Background: The inflammatory-cell and cytokine profiles of chronic sinusitis (CS) are well documented in the literature. In contrast, little is known about the pathogenesis of this condition in patients with cystic fibrosis (CF).

Objective: To determine whether patients with CF have inflammatory-cell and cytokine profiles that differ from other patients with CS.

Methods: Patients with CF (n=7) and adults with CS (n=7) undergoing functional endoscopic sinus surgery were recruited for the study. Patients with no allergies or sinus disease (n=6) were used as controls. Using immunohistochemical analysis, we assessed sinus mucosal specimens for the presence of T lymphocytes, eosinophils, macrophages, and neutrophils. Using in situ hybridization, we assessed the expression of interleukin (IL) 4, IL-5, IL-8, IL-10, and interferon γ.

Results: There was a higher number of neutrophils, macrophages, and cells expressing messenger RNA for interferon γ and IL-8 in patients with CF than in patients with CS or in controls (P<.01). The number of eosinophils and cells expressing messenger RNA for IL-4, IL-5, and IL-10 was higher in patients with CS than in those with CF and controls (P<.01).

Conclusions: Sinus disease in patients with CF presents different inflammatory-cell and cytokine profiles than that seen in other patients with CS. These results may explain the difference in response to treatment in the CF group.


THE INFLAMMATORY-CELL
and cytokine profiles of allergic chronic sinusitis (CS) are well documented in the literature. Eosinophils, T lymphocytes, and cells expressing Th2-like cytokines such as interleukin (IL) 4, IL-5, and IL-13 are found in higher numbers in mucosal specimens of atopic patients with CS than in controls.1-4

Most patients with cystic fibrosis (CF) develop CS, with 90% to 100% of patients older than 8 months demonstrating radiologic evidence of the disease.5 The management of CS in patients with CF is often challenging, with many patients having a poor response to standard medical therapies.6 Although studies have elucidated the pathogenesis of lung disease in patients with CF,7-14 little is known about the pathogenesis of sinus disease in these patients. This is primarily because this group represents only a small proportion of the population with CS.

In this study, we investigated whether patients with CF and sinonasal disease have a different pattern of sinus mucosal inflammation than patients with CS and controls. Specifically, we evaluated nasal mucosal specimens from each group of patients for the presence of eosinophils, neutrophils, macrophages, and T lymphocytes, as well for the expression of IL-4, IL-5, IL-8, IL-10, and interferon (IFN) γ.

METHODS

PATIENT SELECTION

Patients were recruited from the Department of Otolaryngology at the McGill University Hospital Center and the Sir Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, over a 2-year period. Seven patients with CF undergoing functional endoscopic sinus surgery for CS were included in the study. Cystic fibrosis was diagnosed by abnormal findings on a sweat-chloride test and/or characteristic genotype abnormalities. An equal number of patients with CS undergoing functional endoscopic sinus surgery were selected as a comparison group. Six patients undergoing endoscopic orbital decompression surgery who were not atopic and had no evidence of sinus disease were included as controls.
used as controls. Exclusion criteria included the presence of a known immunodeficiency disorder or use of topical or systemic steroids within 2 weeks prior to surgery. The indications for surgery in these patients were symptomatic CS (congestion, rhinorrhea, olfactory disturbance, facial pain/headache) for at least 6 months that was unresponsive to standard medical management (antibiotics and/or nasal steroid sprays). All procedures were performed under general anesthesia using an endoscopic approach.

Skin testing for perennial and seasonal allergens was done with a standard panel including Alternaria, Aspergillus, Cladosporium, grass, Penicillium, ragweed, trees, cat, cockroach, dog, and dust mites Dermatophagoides farinae and Dermatophagoides pteronyssinus. Histamine (1 mg/mL) was used as a positive control and isotonic sodium chloride as a negative control. A wheal 3 mm or more larger than the negative control was considered a positive skin reaction.

