

Cysteinyl leukotriene expression in chronic hyperplastic sinusitis–nasal polyposis: Importance to eosinophilia and asthma

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Background: Chronic hyperplastic eosinophilic sinusitis (CHS) results from the unregulated proliferation of eosinophils, T_H2-like lymphocytes, goblet cells, mast cells, and fibroblasts and is present in most patients with asthma. The frequent coexpression of these disorders and their shared pathophysiology suggests that these are similar disorders affecting the upper and lower airways.

Objective: We evaluated the expression of cysteinyl leukotrienes (CysLTs) in sinus tissue from subjects with CHS compared with that seen in healthy sinus tissue.

Methods: Nasal polyp and sinus tissue was evaluated from 58 individuals undergoing elective functional endoscopic sinus surgery. The diagnosis of CHS was demonstrated through the presence of eosinophilia and activated (EG2⁺) eosinophils, as determined by means of tissue immunohistochemistry. Data were compared with those from both nasal polyp tissue without eosinophilic inflammation and healthy control sinus tissue obtained from the sinus ostiomeatal complex at the time of surgery for unrelated disorders. CysLTs were quantified by means of ELISA in lipid-extracted tissue. Activation of the metabolic pathway leading to CysLT synthesis was demonstrated by ribonuclease protection. Subjects were genotyped for leukotriene C₄ (LTC₄) synthase C-to-A promoter polymorphism.

Results: CysLT concentrations were significantly higher in tissue obtained from subjects with CHS (776.7 ± 201.9 pg/g tissue) compared with that seen in healthy sinus tissue (355.7 ± 101.6 pg/g tissue, *P* < .03). CysLT concentrations within noneosinophilic nasal polyps (328.0 ± 116.4 pg/g tissue) were similar to those in control tissue. The presence of CysLTs in CHS was associated with increased expression of LTC₄ synthase mRNA. The C-to-A promoter polymorphism was associated with trends toward the increased presence of CHS and CysLTs. **Conclusions:** CHS is characterized by the increased presence of CysLTs when compared with concentrations seen in tissue from patients with chronic inflammatory sinusitis or healthy

sinus tissue. These studies support the use of LT modifiers as anti-inflammatory agents that might have clinical benefit in patients with these disorders. (*J Allergy Clin Immunol* 2003;111:342-9.)

Key words: Asthma, chronic hyperplastic sinusitis, cysteinyl leukotrienes, eosinophils, leukotriene C₄ synthase, nasal polyps, polymorphisms

Chronic hyperplastic eosinophilic sinusitis (CHS), which is often present in conjunction with nasal polyposis (NP), is an immune inflammatory disease characterized by the accumulation of eosinophils, fibroblasts, mast cells, and goblet cells.¹⁻⁴ The accumulation of activated eosinophils is considered to be the primary hallmark of this condition.⁵⁻⁷ CHS is frequently associated with asthma. When adult asthmatic patients are evaluated by means of computed tomographic scanning, approximately 74% to 90% have some degree of mucosa hyperplasia,⁸⁻¹⁰ which is often asymptomatic. A smaller percentage of individuals with CHS have asthma, although the presence of CHS is considered to define a cohort at risk for the development and severity of asthma.⁷ This association of CHS with asthma might reflect the shared pathophysiology of these disorders of the upper and lower airways, respectively. The inflamed sinus tissue demonstrates a marked increase in cells expressing mRNA for cytokines responsible for allergic inflammation. As with asthma, this disorder is associated with the activation of T_H2-like lymphocytes characterized by the production of IL-3, IL-4, IL-5, IL-13, eotaxin, and GM-CSF.^{1,11-15} Eosinophils are an additional source of these cytokines, suggesting that CHS can become a disease of unregulated accumulation of eosinophils, with eosinophils themselves providing, in an autocrine fashion, the growth factors necessary for their own recruitment, proliferation, and activation. The production of transforming growth factor α , transforming growth factor β , platelet-derived growth factors, heparin-binding epidermal growth factor, fibroblast growth factor, and other growth factors by activated eosinophils and these other cells might contribute to the proliferation of fibroblasts and deposition of connective tissue in patients with CHS.¹⁶

