
Lysozyme levels in the nasal secretions of patients with perennial allergic rhinitis and recurrent sinusitis

V. Cunejt Kalfa, MD*; Sheldon L. Spector, MD†; Tomas Ganz, PhD, MD‡; Alexander M. Cole, PhD§

Background: The association of perennial allergic rhinitis (PAR) with recurrent sinusitis (RS) is well recognized. Anatomic abnormalities at the osteomeatal complex or ciliary dysfunction may play a significant role in some patients. However, for most patients with allergy, the determinants of RS are unknown.

Objective: To determine whether altered concentrations of antimicrobial peptides and proteins, such as lysozyme, lactoferrin, human β -defensin-2 (HBD-2), and human neutrophil peptides 1 to 3 (HNP-1 to 3), contribute to the development of RS in patients with PAR.

Methods: Nasal secretions were collected by vacuum aspiration from 15 individuals with PAR+RS, 16 with PAR alone, and 16 controls. Lysozyme and lactoferrin levels were determined in nasal secretions by using quantitative enzyme-linked immunosorbent assay, and HBD-2 and HNP-1 to 3 levels were determined in nasal secretions by using semiquantitative Western blot analysis. Eosinophil-derived neurotoxin (EDN) levels were measured by using enzyme-linked immunosorbent assay as a marker of nasal eosinophilia in all 3 groups.

Results: Levels of EDN were elevated significantly in patients with PAR+RS compared with controls. Lysozyme levels were decreased significantly in patients with PAR+RS compared with PAR alone or controls. Mean lysozyme levels were significantly lower in patients with EDN levels greater than 1,000 ng/mL vs those with levels of 1,000 ng/mL or less in the PAR+RS group. There were no statistically significant differences in lactoferrin, HBD-2, and HNP-1 to 3 levels among the 3 groups.

Conclusions: The presence of eosinophils and their products and reduced lysozyme concentrations may be critical factors that predispose the airways of patients with PAR to RS.

Ann Allergy Asthma Immunol. 2004;93:288–292.

INTRODUCTION

The association between allergy and sinusitis has been reported in various studies,^{1,2} but the exact role of allergy in the predisposition to sinusitis is not clear. Anatomic abnormalities at the osteomeatal complex or ciliary dysfunction may play a significant role in the development of recurrent sinusitis (RS) or chronic sinusitis (CS) in these patients. However, Berrettini et al¹ did not show any correlation between increased nasal resistance in a single nasal fossa and signs of sinusitis by computed tomography. Decreased ciliary activity has also been shown in CS, but this abnormality may be reversed with treatment.^{3,4}

There is evidence for a crucial role of ongoing allergic inflammation in the pathogenesis of tissue damage in the airways. Granules of eosinophils contain cytotoxic cationic

proteins, including eosinophil cationic protein, eosinophil-derived neurotoxin (EDN), and major basic protein, that may contribute to the irreversible airway tissue damage in patients with bronchial asthma^{5–8} and CS.⁹ Bascom et al¹⁰ previously showed that the concentration of EDN in nasal lavage fluid following antigen challenge is closely correlated with the number of eosinophils.

Of multiple antimicrobial proteins and peptides, lysozyme and lactoferrin are present at the highest concentrations in the airways,^{11,12} and studies^{11–14} have reported that these proteins are an important component of innate host defense in the airways. Lysozyme is a bacteriolytic enzyme that hydrolyzes the bacterial cell wall,¹⁵ modifies the viscoelasticity of the mucus,¹⁶ and, in vitro, directly promotes ciliary beating in the nasal epithelium.¹⁷ Lactoferrin has also been suggested to have a direct bactericidal action and an ability to withhold iron, which is essential for bacterial growth.¹⁸ Defensins, a prominent group of antimicrobial peptides, are thought to be an important component of the innate immune response, particularly at mucosal surfaces that are vulnerable to colonization by potential pathogens. Human β -defensin (HBD)-1 and HBD-2 are produced by the epithelia of the nasal mucosa; HBD-1 purportedly plays a constitutive role in nasal host defenses, whereas HBD-2 expression is induced in re-

* Division of Pediatric Immunology, Allergy, Rheumatology, Department of Pediatrics, University of California at Los Angeles, Los Angeles, California.

