

Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: Is the histopathology similar to asthma?

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Background: Asthma and chronic rhinosinusitis (CRS) coexist clinically in >50% of patients with CRS. Although epithelial damage and basement membrane thickening are well-known features of airway remodeling in asthma, they have not been described in CRS.

Objective: In this study, we tested the hypothesis that histopathologic features of asthma, namely, the chronic eosinophilic inflammation, epithelial damage, and basement membrane thickening of the airway mucosa, are also present in sinonasal specimens from patients with CRS.

Methods: We examined histologic specimens from 22 randomly selected patients with refractory CRS undergoing endoscopic sinus surgery and 4 healthy control subjects. The shedding of the epithelium and basement membrane thickening were evaluated by 3 independent observers' scores of hematoxylin-and-eosin staining. Eosinophilic inflammation was monitored with immunohistochemistry for eosinophil major basic protein. A novel, computerized method objectively analyzed confocal microscopic images of major basic protein immunofluorescence to determine areas with the least and most inflammation per specimen.

Results: Specimens from all patients with CRS (22/22) revealed epithelial damage (shedding) and basement membrane thickening. Strikingly heterogeneous eosinophilic inflammation, which did not differ between allergic and nonallergic patients, was detected in all patients with CRS and was absent in all healthy control subjects.

Conclusion: The histopathologic findings of asthma, namely, heterogeneous eosinophilic inflammation and features of airway remodeling, are also present in CRS. These findings, coupled with the common clinical coexistence of both diseases, suggest that the same pathologic disease process is manifest as CRS in the sinonasal tissue and as asthma in the lower airway. (*J Allergy Clin Immunol* 2003;112:877-82.)

Key words: Chronic rhinosinusitis, histopathology, eosinophil, remodeling, sinusitis

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa. Patients with CRS are symptomatic for longer than 3 months and have associated inflammatory mucosal thickening and polypoid changes seen on endoscopy or computed tomography.^{1,2} CRS accounts for >90% of all cases of rhinosinusitis, and with a prevalence of 16.8% (32 million patients) in the adult population in the United States, it is one of the most common chronic diseases.³ Adding to the burden of CRS, both a plausible etiology and an effective treatment do not exist. Because no medical intervention has ever been efficacious in a controlled, clinical trial, no drug has been approved by the US Food and Drug Administration to treat CRS.

A clinical relationship between CRS and asthma has been a growing health concern for many years, with a reported incidence of asthma within the CRS population of at least 50%.⁴ Eosinophilic inflammation is the major histologic hallmark of both diseases.^{5,6} The cytokine patterns found in CRS sinus tissue are similar to those of asthma lung tissue and explain the presence of eosinophils.⁷ The damaging effects of eosinophil granule proteins, especially eosinophil granule major basic protein (MBP), on respiratory mucosa have been well established.⁸⁻¹⁰ It is thought that MBP mediates the tissue damage seen in CRS as well as in asthma.^{9,11}

Airway remodeling is a pathologic process that is characteristic of asthma and involves smooth muscle hypertrophy, basement membrane thickening, epithelial damage (shedding), and deposition of matrix proteins.¹²⁻¹⁴ Given the high incidence of asthma in patients with CRS, we wanted to determine whether certain histologic features characterizing asthma (eosinophilic inflammation, epithelial shedding, and basement membrane thickening) were also detectable in sinonasal mucosal specimens from patients with CRS.