CHS is distinguished from noneosinophilic chronic sinusitis, which is generally more responsive to surgical intervention.^{17,18} Chronic inflammatory sinusitis is less well characterized but is thought to result from chronic or recurrent occlusion of the sinus ostia as a result of viral rhinitis, allergic rhinitis, anatomic predisposition, or

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Abbreviations used

CHS: Chronic hyperplastic eosinophilic sinusitis
COX: Cyclooxygenase
CysLT: Cysteinyl leukotriene
EIA: Enzyme immunoassay
FLAP: 5-Lipoxygenase activating protein
5-LO: 5-Lipoxygenase
LT: Leukotriene
NP: Nasal polyposis
OMC: Ostiomeatal complex
RPA: Ribonuclease protection assays

other causes. These processes lead to recurrent acute bacterial infections, possibly in association with barotrauma of the sinus cavities, with damage to the respiratory epithelium, ciliary damage, mucous gland and goblet cell hyperplasia, bacterial colonization, and, ultimately, chronic inflammatory changes. Activated eosinophils are not a feature of chronic inflammatory sinusitis, although this disorder might produce nasal polyps. Less commonly, chronic sinusitis might also be a chronic infectious disease characterized by the presence of bacteria at high titer and prominent neutrophils.¹⁹

The pathologic and immune similarities between CHS and asthma suggest that, similar to asthma, cysteinyl leukotrienes (CysLTs; leukotriene [LT] C₄, LTD₄, and LTE₄) might be expressed in this disorder. The current studies were designed to measure CysLT concentrations in surgically derived nasal polyp tissue. We compared data derived from nasal polyp tissue from patients with CHS with that derived from polyp tissue from patients with chronic inflammatory noneosinophilic sinusitis, as well as with data from healthy uninvolved sinus tissue obtained from subjects undergoing functional endoscopic sinus surgery for disease elsewhere in their sinuses. We also evaluated the expression of the CysLT metabolic cascade, including the enzymes 5-lipoxygenase (5-LO), 5-LO activating protein (FLAP), and LTC₄ synthase. This involved quantification of mRNA transcripts of these enzymes through ribonuclease protection assays (RPAs). Finally, overexpression of LTC₄ synthase in aspirin-sensitive asthma has been associated with an A-to-C promoter polymorphism 444 nucleotides upstream from the translation start site that leads to enhanced promoter strength.²⁰⁻²² The presence of this polymorphism was evaluated in our study subjects.

METHODS

Subjects

Polyp tissue was obtained from subjects (n = 58) referred to the University of Virginia Health System for sinus surgery. Subjects were asked their permission, and informed consent was given to study pathologic specimens under a protocol approved by the human investigations committee at the University of Virginia Health System. No individual was studied unless he or she had been recommended for and agreed to undergo sinus surgery. Study subjects were selected solely on the basis of a medical history consistent with chronic sinusitis, consistent findings on rhinoscopy, and documented disease in their sinuses, as shown through computed tomo-

TABLE I. Subject characteristics

	Control	Noneosinophilic polyposis	CHS
n	12	14	32
Age (y)	49 ± 4	33 ± 5	40 ± 3
Sex (F:M)	8:4	8:6	14:18
Allergy*	0	4	9
Asthma	1	2	13
Aspirin sensitivity	0	0	5

*Defined as 3 or more positive prick skin test responses (wheal ≥5 mm) to the Virginia inhalant panel or a positive (≥class I) IgE immunoassay result.

graphic scanning. Infectious sinusitis, as determined by evaluating sinus tissue for neutrophils, was an exclusion criterion. Similarly, additional exclusion criteria were the presence of cystic fibrosis or an immunodeficiency. Patients were not enrolled if they received oral steroids or an LT modifier within 4 weeks of surgery. Control subjects were characterized by the presence of sinusitis documented on their computed tomographic scans and by the absence of polyps on rhinoscopic evaluation.

