

Chronic hyperplastic sinusitis: Association of tissue eosinophilia with mRNA expression of granulocyte-macrophage colony-stimulating factor and interleukin-3

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Background: We investigated the association among tissue eosinophilia, cellular infiltration, and cytokine mRNA expression in chronic hyperplastic sinusitis (CHS).

Methods: Percutaneous biopsies of the maxillary sinuses and nasal polyps were performed in 12 adult patients (six men and six women) of whom seven were nonallergic and 11 were asthmatic. Tissues were compared with biopsy specimens from the inferior and middle turbinates of normal control subjects.

Results: Histologically, an eosinophil-predominant inflammatory infiltrate was seen in 10 of 12 patients, whereas a mild to moderate neutrophilic infiltrate was seen in 4 of 12 patients. As determined by immunocytochemistry, diseased tissues and normal control tissues differed significantly in terms of the number of activated (EG2⁺) eosinophils ($p = 0.005$) but not in terms of CD3⁺ or CD4⁺ T lymphocytes, elastase-positive neutrophils or CD68⁺ macrophages. The number of eosinophils did not correlate with that of any other cell type. By *in situ* hybridization, CHS tissues showed significantly higher numbers of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-3 mRNA-positive cells than normal control tissues ($p = 0.002$ and 0.0005 , respectively) per high-powered field. There was a significant correlation between the number of infiltrating EG2⁺ eosinophils and cells that expressed mRNA for GM-CSF ($r = 0.60$, $p = 0.041$) or IL-3 ($r = 0.69$, $p = 0.013$). Furthermore, epithelial cells did not show detectable mRNA expression for GM-CSF or IL-3. No significant correlation was found between IL-5 mRNA expression and infiltrating EG2⁺ eosinophils in diseased tissues. However, the IL-5 density was significantly higher in the five patients with CHS who had positive allergy skin test results than in the seven patients with negative skin test results ($p = 0.017$) or in normal control subjects.

Conclusions: Our data support a role for GM-CSF and IL-3 in the eosinophilia characteristic of CHS and show that IL-5 mRNA expression is not a prominent feature of nonallergic inflammation. The cellular sources of GM-CSF and IL-3 in CHS remain to be definitely determined. (*J ALLERGY CLIN IMMUNOL* 1993;92:39-48.)

Key words: Sinusitis, nasal polyps, eosinophilia, GM-CSF, IL-3, *in situ* hybridization, cytokines

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Abbreviations used

CHS:	Chronic hyperplastic sinusitis
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL:	Interleukin
SSC:	Standard saline citrate

Chronic hyperplastic sinusitis (CHS) is characterized by inflammatory mucosal thickening and polypoid changes of the paranasal sinuses and nasal polyp formation. It is a common cause for referral to allergists and ear, nose, and throat physicians, and it is especially common in patients with asthma. More than one third of the 1800 patients with asthma who were admitted to the inpatient service of our institution over the past 3 years were given a secondary diagnosis of chronic sinusitis. Patients have refractory symptoms and repeated exacerbations of "sinusitis" for which they receive treatment with antibiotics and systemic steroids. Frequently, exacerbations of sinusitis precede exacerbations of asthma.¹ A large percentage of patients have undergone one or more sinus surgical procedures. Therapy for chronic sinusitis frequently yields unsatisfying results, and surgery often affords only temporary relief from symptoms and recurrent infections.¹

The most prominent histologic feature of CHS is the striking accumulation of eosinophils in the mucosa and submucosa.² Eosinophils are also likely to play a pivotal role in the pathophysiology of this disease. Local activation and degranulation of eosinophils with release of basic proteins, such as major basic protein and eosinophil cationic protein, are thought to contribute strongly to epithelial injury and sloughing in CHS.³

Recent evidence suggests that production of cytokines by inflammatory cells is associated with the accumulation and activation of eosinophils in the site of allergen-triggered inflammation in the skin,^{4, 5} in the nasal mucosa,⁶ and in bronchial biopsy specimens of patients with asthma.⁷ In allergic individuals, the most strongly implicated cytokines are GM-CSF, interleukin (IL)-3, and IL-5, which contribute to local eosinophil accumulation, activation, and prolonged survival.⁸⁻¹¹ These cytokines, along with IL-4, are produced by cells that are thought to be "TH₂-type" T lymphocytes in allergic individuals after allergen stimulation.^{6, 7, 12} In nonallergic inflammation, which is seen in CHS and intrinsic asthma, the nature of cytokines promoting eosinophilia and their cellular sources are less clear.¹³⁻¹⁶

The purpose of the current study was to examine the association between tissue eosinophilia and infiltration of other cell types including neutrophils, T lymphocytes, and macrophages and to investigate these in relationship to the presence of cytokine-specific mRNA species detected in the tissues by in situ hybridization.

