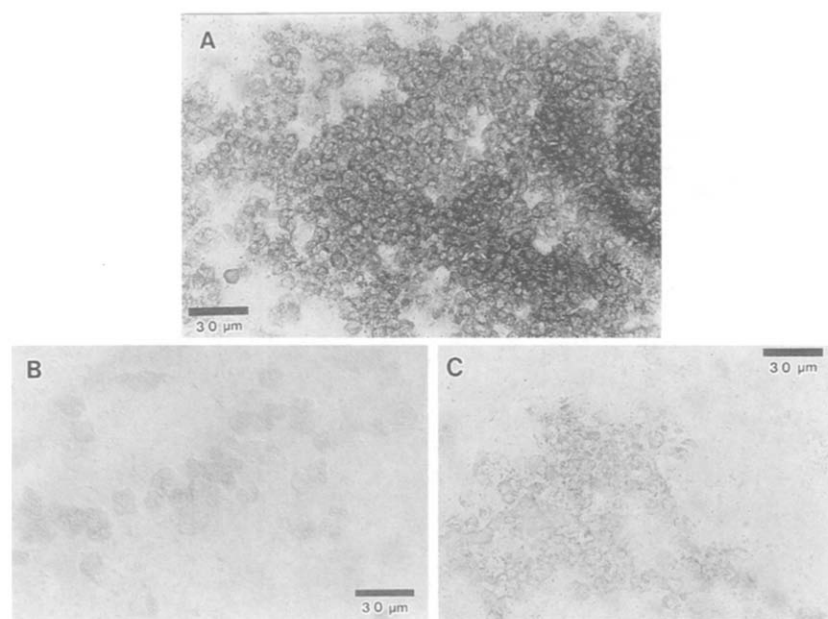


FIG. 5. In situ hybridization for IL-8 mRNA of the nasal smear. **A**, Chronic sinusitis. Polymorphonuclear cells exhibit positive staining. **B**, Allergic rhinitis. **C**, Chronic sinusitis. Negative control.

were examined. IL-8 was found to be localized in the nasal gland duct cells of the chronic sinusitis group (Fig. 3, *A*). Mucus in the nasal gland of patients with chronic sinusitis also stained positively (Fig. 3, *B*). Meanwhile, the same structures in patients with allergic rhinitis showed little or no staining (Fig. 3, *C*). Infiltrated neutrophils and eosinophils were negative or only weakly positive in both groups, and there was no apparent difference between the two. Epithelial cells of the chronic sinusitis group showed positive staining in one case and little or no staining in two cases; however, the epithelium was severely damaged, and no definitive results could be obtained in the other eight cases of chronic sinusitis.

Expression of IL-8 messenger RNA

Six specimens of the ethmoidal mucosae and eight specimens of the nasal smear of patients with chronic sinusitis, and six specimens of the inferior turbinate mucosae and four specimens of the nasal smear of patients with allergic rhinitis were subjected to in situ hybridization for IL-8 mRNA. The results showed patterns of localization similar to those observed in the immunohistochemical study; that is, in mucosal specimens IL-8 mRNA was expressed in nasal gland duct cells (Fig. 4, *A*), and in nasal smear polymorphonuclear cells were stained positively (Fig. 5, *A*) in specimens from patients with chronic sinusitis. In contrast, IL-8

mRNA was detected in neither the inferior turbinate mucosa nor the nasal smear of patients with allergic rhinitis (Fig. 4, *B*, and Fig. 5, *B*). As a control, only faint or no staining was observed when sense IL-8 oligonucleotide probe was used (Fig. 4, *C*, and Fig. 5, *C*).

IL-8 level in nasal discharge

IL-8 level in the nasal discharge of patients with chronic sinusitis was 23.9 ± 5.4 nmol/L ($n = 16$), which was significantly higher than that of the allergic rhinitis group (2.1 ± 0.6 nmol/L, $n = 13$) (Fig. 6).