Clinical evaluation

All enrolled subjects subsequently underwent a detailed medical history and examination to evaluate the presence of allergic rhinitis or asthma and were questioned regarding the use of medications, especially systemic and topical intranasal corticosteroids. A history of aspirin–nonsteroidal anti-inflammatory drug sensitivity was obtained but was unequivocal on the basis of history in only 5 subjects. Aspirin sensitivity was defined as either acute asthma exacerbation or upper respiratory symptoms, including nasal congestion, rhinorrhea, paroxysmal sneezing, and “sinus fullness.” Isolated urticaria–angioedema or nonallergic side effects were not included as aspirin sensitive. Subjects were evaluated for the presence of atopy (positive skin test response defined as ≥5-mm wheal on prick test with 3 or more components of the Virginia aeroallergen panel). Subjects underwent spirometry on a Koko spirometer, and the diagnosis of asthma was based on the presence of reversible airway obstruction (≥12% increase in FEV₁ after administration of a bronchodilator). Fifteen subjects were given a diagnosis of asthma. Subject characteristics are summarized in Table I.

Pathologic examination

Polyp tissue underwent blinded pathologic examination, and one specimen with numerous neutrophils, which was suggestive of infection, was excluded from further analyses. Eosinophilia was evaluated in a blinded fashion by using a semiquantitative scoring system: 0, no eosinophils; 1, rare eosinophils (<1/high-power field [400×], 0.238 mm²); 2, few eosinophils; 3, several eosinophils; and 4, numerous eosinophils. In addition, tissue underwent immunohistochemistry for expression of activated (EG2⁺) eosinophils. The diagnosis of CHS was based on the presence of “several” or “numerous” eosinophils per high-powered field and the expression of EG2.^{1,15} Subjects with few or no eosinophils (all of whom had no staining for EG2) were considered to have chronic inflammatory sinusitis. Control tissue obtained from the ostiomeatal complex (OMC) of subjects undergoing functional endoscopic sinus surgery for chronic inflammatory disease of the sinuses was also confirmed to be without eosinophil, neutrophil, mononuclear cell infiltrate, or other inflammatory changes.

Tissue immunohistochemistry

Blinded tissue immunohistochemistry was performed at the National Jewish Medical and Research Center. OMC and nasal polyp tissue were fixed in either RNase-free acetone for subsequent

glycol methacrylate embedding or in 4% paraformaldehyde for subsequent paraffin embedding. Glycol methacrylate and paraffin blocks were tested for best available staining with anti-human EG2 standardized at a 1:150 dilution (Pharmacia). Immunostaining was carried out by using a standard Vectastain Avidin-Biotin complex kit (Vector Laboratories). Morphometric analysis was accomplished by the use of a CAST system (Olympus Denmark A/S). Briefly, sections were cut at 2- μ m thickness 4 μ m apart and imaged with the CAST system. Fields were chosen randomly, and a cycloid grid was overlaid to count the positive cells that intersected the grid. A minimum of 10 fields were analyzed for each sample. The volume density (cubic millimeters of EG2 per cubic millimeters of reference volume) of EG2 (Vv) was calculated with the following formula:

$$Vv = \text{Positive grid points} / \text{Total countable grid points} \times 100.$$

Quantification of CysLTs in CHS tissue

Sinus and nasal polyp tissue was homogenized in ethanol to extract the lipid fraction, stabilize the LTs, and remove cellular protein. After centrifugation of precipitated proteins, the supernatant was dried, resuspended in 80% ethanol, and loaded on a C-18 reverse-phase cartridge (Accubond columns, J&W Scientific). The C-18 cartridge was rinsed with ultrapure water, followed by HPLC-grade hexane, and the CysLTs were subsequently eluted with ethanol/water (90:10). The CysLTs were quantified by using a high-sensitivity competitive enzyme immunoassay (EIA; Cayman Chemical), according to the manufacturer's directions. This EIA is a competitive sandwich technique, and data were determined in comparison with CysLT standards. This EIA detects LTC₄, LTD₄, and, to a lesser extent, LTE₄ and has a sensitivity of 5 to 10 pg. Data are expressed as picograms of CysLTs per gram of sinus tissue in the original homogenate.

Ribonuclease protection assay

The RPA was used to quantify mRNA in tissue samples. Total RNA was extracted from the polyp tissue in RNeasy (Tel-Test, Inc). The purity of RNA was evaluated on the basis of preliminary electrophoresis on a formaldehyde agarose gel and evaluation of the stability of 18S and 28S RNA. Single-stranded anti-sense RNA probes specific for phospholipase A2, cyclooxygenase (COX) 1, COX-2, 5-LO, FLAP, and LTC₄ synthase, which are all of unique size, were synthesized and internally labeled with α -phosphorous 32-labeled uridine triphosphate. The plasmid necessary for generating these antisense probes was produced for this study through a contract with Pharmingen. Probe and RNA preparations were allowed to hybridize overnight, after which samples were exposed to ribonuclease A for 1 hour. Samples were washed, denatured, and electrophoresed on a polyacrylamide urea denaturing gel. Protected probes of an appropriate size were identified by means of comparison with a labeled ladder and quantified with a Phosphorimager (Molecular Dynamics). Data were normalized to the expression of the housekeeping probe present in the plasmid (*L32*).