MATERIALS AND METHODS

The Institutional Review Board of Mayo Clinic Rochester approved the study. The diagnostic guidelines established by the American Academy of Otorhinolaryngology—Head and Neck Surgery were met in each patient for the diagnosis of CRS.² In addition, a coronal computed tomography scan was obtained in all

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TABLE I. Assessment of epithelial damage and basement membrane thickening

Staging of epithelial damage (shedding) with erosion (0-3)	
Stage 0:	Epithelium intact
Stage 1:	Cilia absent
Stage 2:	Upper cell layer eroded, basal cell layer intact
Stage 3:	Epithelium eroded away
Staging of basement membrane thickening (0-2)	
Stage 0:	No basement membrane visible
Stage 1:	Basement membrane visible, but not thicker than 20µm
Stage 2:	Basement membrane visible, thicker than 20µm

Abbreviations used

CRS: Chronic rhinosinusitis
H&E: Hematoxylin and eosin
MBP: Eosinophil granule major basic protein

patients demonstrating inflammatory mucosal thickening of >5 mm in >2 sinuses, consistent with CRS. Histologic specimens were processed from 22 randomly selected patients with CRS undergoing endoscopic sinus surgery. Patients who were taking systemic steroids within the 6 weeks before surgery were excluded. During surgery, the maximal amounts of tissue and mucus were carefully and gently collected to minimize damage to the specimens and to preserve the attachment of the mucus to the tissue. Because it is generally accepted that eosinophils^{11,15} and features of airway remodeling are absent in healthy control subjects, we decided that specimens from 4 otherwise healthy individuals undergoing septoplasty procedures were an adequate number to serve as negative controls. In addition, serial sections of a lung specimen obtained at autopsy from a patient who died in status asthmaticus were also used to document typical asthma pathology.

Formalin-fixed, paraffin-embedded tissue blocks were cut into 5-µm-thick sections and mounted on positively charged slides. Serial sections from each block were stained with 1) hematoxylin and eosin (H&E), 2) rabbit antibody to human eosinophil granule MBP, or 3) negative control for MBP (normal rabbit IgG), as previously described.^{6,16} In brief, sections were deparaffinized and rehydrated. After a partial tryptic digestion to unmask antigenic sites, the specimens were incubated in 10% normal goat serum to block nonspecific binding by the second-stage antibody. The next day, the slides were washed and overlaid with equal concentrations of either normal rabbit IgG or affinity-purified rabbit anti-human MBP.¹⁷ After incubation at 37°C for 30 minutes, the sections were washed and incubated in 1% chromotrope 2R at room temperature for 30 minutes to block nonspecific binding of fluorescein dye to the eosinophils. After another wash, the sections were overlaid with fluoresceinated goat anti-rabbit IgG and incubated at 37°C for 30 minutes. Subsequent to a final wash, the slides were mounted, coverslipped, and sealed.

Evaluation of airway remodeling

The damage to the epithelium and basement membrane thickening were assessed on H&E-stained sections with the staging system described (Table I and Fig 1). Three experienced examiners separately and independently evaluated the entire specimen. The mean of their combined scores was recorded. To exclude artifacts from trauma caused by the removal of the specimens during surgery, only areas of untouched mucosa that were still covered with mucus were evaluated. In addition, areas with obvious tears or with obvious influx of red blood cells, indicating trauma with bleeding, were excluded. After inspection of the entire specimen, the areas with the

maximal amount of basement membrane thickening as well as the areas with the maximal epithelial damage (shedding) were scored.

Evaluation of tissue inflammation

To objectively quantify the least and most inflammation per specimen, we developed a novel method for computer analysis of digital images (512 × 512 pixels) at 488-nm excitation wavelength from each MBP immunofluorescence specimen. We used a confocal microscope (LSM510, Carl Zeiss, Inc, Oberkochen, Germany) to survey and to select both the least and most intense areas of MBP immunofluorescence staining. First, digital images (512 × 512 pixels, ×400) of areas on the MBP immunofluorescence-stained section that contained maximal fluorescence staining (most intense accumulation of eosinophils) were obtained. Second, digital images of the corresponding areas on the serial section, which was stained with normal rabbit IgG as the negative control, were recorded. Third, with image-analyzing software (KS400 Image Analysis System, Carl Zeiss, Inc, Oberkochen, Germany), the threshold for each negative control image was calibrated to a baseline value that showed no positive pixels. Fourth, this “background” threshold was used to analyze the corresponding area on the MBP immunofluorescence-stained specimen. Any pixels recorded were quantified as a percentage of an area (512 × 512 pixels) positive for MBP. Finally, the image-analyzing software then compared the different areas within the specimen and determined the area with the highest percentage of positive pixels; this percentage indirectly indicated the intensity of the maximal inflammatory eosinophilic infiltrate. A similar survey was made to find the area in the MBP immunofluorescence-stained specimen with the least fluorescence; once located, it was compared with the corresponding area in the negative control serial section and analyzed as described earlier.