METHODS

Patient selection

Adult patients (age range, 18 to 50 years) at the National Jewish Center for Immunology and Respiratory Medicine were selected on the basis of a history of more than 1 year of chronic perennial sinusitis with one or more of the following symptoms: chronic perennial nasal congestion, chronic rhinorrhea, chronic post-nasal drip, anosmia, ageusia, and pain or discomfort in the regions of the sinuses. Each patient signed an informed consent form that was approved by the National Jewish Institutional Review Board. Most patients had received frequent courses of antibiotics and orally administered steroids for flare-ups of sinusitis, and seven of the 12 patients had undergone sinus surgery within the previous 3 years. As determined by nasal examination, all patients had nasal polyps. All patients had undergone a recent sinus computed tomographic scan taken in the coronal plane, which demonstrated involvement of both ethmoid and maxillary sinuses by either mucosal thickening of at least 6 mm or complete or near complete opacification of the sinus.

Patients with documented hypogammaglobulinemia or those with symptoms that were strongly suggestive of cystic fibrosis, bronchiectasis, immotile-cilia syndrome, systemic granulomatous disease, or significant cocaine use were excluded. All patients selected for study had normal quantitative immunoglobulin, IgG subclass, and salivary IgA levels. Patients were excluded if they had received any dose of systemic steroids greater than their average daily dose (≤ 5 mg/day of prednisone or equivalent) during the month before the study. Antibiotics were withheld for 4 weeks, and inhaled nasal steroids were withheld for 2 weeks before the study.

Initial evaluation

Each patient completed a questionnaire regarding history of sinusitis, allergy, environmental exposures, immunotherapy, and medical treatment. Allergy skin tests were performed by the prick technique with a panel of 42 aeroallergens that are prevalent throughout the United States, including 12 trees, 7 grasses, 10 weeds, 9 molds, standardized cat, mixed breed dog, and two species of mite (*Dermatophagoides pteronyssinus* and *D. farinae*). Results of prick tests that elicited a wheal of 3 mm or more in diameter were considered positive. Patients who had negative prick test results underwent intradermal skin tests to a panel of 17 aeroallergens, including 3 trees, 3 grasses, 5 weeds, 3 molds, standardized cat, and 2 species of mite. No

TABLE I. Patient characteristics

Patient	Sex	Age (yr)	Sinus surgery in past 3 yr	Allergy skin tests	Aspirin sensitivity	Asthma	Circulating eosinophil count
SC	M	41	+	—	+	+	123
RF	F	39	+	—	+	+	1038
NH	F	34	+	—	—	+	493
CS	M	47	—	+	ND*	+	317
HH	M	22	—	—	+	+	546
DS	M	56	—	+	—	+	616
BC	M	26	—	+	—	—	827
LS	F	39	+	+	+	+	651
DG	M	39	—	—	—	+	158
JD	F	21	+	—	+	+	581
KB	F	32	+	+	—	+	106
MS	F	41	+	—	+	+	1056

*ND indicates that patient CS completely avoided aspirin ingestion but had no history of reaction to aspirin. All other patients had a positive or negative history of reactions.

additional atopic patients were identified by intradermal skin tests.

A circulating eosinophil count was obtained between 8:00 and 10:00 AM on the morning of the sinus procedure or sinus surgery. Cultures were obtained from the maxillary sinuses when possible (8 of 12 patients). Only two patients had positive aspirates, and both had significant aerobic gram-negative bacterial growth (patient DG: *Enterobacter aerogenes*; patient MS: *Pseudomonas aeruginosa*). Both patients showed an intense inflammatory tissue response, with patient DG showing a mixed neutrophilic and eosinophilic infiltrate and patient MS showing an intense eosinophilic infiltrate.

Normal control nasal biopsies

Nasal mucosal biopsy specimens were obtained from the inferior or middle turbinates of 10 individuals with no history of allergies, asthma, sinus disease, previous sinus surgery, recent upper respiratory infections, or cigarette smoking within the past 3 years. These biopsy specimens were used as normal controls.

Tissue preparation

Percutaneous 2 to 3 mm biopsy specimens of nasal polyps and maxillary sinus tissue were obtained with 5 mm cup biopsy forceps. Four percent xylocaine solution was used to anesthetize the nasal mucosa. The maxillary sinus biopsy specimens were obtained after the gums above the upper teeth were anesthetized with 2% xylocaine solution with epinephrine and after the maxillary sinus was entered via trocar puncture through the canine fossae. Generally, two to six specimens were obtained from both right and left nasal polyps and both maxillary sinuses.

Tissue biopsy specimens from each area were processed in four ways. Specimens for routine histologic examination were fixed in 4% paraformaldehyde in

phosphate-buffered saline for a minimum of 24 hours, cut at a thickness of 5 μ m, and stained with hematoxylin and eosin. Each specimen was also stained with Gomori's methanamine silver. No fungal elements were seen. A second set of specimens was fixed in Mota's basic lead acetate for a minimum of 24 hours for staining of metachromatic cells, cut at a thickness of 5 μ m, and stained with 0.5% toluidine blue in 0.7 N HCl (pH 0.5) for 15 to 20 minutes. A third set of specimens was frozen in 95% ethanol plus dry ice and stored at -70° C until processing for immunocytochemistry. The fourth set of specimens was fixed in 4% paraformaldehyde for 4 hours, then washed and stored in phosphate-buffered saline with 15% sucrose at 4° C for up to 4 weeks, blocked and cut at a thickness of 10 μ m, and processed for in situ hybridization.