Identification of polymorphism in the LTC₄ synthase promoter

We used real-time PCR to genotype the single nucleotide polymorphism at nucleotide -444 of the LTC₄ synthase promoter. DNA was extracted from either anticoagulated blood or from the polyp tissue itself by using a commercial DNA extraction kit (Gentra Systems, Inc). We analyzed promoter fragments 84 bp in length that contained the LTC₄ synthase gene polymorphism.²⁰⁻²² Amplification of the gene encoding LTC₄ synthase was performed by using the following primers: CAGAGGAGGGTTTGG and TCCCGGCTAACTCCT. Detection of the C and A alleles was accomplished through the differential hybridization of fluorescently labeled probes to the amplified product. The probe for the C allele was

5'-FAM-AATGGGGACCGGGAACAGA-BHQ-1, and the probe for the A allele was 5'-HEX-AATGGGGACAGGGAACAGA-BHQ-1 (Integrated DNA Technologies, Inc). PCR reactions consisted of 40 cycles of 30 seconds each at 95°C, 55°C, and 72°C in a Bio-Rad iCycler thermocycler (Bio-Rad), with real-time detection occurring at 72°C. Many sequences were confirmed by using direct sequencing on both the sense and antisense strands. PCR was performed on genomic DNA (1 μ g) and internally labeled with fluorescent probes and appropriate dideoxy termination mixes to perform the sequencing reaction. DNA sequences were analyzed on an Applied Biosystems DNA 377 Prism sequencer.

Statistical analyses

We quantified CysLTs in nasal polyp tissue as picograms per gram of tissue. Expression of 5-LO, FLAP, and LTC₄ synthase mRNA transcripts were quantified by using the phosphorimager and normalized to expression of the housekeeping gene *L32*. Data were contrasted between tissue from patients with CHS, tissue from patients with NP and sinusitis without eosinophilic inflammation, and healthy OMC tissue from control subjects by using ANOVA for parametric data and χ^2 statistics for nonparametric data or regression analyses with JMP 3.2 software on a Macintosh G4 computer.

RESULTS

Pathology

Only one subject had numerous neutrophils identified in polyp tissue, and this individual was excluded from further analyses. Microscopic examination of the polyp tissue in the remaining 57 subjects was characterized by a nonspecific mononuclear cell inflammatory infiltrate. Thirty-two pathologic specimens on blinded examination of multiple high-powered fields were identified as having "several" or "numerous" tissue eosinophils and were therefore considered to represent CHS. Tissue immunohistochemistry was performed and confirmed the presence of activated (EG2⁺) eosinophils in these samples. The remaining polyp tissue samples had few or no eosinophils per high-powered field on blinded examination, did not stain for EG2, and were given a diagnosis of chronic inflammatory sinusitis. None of the control sinus tissue had either eosinophils or stained for EG2. Neither the presence of allergy (skin test reactivity) or aspirin sensitivity nor the use of nasal corticosteroids influenced tissue eosinophilia or eosinophil cationic protein expression. CHS significantly correlated with underlying asthma ($P < .05$, Table II). CHS was dramatically associated with circulating eosinophilia (Fig 1). Absolute eosinophil counts were $400 \pm 51/\mu\text{L}$ in subjects with CHS, $181 \pm 50/\mu\text{L}$ in subjects with noneosinophilic chronic sinusitis with nasal polyps, and $160 \pm 42/\mu\text{L}$ in subjects without NP or OMC disease ($P < .01$). All but 2 of the subjects with greater than 350 circulating eosinophils per microliter had CHS. This trend was not related to the increased prevalence of asthma in the CHS cohort because total eosinophil counts were approximately equal in patients with CHS without and with asthma ($339 \pm 71/\mu\text{L}$ and $423 \pm 97/\mu\text{L}$, respectively).