RESULTS**Epithelial damage and basement membrane thickening**

The demographics of the patients are shown in Table II. All patients with CRS demonstrated epithelial damage (Fig 2). In fact, 91% (20/22) of patients with CRS had substantial areas of the sinonasal epithelium completely eroded away (stage 3) down to the level of the basement membrane, whereas 9% (2/22) had only the upper layers eroded away (stage 2).

Basement membrane thickening was pathologic in all (22/22) of the patients with CRS. In 95% (21/22) of the patients with CRS, substantial areas of the specimens had basement membrane thickening that was >20 µm (stage 2). In 1 patient (5%), the basement membrane was thickened <20 µm (stage 1). No apparent differences in the severity of the epithelial damage and basement membrane thickening of the sinus tissue were seen between allergic and nonallergic patients, nor were there differences between patients with or without asthma.

Eosinophilic inflammation of CRS tissue

The eosinophilic inflammation (infiltrate) in the tissue specimens from the patients with CRS was very heterogeneous within any given specimen and contained intact cells as well as extracellular granules. Each specimen showed areas of abundant eosinophilia and areas without eosinophils. Thus, the digitized areas with the greatest

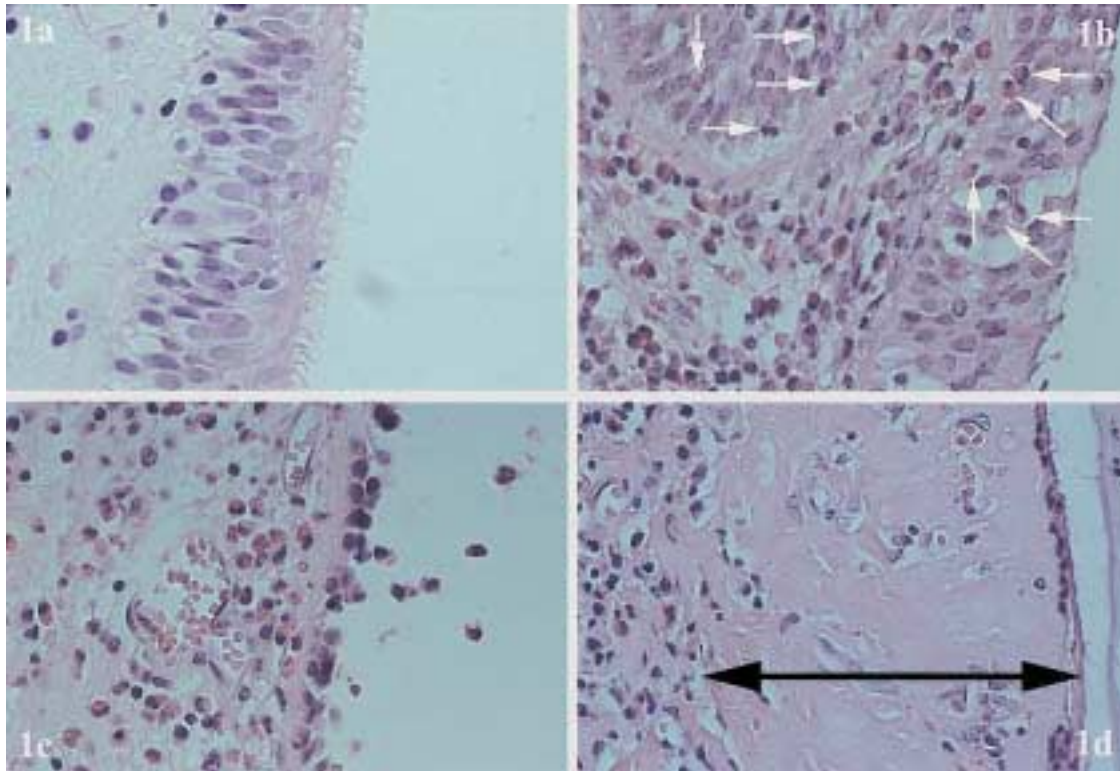


