

Expression of IgE Heavy Chain Transcripts in the Sinus Mucosa of Atopic and Nonatopic Patients with Chronic Sinusitis

Omar Ghaffar, Stephen R. Durham, Khalid Al-Ghamdi, Erin Wright, Peter Small, Saul Frenkiel, Hannah J. Gould, and Qutayba Hamid

Meakins-Christie Laboratories, SMBD-Jewish General Hospital, McGill University, Montreal, Quebec, Canada; Division of Upper Respiratory Medicine, Imperial College School of Medicine at National Heart and Lung Institute; and Randall Institute, King's College, London, United Kingdom

We have recently shown the increased mRNA expression of interleukin (IL)-4 and IL-13 in sinus biopsies from allergic subjects with chronic sinusitis (ACS), whereas only IL-13 mRNA was elevated in biopsies obtained from nonallergic subjects with chronic sinusitis (NCS). In the lymph nodes and spleen, these cytokines may promote IgE production through transcriptional activation of the germline IgE heavy chain promoter, an event which precedes immunoglobulin isotype switching to IgE in B cells. We hypothesized that local expression of IL-4 and/or IL-13 might act by inducing germline IgE heavy chain transcript expression locally in the sinus mucosa of chronic sinusitis patients. Mucosal sinus biopsies were obtained from 13 patients with ACS, 12 subjects with NCS, and 11 normal control individuals. The numbers of B cells in the sinus mucosa were studied by immunocytochemistry with anti-CD20 monoclonal antibodies. *In situ* hybridization was performed using antisense radiolabeled riboprobes complementary to the IgE ϵ -heavy chain germline ($I\epsilon$) and heavy chain constant region ($C\epsilon$) gene transcripts. Riboprobes specific for the IgG γ -heavy chain constant region ($C\gamma$) were used as an isotype control. Immunocytochemical analysis indicated augmented numbers of CD20-positive B cells in the biopsies obtained from ACS patients compared with NCS subjects ($P < 0.05$) and normal control subjects ($P < 0.01$). Statistically significant increases were observed in the numbers of cells expressing $I\epsilon$ and $C\epsilon$ transcripts in the sinus mucosa of ACS patients compared with those with NCS ($P < 0.001$) and normal controls ($P < 0.001$), while $C\gamma$ RNA expression did not differ significantly between the groups. In three randomly selected ACS biopsies, 92–100% of cells expressing $C\epsilon$ transcripts and 100% of $I\epsilon$ RNA-positive cells coexpressed CD20 immunoreactivity. Cells expressing $C\epsilon$ transcripts were also significantly increased in NCS compared with normal controls ($P < 0.05$). The results of this study suggest that local IgE class switching occurs in the pathogenesis of ACS and that ACS and NCS are both associated with increased expression of $C\epsilon$ transcripts. **Ghaffar, O., S. R. Durham, K. Al-Ghamdi, E. Wright, P. Small, S. Frenkiel, H. J. Gould, and Q. Hamid. 1998. Expression of IgE heavy chain transcripts in the sinus mucosa of atopic and non-atopic patients with chronic sinusitis. *Am. J. Respir. Cell Mol. Biol.* 18:706–711.**

Chronic sinusitis is a disease of significant morbidity in patients with and without allergy (1). It is associated with cellular infiltration of the sinus mucosa with lymphocytes, monocyte/macrophages, and eosinophils (2, 3). The recruitment and activation of these inflammatory cells are believed to be mediated by T cell-derived cytokines. We

have recently shown the increased density of cells expressing interleukin (IL)-4 mRNA in the sinus mucosa of allergic subjects with chronic sinusitis (ACS), but not in nonallergic subjects with chronic sinusitis (NCS). In contrast, IL-13, a cytokine with functional similarities to IL-4, was augmented in the sinus mucosa of both ACS and NCS patients (3).

In vitro studies have suggested an important role for IL-4 and IL-13 in allergic inflammation through the induction of immunoglobulin isotype switching to IgE in B cells (4, 5). This site-specific DNA recombination results in rearrangement of the immunoglobulin heavy chain genes so as to activate production of IgE in lieu of other immunoglobulin classes. IL-4 and IL-13 are believed to be crucial in the events that precede IgE isotype switching by stimulating transcription from the germline gene promoter lo-

(Received in original form May 23, 1997 and in revised form October 15, 1997)

Address correspondence to: Q. Hamid, Meakins-Christie Laboratories, McGill University, 3626 St. Urbain St., Montreal, QC, H2X 2P2 Canada. E-mail: hamid@meakins.lan.mcgill.ca

Abbreviations: allergic subjects with chronic sinusitis, ACS; alkaline phosphatase anti-alkaline phosphatase, APAAP; interleukin, IL; nonallergic subjects with chronic sinusitis, NCS; vascular cell adhesion molecule, VCAM.

cated upstream of the unrearranged IgE ϵ -heavy chain constant region coding gene segments (C ϵ) (4–6). Transcription from the germline gene promoter has two consequences: (1) the production of untranslatable transcripts containing the IgE ϵ -heavy chain germline (I ϵ) and C ϵ sequences, and (2) the generation of the conditions required for operation of the putative switch recombinase (7–10). In the process of isotype switching, I ϵ is deleted and the C ϵ gene is positioned directly downstream of the variable region genes encoding antigen specificity. Cells expressing I ϵ transcripts are therefore necessary precursors to IgE-producing B cells.