CysLT expression

CysLT concentrations were significantly higher in tissue obtained from patients with CHS (776.7 ± 201.9 pg/g

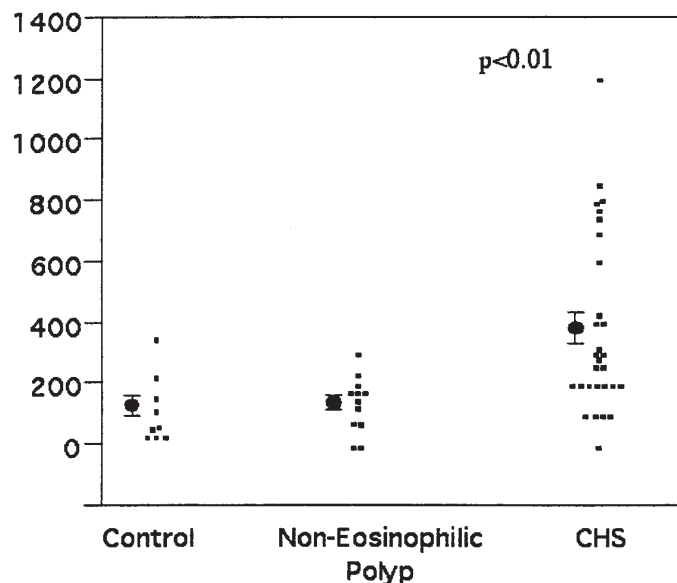


FIG 1. Circulating eosinophils, as determined by means of sinus tissue histology. Control subjects were without nasal polyps and had no inflammation in their surgically derived sinus tissue. Nasal polyps were categorized as CHS or inflammatory sinusitis according to the presence of numerous intact eosinophils or eosinophil cationic protein. Absolute circulating eosinophil counts (cells per microliter) were determined by using an automated system.

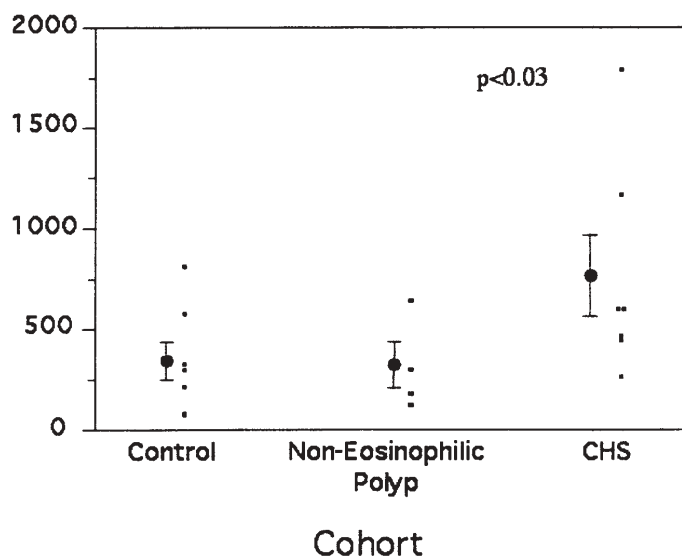


FIG 2. Concentration of CysLTs in nasal polyp tissue. CysLTs were quantified in nasal polyp tissue by using a high-sensitivity competitive EIA that detects LTC₄, LTD₄, and, to a lesser extent, LTE₄. Data are expressed as picograms of CysLTs per gram of sinus tissue in the original homogenate.

tissue) compared with in healthy sinus tissue (355.7 ± 101.6 pg/g, $P < .03$; Fig 2). CysLT concentrations within nasal polyps derived from tissue without eosinophils or EG2-staining cells (328.0 ± 116.4 pg/g) were similar to those of control tissue. CysLT expression was not influenced by either the use of nasal corticosteroids or by the presence of either inhalant allergy or aspirin sensitivity.