FIG 1. Staging system for epithelial damage (shedding) with erosion and for basement membrane thickening. To illustrate the wide variability in the histopathology of a given specimen, the photographs shown were all obtained from the same specimen from 1 patient. **1a**, Normal, healthy epithelium (stage 0) and absence of basement membrane thickening (stage 0). **1b**, Cilia absent (stage 1) and basement membrane thickening <20 μm (stage 1); white arrows point to eosinophils within the epithelium. **1c**, Upper layers of the epithelium eroded away, basal layer intact (stage 2), and basement membrane thickening <20 μm (stage 1). **1d**, Complete erosion of epithelium (stage 3) and massive thickening of the basement membrane (black double arrow, stage 2). Original magnification $\times 800$.

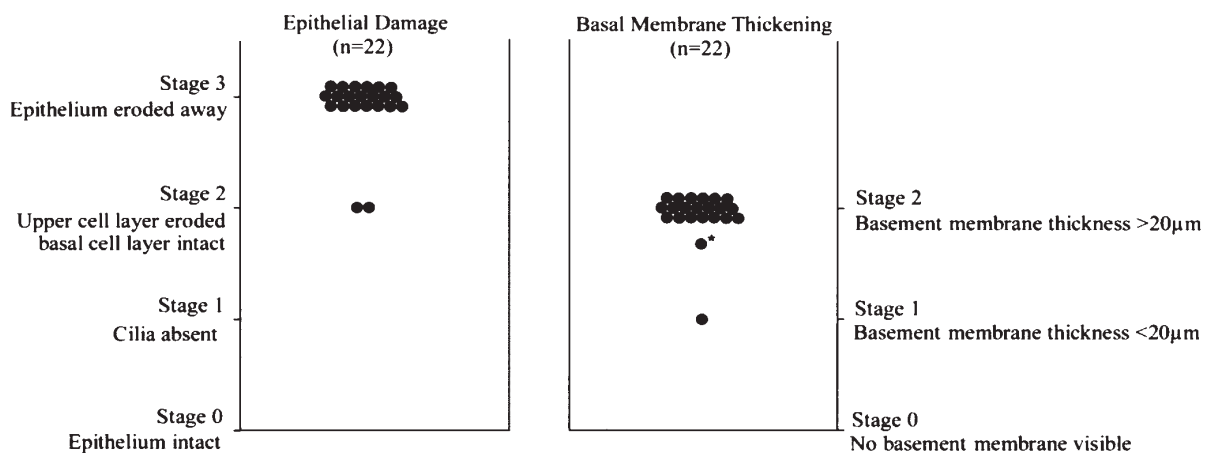


FIG 2. Maximal epithelial damage and basement membrane thickening of the epithelium in patients with CRS. Data were recorded as the mean score of 3 independent examiners. All the healthy control subjects (not shown) had an intact epithelium (4/4, stage 0) and no thickening of the basement membrane (4/4, stage 0). *This point results from 1 examiner grading 1 specimen as stage 1 and the other 2 examiners grading it as stage 2.

eosinophilic inflammation were quantified and compared with the digitized areas with the least inflammation. Within the same specimen, the areas showing the presence of MBP immunofluorescence varied from a mini-

mum mean of 0.16% ($\pm 0.19\%$) to a maximum mean of 21.89% ($\pm 16.85\%$; Figs 3 and 4). The eosinophilic inflammation was similar between allergic and nonallergic patients with CRS ($P = .44$; Fig 4).