DISCUSSION

Major inflammatory sites of chronic sinusitis are located in the paranasal mucosa and sinus effusion. Several authors have investigated IL-8 expression and secretion in normal and inflammatory nasal and paranasal mucosa.^{3, 33} However, the functional importance of emigrated neutrophils in the sinus effusion has been overlooked. Considering that neutrophils are not only terminal effector cells but also immunocompetent cells that play an active role in the regulation of the inflammatory process by secreting a variety of cytokines such as IL-1 α , IL-1 β , IL-6, interferon- α , tumor necrosis factor,¹¹⁻¹⁴ and IL-8,¹⁵⁻¹⁷ the large number of neutrophils recruited in the paranasal sinus effusion of patients with chronic sinusitis is likely to be significant. Sinus effusion flows out into the nasal cavity

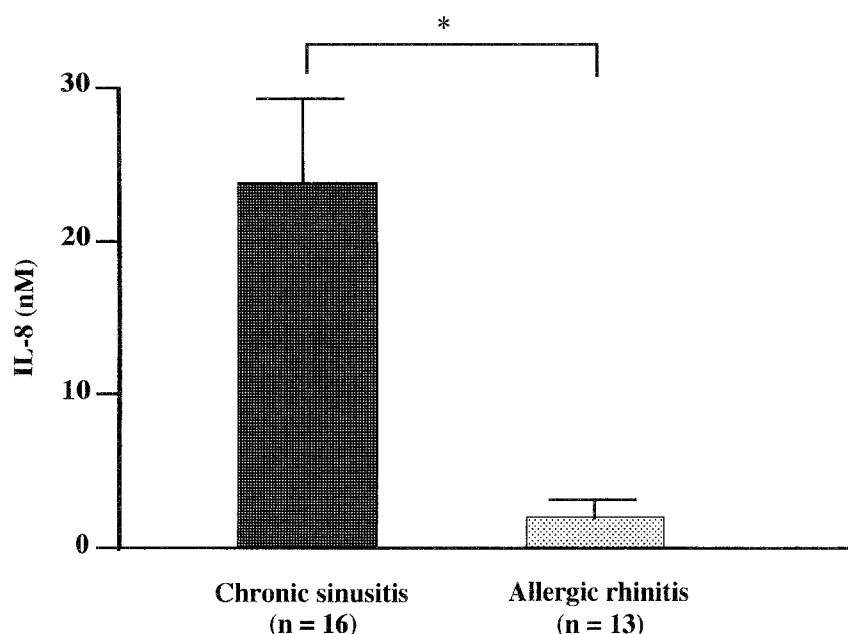


FIG. 6. IL-8 level in nasal discharge. Nasal discharge was collected by suction, diluted 5 to 10 times with saline solution and centrifuged at 1000 *g* for 5 minutes. The supernatant was then subjected to enzyme immunoassay for IL-8 with Biotrak IL-8 ELISA system (Amersham International plc.) according to the manufacturer's instructions. **p* < 0.002.

to become nasal discharge, which is easily accessible and should accurately reflect the quality of the sinus effusion. We therefore examined nasal discharge instead of sinus effusion in this study.

First of all, we have shown that most of the polymorphonuclear cells in the nasal discharge were neutrophils, whereas their population was minor in the ethmoidal mucosa in the chronic sinusitis group (Fig. 1). It has been previously shown that eosinophils are much more predominant than neutrophils in sinus mucosa and nasal polyps of patients with nonallergic sinusitis, as well as in those patients with allergic sinusitis,^{4, 34} which is consistent with our results. This implies that neutrophils migrate from the mucosa into the sinus effusion immediately after extravascular transmigration.

Immunohistochemical analysis revealed that IL-8 was localized in emigrated polymorphonuclear cells in the nasal discharge of patients with chronic sinusitis (Fig. 2, *A*). In contrast, polymorphonuclear cells in the nasal discharge of the allergic rhinitis group lack IL-8 in most cases (Fig. 2, *B*), indicating that neutrophil prevalence in nasal discharge correlates with the IL-8 expression of the emigrated cells. Taking into account the studies that have shown that IL-8 is a potent neutrophil chemotactic factor^{18, 19} and that neutrophils themselves are capable of producing IL-8 in response to

several kinds of stimuli,¹⁵⁻¹⁷ it is likely that the neutrophils that have migrated into sinus effusion produce and secrete IL-8, and therefore further neutrophil accumulation occurs in the sinus effusion. The expression of IL-8 mRNA, revealed by in situ hybridization (Fig. 5, *A*), strongly suggests that the neutrophils in the nasal discharge of patients with chronic sinusitis actually produce IL-8, and this supports the positive feedback mechanism of neutrophil recruitment. There are two possible explanations for the discrepancy in the IL-8 expression of neutrophils between the mucosa and the secretions. One is that neutrophils are triggered to produce IL-8 in the paranasal sinus effusion, possibly by lipopolysaccharide or cytokines, such as IL-1 β and tumor necrosis factor.¹⁷ The other possibility is that neutrophils in the mucosa are already activated but quickly emigrate into the effusion before they start to produce IL-8.