TISSUE PREPARATION

In preparation for immunohistochemical analysis, biopsy specimens were placed in ornithine carbamoyltransferase (OCT) medium, snap-frozen in liquid nitrogen-cooled isopentane, and stored at −80°C. Cryostat sections were cut 3-µm thick and maintained at −20°C until use for immunohistologic analysis. In preparation for in situ hybridization, the biopsy specimens were immediately fixed in 4% paraformaldehyde for 2 hours. After washing in phosphate-buffered saline with 15% sucrose, they were stored overnight at 4°C. The following day the tissue was blocked in OCT medium and frozen in isopentane cooled in liquid nitrogen. Cryostat sections (10 µm) were then cut from the specimens, placed on poly-L-lysine-coated slides, allowed to dry for 12 hours at 37°C, and stored at −80°C prior to further processing.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical analysis was performed using the alkaline phosphatase–anti-alkaline phosphatase method, as previously described. Monoclonal antibodies including anti-CD3, MBP (supplied by R. M. Moqbel, MD, University of Alberta, Edmonton), CD68, and elastase were used to detect T lymphocytes, eosinophils, macrophages, and neutrophils, respectively. Slides were developed using fast red substrate for alkaline phosphatase. A negative control slide was included in each immunohistochemistry experiment.

IN SITU HYBRIDIZATION

Radiolabeled complementary riboprobes (cRNA antisense) for IL-4, IL-5, IL-8, IL-10, and IFN-γ were used to identify the presence of messenger RNA (mRNA) as previously reported. Cytopsins were permeabilized, treated with acetic anhydride and triethanolamine. Sulfur 35 (35S)–labeled antisense probes were used, followed by high-stringency posthybridization washings. Unhybridized single-stranded RNA was removed by treating the preparation with a solution of RNase. To ensure specificity, sections and cytopsins were hybridized with the sense probe or pretreated with an RNase solution.

QUANTIFICATION

Biopsy specimens were coded, and the number of positive cells for protein and mRNA were counted “blind” using a microscope with an eyepiece graticule at ×200 magnification. Results were expressed as the mean ± SEM number of positive cells per square millimeter.

STATISTICAL ANALYSIS

Cell counts were compared among the 3 groups using a Mann-Whitney test. A P value less than .01 was regarded as statistically significant.

RESULTS

The mean age of the patients with CF was 32.1 years (range, 22–36 years) vs 39.6 years (range, 30–52 years) in the CS group and 43.5 years (range, 39–53 years) in controls. None of the patients with CF or controls were atopic, whereas 86% of patients with CS had at least 1 positive skin test finding.

IMMUNOHISTOCHEMICAL ANALYSIS

Our immunohistochemical findings are illustrated in Figure 1. The numbers of neutrophils and macrophages were significantly higher in the nasal mucosa of patients with CF (68.0 ± 2.6 and 51.1 ± 4.0 cells/mm², respectively) than in patients with CS (16.6 ± 3.3 and 16.8 ± 2.2 cells/mm², respectively) (P < .01). There were significantly higher numbers of eosinophils in the sinus mucosa of patients with CS (36.2 ± 4.7 cells/mm²) than patients with CF (3.0 ± 1.0 cells/mm²) (P < .01) and controls (1.7 ± 0.5 cells/mm²) (P < .01). The number of T lymphocytes was significantly higher in the nasal mucosa of patients with CF (26.4 ± 4.1 cells/mm²) and CS (53.0 ± 6.0 cells/mm²) than in controls (12.1 ± 1.9 cells/mm²) (P < .01). Although elevated in both groups of patients, the number of T lymphocytes was significantly higher in the CS than in the CF group (P < .01).

IN SITU HYBRIDIZATION

Our in situ hybridization findings are illustrated in Figure 2. The numbers of cells positive for IL-4, IL-5, and IL-10 were significantly higher in the nasal mucosa of the CS group (12.9 ± 1.6, 16.2 ± 1.8, and 20.0 ± 2.8...
cells/mm², respectively) than in the patients with CF (1.7±0.5, 3.2±0.9, and 3.4±0.6 cells/mm², respectively) (P < .01) and controls (1.5±0.5, 2.0±0.6, and 4.3±0.8 cells/mm², respectively) (P < .01). The number of IFN-γ-positive cells was significantly higher in the nasal mucosa of the CF group (7.4±0.7 cells/mm²) than in patients with CS (2.1±0.7 cells/mm²) (P < .01) and controls (3.0±0.6 cells/mm²) (P < .01). The number of IL-8-positive cells was significantly higher in the nasal mucosa of patients with CF (22.6±2.8 cells/mm²) and CS (9.6±0.8 cells/mm²) than in controls (3.9±1.0 cells/mm²) (P < .01). Although elevated in both groups of patients, the number of IL-8-positive cells was significantly higher in patients with CF than in those with CS (P < .01).