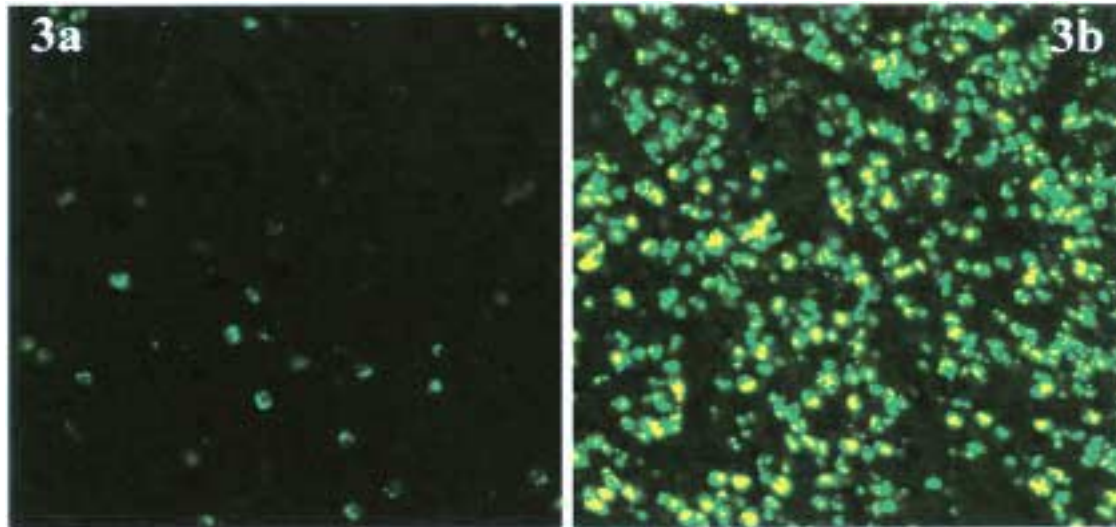


FIG 3. Range of eosinophilic inflammation within a patient with CRS. These photomicrographs of MBP immunofluorescence are taken from the same tissue specimen and are <1 mm apart. **3a**, An example of a minimal area positive for MBP (0.28%). **3b**, An example of a maximal area positive for MBP (20.04%). Original magnification $\times 400$.

