Published in final edited form as:

Ann Otol Rhinol Laryngol. 2012 September ; 121(9): 579–586.

Etiology of Nasal Polyps in Cystic Fibrosis: Not a Unimodal Disease

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Abstract

Objectives—The objective was to determine whether the polyp subtypes observed in cystic fibrosis (CF)–related sinusitis were similar to those observed in non–CF-related sinusitis.

Methods—Polyp and mucus samples were collected from CF patients who presented for sinus surgery. The polyps underwent histologic and cytochemical evaluation for the presence of lymphocyte cell populations and their respective cytokine markers. The mucus samples were evaluated for DNA content.

Results—Of the polyps, 42% had an eosinophilic infiltrate, of which 80% had an additional mixed neutrophilic infiltrate. Of the remaining polyp samples, 42% did not have a granulocytic infiltrate, consistent with non-eosinophilic polyps. All samples had CD138-positive plasma cells. The mucus samples from the patients with CF showed higher extracellular DNA concentrations than did the mucus samples from patients with non-CF sinus disease.

Conclusions—Cystic fibrosis–related polyps demonstrated an eosinophil-based dichotomy similar to that of idiopathic non–CF-related polyps. Many also demonstrated neutrophilic infiltrate, indicating that chronic mucus stasis and infection complicate the disease. Agents capable of reducing extracellular DNA may help manage sinusitis in CF patients.

Keywords

cystic fibrosis; DNA; eosinophil; histology; mucolytic; nasal polyp; neutrophil

INTRODUCTION

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease; most cases result from genetic mutations of the cystic fibrosis transmembrane receptor (*CFTR*) chloride channel gene. The prevalence of CF is 1 in 3,500 live births, and the carrier frequency is 1 in 25.¹ Chronic sinusitis (CS) with or without nasal polyps (NPs) is virtually universal in patients with CF, and NPs are often the presenting complaint.² The tendency to form NPs is a stereotypic feature of the sinuses that results from chronic inflammation–induced differentiation and hyperplasia of the sinus tissue. It is recognized that several distinct histologic forms of NPs exist.^{3–5}

Although it is infrequently associated with NP formation, the most common form of CS is noneosinophilic sinusitis. Non-eosinophilic sinusitis results from chronic or recurrent occlusion of the sinus ostia secondary to frequent viral rhinitis, allergic rhinitis, anatomic predisposition, or other causes. Obstruction leads to recurrent bacterial infections, damage to respiratory epithelium, ciliary destruction, mucous gland and goblet cell hyperplasia, bacterial colonization with the formation of biofilms, and, ultimately, chronic inflammatory changes. Inflammatory infiltrate includes primarily mono-nuclear cells, including activated B cells and plasma cells with secondary germinal center formation.^{3,4} Other distinct forms of NPs have been characterized, presenting as disorders with prominent eosinophilia — specifically, chronic hyperplastic eosinophilic sinusitis, allergic fungal sinusitis, and aspirin-exacerbated respiratory disease.^{3,6-8}

Little is known regarding the pathology of the CS that complicates CF, but it was historically assumed that CF patients' CS reflected an infectious disorder. Thus, the standard of care has primarily been antibiotics and surgical drainage procedures. However, the sinus disease seems more consistent with non-eosinophilic sinusitis, reflecting the excessive inspissated mucus produced in this disorder, followed secondarily by colonization of the sinuses with bacteria and biofilm formation. This idea is consistent with a report that the NPs in CF have the same histologic architecture as that of NPs from patients without CF.⁹ Since that report, little has been written regarding the histology of NPs in patients with CF. The appearance of chronic hyperplastic eosinophilic sinusitis reflects the predisposition of the human airway to produce T helper type 2 (Th2) inflammatory responses. Chronic hyperplastic eosinophilic sinusitis often occurs in association with allergy and asthma, and as in these disorders, the sinus tissue demonstrates a marked increase in cells expressing cytokines, chemokines, and pro-inflammatory lipid mediators responsible for eosinophilia.^{3,6,10-12} Allergic fungal sinusitis is a form of chronic hyperplastic eosinophilic sinusitis in which T lymphocyte activation is driven by the colonization of the sinuses with fungi to which the patient develops allergic hypersensitivity. Patients with allergic fungal sinusitis display prominent eosinophilia and high titers of specific immunoglobulin E targeting the fungal allergen.¹³ We speculated that the pathologic characteristics of CF would be heterogeneous, with some cases presenting as either non-eosinophilic sinusitis, chronic hyperplastic eosinophilic sinusitis, or allergic fungal sinusitis. As distinct diseases, these subtypes have differing treatment regimens, making the correct diagnosis critically important.