† David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California.

‡ Division of Pulmonary and Critical Care Medicine, Department of Medicine, and Will Rogers Institute Pulmonary Research Laboratory, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California.

§ Department of Molecular Biology and Microbiology, Biomolecular Science Center, University of Central Florida, Orlando, Florida.

sponse to local infection or inflammation.^{19,20} It has been shown that certain α -defensins, human neutrophil peptides (HNPs) 1 to 3, are actually released into nasal secretions primarily by neutrophils and that increased levels of HNP-1 to 3 have been noted in purulent sputum from patients with cystic fibrosis or obstructive pulmonary disease.²¹

Pathologic alterations in the antimicrobial properties of airway secretions caused by either impaired mucosal surfaces or chronic inflammation may contribute to epithelial colonization by bacteria in disorders such as RS or CS, cystic fibrosis, and chronic bronchitis.^{22,23} To examine the effects of allergy on innate mucosal immunity and the development of RS in patients with perennial allergic rhinitis (PAR), we compared the concentrations of antimicrobial peptides and proteins in nasal secretions from healthy donors and patients with PAR with or without RS.

MATERIALS AND METHODS

Participants

Fifteen patients with PAR and RS (9 men and 6 women; mean $\pm$ SEM age, 25.9 $\pm$ 3.2 years) and 16 patients with PAR without a history of RS (8 men and 8 women; mean $\pm$ SEM age, 32.4 $\pm$ 3.6 years) were studied. Patients had a documented history of PAR of at least 2 years' duration. Eligible patients exhibited a positive skin prick test reaction (wheal diameter $\geq$ 3 mm greater than saline control) to house dust mite, dog, or cat allergens or molds (*Alternaria* species or *Cladosporium* species). In addition, 16 healthy individuals with no history of allergy or RS (7 men and 9 women; mean $\pm$ SEM age, 27.3 $\pm$ 2.7 years) were studied as control subjects. The protocol received institutional review board approval, and informed consent was obtained from each participant before the study. Many patients required continuous medical management to control their symptoms, and, therefore, the study was often performed in the setting of concurrent treatment (12 of 15 patients with PAR+RS and 14 of 16 patients with PAR were using nasal corticosteroids), and sampling was performed during relatively quiescent periods. Except for 2 patients in the PAR+RS group who were prescribed 400 μ g/d of fluticasone propionate, all patients were prescribed 200 or 220 μ g/d of nasal corticosteroids.

Recurrent sinusitis was defined as 2 or more episodes of sinusitis per year for 2 or more years. An episode of sinusitis was diagnosed when the following symptoms were present: an increase in nasal congestion, purulent nasal discharge, cough, and facial pain or tenderness and abnormal findings on sinus radiographs. Each participant had at least 1 episode of acute sinusitis documented by abnormal findings on a sinus radiograph.²⁴ Positive findings on the Waters view were defined as complete opacification or maxillary mucoperiosteal thickening of more than 4 mm in 1 or both maxillary antra. Care was taken not to study any patient within 3 weeks of an upper respiratory tract infection or any increase in symptoms related to their sinus disease. Chronic sinusitis or RS was excluded in patients with PAR by the absence of a

history of sinusitis or clinical findings, including an increase in nasal congestion, purulent nasal discharge, and facial pain or tenderness.