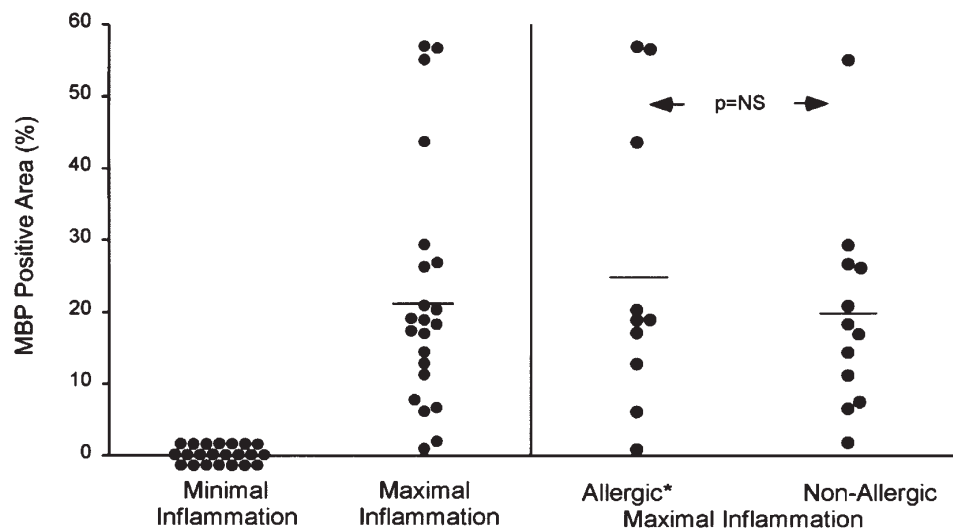


FIG 4. Comparison of digitized areas of minimal and maximal eosinophilic inflammation in patients with CRS. In the *left panel*, the data points represent the minimal and maximal percentages of tissue area positive for MBP immunofluorescence in each of the 22 patient specimens; the horizontal lines show the mean values in each group. Within the same specimen, the eosinophilic inflammation in the sinus tissues, as measured by the area positive for MBP, is very heterogeneous. In the *right panel*, the data points represent the maximal percentage of tissue area positive for MBP immunofluorescence in allergic versus nonallergic patients; no statistically significant differences were evident. *Defined as a positive skin prick test to at least 1 allergen in a panel of 16 common aeroallergens.

None of the specimens from healthy control subjects (0/4) stained positive for MBP immunofluorescence, indicating an absence of eosinophils as well as free eosinophil granules.

DISCUSSION

The predominantly eosinophilic inflammation present in CRS is recognized as playing an important role in the

pathogenesis of CRS.^{1,11} Because less than half of the patients with CRS in this study and in prior studies have elevated specific IgE or positive skin tests, this eosinophilic inflammation is not likely driven by an IgE mechanism. Furthermore, the eosinophilic inflammation is clearly heterogeneous in any given specimen; therefore, careful evaluation of numerous sites of the same relatively large tissue specimen is needed to avoid false-negatives with erroneous conclusions. For example,