IL-8 and mRNA for IL-8 were also localized in nasal gland duct cells in the ethmoidal mucosae of patients with chronic sinusitis (Fig. 3, *A*, and Fig. 4, *A*), as demonstrated in the lower respiratory tract in a previous study.³⁵ Because mucus in the nasal gland duct showed strong immunoreactivity to IL-8 (Fig. 3, *B*), it is very likely that these cells produce and secrete IL-8 and elicit neutrophil migration into the sinus effusion. Although the presence of IL-8 in epithelial cells was not observed in all the

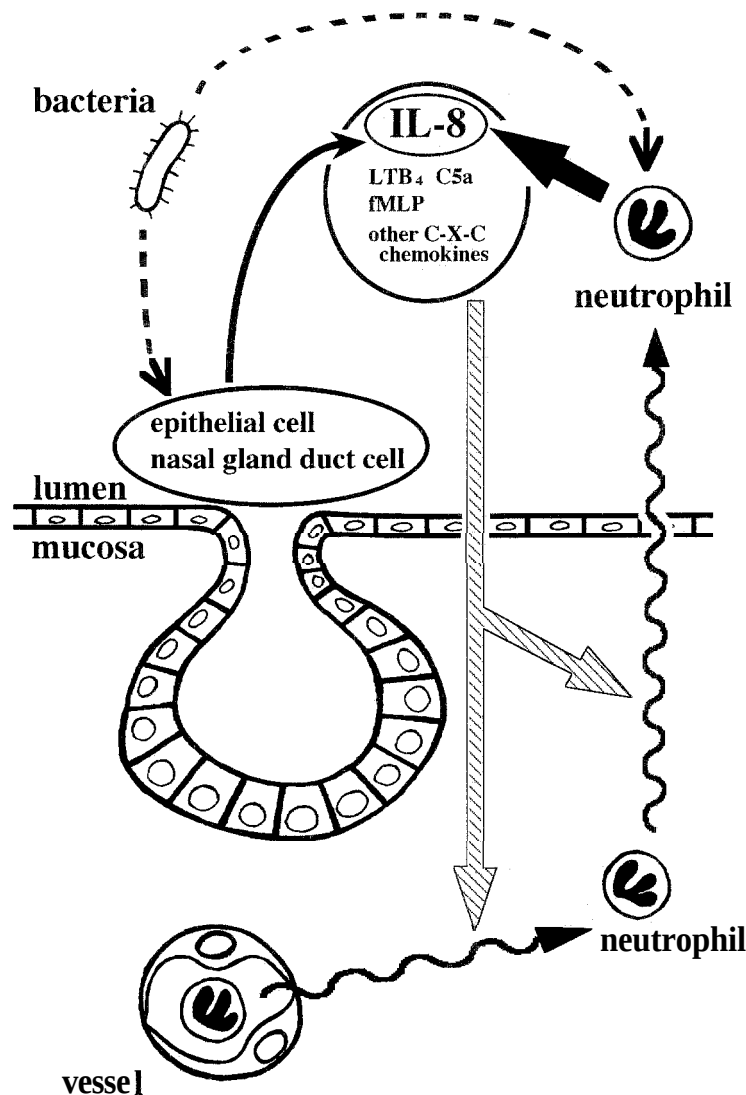


FIG. 7. Hypothesis of neutrophil recruitment in chronic sinusitis. Neutrophil chemotactic substances including IL-8 released from nasal gland duct cells and epithelial cells (*thin solid arrow*) initiate neutrophil migration (*striped arrows*) out of vessels to the luminal side and eventually into sinus effusion (*wavy arrows*). Emigrated neutrophils produce and secrete IL-8 (*thick solid arrow*), which further induces neutrophil migration and accumulation in sinus effusion. Bacteria and bacterial components may stimulate neutrophils, epithelial cells, and nasal gland duct cells to secrete IL-8 (*dotted arrows*). Accumulated neutrophils in sinus effusion probably release proteases and superoxides, which impair mucociliary function of the paranasal epithelium, and this causes retention of sinus effusion. As a result, the whole inflammatory process is prolonged. *LTB₄*, Leukotriene B₄; *fMLP*, *N*-formyl-methionyl-leucyl-phenylalanine.

cases of chronic sinusitis in our study, it is quite possible that these cells also release IL-8 into the sinus effusion as shown in previous investigations.^{33, 36} Our study also revealed that the IL-8 level in the nasal discharge of patients with chronic sinusitis was more than 20 nmol/L (Fig. 6), being about 10 times higher than that which exhibits strong chemotactic activity for human neutrophils *in vitro*.²⁸ This evidence indicates that IL-8 in sinus

effusion is potent as a neutrophil chemoattractant *in vivo*.