We have previously shown increased expression of C ϵ and I ϵ transcripts in allergen-induced rhinitis, suggesting that immunoglobulin isotype switching to IgE may occur in the nasal mucosa and not exclusively in the lymph nodes and spleen (11). In the present study, we investigated IgE switch recombination within the sinus mucosal microenvironment in allergic and nonallergic chronic sinusitis patients. *In situ* hybridization was employed to compare C ϵ and I ϵ transcript expression in sinus biopsies obtained from ACS, NCS, and normal non-atopic controls. Riboprobes corresponding to the IgG γ -chain constant region (C γ) were used as an isotype control. B-cell infiltration in the sinus mucosa was examined by immunocytochemistry.

Materials and Methods

Study Design

Twenty-five patients were recruited from the SMBD-Jewish General Hospital, Department of Otolaryngology (Montreal, Quebec, Canada) with the approval of the Jewish General Hospital Ethics Committee. Patients had a history of chronic sinusitis for at least 6 mo with one or more of the following symptoms: chronic perennial nasal congestion, anosmia, ageusia, rhinorrhea, postnasal drip, headache, or discomfort in the regions of the sinuses. Patients underwent computerized axial tomographic examinations taken in the coronal plane prior to their surgeries, which demonstrated variable pathologic changes ranging from mucosal hypertrophy at least 6 mm in thickness in one or more of the sinuses to complete opacification of the sinuses.

Patients who had hypogammaglobulinemia, a history of cystic fibrosis, bronchiectasis, immotile cilia syndrome, systemic granulomatous disease, cocaine use, or immunodeficiency were excluded. Antibiotics were withheld for 4 wk prior to the study.

Initial Evaluation

A questionnaire regarding history of sinusitis, allergy, environmental exposure, previous sinus surgeries, immunotherapy, and medical treatment was filled out by each patient. Allergen skin-prick tests were performed with a panel of aeroallergens prevalent in Canada, including trees, grasses, weeds, molds, standardized cat, mixed-breed dog, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*. A skin test was considered positive if a wheal of 3 mm or more in diameter was elicited. Negative and positive controls for the skin tests were performed with diluent and histamine, respectively. Patients were thus categorized as having chronic sinusitis with allergy or chronic sinusitis without allergy.

Normal Control Sampling

Biopsies representing the normal sinus mucosa were collected from the anterior ethmoid air cells of 11 subjects without chronic sinusitis undergoing surgical procedures such as septoplasty or rhinoplasty. Normal subjects had negative skin-test results and no history of allergies, asthma, aspirin sensitivity, previous sinus surgery, sinus disease, or recent attack of upper respiratory-tract infection.

Tissue Collection

Tissue was obtained from patients and control subjects under general anesthesia during the planned surgical procedures, functional endoscopic sinus surgery, septorhinoplasty, or septoplasty. For patients, biopsies from the ethmoid sinuses were used and right or left sides were biopsied randomly. The specimens obtained from each patient were processed for *in situ* hybridization and immunocytochemical analyses.

For *in situ* hybridization, tissue was immediately fixed in 4% paraformaldehyde for 2 h then washed in phosphate-buffered saline (PBS) with 15% sucrose at 4°C overnight. Tissue was then blocked in ornithine carbamyl transferase (OCT) medium and frozen in liquid nitrogen-cooled isopentane. Cryostat sections, 8 μ m, were mounted on poly-L-lysine-coated slides and stored at -80°C prior to use.

Tissue for immunocytochemistry was immersed in 15% PBS on ice for 15–20 min, then blocked in OCT medium and snap-frozen. Cryostat sections of 5- μ m thickness were mounted on slides and stored at -20°C until use.

Immunocytochemistry

We employed the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique, as previously described (12). The slides were retrieved from the -20°C freezer and allowed to acquire room temperature before incubation with the primary antibodies. Staining was performed with a mouse antihuman CD20 monoclonal antibody (Becton-Dickenson, Mississauga, Ontario, Canada) diluted in Tris-buffered saline. This was followed with application of rabbit antimouse immunoglobulin, and then the APAAP complex. The reaction was visualized with Fast Red (Sigma Chemical Co., St. Louis, MO). Negative controls involved the replacement of the primary antibody with Tris-buffered saline or an irrelevant mouse isotype-matched antibody, and none exhibited positive staining.