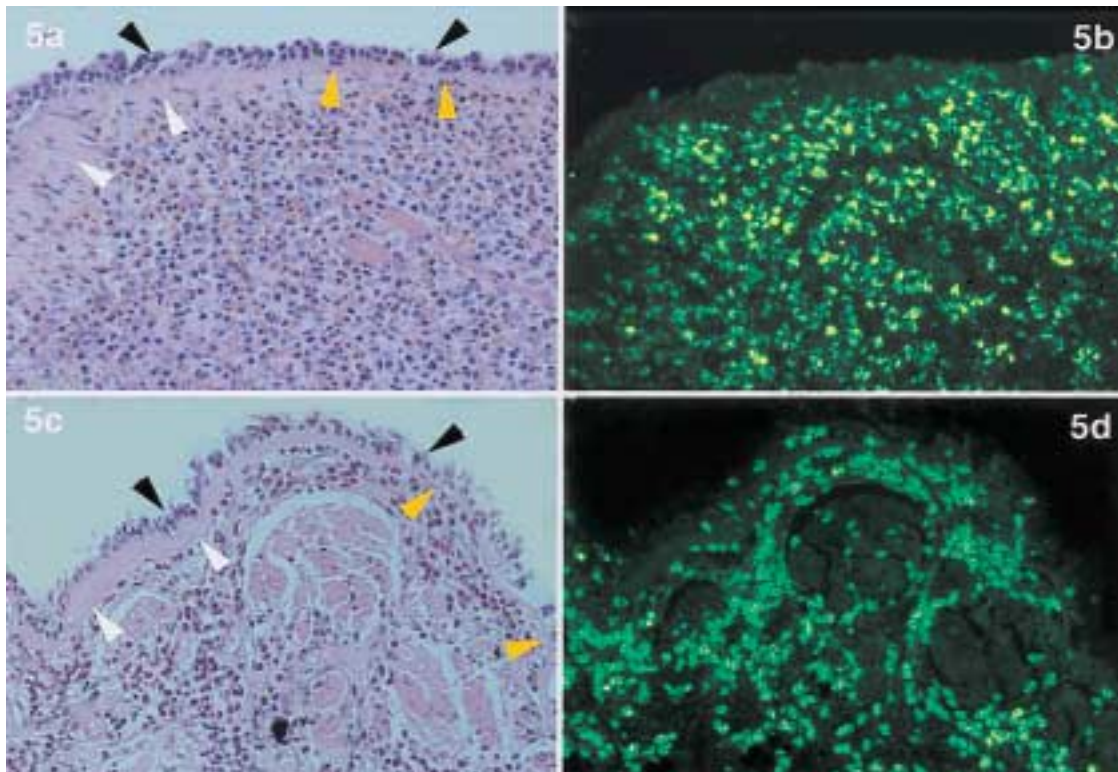


FIG 5. Comparison of histopathology in CRS sinus and asthma lung specimens. The striking similarities in the histopathology of specimens from patients with CRS (**5a** and **5b**) and fatal asthma (**5c** and **5d**) are illustrated. H&E-stained sections (**5a** and **5c**) show erosion of the epithelium (stage 2, black arrowheads) as well as basement membrane thickening (stage 1, yellow arrowheads; stage 2, white arrowheads). MBP immunofluorescence-stained serial sections (**5b** and **5d**) show that the intensity of the eosinophilic infiltration appears to be similar in CRS (**5b**) and in asthma (**5d**). Original magnification $\times 400$.

TABLE II. Patient demographics

Age (mean, range)	47.4 years	16-86 years
Gender	11 Female	11 Male
Number of previous sinus surgeries (mean, range)	1.8	0-7
Duration of disease (mean, range)	8.6 years	2-27 years
Incidence of asthma or increased airway hyperreactivity*	91%	(20/22)
Incidence of aspirin sensitivity	41%	(9/22)
Total IgE (mean, range)	339 KU/L	9-1483 KU/L
Incidence of elevated total IgE (>128 KU/L)	50%	(11/22)
Incidence of allergy†	45%	(10/22)

*Fifteen of 22 patients had physician-diagnosed asthma, and the remaining 5 patients had increased airway hyperreactivity, as demonstrated by methacholine challenge test.

†Defined as a positive skin prick test to at least 1 allergen in a panel of 16 common aeroallergens. Of the 15 patients with asthma, 7 had allergy; of the 5 patients with increased airway hyperreactivity, 2 had allergy; and of the 2 patients without asthma or increased airway hyperreactivity, 1 had allergy.

small biopsy specimens of nasal tissue, which reveal no eosinophils or MBP, might be vulnerable to sampling errors and give a false-negative result. To minimize sample artifacts, we also used meticulous techniques in obtaining the specimens. In addition, in prior studies, the patients' use of systemic or topical corticosteroids before obtaining the specimen might explain the absence of eosinophils in a small percentage of cases. Thus, an examination of the entire tissue specimen as well as avoidance of corticosteroids before obtaining the specimen are likely required to optimize the detection of the eosinophilic inflammation.

Striking epithelial damage and basement membrane thickening were present in all 22 patients with CRS, a finding that had not been previously recognized in CRS. Moreover, CRS and asthma share similar histopathologic features, namely, an intense eosinophilic inflammation, basement membrane thickening, and erosion of the epithelium (Fig 5). It is well known that CRS and asthma occur concomitantly in many patients and clinically flare at the same time. Because the histopathologic changes are strikingly similar, these airway disorders might be tissue-specific regional manifestations of the same disease.

The severe erosion and prominent damage to the epithelial layer could also explain the acute bacterial exacerbations seen in patients with CRS. Because there is no consistent difference between the bacterial populations of patients with CRS and healthy control subjects,¹⁸ the bacteria themselves cannot be considered pathogenic. The damaged epithelium might provide an entry port for the colonizing bacteria to invade the nasal and paranasal tissues and establish an infection. Healthy control subjects, as shown in our study, consistently maintain an intact epithelial barrier, thereby denying an entry port for colonizing bacteria. In contrast to the sinuses, the relative sterility of the lung might explain the lack of frequent bacterial infections in patients with asthma.