On the basis of these findings, we propose a hypothesis of neutrophil recruitment in chronic sinusitis as shown in Fig. 7. Viral and bacterial infections, which are common triggers of sinusitis, induce IL-8 and arachidonic acid metabolites including leukotriene B₄ from epithelial cells.^{33, 35-38} A potent neutrophil chemotactic peptide, C5a,

may be generated by complement activation, as occurs in the lung.³⁹ In addition, bacteria would release chemotactic oligopeptides such as *N*-formyl-methionyl-leucyl-phenylalanine. These chemotactic substances initiate neutrophil migration out of vessels in a luminal direction and eventually into the sinus effusion. The emigrated neutrophils then produce and secrete IL-8, and this induces further neutrophil migration and accumulation in the sinus effusion. Bacteria and bacterial components may stimulate neutrophils, epithelial cells, and nasal gland duct cells to secrete IL-8.^{17, 35, 36} Accumulated neutrophils in the sinus effusion probably release proteases and superoxides, which impair the mucociliary function of the paranasal epithelium,⁴⁰⁻⁴² and this causes retention of the sinus effusion. As a result, the whole process is prolonged, and eventually a chronic inflammatory state is established.

Vascular adhesion and transendothelial migration of neutrophils would be mediated by adhesion molecules, particularly intercellular adhesion molecule-1 and E-selectin, which is known to participate in extravasation of neutrophils and lymphocytes⁴³⁻⁴⁵ and to be expressed in vascular endothelial cells of the paranasal mucosa of patients with chronic sinusitis.^{46, 47} Expression of adhesion molecules in the paranasal mucosa would be regulated by another cytokine network including IL-1 β .² Although C-C chemokines such as RANTES, monocyte chemotactic protein-3, eotaxin, and macrophage inflammatory protein-1 α are known to be potent eosinophil chemoattractants *in vitro* and are expressed in eosinophil-infiltrated allergic tissues,⁴⁸⁻⁵⁰ these chemokines have not been demonstrated in nonallergic sinusitis mucosa. The considerable number of eosinophils in chronic sinusitis mucosa can be explained differently. Intercellular adhesion molecule-1 and E-selectin can induce extravasation of eosinophils,⁵¹ as well as neutrophils and lymphocytes. If neutrophils immediately move out to the lumen, where neutrophil chemotactic factors such as IL-8 are present, the absence of eosinophil-specific chemoattractant would result in an apparent dominance of eosinophils over neutrophils in the mucosa.

It has been disclosed in recent years that the C-X-C chemokine family includes a series of neutrophil chemotactic peptides such as neutrophil-activating peptide-2,^{52, 53} growth-related proteins α , β , and γ ,⁵³⁻⁵⁵ epithelial-derived neutrophil-activating factor-78,^{56, 57} macrophage inflammatory protein-2,^{58, 59} granulocyte chemotactic protein-2,^{60, 61} as well as IL-8. Participation of these chemokines in neutrophil recruitment in paranasal

sinuses is to be further investigated. Another question arising from this study is: To what extent is IL-8 responsible for the entire chemotactic activity of the nasal discharge of patients with chronic sinusitis? This will be studied by *in vitro* and *in vivo* neutrophil chemotaxis assay.

According to our hypothesis, paranasal sinus effusion plays an essential role in the prolongation of the inflammatory process as previously speculated,² and this is in agreement with a clinical feature of chronic sinusitis. In recent years, functional endoscopic sinus surgery has been reported to yield satisfactory results⁶²⁻⁶⁶ and is currently the prevailing surgical treatment for chronic sinusitis, taking the place of conventional radical sinus surgery. In functional endoscopic sinus surgery, passages connecting paranasal sinuses with the nasal cavity are widely opened to allow the drainage of sinus effusion while most of the inflamed paranasal mucosa is preserved. Endoscopic observation of the sinus suggests that mucociliary clearance becomes reestablished several weeks after the surgery.⁶⁷ The mechanism of recovery can be reasonably explained by our hypothesis; that is, the positive feedback loop of neutrophil recruitment is interrupted by the absence of the sinus effusion, and the inflammation process subsides. Nevertheless, if the mucosal damage is too severe, ciliary function may become irreversibly impaired, and simply opening the inflamed sinuses by surgery would not cure the patient.