**COMMENT**

This study demonstrates that the nasal mucosa of patients with CF and CS is characterized by an infiltration of neutrophils and macrophages as well as IFN-γ and IL-8-positive cells. Conversely, sinus specimens from this group of patients did not demonstrate high numbers of eosinophils or T$_{h2}$-positive cells. This pattern of inflammation is consistent with that found in lower airway specimens of patients with CF.

Studies have consistently demonstrated increased numbers of neutrophils and IL-8-positive cells in bronchoalveolar lavage and sputum samples of patients with CF compared with disease controls. Interleukin 8 is known to be a potent neutrophil chemoattractant and is thought to be critical in the development of the characteristic neutrophil infiltrate in patients with CF. Interleukin 10 is thought to be a potentially important anti-inflammatory cytokine in normal lungs, and it is known to suppress IL-8 production. The relative absence of IL-10 in lung specimens of patients with CF has been thought to play an important role in the pathogenesis of this disease in that IL-8 production remains unopposed. We have demonstrated that diseased nasal specimens in patients with CF predominantly consist of neutrophilic infiltrates in the presence of IL-8-positive cells and in the absence of IL-10. Thus, the inflammatory pattern of sinus disease in patients with CF appears to be similar to that found in diseased lung specimens.

In a study assessing the inflammatory profile of nasal lavage specimens in patients with CF without nasal disease and healthy controls, Noah et al. found that there was no evidence of neutrophil predominance or increased levels of IL-6, IL-8, or IL-10. Members from this same research group stimulated nasal epithelial cell cultures established from patients with and without CF with TNF-α or respiratory syncytial virus and found no difference in the subsequent expression of IL-8. They concluded that the nasal mucosa of patients with CF was not more susceptible than that of patients without the disease to exaggerated inflammatory responses as seen in the bronchial mucosa. Danel et al. noted similar results in nasal brush biopsy specimens in adult patients with CF and controls. It is important to note, however, that these studies evaluated the nasal mucosa of patients with CF who had no manifestations of sinonasal disease.

The patients with CS in our study demonstrated mucosal-cell and cytokine profiles consistent with those found in other studies. The number of eosinophils was higher in sinus specimens from this patient group than it was in the CF group and controls. Moreover, the cells expressing T$_{h2}$ cytokines, including IL-4, IL-5, and IL-10, were found in higher numbers in patients with CS than in controls. Thus, unlike in the CF group, the inflammatory profile of CS in our atopic patients appears to be mediated by eosinophilic infiltrates in the presence of T$_{h2}$-like cytokines.

The observed inflammatory pattern in our CF group may explain the poor response of these patients to topical corticosteroids. Steroid administration is thought to coincide with an alteration in the number and activity of inflammatory cells and with a reduction in the number of T$_{h2}$-type cytokines within the nasal mucosa of patients with allergic rhinitis and CS. Aside from directly reducing the synthesis of T$_{h2}$-type cytokines, steroids also increase the level of T$_{h1}$-type cytokines, particularly IFN-γ and IL-12, which can suppress the transcription of IL-4. The predominance of neutrophils and paucity of T$_{h2}$ cytokines in diseased sinus specimens from patients with CS make the use of topical steroids in these patients questionable. Clinical and molecular studies need to be carried out in this patient group to assess the usefulness of and potential morbidity associated with nasal steroids.

In summary, we have found that the inflammatory pattern of CS in CF is similar to that seen in lower airway specimens, consisting of macrophages and neutrophilic infiltrates in the presence of IFN-γ and IL-8. The eosinophilic infiltrates and increased expression of T$_{h2}$ cytokines characteristic in patients with CS are not found in patients with CF. The different inflammatory pattern seen in diseased sinus specimens of patients with CF may explain the relatively poor response to nasal steroids in this patient group compared with other patients with CS.

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REFERENCES