Immunocytochemistry

The alkaline phosphatase-antialkaline phosphatase procedure was used as previously described.¹⁷ Cryostat sections (6 μ m) were cut from the frozen biopsy specimens, air-dried for 1 hour, fixed in a mixture of equal parts acetone and methanol for 2 minutes, and stained with the following antibodies: anti-neutrophil elastase, anti-CD8 and anti-CD68 (DAKO Ltd., High Wycombe, Buckinghamshire, England); and anti-CD3 (Leu-4), anti-CD4 (Leu-3A), anti-CD25 (anti-IL-2 receptor), anti-CD45 (HLe-1) (Becton Dickinson, Ltd., Cowley, Oxford, England) and EG2 (Bradsure Biologicals, Loughborough, Leicestershire, England). EG2 recognizes specifically the cleaved, actively secreted form of eosinophil cationic protein^{18, 19} and hence identifies activated eosinophils within the tissues.

In situ hybridization

The tissue was used for in situ hybridization as previously described.⁷ A total of 20 cryostat sections (10

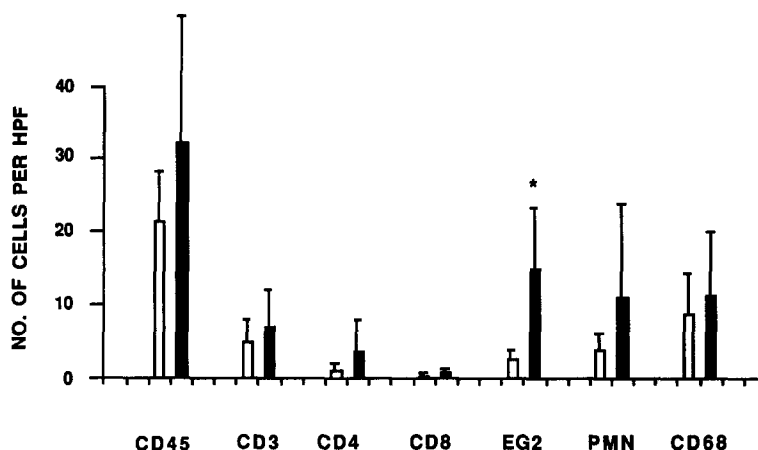


FIG. 1. Comparison of the density of CD45⁺, CD3⁺, CD4⁺, CD8⁺, EG2⁺ eosinophils, neutrophils, and CD68⁺ (macrophages) by immunocytochemistry in normal control subjects ($n = 10$) (open bars) and patients with CHS ($n = 12$) (shaded bars). Results are expressed as positive cells per high power field (0.202 mm²). The difference in EG2⁺ eosinophil number is statistically significant (* $p = 0.005$).

μm) were cut from each biopsy section, dried for 12 hours at 37° C, and stored at -80° C until processing. Sections from biopsy specimens were first made permeable with 0.3% solution of Triton X-100 in phosphate-buffered saline for 10 minutes and then with proteinase K (1 $\mu\text{g}/\text{ml}$) in 0.1 mol/L Tris that contained 50 mmol/L ethylenediaminetetraacetic acid for 20 to 30 minutes at 37° C. The reaction was terminated by immersion of the preparations in 4% paraformaldehyde for 5 minutes. The preparations were then treated in 0.25% acetic anhydride in 0.1 mol/L triethanolamine for 10 minutes to reduce nonspecific binding and prehybridized in 50% formamide and 2 $\times$ standard saline citrate (SSC) for 30 minutes at 40° C. Riboprobes, both antisense (complementary to mRNA) and sense (having an identical sequence to mRNA) were prepared from complementary DNA for GM-CSF, IL-3, and IL-5 as previously described.^{5, 20} Complementary DNA was inserted into different pGEM vectors and linearized with appropriate enzymes before transcription. Transcription was performed in the presence of phosphorous-32-labeled uridine triphosphate and the appropriate T7 or SP6 RNA-polymerases. For hybridization, a radiolabeled antisense or sense probe (10⁶ cpm/section) diluted in hybridization buffer²¹ was used. The preparations were covered with dimethyldichlorosilane-coated cover slips, and hybridization was performed in a humid chamber for 12 hours at 40° C. Posthybridization washing was performed in decreasing concentrations of SSC (4 $\times$ SSC to 0.05 $\times$ SSC) at 43° C. Unhybridized single-stranded RNAs were removed by treating the preparations with a solution that contained RNase A (20 $\mu\text{g}/\text{ml}$), 0.5 mol/L NaCl, 10 mmol/L Tris, and 1 mmol/L ethylenediaminetetraacetic acid for 30 minutes at 42° C. After dehydration, the sections were immersed in K-5 emulsion (Ilford, England), and exposed for 72 hours. The autoradiographs were developed in Kodak

D-19 (Eastman Kodak Company, Rochester, N.Y.), fixed with Hypam (Ilford), and counterstained with hematoxylin.