Chronic sinusitis is frequently associated with lower respiratory tract diseases, such as diffuse panbronchiolitis. This association is referred to as sinobronchial syndrome.⁶⁸ Both diseases have some characteristics in common. Both of them affect the respiratory tract and manifest mucosal thickening^{68, 69} and persistent purulent secretion with marked neutrophil emigration.^{21, 70} Moreover, a high level of IL-8 is detected in the bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis,^{21, 70} and recruited neutrophils express IL-8 mRNA in a lower respiratory tract infection.³⁵ It is, accordingly, conceivable that chronic sinusitis and diffuse panbronchiolitis may share the same mechanism of neutrophil recruitment. Recently, low-dose and long-term erythromycin treatment has proved to be effective for sinobronchial syndrome^{9, 10} and diffuse panbronchiolitis.^{8, 69-72} Nagai et al.⁸ suggested that this drug does not act as a simple bactericide because they observed that the maximum serum and sputum levels of erythromycin during low-dose erythromycin therapy were below the minimum inhibitory concentrations of

clinically pathogenic bacteria. It has been shown that the number of neutrophils, neutrophil chemotactic activity, and IL-8 level in the bronchoalveolar lavage fluid were reduced after the administration of erythromycin in patients with diffuse panbronchiolitis.^{70, 72} It is also known that erythromycin inhibits the motile ability of neutrophils.^{70, 73, 74} These lines of evidence suggest that this drug may inhibit the positive feedback loop of IL-8 and neutrophil recruitment. The mechanism of the inhibition of IL-8 and neutrophil migration by macrolide antibiotics remains to be investigated and may lead to the development of a cytokine therapy for chronic sinusitis, as well as for diffuse panbronchiolitis.