METHODS

Subjects and Specimen Collection

Sixteen consecutive patients with CF and NPs were enrolled under a protocol approved by the University of Virginia Institutional Review Board. The diagnosis of CF was confirmed in all patients on the basis of positive pathognomonic sweat tests or the presence of homozygous CF mutations. The patients had extensive sinus disease and were referred by their physicians for polypectomy. The patients gave consent for the use of discarded tissue; however, the conditions of approval from the University of Virginia Institutional Review Board precluded obtaining personalized information. Limitations of NP size and cellular content precluded performing all studies in all subjects. Twelve NP specimens underwent histologic evaluation, 3 NP samples provided enough tissue for ImageStream flow cytometry, and mucus was collected from 4 patients undergoing rhinoscopic sinus debridement.

Histologic Evaluation

Histologic evaluation was performed as previously described.³ Briefly, a portion of each polyp was placed in 4% paraformaldehyde (Sigma, St Louis, Missouri) overnight at 4°C. The next day these specimens were washed in phosphate-buffered saline solution and stored in 70% ethanol until paraffin embedding. Paraffin embedding, tissue sectioning, and hematoxylin-eosin (H&B) and Gomori's trichrome staining were performed by the Histology Core Laboratory of the University of Virginia. For chloroacetate esterase staining, samples were deparaffinized and hydrated in distilled water. Chloroacetate esterase staining was performed as previously described,³ and sections were counter-stained with Mayer's hematoxylin (Vector Laboratories, Burlingame, California). Samples were washed in distilled water and dehydrated through alcohol and xylenes before mounting. Immunohistochemical analysis for the plasma cell-specific marker CD138 was performed on 7 NPs with commercial antibodies (Clone MI15; 1:100; Dako, Carpinteria, California) and the Dako Automated Plus immunostaining machine. Antigen retrieval was performed with the Dako Target Retrieval System. Scoring was performed as described for eosinophils and polymorphonuclear cells (PMNs).

Histologic Scoring

Nasal polyps were scored for eosinophils and PMNs on the basis of the numbers of each granulocyte in H&E-stained sections. Similarly, scoring for mast cells was performed on the chloroacetate esterase-stained samples. Sections were examined under 400× magnification with an Olympus BX51 microscope (Center Valley, Pennsylvania) by a single blinded reviewer, and positive cells were counted in 10 random sections for each sample, with the final number being the average number of cells per 10 high-power fields (HPFs).

ImageStream Flow Cytometry

Flow cytometry was performed with the ImageStream (Amnis Corp, Seattle, Washington) cytometer, which combines flow cytometry with confocal microscopy. In addition to staining cell surface markers, we added a nuclear dye to allow visual confirmation of the cellular identification suggested by surface marker staining. Nasal polyps were minced and digested with Accutase (Innovative Cell Technologies, San Diego, California) for 1 hour at 37°C, and the leukocyte-containing fraction was collected by passing the cell suspension through a 70-μm nylon mesh strainer (BD Falcon, Bedford, Massachusetts). Red blood cells were lysed by resuspending in 2 mL ACK lysis buffer for 2 minutes followed by addition of 8 mL RPMI (Roswell Park Memorial Institute) medium (Invitrogen, Carlsbad, California). Two-color immunofluorescence was performed, with all cells stained with a pan-leukocyte marker (CD45). Infiltrating CD45+ leukocytes were then assigned to their specific cell type. Cells were permeabilized, and the nuclei were stained with 4',6-diamidino-2-phenylindole (Sigma). Leukocytes were fractionated with the following markers: CD4 (T helper), CD8 (T cytotoxic), CD19 (B cells), CCR3 (eosinophils and others), and CD11b (mononuclear phagocytes, PMNs, and others). Data are presented as the percentage of each population after visual confirmation of each cell type among representative cells.