Ribonuclease protection assay

The RPA was performed on total RNA derived from nasal polyp tissue from 15 subjects without and with eosinophilia in whom sufficient RNA could be obtained (≥ 10 µg) that was free of ribonuclease activity. Invalidation of the remaining samples reflected either the high concen-

Genotyping

Genotyping was performed on 12 control patients and 29 patients with CHS, and the C-444 allele was expressed in 17 subjects (14 heterozygotes and 3 homozygotes). The frequency of the C allele in the control group was 0.17 compared with 0.28 in the patients with CHS, although these differences were not statistically significant ($P = .16$). Similarly, subjects with the mutation demonstrated trends toward increased CysLT concentrations in their sinus tissue (597.3 ± 188.2 pg/g) in comparison with concentrations in those who were of the homozygous wild type (285.4 ± 72.5 pg/g) and had increased concentrations of LTC₄ synthase transcripts (data not shown). These differences, however, again did not achieve statistical significance. There were no additive effects observed between subjects who were heterozygous or homozygous for the polymorphism. There were insufficient numbers of aspirin-insensitive subjects ($n = 5$) to evaluate a linkage to the LTC₄ synthase polymorphism.

DISCUSSION

These studies compared the expression of CysLTs and metabolic pathways involved in CysLT synthesis between tissue from patients with CHS, noneosinophilic inflammation, or uninvolved healthy sinuses. The pathology and immunology of CHS share many features with asthma, and these conditions are frequently coincidentally expressed in patients.⁵⁻⁷ We therefore reasoned that these similarities would extend toward a tendency to express CysLTs. Subjects with CHS were defined by the presence of prominent eosinophilia on pathologic examination and the presence of activated (EG2⁺) eosinophils. As previously shown,^{5,8-10,23,24} CHS correlated with the presence of asthma, and 12 of 15 of the subjects with asthma were in the CHS cohort (Table II). In contrast, the presence of skin test reactivity did not distinguish CHS from noneosinophilic sinus disease. This is consistent with the proposed concept that chronic inflammatory sinusitis might develop in part as a result of chronic or recurrent obstruction of the sinus ostia and that allergic rhinitis represents one mechanism for this occlusion. Alternatively, skin test reactivity might represent an irrelevant coincidental finding in these subjects.

As has been previously shown, the presence of CHS was strongly associated with the presence of circulating eosinophils (Fig 1).^{8,9,24-26} It is surprising, however, that this trend cannot be explained by the increased prevalence of asthma in the CHS cohort insofar as total eosinophil counts were approximately equal in patients with CHS without and with asthma. Thus these observations argue against the hypothesis that asthma-mediated eosinophilia contributes to the tissue eosinophilia of CHS. The presence of roughly equal numbers of eosinophils in subjects without and with asthma suggests that it is the CHS-polyp tissue itself that is responsible for driving the increased absolute eosinophil counts. Indeed, these data support the opposite hypothesis,

TABLE II. Polyp pathology and asthma

Cohort	Nonasthmatic patients	Asthmatic patients
Control	8	1
Noneosinophilic NP	10	2
CHS	14	12*

* $P < .05$, compared with control or noneosinophilic NP.

specifically that eosinophilia caused by CHS might contribute to the severity of asthma. Although a causal link between sinusitis and asthma has been suggested, explanations for this linkage, such as the presence of a sinus-bronchial reflex or aspiration of sinus secretions into the lung, are not satisfactory.²⁷ These data suggest that the expression of cytokines, such as IL-5, in the CHS tissue¹²⁻¹⁵ and the local production of eosinophil precursors²³ contribute to asthma severity. The ensuing eosinophilia will lead to selective recruitment of eosinophils into the asthmatic lung. However, this will only occur in subjects with established asthma who have vascular cell adhesion molecule 1 and other eosinophil-specific adhesion molecules expressed on their lung tissue. This model is supported by recent data demonstrating that the local production of granulocyte colony-stimulating factor in the upper airway during a rhinovirus infection is capable of driving systemic neutrophilia and recruitment of those neutrophils into the asthmatic lung.²⁸ Similarly, recent studies have shown that severity of CHS links to absolute eosinophil counts and asthma severity.^{8,24}