As a control for in situ hybridizations, a separate set of sections from patients with sinusitis was hybridized with sense probes and treated with RNase-A solution (10 $\mu\text{g}/\text{ml}$) at 37° C for 40 minutes before the prehybridization step. Hybridization was subsequently performed with labeled cRNA probes as described above. No hybridization signals were observed in any of these tissue specimens, thus confirming the specificity of the results obtained during hybridization with complementary RNA probes. As positive control for GM-CSF, IL-3 and IL-5, a peripheral blood T-lymphocyte clone was obtained from a patient with hyperimmunoglobulinemia E syndrome²² as previously described.⁷

Quantitation

Histologic tissue sections were examined by two observers, and the density of individual cell types within the infiltrate was graded on a 3-point scale: 1 = absent or sparse; 2 = moderate infiltrate, and 3 = dense infiltrate. The two observers first graded the histologic sections independently. Subsequently, all histologic sections were reviewed jointly in order to arrive at a consensus opinion independent of the results of immunocytochemistry.

For immunohistology and in situ hybridization, sections were counted in blinded fashion in coded random order with an Olympus microscope with an eyepiece graticule at 200 $\times$ magnification. The graticule was oriented along the epithelial basement membrane, and subepithelial counts were expressed as mean counts per high power field (0.202 mm²). At least two sections were immunostained or hybridized from which six to eight fields were counted. Results were expressed as positive cells per field. The within-observer coefficient

cient of variation for repeated counts of immunoreactive cells was 8% and for hybridized sections less than 5%.

Statistics

For analysis of histologic specimens, a total of 20 nasal polyp and 16 maxillary sinus biopsy specimens were analyzed from 12 patients, including 14 paired biopsy specimens from eight patients. For analysis of relationships in the inflammatory infiltrate in nasal polyps and the ipsilateral maxillary sinuses, each of the 14 paired biopsy specimens were treated as independent samples and analyzed by Pearson's chi square statistic with a 2×2 contingency table that was based on histologic grading categories. The strength of the association between infiltrating cells as determined by immunocytochemistry and the density of in situ positivity for the cytokines GM-CSF, IL-3, and IL-5 was determined by linear regression.²³

RESULTS

Patient characteristics

The patient characteristics are summarized in Table I. There were six men and six women with a mean age of 36.4 years. Five of the 12 patients had positive allergy skin test results. A history of a serious exacerbation of asthma or sinusitis after ingestion of aspirin was noted in six of 12 patients. One patient completely avoided aspirin. Eleven of the 12 patients had well-documented asthma. The sole nonasthmatic patient did not undergo formal testing to rule out asthma but had never had symptoms suggestive of asthma. The mean circulating eosinophil count for the entire group was 543 (SD = 313).

Histologic appearance of specimens from control subjects and patients with CHS

Biopsy specimens from the middle turbinates of five normal control subjects revealed minimal inflammatory infiltrate (graded 1+) consisting predominantly of plasma cells and mononuclear cells. A minimal infiltrate of eosinophils or neutrophils (graded 1+) was seen in two and one of the subjects, respectively. Metachromatic cells were sparse (graded 0 to 1+) in all subjects. The fibroblasts in these biopsy specimens consistently showed a compact nucleus with dense heterochromatin consistent with relatively quiescent cells.

A moderate to intense inflammatory infiltrate (graded 2 to 3+) was seen in 10 of the 12 patients with CHS. The predominant inflammatory cell type was eosinophils. Two patients (KB and CS) showed relatively mild inflammation and minimal eosinophilic infiltration. Neutrophilic infiltration

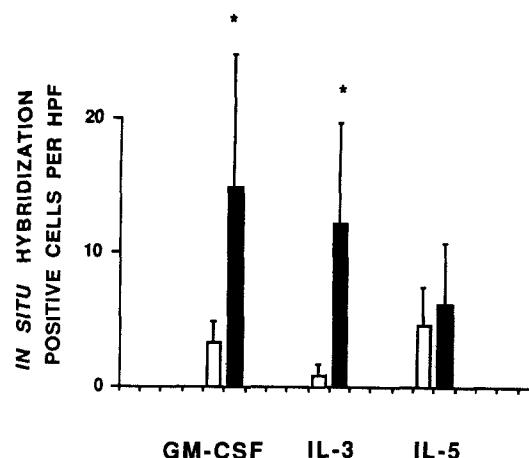


FIG. 2. Comparison of the density of cells positive by in situ hybridization for the cytokines GM-CSF, IL-3, and IL-5 in normal control subjects ($n = 10$) (open bars) compared with patients with CHS ($n = 12$) (shaded bars). Results are expressed as positive cells per high power field (0.202 mm²). The numbers of GM-CSF-positive and IL-3-positive cells are significantly greater in patients with CHS ($p = 0.002$ and 0.0005 , respectively).

was moderately intense (graded 2 to 3+) in only four of 12 patients, and in three of these patients eosinophils were also moderately intense (2 to 3+). Metachromatic cells were increased (graded 2 to 3+) in six of 12 patients. Increased fibroblast activity, as evidenced by nuclear enlargement and dispersed heterochromatin, was seen in 10 of 12 patients.