Neutrophil- and Eosinophil-Derived Mediators

Myeloperoxidase (Assay Designs, Plymouth Meeting, Pennsylvania) and eosinophil-derived neurotoxin (MBL, Nagoya, Japan) were quantified in the mucus samples by enzyme-linked immunosorbent assay according to the manufacturers' instructions. The detection limits for the assays were 19 pg/mL for myeloperoxidase and 0.62 ng/mL for eosinophil-derived neurotoxin. DNA was quantified by fluorometry via incorporation of the fluorescent dye bisbenzimidazole (Sigma) into the double-stranded DNA. Excitation was performed at 360 nm,

and fluorescence emission was detected at 460 nm. A DNA standard curve was generated to allow comparison of patient samples.

RESULTS

Histologic Appearance

Similar to idiopathic NPs, the NPs in CF largely comprised loose collagenous fibrils surrounding markedly edematous tissue when viewed with H&E staining (Fig 1A,B). This pattern was also observed in trichrome-stained specimens (Fig 2A). Scattered among these edematous regions, in most samples (8 of 10), were areas of dense stroma that gave the appearance of collagenous “stalks” (Fig 2B). As with idiopathic NPs, these dense collagenous stalks were observed contiguous to areas of glandular hypertrophy (Fig 2B). Consistent with glandular hypertrophy, these regions displayed thick, inspissated mucin (not shown). In general, the epithelial lining was intact, although it frequently displayed hypertrophy.

Inflammatory Infiltrate

Samples were initially evaluated for inflammatory cellular infiltration by H&E staining. All specimens contained a chronic mononuclear cell infiltrate. The H&E-stained specimens were specifically scored for the presence of eosinophils and neutrophils. We considered an average of at least 1 cell per 10 HPFs (400×) to be meaningful (see Table). As with idiopathic NPs, we readily defined eosinophilic and non-eosinophilic presentations, with significant eosinophilia observed (at least in some regions) in 5 of the 12 NPs. In contrast to idiopathic NPs, in which we only rarely observe neutrophilic infiltration, 6 of these 12 NPs displayed significant neutrophilia (Fig 1B). We did not identify intact granulocytes in 5 of the 12 NPs, and describe these NPs as being paucigranulocytic (Fig 1A). Of the 7 NPs with prominent numbers of granulocytes, 1 had only eosinophils (Fig 1C), 2 displayed only PMNs, and the remaining 4 had a combined infiltrate (Fig 1D). It should be noted, however, that as suggested by the ranges delineated in the Table and as seen in Fig 1, these NPs were extremely heterogeneous, with distinct areas differentially displaying intense neutrophilic, eosinophilic, or combined infiltrates scattered among paucigranulocytic regions.

Mast Cells

Tissue samples underwent chloroacetate esterase staining for the chymase-positive mast cell subtype that is observed in NPs.³ Six of 10 NPs had elevated numbers of chymase-positive mast cells (Fig 3). There was no correlation with the pattern of granulocyte expression. However, as we have previously noted for idiopathic NPs, the presence and location of mast cells correlate with fibrosis, specifically with the presence of regions of dense stroma.

Granulocyte Secretion Markers

We investigated for evidence of activation of PMNs or eosinophils through the expression of their associated secretory products in sinonasal secretions: PMN-derived myeloperoxidase (also derived from mononuclear phagocytes) and eosinophil-derived neurotoxin. Additionally, we quantified expression of extracellular DNA as a biomarker reflecting a neutrophil- or eosinophil-mediated antibacterial response (mitochondrial DNA net formation).¹⁴ Similar to idiopathic NPs, 50% of the CF mucus samples displayed striking concentrations of eosinophil-derived neurotoxin, consistent with the concept that there are eosinophilic and non-eosinophilic presentations of nasal polyposis. In contrast to idiopathic NPs, in which myeloperoxidase secretions are seldom elevated, again, 50% of the CF patients demonstrated increased levels of myeloperoxidase. As with the histologic studies, we identified patients with isolated eosinophilic or neutrophilic biomarkers, or neither

biomarker. The most impressive data were derived from quantification of extracellular DNA in the sinus secretions; it was markedly elevated in all of the CF specimens — higher than we observed in any non-CF sample. The DNA concentration correlated with that of myeloperoxidase, suggesting the presence of a neutrophil-mediated antibacterial mitochondrial DNA response.