REFERENCES

- Carenfelt C. Pathogenesis of sinus empyema. *Ann Otol Rhinol Laryngol* 1979;8:16-20.
- Itoh K, Katahira S, Matsuzaki T, Ohya M, Fukuda K. Retention fluids of chronic sinusitis induce neutrophil adherence to microvascular endothelial cells. *Acta Otolaryngol* 1992;112:882-9.
- Takeuchi K, Yuta A, Sakakura Y. Interleukin-8 gene expression in chronic sinusitis. *Am J Otolaryngol* 1995;16:98-102.
- Demoly P, Crampette L, Mondain M, Campbell AM, Lequeux N, Enandes I, et al. Assessment of inflammation in noninfectious chronic maxillary sinusitis. *J Allergy Clin Immunol* 1994;94:95-108.
- Wald ER. Sinusitis in children. *N Engl J Med* 1992;326:319-23.
- Mabry RL. Allergic and infective rhinosinusitis: differential diagnosis and interrelationship. *Otolaryngol Head Neck Surg* 1994;111:335-9.
- Legent F, Bordure P, Beauvillain C, Berche P. A double-blind comparison of ciprofloxacin and amoxicillin/clavulanic acid in the treatment of chronic sinusitis. *Chemotherapy* 1994;40(Suppl 1):8-15.
- Nagai H, Shishido H, Yoneda R, Yamaguchi E, Tamura A, Kurashima A. Long-term low-dose administration of erythromycin to patients with diffuse panbronchiolitis. *Respiration* 1991;58:145-9.
- Iino Y, Toriyama M, Kudo K, Natori Y, Yuo A. Erythromycin inhibition of lipopolysaccharide-stimulated tumor necrosis factor α production by human monocytes *in vitro*. *Ann Otol Rhinol Laryngol* 1992;Suppl 157:16-20.
- Iino Y, Sugita K, Toriyama M, Kudo K. Erythromycin therapy for otitis media with effusion in sinobronchial syndrome. *Arch Otolaryngol Head Neck Surg* 1993;119:648-51.
- Lindemann A, Riedel D, Oster W, Meuer SC, Blohm D, Mertelsmann RH, et al. Granulocyte/macrophage colony-stimulating factor induces interleukin 1 production by human polymorphonuclear neutrophils. *J Immunol* 1988;140:837-9.
- Shirafuji N, Matsuda S, Ogura H, Tani K, Kodo H, Ozawa K, et al. Granulocyte colony-stimulating factor stimulates human mature neutrophilic granulocytes to produce interferon- α . *Blood* 1989;75:17-9.
- Cicco NA, Lindemann A, Content J, Vandenbussche P, Lübbert M, Gauss J, et al. Inducible production of interleukin-6 by human polymorphonuclear neutrophils: role of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor- α . *Blood* 1990;75:2049-52.
- Dubravac DB, Spriggs DR, Mannick JA, Rodrick ML. Circulating human peripheral blood granulocytes synthesize and secrete tumor necrosis factor α . *Proc Natl Acad Sci USA* 1990;87:6758-61.
- Strieter RM, Kashara K, Allen R, Showell HJ, Standiford TJ, Kunkel SL. Human neutrophils exhibit disparate chemotactic factor gene expression. *Biochem Biophys Res Commun* 1990;173:725-30.
- Bazzoni F, Cassatella MA, Róssi F, Ceska M, Dewald B, Baggiolini M. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. *J Exp Med* 1991;173:771-4.
- Fujishima S, Hoffman AR, Vu T, Kim KJ, Zheng H, Daniel D, et al. Regulation of neutrophil interleukin 8 gene expression and protein secretion by LPS, TNF- α , and IL-1 β . *J Cell Physiol* 1993;154:478-85.
- Mukaida N, Shiroo N, Matsushima K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. *J Immunol* 1989;143:1366-71.
- Yuo A, Kitagawa S, Kasahara T, Matsushima K, Saito M, Takaku F. Stimulation and priming of human neutrophils by interleukin-8: cooperation with tumor necrosis factor and colony-stimulating factors. *Blood* 1991;78:2708-14.
- Richman-Eisenstat JB, Jorens PG, Hebert CA, Ueki I, Nadel JA. Interleukin-8: an important chemoattractant in sputum of patients with chronic inflammatory airway diseases. *Am J Physiol* 1993;264:L413-8.
- Koga T. Neutrophilia and high level of interleukin 8 in the bronchoalveolar lavage fluid of diffuse panbronchiolitis. *Kurume Med J* 1993;40:139-46.
- Brennan FM, Zachariae COC, Chantry D, Larsen CG, Turner M, Maini RN, et al. Detection of interleukin-8 biological activity in synovial fluids from patients with rheumatoid arthritis and production of interleukin-8 mRNA by isolated synovial cells. *Eur J Immunol* 1990;20:2141-4.
- Piechl P, Ceska M, Effenberger F, Haberhauer G, Broell H, Lindley IJD. Presence of NAP-1/IL-8 in synovial fluids indicates a possible pathogenic role in rheumatoid arthritis. *Scand J Immunol* 1991;34:333-9.
- Seitz M, Dewald B, Gerber N, Baggiolini M. Enhanced production of neutrophil-activating peptide-1/interleukin-8 in rheumatoid arthritis. *J Clin Invest* 1991;87:463-9.
- Camp RDR, Fincham NJ, Cunningham FM, Greaves MW, Morris J, Chu A. Psoriatic skin lesions contain biologically active amounts of an interleukin-1-like compound. *J Immunol* 1986;137:3469-74.
- Fincham NJ, Camp RDR, Gearing AJH, Bird CR, Cunningham FM. Neutrophil chemoattractant and IL-1 like activity in samples from psoriatic lesions: further characterization. *J Immunol* 1988;140:4294-9.
- Nickoloff BJ, Karabin GD, Barker JNWN, Griffiths CE, Sarma V, Mitra RS, et al. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor- α in psoriasis. *Am J Pathol* 1991;138:129-40.
- Takematsu H, Tagami H. Quantification of chemotactic peptides (C5a anaphylatoxin and IL-8) in psoriatic lesional skin. *Arch Dermatol* 1993;129:74-80.
- Raab Y, Gerdin B, Ahlstedt S, Hallgren R. Neutrophil mucosal involvement is accompanied by enhanced local