Correlation of histologic grading in polyps and ipsilateral sinus biopsy specimens

According to Pearson's chi square analysis, the densities of eosinophils in the nasal polyp and the ipsilateral maxillary sinus were significantly related ($p = 0.05$). The association of polyp and ipsilateral sinuses with respect to density of neutrophils, mast cells, or the degree of fibroblast activation did not reach statistical significance ($p = 0.10$, 0.138 , and 0.406 , respectively). No correlation was seen between the density of eosinophils and metachromatic cells within the same tissue specimen. However, there was a significant correlation between neutrophil density and fibroblast activation within the maxillary sinuses ($p = 0.01$) but not within nasal polyps ($p = 0.09$) (data not shown). There was no significant association between clinical history of aspirin sensitivity (six patients) or sinus surgery in the preceding 3 years (seven patients) and histologic pattern (data not shown).

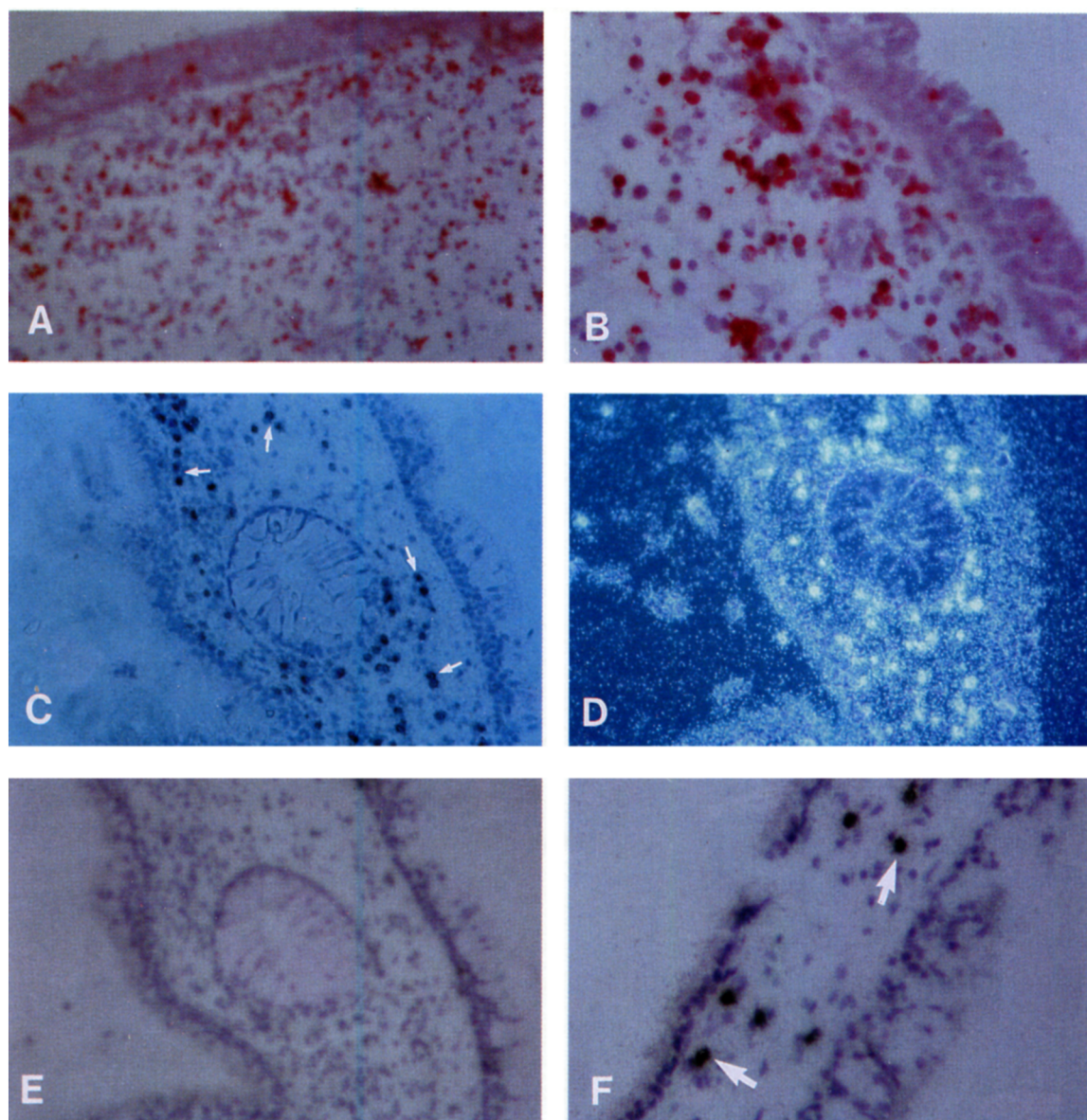


FIG. 3. Examples of immunocytochemical staining and in situ hybridization in samples from CHS. **A**, Low ($\times 100$) and **B**, high ($\times 400$) power light microscopic photomicrographs showing EG2 immunoreactivity in CHS with APAAP technique. Note the large number of eosinophils. Autoradiographs of adjacent sections of a CHS sample hybridized with GM-CSF antisense (**C**, **D**) and sense probe (**E**). **C**, phase-contrast illumination; **D**, darkfield illumination. Note the large number of cells with positive hybridization signal in **C** and **D** and the absence of hybridization signal in **E**. **F**, an example of IL-3 mRNA positivity in a polyp from a CHS sample. ($\times 400$.)

Analysis of specimens from control subjects and patients with CHS by immunocytochemistry

The immunocytochemical quantitation of CD45+, CD3+, CD4+, CD8+, EG2+ eosinophils, elastase+ neutrophils, and CD68+ macrophages in biopsy specimens from normal control subjects and patients with CHS is shown in Fig. 1. Increased numbers of EG2+ eosinophils,

CD45+ cells, and neutrophils were seen in patients with CHS compared with control subjects. However, the only significant difference between the two groups was the number of EG2+ eosinophils (CHS group: 14.7 ± 8.8 ; normal control group: 2.2 ± 1.5 ; $p = 0.005$). The number of EG2+ eosinophils in patients with CHS did not correlate significantly with the presence of any other cell type.