Mononuclear Cell Infiltrates

As noted, all of the tissue samples displayed a mononuclear cell infiltrate. On H&E staining, the greatest proportion of these mononuclear cells had the appearance of plasma cells. To better characterize this finding, we performed immunohistochemical staining using the plasma cell-specific marker CD 138 (Fig 4). These studies confirmed large numbers of plasma cells in all 7 of the samples studied (mean, 28.6 cells per HPF; range, 1 to 118 cells per HPF). To characterize the remaining mononuclear cell infiltrate, we performed ImageStream analysis. This was done on only 3 samples, as these provided sufficiently large NPs to permit flow cytometric analysis. The staining characteristics varied widely among the 3 samples, consistent with the heterogeneity of the inflammatory processes. As with this subject's H&E analysis, the sample from subject 4 demonstrated a neutrophilic profile with few CD4 (0%), CD8 (3.9%), or CD19 (1.2%) cells but with strong staining for CD11b (82.9%). Imaging confirmed that these cells were granular, with multilobed nuclei confirming them as PMNs (Fig 5). Subject 5, whose sample had a paucigranulocytic appearance on H&E staining, demonstrated both CD4 (21.1%) and CD8 (10.5%) surface staining. Further, this subject's CD11b (21.4%) cells when observed were not neutrophils and instead represented mononuclear phagocytes (Fig 6). Finally, subject 11 also displayed a paucigranulocytic environment, with flow cytometry demonstrating multiple cell types present, including CD4 (37.3%), CD8 (41.2%), and CD11b (55.4%) cells. As in subject 5, this CD11b-positive population comprised mostly mononuclear phagocytes.

DISCUSSION

As with idiopathic nasal polyposis, the NPs observed in CF comprise a heterogeneous range of disorders. A weakness of this investigation is that these data represent a modest pilot study with few subjects and thus do not enable us to accurately predict the relative frequency of each condition. It remains possible that other presentations may also occur. This is important to consider in making the correct diagnosis and determining treatment options. In general, these studies suggest that as with idiopathic NPs, the most common presentations are eosinophilic and non-eosinophilic disorders, with slightly more than half of patients presenting without eosinophils. The presence of eosinophilia in CF NPs has previously been noted; Neely et al¹⁵ commented that CF NPs “frequently” had marked eosinophilia. In contrast to these 2 studies, Tos et al⁹ found predominantly plasma cells and lymphocytes in their 11 CF NPs, with only 1 sample demonstrating eosinophilia. As noted above, non-eosinophilic NPs in patients without CF are thought to result from anatomic defects or inflammatory processes of the nares that obstruct the sinus ostia. This obstruction leads to recurrent protracted infections, ultimately leading to remodeling of the airways with glandular hyperplasia, fibrosis, and the presence of a chronic inflammatory (mononuclear cell) infiltrate.³ This disease frequently responds to surgery or other interventions that open the sinus ostia and prevent recurrent infection. This pathology is consistent with the non-eosinophilic disease observed in more than half of our CF NP samples. In patients with CF, this disease could reflect either the occlusion of the sinus ostia by inspissated mucus or, more likely, the inability to clear the thickened mucus characteristic of CF and the secondary development of a vicious circle involving infection, remodeling, glandular hyperplasia, and recurrent infection.