Collection of Nasal Fluids

Nasal secretions were collected by vacuum-aided suction without chemical stimulation. The major advantage of this technique over the nasal lavage technique is that it permits the collection of whole nasal fluid without the unpredictable effects of dilution in saline or other lavage fluid. Gentle manipulation of a narrow rubber-tipped vacuum device inside the nasal passageways stimulated nasal secretions. Secretions were stored at 4° C for prompt use or at -20° C for longer-term storage. As shown previously,¹¹ secretions could be stored for several months at -20° C without alteration of antimicrobial properties or protein profiles. When necessary, nasal secretions were fluidized by brief (10-second) microtip sonication, which does not alter the antimicrobial properties of nasal fluid.²⁵

Semiquantitative Western Blot Analysis

Nasal secretions were separated by acid-urea polyacrylamide gel electrophoresis and then immunoblotted to Immobilon P-PVDF membranes (Millipore, Bedford, MA) for 20 minutes in 0.7% acetic acid-10% methanol, as described previously.²⁶ The membranes were then incubated with a 1:1,000 dilution of rabbit polyclonal anti-HBD-2 antisera²⁷ or monoclonal anti-HNP-1 to 3 antisera²⁸ overnight at room temperature. After several washings in 1 $\times$ TTBS (Tris-buffered saline + 0.05% Tween 20), membranes were incubated with a 1:2,000 dilution of alkaline phosphatase-conjugated polyclonal goat anti-rabbit secondary antibody (HBD-2 and HNP; Pierce, Rockford, IL) for 1 hour at room temperature. The membranes were then developed by using nitroblue tetrazolium-5-bromo-4-chloro-3-indolylphosphate as a chromogenic substrate. Serial dilutions of HBD-2, produced recombinantly in baculovirus,²⁹ and HNP-1, isolated from human neutrophils,²⁷ were used as positive controls and for semiquantitative studies.¹¹

Quantitation of EDN, Human Lactoferrin, and Human Lysozyme

Nasal fluids were assayed for EDN (MBL Medical & Biological Laboratories, Nagoya, Japan), human lactoferrin (Oxford Biomedical Research Inc, Oxford, MI), and human lysozyme (Biomedical Technologies Inc, Stoughton, MA) using commercially available enzyme-linked immunosorbent assay kits.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Comparisons between groups were evaluated using the 2-tailed *t* test. Correlations between parameters were analyzed using the Pearson correlation coefficient.

RESULTS

A significant difference in EDN levels was only found between patients with PAR+RS and controls (*P* < .01; Fig 1).

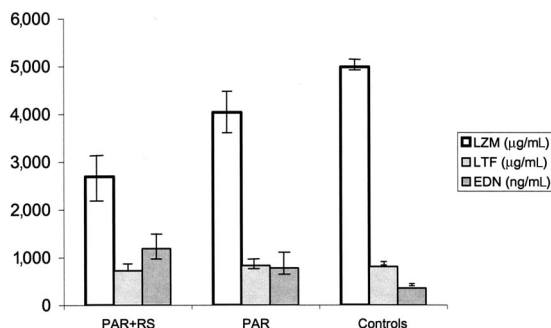


Figure 1. Levels of lysozyme (LZM), lactoferrin (LTF), and eosinophil-derived neurotoxin (EDN) in nasal secretions of patients with perennial allergic rhinitis with recurrent sinusitis (PAR+RS), patients with PAR alone, and controls. Patients with PAR+RS had a lower mean LZM level than those with PAR alone ($P < .05$) and controls ($P < .001$). Patients with PAR had a reduced concentration of LZM compared with controls; however, the difference was not significant ($P > .05$). Levels of EDN were elevated in patients with PAR+RS and PAR compared with controls, but a significant difference was found only between patients with PAR+RS and controls ($P < .01$). Concentrations of LTF were not different statistically among the 3 study groups ($P > .05$). Error bars represent SEM.

Concentrations of lactoferrin in nasal secretions were not different statistically among the 3 study groups ($P > .05$; Fig 1). However, patients with PAR+RS had a lower mean lysozyme level than those with PAR alone ($P < .05$) and controls ($P < .001$). Although patients with PAR had a reduced concentration of lysozyme compared with controls, the difference was not statistically significant ($P > .05$) (Fig 1). To analyze the relationship between EDN and lysozyme levels, patients with PAR+RS were categorized according to their EDN levels. Nine of 15 patients had EDN levels of 1,000 ng/mL or less and 6 had levels greater than 1,000 ng/mL. Patients with lower EDN levels had higher lysozyme levels ($P < .05$; Fig 2).