Analysis of specimens from control subjects and patients with CHS by in situ hybridization

By in situ hybridization, GM-CSF, IL-3, and IL-5 mRNA species were detected within inflammatory cells in the tissues (Fig. 2). Examples of immunocytochemical staining and in situ hybridization in samples from patients with CHS are shown in Fig. 3. In all cases, the autoradiographic signal associated with a positive in situ hybridization was cell-associated and could be easily distinguished from background (Fig. 3, C-F). Normal control tissues revealed very few positive cells. Diseased tissues, however, contained significantly greater numbers of cells expressing mRNA for GM-CSF (diseased: 14.8 ± 9.8 ; normal control: 3.3 ± 1.6 ; $p = 0.002$) and IL-3 (diseased: 12.0 ± 7.7 ; normal control: 0.9 ± 0.9 ; $p = 0.0005$). IL-5 mRNA-positive cells were seen with the lowest frequency and with the least consistency in patients with CHS, and the levels detected did not differ from those seen in normal control subjects (CHS group: 6.08 ± 4.64 ; normal control group: 4.40 ± 3.10). However, IL-5 mRNA-positive cells were significantly elevated in the patients with CHS who had positive allergy skin test results (9.6 ± 4.9 , $n = 5$) compared with those who had negative skin test results (3.6 ± 2.4 , $n = 7$) ($p = 0.017$). In contrast, IL-5 mRNA expression in the patients with negative skin test results was similar to that in normal control subjects (4.4 ± 3.1).

Correlation between immunocytochemistry and in situ cytokine mRNA in CHS

The number of cells expressing mRNA for GM-CSF and IL-3 in diseased tissues correlated strongly with the number of EG2⁺ eosinophils ($r = 0.60$, $p = 0.041$ and $r = 0.69$, $p = 0.013$, respectively) (Figs. 4 and 5). Neither GM-CSF nor IL-3 number correlated with the number of CD3⁺ T cells ($p = 0.156$ and 0.296 , respectively), the number of CD4⁺ T cells ($p = 0.712$ and 0.595 , respectively), or the number of cells of any other inflammatory type. The ratio of GM-CSF mRNA⁺ cells to CD45⁺ cells is increased threefold in patients with CHS (control subjects = 0.15; patients with CHS = 0.46). Similarly, the ratio of IL-3 mRNA⁺ cells to CD45⁺ cells is increased ninefold (control subjects = 0.04; patients with CHS = 0.38). In contrast, the ratio of IL-5 mRNA⁺ to CD45⁺ cells is not increased (control subjects = 0.21; patients with CHS = 0.19). Hence, the presence of GM-CSF and IL-3 mRNA