We initiated these studies with the expectation that we would identify eosinophilia in at least a subset of CF patients. There was heterogeneity in the eosinophil numbers we observed in the CF NPs. Five of the subjects displayed eosinophilic infiltrate with an average of 5 eosinophils per HPF (see Table), which is similar to our previous findings in non-CF NPs, in which we observed an average of 9.3 eosinophils per HPF in samples with a marked eosinophilic infiltrate.³ In the remaining 7 CF NPs, the eosinophil average was 0.26 eosinophils per HPF (see Table), consistent with the non-eosinophilic group (2.9 eosinophils per HPF) of non-CF NPs we identified.³ In non-CF NPs, the presence of eosinophilia often correlates with the presence of allergic rhinitis or asthma. As allergens do not readily enter the sinus space,¹⁶ the mechanism driving eosinophilia is not obvious. It has been argued that the eosinophilia reflects an underlying tendency of the sinuses to produce a Th2 inflammatory reaction to immune insults.^{3,11,17} Patients with CF are uniquely predisposed to develop a Th2 immune response to fungi that colonize their lower airways, and up to 20% of CF patients, as noted, develop allergic bronchopulmonary aspergillosis.¹³ Although none of our patients presented with allergic fungal sinusitis, it remains possible that the subjects with eosinophilia could still be displaying a Th2 response to pathogens, including fungi, that had colonized their sinus cavities.

The most striking feature in these NPs was the presence of neutrophils in 4 of 10 samples — something that we almost never observe in idiopathic NPs of non-CF patients. The presence of neutrophils likely reflects trafficking of the cells in response to chemotactic stimuli different from those found in the NPs of non-CF patients. The distinguishing feature of CF lower airway disease is the almost universal colonization with bacteria — typically, gram-negative organisms. Although this is not the focus of the present study, it is reasonable to argue that CF similarly predisposes patients to colonization of the sinuses with bacteria and biofilm formation. Neutrophilia would then reflect the migration of these cells from the vasculature in response to this process. A role for PMNs is further supported by our studies regarding granulocyte biomarker expression in sinus secretions. As sinus secretions were obtained during routine clinic visits and not as a part of the polypectomy, we cannot directly correlate expression of mediators in mucus samples with polyp histology. Despite this caveat, as with the NPs, markedly elevated concentrations of myeloperoxidase were seen in half of these specimens, and again, this is something not routinely observed in non-CF chronic sinus disease. Van Zele et al¹⁸ found elevated levels of myeloperoxidase (as well as CXCL8 [IL-8]) in CF-derived sinus secretions, also pointing to a prominent role for PMNs. Even more impressive were our extracellular DNA concentrations, which were elevated in all of the CF specimens and to an extent higher than those we have observed in non-CF disease. Extracellular DNA is primarily derived from granulocytes, secreted as a component of neutrophils and eosinophils (mitochondrial-derived and nuclear-derived DNA nets, respectively) as part of their antibacterial response.^{14,19} In addition, extracellular DNA reflects cellular necrosis and the presence of inhibitors of phagocytosis of apoptotic bodies.²⁰ These striking elevations in DNA content contribute to the viscosity of secretions and the inability to clear them (even with surgery and postsurgical irrigations) and will thereby contribute to the infection-remodeling-infection process. Elevations in DNA content are also consistent with reports that in attempts to reduce viscosity in the lung, CF secretions respond best to DNase.²¹⁻²³ This approach is being tested as a therapeutic option following sinus surgery. Administration of dornase alpha between 4 weeks and 12 months after surgery was associated with improved nasal symptoms and rhinoscopic findings.²² A recent double-blind placebo-controlled study found that dornase alpha improved quality-of-life outcome measures in subjects who had previously undergone sinus surgery.²⁴

As found in non-CF NPs, these samples all demonstrated a mononuclear infiltrate. As noted, Tos et al⁹ also prominently found plasma cells in CF NPs. These are also seen in non-CF NPs, implying the development of secondary lymphoid tissue and antibody production. The

target of these antibodies was not the subject of this study, but could include colonizing bacteria or other pathogens. Along with plasma cells and lymphocytes, large numbers of mast cells were seen in many of these NPs (6 of 10). This finding is consistent with those of Henderson and Chi²⁵ demonstrating the presence of mast cells in CF NPs, 30% of which appeared to have undergone spontaneous degranulation. Although typically associated with allergic processes, mast cells are a component of fibrotic disorders reflecting a synergistic feedback process wherein mast cells provide fibroblast-targeting growth factors and fibroblasts respond in kind as sources of mast cell growth factors. This process is consistent with our finding of mast cells within areas of thickened stroma (Fig 3).