CysLT concentrations, as measured by means of ELISA, were significantly greater in CHS tissue compared with in tissue from either of the control cohorts, and very similar concentrations were observed in both the noneosinophilic polyp tissue and the uninvolved control sinus tissue (Fig 2). The similarity of the CysLT concentrations in the nasal polyp tissue without eosinophils and in the sinus tissue from subjects with chronic sinusitis without polyps suggests that these might represent the same condition. In addition, we used the RPA to evaluate activation of the metabolic cascade leading to CysLT production, including the rate-limiting enzyme LTC₄ synthase. Using this technique, we were able to show that subjects with CHS demonstrated greater expression of mRNA transcripts for LTC₄ synthase (Fig 3, B). CysLTs are produced by eosinophils, mast cells, basophils, and monocytes. Insofar as CHS was defined in these studies through the presence of eosinophilic inflammation, it is plausible that the increased expression of CysLTs in these subjects merely represents the presence of activated eosinophils. Alternatively, CysLTs are known to be chemotactic for eosinophils and to promote eosinophilic inflammation through their ability to promote adhesion molecule (P-selectin and CR3) expression and to inhibit eosinophil apoptosis.²⁹⁻³⁴ As such, the enhanced presence of CysLTs might be responsible for the eosinophilia that is pathognomic for this disease. In addition, influences of CysLTs on fibroblast proliferation³⁵ might also

contribute to the pathology of CHS. Irrespective of whether CysLTs are an epiphenomenon of the eosinophilia, given the myriad of their pro-inflammatory influences,²⁹⁻³⁵ they are likely to contribute to the severity of CHS.

Neither the presence of inhalant allergy or aspirin sensitivity nor the use of nasal corticosteroids influenced tissue eosinophilia, although the number of subjects with aspirin sensitivity in these studies was too few to be statistically meaningful. We cannot exclude the possibility that the absence of a difference in eosinophil prevalence or eosinophil cationic protein concentrations between subjects exposed and those not exposed to topical corticosteroid precluded an influence by the steroids on these parameters.

Previous studies have addressed the possibility of CysLT production in CHS; however, this has not been categorically established. CysLTs have been identified in the nasal lavage fluid of subjects with allergic rhinitis and nasal polyps or in the supernatants of stimulated polyp tissue in some,³⁶⁻³⁹ although not all,^{40,41} studies. These studies are not clear as to whether these LTs are derived from the nasal epithelium directly stimulated by allergen or from the nasal polyps or are difficult to interpret secondary to the difficulties in normalizing the data. Because nasal polyps are covered by epithelium, we cannot completely exclude the possibility that this might have been the source of some of the measured CysLTs in our studies as well.

A single nucleotide polymorphism at nucleotide -444 in the LTC₄ synthase gene promoter has been associated with overexpression of this gene. In the study of Sanak et al,²⁰ the C -444 allele was present in 44% of patients with aspirin-intolerant asthma but only in 23% of normal control subjects. This compares with a recent study by Kawagishi et al,²¹ who identified the gene frequency (q) of this polymorphism more frequently in subjects with aspirin-intolerant asthma (q = 0.192) than in those with aspirin tolerance (q = 0.110). Similarly, Sampson et al²² reported this polymorphism in 32% of normal subjects in comparison with 56% of subjects with severe asthma. The C -444 allele appeared to be overexpressed in our cohort, with a frequency of 0.28 in our subjects with CHS-NP but only 0.17 in the control cohorts. That this did not reach statistical significance was most likely caused by low numbers of patients in the control group. As would be expected given the linkage of this polymorphism to the presence of CHS, there were trends between the presence of this polymorphism and both the level of expression of LTC₄ synthase transcripts and CysLTs. This is further evidence that the increased expression of CysLTs is a primary event in CHS and not an epiphenomenon of the eosinophilic inflammation.

In summary, our studies demonstrate the association of CHS with the production of CysLTs. Whether the CysLTs are responsible for the eosinophilic inflammation or are just markers of the presence of activated eosinophils does not preclude the recognition that these compounds are pro-inflammatory and are likely to be contributing to the symptoms of this condition. CysLTs

could contribute to and exacerbate CHS through their ability to induce microvascular leakage, stimulate goblet cell secretion, stimulate myofibroblast proliferation, and mediate eosinophil recruitment, adhesion, hematopoiesis, and survival.²⁸⁻³⁴ In support of this concept, patients who received LT modifiers for their asthma have noted improvement in their sinus disease, and in some cases their sense of smell was restored.^{42,43} Finally, these data support the hypothesis that pro-inflammatory events in the sinus and polyp tissues of patients with CHS might contribute to the heightened production of eosinophils, which, in subjects with pre-existing lung disease, will contribute to the severity of asthma.

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