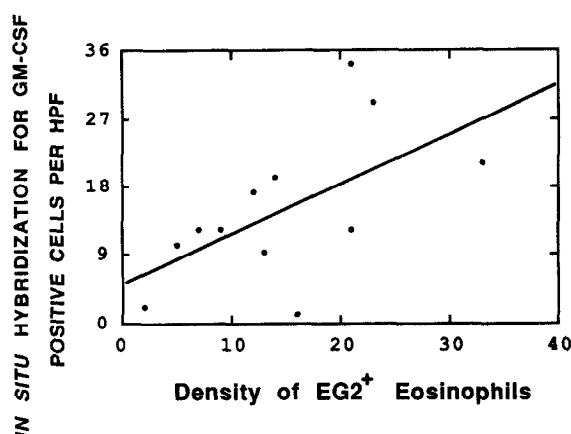


FIG. 4. Regression analysis of the relationship between the density of EG2⁺ eosinophils by immunocytochemistry and cells positive for the cytokine GM-CSF by in situ hybridization in patients with CHS ($n = 12$). The relationship is statistically significant ($r = 0.60$, $p = 0.041$).

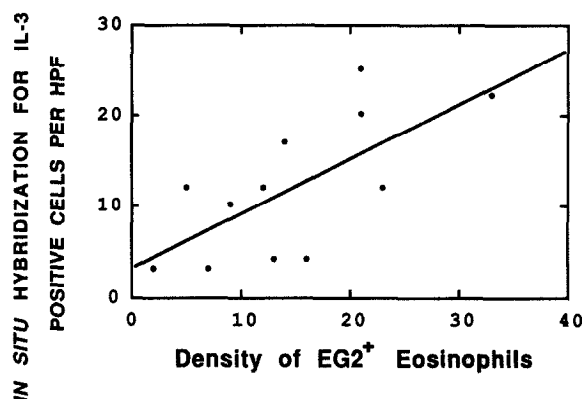


FIG. 5. Regression analysis of the relationship between the density of EG2⁺ eosinophils by immunocytochemistry and cells positive for the cytokine IL-3 by in situ hybridization in patients with CHS ($n = 12$). The relationship is statistically significant ($r = 0.69$, $p = 0.013$).

NA⁺ in patients with CHS is not simply a reflection of increased numbers of inflammatory cells.

In contrast, IL-5 mRNA expression did not correlate with the number of EG2⁺ eosinophils. Rather, IL-5 mRNA expression correlated with the number of CD3⁺ T cells ($r = 0.85$, $p = 0.0005$) (Fig. 6) and showed a trend toward a correlation with the number of CD4⁺ T cells ($p = 0.083$).

Most of GM-CSF mRNA-positive cells were found in the subepithelial tissue; however, a few positive cells were seen within the epithelium. In contrast, IL-5 and IL-3 mRNA-positive cells were consistently found in the subepithelial tissue.

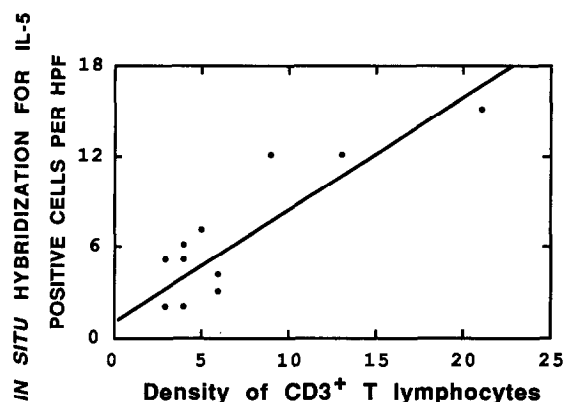


FIG. 6. Regression analysis of the relationship between the density of CD3⁺ T lymphocytes by immunocytochemistry and cells positive for the cytokine IL-5 by in situ hybridization in patients with CHS ($n = 12$). The relationship is statistically significant ($r = 0.85$, $p = 0.0005$).

DISCUSSION

Our current study confirms the importance of tissue eosinophilia as a pathologic feature of CHS and provides evidence that this phenomenon is strongly correlated with potential local cytokine production, particularly production of GM-CSF and IL-3. The patients with CHS in this study were similar to those described by other investigators¹ in terms of symptoms, radiographic findings, and histologic patterns. In addition, 11 of 12 of our patients had associated asthma. The high prevalence of asthma may be explained by the type of patients seen in our institution, although the coexistence of CHS and asthma is well recognized.¹ The major histologic feature of CHS, namely the presence of chronic eosinophilic inflammation, was highly correlated in polyps and maxillary sinuses. Furthermore, CHS tissue biopsy specimens were most clearly distinguished from normal control biopsy specimens by the presence of eosinophilic infiltration, and to a lesser extent, by increased metachromatic cells and signs of fibroblast activation. We did not find a strong correlation between the density of metachromatic cells and eosinophils within the CHS tissue. Although multipotent hematopoietic progenitor cells give rise to mature mast cells, basophils, and eosinophils²⁴⁻²⁶ and metachromatic cells and eosinophils accumulate in the nasal mucosa after allergen challenge,^{6, 27} the local proliferation and survival of these mature cells within inflammatory tissue are differentially regulated by cytokines. We hypothesize that these local effects account for the lack of correlation between metachromatic cells and eosinophils in the chronically inflamed CHS tissues.