Defensin concentrations were also quantitated by Western blot analyses. Levels of HBD-2 were decreased in patients with PAR+RS and PAR alone compared with controls, and HNP-1 levels were found to be higher in patients with

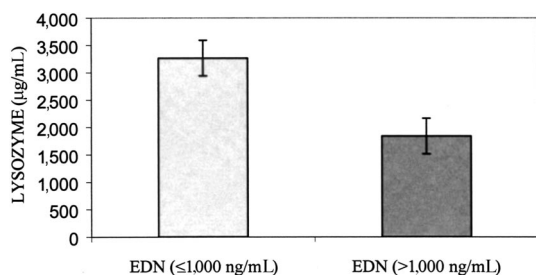


Figure 2. Comparison of lysozyme levels in patients with perennial allergic rhinitis with recurrent sinusitis and eosinophil-derived neurotoxin (EDN) levels of 1,000 ng/mL or less and greater than 1,000 ng/mL. Patients with EDN levels of 1,000 ng/mL or less had a higher mean concentration of lysozyme than those with EDN levels greater than 1,000 ng/mL ($P < .05$). Error bars represent SEM.

PAR+RS than in patients with PAR alone and controls. However, none of these differences were statistically significant ($P > .05$; data not shown). In summary, only EDN and lysozyme concentrations were significantly altered in patients with PAR+RS.

DISCUSSION

The presence of EDN, an eosinophil granule protein, in nasal lavage fluid following antigen challenge was closely correlated with the number of eosinophils.¹⁰ Accordingly, there was a significant increase in EDN levels in patients with PAR+RS vs controls. Although eosinophilia in the nasal secretions of patients with PAR is well documented,^{30,31} in our study the concentration of EDN in patients with PAR compared with controls did not reach statistical significance. Many patients in our study were using nasal corticosteroids, and sampling was performed during relatively quiescent periods, but patients had persistent symptoms, even during these times. Thus, in the PAR group, 14 (88%) of 16 patients were using nasal corticosteroids, and in the PAR+RS group 12 (80%) of 15 patients were using nasal corticosteroids. There is evidence that topical corticosteroids suppress cellular infiltrates in the edematous nasal tissues³² and the release of inflammatory mediators.³³ Because relatively more patients were using topical corticosteroids, it is possible that the eosinophilic activity of patients in the PAR group may be suppressed more than that of patients in the PAR+RS group. However, because our sample population did not have enough individuals who were not using nasal corticosteroids, we could not probe separately at the effect of topical corticosteroids.

The association between extensive sinus disease, as diagnosed by computed tomography, and an increase in the peripheral eosinophil count has been shown previously.^{34,35} The damaging effects of eosinophil granule proteins on respiratory mucosa have been well established in patients with chronic rhinosinusitis (CRS),^{36,37} including intense eosinophilic inflammation, basement membrane thickening, and erosion of the epithelium. The severe erosion and prominent damage to the epithelial layer were proposed to explain the acute bacterial exacerbations seen in patients with CRS. Because there was not a consistent difference between the bacterial populations in CRS and controls, the decreased defense of sinonasal mucosa with damaged epithelium was reported as a potential cause.³⁸ It is possible that sustained eosinophilia induces mucosal damage and impairs innate immunity, which leads to CS or RS.