Our findings demonstrate that CF NPs are not a distinct subtype, but rather, exhibit features of the well-described subtypes of non-eosinophilic sinusitis and chronic hyperplastic eosinophilic sinusitis. The striking feature of CF NPs was the frequent association of both eosinophilic and non-eosinophilic diseases with the presence of neutrophilia — something virtually never observed in idiopathic NPs. Even when not present in the NP itself, evidence of neutrophilic inflammation was universally present in the mucous secretions, as manifested through the presence of extracellular DNA. These data suggest the need for investigating appropriately targeted therapeutic approaches based upon each patient's unique histologic presentation.

Acknowledgments

Supported by NIH grants AI057483, AI090413, and AI070364 and by a medical school basic research grant from Genentech. Genentech is a manufacturer of products; however, this study is not a clinical trial, nor does it involve use of any of the products manufactured by Genentech. There is no basis for there to be any bias.

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Fig 1.

Hematoxylin-eosin (H&E) staining of cystic fibrosis (CF) polyps. Arrows indicate relevant cell types. **A)** Paucigranulocytic nasal polyp (NP; original $\times 200$). **B)** Polymorphonuclear cell (PMN) infiltration without eosinophils (original $\times 200$). **C)** Eosinophilic infiltration without PMNs (original $\times 200$). **D)** Both eosinophils and PMNs within same field (original $\times 400$).

Fig 2.
Trichrome staining of CF polyps (original $\times 200$). **A)** Marked tissue edema and loose stroma.
B) Dense collagenous stalk adjacent to area showing glandular hypertrophy.

Fig 3. Mast cell staining in CF polyp tissue (original $\times 200$). Chloroacetate stain demonstrates numerous mast cells scattered among areas with dense stromal tissue.

Fig 4.
Immunohistochemistry of CD138-staining plasma cells.

Fig 5.

CD11b (CR3) ImageStream flow cytometry analysis of cells isolated from CF polyp. Dark field (DF) examination reveals cytoplasmic granules, defining these cells as granulocytes. Staining of nucleus with 4',6-diamidino-2-phenylindole (DAPI) demonstrates multilobed structure of PMNs. BF — bright field.

Fig 6.

CD11b (CR3) ImageStream flow cytometry analysis of cells isolated from CF polyp. These cells are larger than those in Fig 5, and dark field examination reveals cytoplasmic granules. DAPI staining of nucleus demonstrates mononuclear structure of macrophages.

INFLAMMATORY INFILTRATE IN CYSTIC FIBROSIS-DERIVED NASAL POLYPS

Subject	Eosinophils	Polymorphonuclear Cells
1	0.3 ± 0.1 (0-1)	3.5 ± 1.1 (0-10)
2	0.1 ± 0.0 (0-1)	0.0 ± 0.0 (0)
3	13.9 ± 4.4 (0-25)	3.2 ± 1.1 (0-17)
4	0.9 ± 0.3 (0-3)	12.1 ± 3.8 (1-25)
5	0.3 ± 0.1 (0-3)	0.6 ± 0.2 (0-2)
6	4.7 ± 1.5 (0-9)	0.3 ± 0.1 (0-1)
7	3.7 ± 1.2 (0-11)	7.2 ± 1.1 (0-25)
8	0.0 ± 0.0 (0)	0.1 ± 0.0 (0-1)
9	0.1 ± 0.0 (0-1)	0.5 ± 0.2 (0-5)
10	0.1 ± 0.0 (0-1)	0.2 ± 0.1 (0-2)
11	1.5 ± 0.5 (0-8)	2.5 ± 0.8 (0-9)
12	1.2 ± 0.4 (0-4)	6.2 ± 2.0 (0-16)

Data are mean ± SD (range) of 10 high-power fields.