Using immunocytochemistry, we showed a clear distinction between normal control subjects and patients with CHS in terms of the density of EG2⁺ eosinophils. In contrast, CHS tissues could not be distinguished from normal control tissues in terms of the densities of CD45⁺, CD3⁺, CD4⁺, CD8⁺, elastase⁺ neutrophils, or CD68⁺ macrophages. These results point strongly toward the importance of eosinophils in the pathogenesis of CHS. When in situ hybridization was used, CHS tissues were also clearly distinguishable from normal control tissues in terms of higher numbers of GM-CSF and IL-3 mRNA-positive cells.

Harlin, et al.³ described a subgroup of patients with CHS who did not have associated allergic rhinitis or asthma. This subgroup showed relatively little evidence of tissue eosinophilia or extracellular major basic protein deposition. In our experience, however, such patients are quite rare, and all 12 of the patients with CHS in the present study had positive allergy skin test results, asthma, or both. If such patients could be identified, they might help to confirm the importance of GM-CSF and IL-3 in tissue eosinophilia.

Our results are in agreement with those of other investigators in suggesting a role for GM-CSF in tissue eosinophilia. Jordana et al.¹⁶ reported that explants of nasal polyp epithelium and polyp stromal cells (particularly fibroblasts) from patients with allergic rhinitis produce cytokines when grown in vitro. GM-CSF was the principal cytokine detected. In addition, activated eosinophils have been shown by in situ hybridization techniques to express mRNA for GM-CSF when studied in nasal polyps²⁸ or when isolated from peripheral blood.²⁹ Because there are several potential cellular sources of GM-CSF in the CHS tissues, a clearer elucidation of the local dynamics of GM-CSF production is needed. We can state, however, that with in situ hybridization techniques we found very little evidence for GM-CSF or IL-3 mRNA expression within the epithelial compartment. This suggests that the epithelium is not a major source of these cytokines in CHS.

In addition to GM-CSF and IL-3, IL-5 was studied because of its effects on eosinophil growth, activation, and prolongation of eosinophil survival.¹¹ We found no significant difference in the number of IL-5 mRNA-positive cells in CHS tissues compared with normal control tissues. IL-5 mRNA-positive cells were significantly higher in the five patients with CHS who had positive allergy skin test results than in the seven patients with negative test results or in the 10 normal

control subjects. This is consistent with a possible expansion of IL-4- and IL-5-producing Th₂-type T lymphocytes in these patients, in agreement with results of several recent studies of allergic patients.⁴⁻⁷ Furthermore, IL-5 mRNA-positive cells correlated only with the number of CD3⁺ T lymphocytes in diseased tissues and not with the number of activated eosinophils. This suggests that IL-5 mRNA expression is not a prominent feature of nonallergic inflammation.

Although we found no significant increase in T cells or T-cell subsets in CHS, we cannot rule out their involvement in an earlier stage of the disease. Corrigan et al.,³⁰ Corrigan and Kay,³¹ and others^{12, 32-35} described the association of T-cell activation with acute attacks of asthma, both allergic and nonallergic. Recently, Walker et al.^{32, 35} reported T-cell activation and production of IL-5 by T cells in bronchoalveolar lavage of patients with nonallergic asthma. Given the strong parallels between nonallergic asthma and CHS and their frequent coexistence, it is reasonable to postulate that T-cell activation and cytokine production may be involved in the early phase of CHS. In contrast, the later stages of the disease may be driven by cytokine production from eosinophils and perhaps stromal cells, including GM-CSF and IL-3, which may promote further dysregulation by their effects on the sinus mucosa and epithelium.

Eosinophilic inflammation is a prominent feature of the upper and lower respiratory tract mucosa in patients with allergic disease and in patients with nonallergic CHS, nasal polyposis, and intrinsic asthma.^{1-3, 36} In nonallergic patients, the mechanism of eosinophil influx and accumulation remains unknown. Other investigators have proposed that this process is under the control of local cytokine production^{2, 16, 37} and that dysregulation of cytokine production in the microenvironment of the nasal-sinus mucosa gives rise to the stimulus for influx of metachromatic bone-marrow-derived precursor cells, which can differentiate into mast cells, basophils, or eosinophils.^{13-15, 24, 25} Unraveling this dysregulation at the tissue level is a formidable task, because many cell types, including mast cells, T lymphocytes, epithelial cells, stromal fibroblasts, and eosinophils participate in the inflammatory process and have the potential for cytokine production. By applying the combination of in situ hybridization techniques and immunocytochemistry to the study of tissue eosinophilia in patients with CHS, we were able to establish a strong correlation between GM-CSF and IL-3 and tissue eosinophilia in CHS.

With similar techniques it may be possible in the future to more clearly elucidate the complex cellular interactions in cytokine production that lead to tissue eosinophilia.

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