In the respiratory tract, lysozyme and lactoferrin, important resistant factors for neutralization of bacteria, are produced and stored in the serous cells of nasal submucosal glands.^{39,40} As mentioned previously herein, chronic and progressive inflammation of the sinonasal mucosa results in remodeling, leading to histologic and functional changes in the tissues. In our study, patients with PAR+RS had a significantly lower mean lysozyme level than those with PAR alone and controls. Furthermore, we found that as the level of EDN increases, the

concentration of lysozyme decreases. Patients with PAR also had reduced lysozyme levels compared with controls; however, the difference was not statistically significant, suggesting that a subgroup of patients with PAR remains free of infection owing to relatively high levels of lysozyme. This group of patients (PAR alone) also had lower levels of EDN. Our findings suggest that increased EDN levels in allergic individuals predispose the sinonasal mucosa to CS or RS by decreasing lysozyme levels in nasal secretions. Jeney et al⁴¹ showed increased concentrations of lactoferrin and lysozyme in secretions from a group of patients with CS compared with controls. However, a blunted response was observed in patients with CS compared with controls after cholinergic stimulation. After 4 to 12 months of aggressive medical treatment, which included prolonged use of topical corticosteroids, the methacholine-stimulated mucosal release of lysozyme and lactoferrin significantly improved. Fukami and colleagues³⁹ also showed mucosal abnormalities with atypical gland formation due to the chronic inflammatory process in patients with CS. Increased immunoreactivity to lysozyme by goblet cells and glands was found in all patients with sinusitis. In these 2 studies, dysfunction of the sinus mucosa seemed to decrease mucosal defense even in the presence of lysozyme.

In the present study, concentrations of lactoferrin in nasal secretions were not different statistically among the 3 study groups. In another study,¹⁴ we tested the ex vivo antibacterial activity of cationic peptide-depleted fractions of nasal fluid in the presence or absence of physiologic concentrations of lysozyme, secretory leukocyte protease inhibitor, and lactoferrin (either alone or in combination) in colony-forming unit assays against a mucoid strain of *Pseudomonas aeruginosa* isolated from a patient with cystic fibrosis. Although whole nasal fluid reduced the colony-forming units per milliliter by nearly 10-fold, after 3-hour incubation there was an approximately 5-fold increase with cationic peptide-depleted nasal fluid. The addition of physiologic concentrations of lysozyme alone was sufficient to restore the activity of cationic peptide-depleted nasal fluid. In addition, among the 3 most abundant cationic polypeptides in nasal fluid, lysozyme was substantially more effective than either lactoferrin or secretory leukoprotease inhibitor in restoring the antibacterial activity of the cationic polypeptide-depleted nasal fluid. Taken together, this reinforces the idea that lysozyme is a key protein of the nasal host defense. Decreased levels of lysozyme in patients with PAR may explain the close association of allergy and sinusitis.

Given the purported roles of defensins in the innate host defense of the airways, we hypothesized that dysregulation of HBD-2 and HNP-1 to 3 might also be contributing factors in RS. However, our findings showed that HBD-2 levels were decreased in patients with PAR+RS and PAR alone compared with controls, and HNP-1 levels were found to be higher in patients with PAR+RS than in patients with PAR and controls, but the differences were not statistically significant. These findings might be attributed to most of our patients being treated with intranasal corticosteroids in light

of recent findings of glucocorticoid-induced down-regulation of HBD-2 and neutrophil-derived α -defensins.^{42,43}

CONCLUSION

Higher levels of EDN in nasal fluids were observed in patients with PAR+RS compared with patients with either PAR alone or controls. Of the antimicrobial (poly)peptides tested, lysozyme levels were significantly decreased in patients with PAR+RS compared with those with PAR alone or controls. We also observed lower lysozyme levels with higher EDN levels in patients with PAR+RS. These findings suggest that eosinophilia in the nasal mucosa and decreased lysozyme levels in patients with PAR may contribute to the development of RS. Further studies are needed that examine the relative contribution of each of the factors alone and in combination in the development of RS in PAR.

ACKNOWLEDGMENTS

This work was supported by a fellow-in-training grant from the American College of Allergy, Asthma, & Immunology (to V.C.K.) and by grant HL70876 from the National Institutes of Health (to A.M.C.). We thank Albert Park, Dawn Yoshioka, and Hsiang-I Liao for technical assistance, and we are especially grateful to Dr. Ole Sorensen for critical review of the manuscript.