- production of interleukin-8 in ulcerative colitis. *Gut* 1993; 34:1203-6.
30. Mitsuyama K, Toyonaga A, Sasaki E, Watanabe K, Tateishi H, Nishiyama T, et al. IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. *Clin Exp Immunol* 1994;96:432-6.
31. Mazzucchelli L, Hauser C, Zraggen K, Wagner H, Hess M, Laissue JA, et al. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. *Am J Pathol* 1994;144:997-1007.
32. Miyamasu M, Hirai K, Takahashi Y, Iida M, Yamaguchi M, Koshino T, et al. Chemotactic agonists induce cytokine generation in eosinophils. *J Immunol* 1995;154:1339-49.
33. Becker S, Koren HS, Henke DC. Interleukin-8 expression in normal nasal epithelium and its modulation by infection with respiratory syncytial virus and cytokines, tumor necrosis factor, interleukin-1, and interleukin-6. *Am J Respir Cell Mol Biol* 1993;8:20-7.
34. Hamilos DL, Leung DY, Wood R, Cunningham L, Bean DK, Yasriel Z, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. *J Allergy Clin Immunol* 1995;96:537-44.
35. Inoue H, Massion PP, Ueki IF, Grattan KM, Hara M, Dohrmann AF, et al. *Pseudomonas* stimulates interleukin-8 mRNA expression selectively in airway epithelium, in gland ducts, and in recruited neutrophils. *Am J Respir Cell Mol Biol* 1994;11:651-63.
36. Kennedy JS, Baker C, Welch MR, Altman LC. Synthesis of interleukin-1 α , interleukin-6, and interleukin-8 by cultured human nasal epithelial cells. *J Allergy Clin Immunol* 1994; 93:1060-7.
37. Raz M, Robbins RA, Kelling CL, Stine LC, Leikauf GD, Rennard SI, et al. Viral infection of bovine bronchial epithelial cells induces increased neutrophil chemotactic activity and neutrophil adhesion. *Clin Sci* 1993;85:753-60.
38. Koyama S, Rennard SI, Leikauf GD, Shoji S, Von Essen S, Claassen L, et al. Endotoxin stimulates bronchial epithelial cells to release chemotactic factors for neutrophils. A potential mechanism for neutrophil recruitment, cytotoxicity, and inhibition of proliferation in bronchial inflammation. *J Immunol* 1991;147:4293-301.
39. Milks LC, Brontoli MJ, Cramer EB. Epithelial permeability and the transepithelial migration of human neutrophils. *J Cell Biol* 1983;96:1241-7.
40. Takasaka T, Sato M, Onodera A. A typical cilia of the human nasal mucosa. *Ann Otol* 1980;89:37-45.
41. Ohashi Y, Nakai Y. Reduced ciliary action in chronic sinusitis. *Acta Otolaryngol* 1983;Suppl 397:3-9.
42. Ohashi Y, Nakai Y. Functional and morphological pathology of chronic sinusitis mucous membrane. *Acta Otolaryngol* 1983;Suppl 397:11-48.
43. Nakajima H, Sano H, Nishimura T, Yoshida S, Iwamoto I. Role of vascular cell adhesion molecule 1/very late activation antigen 4 and intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 interactions in antigen-induced eosinophil and T cell recruitment into the tissue. *J Exp Med* 1994;179:1145-54.
44. Bevilacqua MP, Steingelin S, Gimbrone MA Jr, Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 1989;243:1160-5.
45. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 1991;349:796-9.
46. Lund VJ, Henderson B, Song Y. Involvement of cytokines and vascular adhesion receptors in the pathology of frontoethmoidal mucocoeles. *Acta Otolaryngol* 1993;113:540-6.
47. Tokushige E, Itoh K, Ushikai M, Katahira S, Fukuda K. Localization of IL-1 β mRNA and cell adhesion molecules in the maxillary sinus mucosa of patients with chronic sinusitis. *Laryngoscope* 1994;104:1245-50.
48. Ying S, Taborda-Barata L, Meng Q, Humbert M, Kay AB. The kinetics of allergen-induced transcription of messenger RNA for monocyte chemoattractant protein-3 and RANTES in the skin of human atopic subjects: relationship to eosinophil, T-cell, and macrophage recruitment. *J Exp Med* 1995;181:2153-9.
49. Jose PJ, Adcock IM, Griffiths-Johnson DA, Berkman N, Wells TNC, Williams TJ, et al. Eotaxin: cloning of an eosinophil chemoattractant cytokine and increased mRNA expression in allergen-challenged guinea-pig lungs. *Biochem Biophys Res Commun* 1994;205:788-94.
50. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. RANTES and macrophage inflammatory protein 1 α induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;176:1489-95.
51. Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. *Am J Respir Cell Mol Biol* 1993;8:349-55.
52. Malkowski MG, Wu JY, Lazar JB, Johnson PH, Edwards BF. The crystal structure of recombinant human neutrophil-activating peptide-2 (M6L) at 1.9-Å resolution. *J Biol Chem* 1995;270:7077-87.
53. L'Heureux GP, Bourgoin S, Jean N, McColi SR, Naccache PH. Diverging signal transduction pathways activated by interleukin-8 and related chemokines in human neutrophils: interleukin-8, but not NAP-2 or GRO α , stimulates phospholipase D activity. *Blood* 1995;85:522-31.
54. Zagorski J, DeLarco JE. Expression of active human GRO β and GRO γ neutrophil chemotactic peptides in *E. coli*. *Protein Expr Purif* 1994;5:337-45.
55. Nakagawa H, Komorita N, Shibata F, Ikesue A, Konishi K, Fujioka M, et al. Identification of cytokine-induced neutrophil chemoattractants (CINC), rat GRO/CINC-2 α and CINC-2 β , produced by granulation tissue in culture: purification, complete amino acid sequences and characterization. *Biochem J* 1994;301:545-50.
56. Power CA, Furness RB, Brawand C, Wells TN. Cloning of a full-length cDNA encoding the neutrophil-activating peptide ENA-78 from human platelets. *Gene* 1994;151:333-4.
57. Schmodder RL, Strieter RM, Walz A, Kunkel SL. Epithelial-derived neutrophil-activating factor-78 production in human renal tubule epithelial cells and in renal allograft rejection. *Transplantation* 1995;59:118-24.
58. Frevert CW, Farone A, Danaee H, Paulauskis JD, Kobzik L. Functional characterization of rat chemokine macrophage inflammatory protein-2. *Inflammation* 1995;19:133-42.
59. Driscoll KE, Hassenbein DG, Howard BW, Isfort RJ, Cody D, Tindal MH, et al. Cloning, expression, and functional characterization of rat MIP-2: a neutrophil chemoattractant and epithelial cell mitogen. *J Leukoc Biol* 1995;58:359-64.
60. Proost P, Wuyts A, Conings R, Lenaerts JP, Billiau A, Opdenakker G, et al. Human and bovine granulocyte chemotactic protein-2: complete amino acid sequence and functional characterization as chemokines. *Biochemistry* 1993;32:10170-7.
61. Proost P, De Wolf Peeters C, Conings R, Opdenakker G, Billiau A, Van Damme J. Identification of a novel granu-