The clinical significance of these histopathologic findings relates to the clinical associations between these 2 disorders. As noted in Table II, 15 of 22 (68%) of the patients with CRS had been previously diagnosed with asthma. In addition, the remaining 7 patients with CRS were given a methacholine challenge test, and 5 of 7 showed hyperreactive airway disease. Overall, 91% of patients with CRS had either asthma or increased airway hyperreactivity. Thus, patients with CRS should be tested for asthma, as we can anticipate the likely diagnosis of asthma in patients with CRS (and vice versa). Their asthma can be diagnosed and treated at an earlier stage to minimize unwanted complications or severe manifestations.

The previously described histopathologic markers of asthma (eosinophilic inflammation and features of airway remodeling, such as erosion of epithelium and basement membrane thickening) are also present in sinonasal specimens from patients with CRS. These findings along with the high clinical overlap suggest that CRS and asthma are part of the same disease process: an eosinophilic inflammation of airway mucosa stretching from the nostril down to the alveoli. This inflammation has the ability to mediate the epithelial damage seen in CRS; this damage might be a crucial factor in the development of secondary bacterial infections in patients with CRS, which are absent in healthy control subjects.

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REFERENCES

1. Kaliner MA, Osguthorpe JD, Fireman P, Anon J, Georgitis J, Davis ML, et al. Sinusitis: bench to bedside: current findings, future directions. *Otolaryngol Head Neck Surg* 1997;116:S1-20.
2. Lanza DC, Kennedy DW. Adult rhinosinusitis defined. *Otolaryngol Head Neck Surg* 1997;117:S1-7.
3. Blackwell DL, Collins JG, Coles R. Summary health statistics for U.S. adults: National Health Interview Survey, 1997. National Center for Health Statistics. *Vital Health Stat* 2002;10.
4. Hamilos DL. Chronic sinusitis. *J Allergy Clin Immunol* 2000;106:213-27.
5. Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877-84.
6. Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 1982;2:11-6.
7. Hamilos DL, Leung DY, Wood R, Cunningham L, Bean D, Yasruei Z, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. *J Allergy Clin Immunol* 1995;96:537-44.
8. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. *Annu Rev Med* 1993;44:85-101.
9. 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.
10. Hisamatsu K, Ganbo T, Nakazawa T, Murakami Y, Gleich GJ, Makiyama K, et al. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J Allergy Clin Immunol* 1990;86:52-63.
11. Harlin SL, Ansel DG, Lane J, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol* 1988;81:867-75.
12. Chiappara G, Gagliardo R, Siena A, Bonsignore MR, Bousquet J, Bonsignore G, et al. Airway remodelling in the pathogenesis of asthma. *Curr Opin Allergy Clin Immunol* 2001;1:85-93.
13. Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57:309-16.
14. Carter PM, Heinly TL, Yates SW, Lieberman PL. Asthma: the irreversible airways disease. *J Invest Allergol Clin Immunol* 1997;7:566-71.
15. Bhattacharyya N, Vyas DK, Fechner FP, Gliklich RE, Metson R. Tissue eosinophilia in chronic sinusitis: quantification techniques. *Arch Otolaryngol Head Neck Surg* 2001;127:1102-5.
16. Filley WV, Ackerman SJ, Gleich GJ. An immunofluorescent method for specific staining of eosinophil granule major basic protein. *J Immunol Methods* 1981;47:227-38.
17. Peters MS, Schroeter AL, Kephart GM, Gleich GJ. Localization of eosinophil granule major basic protein in chronic urticaria. *J Invest Dermatol* 1983;81:39-43.
18. Nadel DM, Lanza DC, Kennedy DW. Endoscopically guided cultures in chronic sinusitis. *Am J Rhinol* 1998;12:233-41.