REFERENCES

1. Berrettini S, Carabelli A, Sellari-Franceschini S, et al. Perennial allergic rhinitis and chronic sinusitis: correlation with rhinologic risk factors. *Allergy*. 1999;54:242–248.
2. Furukawa CT. The role of allergy in sinusitis in children. *J Allergy Clin Immunol*. 1992;90:515–517.
3. Ohashi Y, Nakai Y. Reduced ciliary action in chronic sinusitis. *Acta Otolaryngol*. 1983;397:3–9.
4. Sakakura Y, Majima Y, Saida S, Ukai K, Miyoshi Y. Reversibility of reduced mucociliary clearance in chronic sinusitis. *Clin Otolaryngol*. 1985;10:79–83.
5. Togias A, Naclerio RM, Proud D, et al. Studies on the allergic and nonallergic nasal inflammation. *J Allergy Clin Immunol*. 1988;81:782–790.
6. Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. *Am Rev Respir Dis*. 1988;139:801–805.
7. Hoekstra MO, Hovenga H, Gerritsen J, Kauffman HF. Eosinophils and eosinophil-derived proteins in children with moderate asthma. *Eur Respir J*. 1996;9:2231–2235.
8. Chivato T, Martinez D, Blasco R, Melgarejo M, Gomez de Terreros FJ, Laguna R. Serum levels of eosinophil cationic protein and eosinophil protein x in pollen atopic patients with stable asthma and its relation with bronchial hyperresponsiveness. *Allergol Immunopathol (Madr)*. 1996;24:243–247.
9. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol*. 1988;81:867–875.
10. Bascom R, Pipkorn U, Proud D, et al. Major basic protein and eosinophil-derived neurotoxin concentrations in nasal-lavage fluid after antigen challenge: effect of systemic corticosteroids and relationship to eosinophil influx. *J Allergy Clin Immunol*.