- locyte chemotactic protein (GCP-2) from human tumor cells. *In vitro* and *in vivo* comparison with natural forms of GRO, IP-10, and IL-8. *J Immunol* 1993;150:1000-10.
62. Rice DH. Endoscopic sinus surgery: results at 2-year follow-up. *Otolaryngol Head Neck Surg* 1989;101:476-9.
63. Levine HL. Functional endoscopic sinus surgery: evaluation, surgery, and follow-up of 250 patients. *Laryngoscope* 1990;100:79-84.
64. Lund VJ, Holmstrom M, Scadding GK. Functional endoscopic sinus surgery in the management of chronic rhinosinusitis: an objective assessment. *J Otol Laryngol* 1991;105:832-5.
65. Kennedy DW. Prognostic factors, outcomes, and staging in ethmoid sinus surgery. *Laryngoscope* 1992;102(Suppl 57):1-18.
66. King JM, Caldarelli DD, Pigato JB. A review of revision functional endoscopic sinus surgery. *Laryngoscope* 1994;104:404-8.
67. Kennedy DW, Zinreich SJ, Shaalan H, Kuhn F, Naclerio R, Loch E. Endoscopic middle meatal antrostomy: theory, technique, and patency. *Laryngoscope* 1987;97(Suppl 43):1-9.
68. Sugiyama Y. Diffuse panbronchiolitis. *Clin Chest Med* 1993;14:765-72.
69. Mukae H, Hirota M, Kohno S, Komori K, Fukushima K, Hiratani K, et al. Elevation of tumor-associated carbohydrate antigens in patients with diffuse panbronchiolitis. *Am Rev Respir Dis* 1993;148:744-51.
70. Kadota J-I, Sakito O, Kohno S, Sawa H, Mukae H, Oda H, et al. A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. *Am Rev Respir Dis* 1993;147:153-9.
71. Akira M, Higashihara T, Sakatani M, Hara H. Diffuse panbronchiolitis: follow-up CT examination. *Radiology* 1993;189:559-62.
72. Oda H, Kadota J, Kohno S, Hara K. Erythromycin inhibits neutrophil chemotaxis in bronchoalveoli of diffuse panbronchiolitis. *Chest* 1994;106:1116-23.
73. Nelson S, Summer WR, Terry PB, Warr GA, Jakab GJ. Erythromycin-induced suppression of pulmonary antibacterial defenses. *Am Rev Respir Dis* 1987;136:1207-12.
74. Torre D, Broggin M, Botta V, Sampietro C, Busarello R, Garberi C. *In vitro* and *ex vivo* effects of recent and new macrolide antibiotics on chemotaxis of human polymorphonuclear leukocytes. *J Chemother* 1991;3:236-9.