- 1989;84:338–346.
11. Cole AM, Dewan P, Ganz T. Innate antimicrobial activity of nasal secretions. *Infect Immun*. 1999;67:3267–3275.
12. Travis SM, Singh PK, Welsh MJ. Antimicrobial peptides and proteins in the innate defense of the airway surface. *Curr Opin Immunol*. 2001;13:89–95.
13. Cole AM, Tahk S, Oren A, et al. Determinants of *Staphylococcus aureus* nasal carriage. *Clin Diagn Lab Immunol*. 2001;8:1064–1069.
14. Cole AM, Liao HI, Stuchlik O, Tilan J, Pohl J, Ganz T. Cationic polypeptides are required for antibacterial activity of human airway fluid. *J Immunol*. 2002;15;169:6985–6991.
15. Chipman DM, Sharon N. Mechanism of lysozyme action. *Science*. 1969;165:454–465.
16. Puchlle E, Jacqot J, Zham JM. In vitro restructuring effect on human airway immunoglobulins A and lysozyme on airway secretions. *Eur J Respir Dis*. 1987;71:117–122.
17. Hisamatsu K, Yamauchi Y, Uchida M, Murakami Y. Promotive effect of lysozyme on the ciliary activity of the human nasal mucosa. *Acta Otolaryngol*. 1986;101:290–294.
18. Arnold RR, Cole MF, McGhee JR. A bactericidal effect for human lactoferrin. *Science*. 1977;197:263–265.
19. Lee SH, Kim JE, Lim HH, Lee HM, Choi JO. Antimicrobial defensin peptides of the human nasal mucosa. *Ann Otol Rhinol Laryngol*. 2002;111:135–141.
20. Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin [letter]. *Nature*. 1997;387:861.
21. Schnapp D, Harris A. Antibacterial peptides in bronchoalveolar lavage fluid. *Am J Respir Cell Mol Biol*. 1998;19:352–356.
22. Kaliner MA. Human nasal respiratory secretions and host defense. *Am Rev Respir Dis*. 1991;144:S52–S56.
23. Kaliner MA. Human nasal host defense and sinusitis. *J Allergy Clin Immunol*. 1992;90:424–430.
24. Barlan IB, Erkan E, Bakir M, Berrak S, Basaran MM. Intranasal budesonide spray as an adjunct to oral antibiotic therapy for acute sinusitis in children. *Ann Allergy Asthma Immunol*. 1997;78:598–601.
25. Cole AM, Wu M, Kim YH, Ganz T. Microanalysis of antimicrobial properties of human fluids. *J Microbiol Methods*. 2000;41:135–143.
26. Valore EV, Park CH, Quayle AJ, Wiles KR, McCray PB Jr, Ganz T. Human β -defensin-1: an antimicrobial peptide of urogenital tissues. *J Clin Invest*. 1998;101:1633–1642.
27. Liu L, Wang L, Jia HP, et al. Structure and mapping of the human β -defensin HBD-2 gene and its expression at sites of inflammation. *Gene*. 1998;222:237–244.
28. Ganz T, Selsted ME, Szklarek D, et al. Defensins: natural peptide antibiotics of human neutrophils. *J Clin Invest*. 1985;76:1427–1435.
29. Bals R, Wang X, Meegalla RL, et al. Mouse β -defensin 3 is an inducible antimicrobial peptide expressed in the epithelia of multiple organs. *Infect Immun*. 1999;67:3542–3547.
30. Lack G. Pediatric allergic rhinitis and comorbid disorders. *J Allergy Clin Immunol*. 2001;108:S9–S15.
31. Hamilos DL. Chronic sinusitis. *J Allergy Clin Immunol*. 2000;106:213–227.
32. Sahay JN, Ibrahim NB, Chatterjee SS, Nassar WY, Lodge KV, Jones CW. Long-term study of flunisolide treatment in perennial rhinitis with special reference to nasal mucosal histology and morphology. *Clin Allergy*. 1980;10:451–457.
33. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. *N Engl J Med*. 1987;316:1506–1510.
34. Newman LJ, Platts-Mills TA, Phillips CD, Hazen KC, Gross CW. Chronic sinusitis: relationship of computed tomographic findings to allergy, asthma, and eosinophilia. *JAMA*. 1994;271:363–367.
35. Bresciani M, Paradis L, Des Roches A, et al. Rhinosinusitis in severe asthma. *J Allergy Clin Immunol*. 2001;107:73–80.
36. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. *Annu Rev Med*. 1993;44:85–101.
37. Frigas E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab Invest*. 1980;42:35–43.
38. Nadel DM, Lanza DC, Kennedy DW. Endoscopically guided cultures in chronic sinusitis. *Am J Rhinol*. 1998;12:233–241.
39. Fukami M, Stierna P, Veress B, Carlsoo B. Lysozyme and lactoferrin in human maxillary sinus mucosa during chronic sinusitis: an immunohistochemical study. *Eur Arch Otorhinolaryngol*. 1993;250:133–139.
40. Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner MA. Pathophysiology of rhinitis: lactoferrin and lysozyme in nasal secretions. *J Clin Invest*. 1989;84:1528–1535.
41. Jeney EV, Raphael GD, Meredith SD, Kaliner MA. Abnormal nasal glandular secretion in recurrent sinusitis. *J Allergy Clin Immunol*. 1990;86:10–18.
42. Tomita T, Nagase T, Ohga E, Yamaguchi Y, Yoshizumi M, Ouchi Y. Molecular mechanisms underlying human β -defensin-2 gene expression in a human airway cell line (LC2/ad). *Respirology*. 2002;7:305–310.
43. van Wetering S, Mannesse-Lazeroms SP, van Sterkenburg MA, Hiemstra PS. Neutrophil defensins stimulate the release of cytokines by airway epithelial cells: modulation by dexamethasone. *Inflamm Res*. 2002;51:8–15.

Requests for reprints should be addressed to:
Alexander M. Cole, PhD
Department of Molecular Biology and Microbiology
University of Central Florida
4000 Central Florida Blvd
Bldg 20, Room 136
Orlando, FL 32816-2364
E-mail: acole@mail.ucf.edu