In Situ Hybridization

The procedure has been described elsewhere in detail (13). Slide specimens were permeabilized with 0.3% Triton X-100 in PBS for 10 min and then with proteinase K (1 μ g/ml) in 0.1 mol/liter Tris that contained 50 mmol/liter ethylenediaminetetraacetic acid (EDTA) for 20–30 min at 37°C . Immersion of the slides in 4% paraformaldehyde for 5 min terminated the reaction. Specimens were then treated in 0.25% acetic anhydride in 0.1 mol/liter triethanolamine for 10 min to reduce nonspecific binding. Prehybridization was performed in 50% formamide and $2\times$ standard saline citrate (SSC) for 30 min at 40°C . Dehydra-

tion of the specimens using ethanol was performed gradually, starting with 70% and ending with 100% ethanol.

Riboprobes, both antisense (complementary to mRNA) and sense (identical in sequence to mRNA), were prepared from cDNA encoding $C\epsilon$ and $I\epsilon$ (11). Sense and antisense riboprobes were also prepared from $C\gamma$ cDNA (corresponding to the $C\gamma 2$ region) to act as an isotype control (11). cDNA were inserted into different pGEM vectors (Promega Corp., Madison, WI) and linearized with appropriate enzymes before transcription. Transcription was carried out in the presence of ^{35}S and the appropriate RNA polymerases.

For hybridization, radiolabeled antisense (10^6 cpm/section) diluted in hybridization buffer was employed. The slides were covered with dimethyldichlorosilane-coated cover slips and hybridization was performed in a humid chamber for 12 h at 40°C .

Posthybridization washing was done in decreasing concentrations of SSC ($4\times$ SSC to $0.05\times$ SSC) at 43°C . Unhybridized, single-stranded RNAs were removed by treating the preparations with RNase A (20 $\mu\text{g}/\text{ml}$), 0.5 mol/liter NaCl, 10 mmol Tris, and 1 mmol/liter EDTA for 30 min at 42°C . After dehydration, the slides were immersed in emulsion and left to dry overnight. The autoradiographs were developed in Kodak D-19 and counterstained with hematoxylin.

As a control for the experiments, slides were hybridized with sense probes or treated with RNase A solution at 37°C for 40 min prior to the prehybridization step. No signals were observed in any of the control experiments, thus confirming the specificity of the results obtained during hybridization with the complementary RNA probes.

Combined Immunocytochemistry–*In Situ* Hybridization

To identify the percentage of $C\epsilon$ and $I\epsilon$ RNA-positive cells coexpressing the B-cell marker CD20, co-localization studies were performed on sinus biopsy sections prepared from three randomly selected ACS subjects. This technique has been described elsewhere (14). The specimens were first immunostained with the anti-CD20 monoclonal antibody using the APAAP technique. To avoid RNase contamination, all solutions used were made up in 0.1% diethylpyrocarbonate-treated distilled water. After immunocytochemical staining, the specimens were processed for *in situ* hybridization using digoxigenin-labeled $C\epsilon$ or $I\epsilon$ antisense riboprobes. Cell counts were expressed as the percentage of $C\epsilon$ or $I\epsilon$ RNA-positive cells that co-expressed immunoreactivity for CD20.

Quantification

For immunocytochemistry and *in situ* hybridization, slides were counted blind in a coded, random fashion using a light microscope with an eyepiece graticulate (0.202 mm^2). At least two sections were assayed, from which three to four fields in the subepithelium were counted. The graticulate was oriented along the epithelial basement membrane and the results were expressed as mean numbers of positive cells per field $\pm$ standard error of mean (SEM). The within-observer coefficient of variation for repeated counts of immunostaining was 8% and for hybridized sections less than 5%. Comparisons of cell counts between groups were

performed by analysis of variance with subsequent Tukey honest significant difference (HSD) multiple comparisons. P values < 0.05 were accepted as statistically significant. Statistical analyses were performed using a standard computer package (Systat v. 6.1; Systat, Inc., Evanston, IL).

Results

Patient Characteristics

Of the 25 patients recruited with chronic sinusitis, 12 were non-atopic (mean age: 48 yr; sex: 6 female, 6 male) and 13 were atopic (mean age: 39 yr; sex: 8 male, 5 female). Eleven normal, healthy subjects (mean age: 34 yr; sex: 1 female, 10 male) were used as controls.

Immunocytochemical Analyses

As shown in Figure 1, immunocytochemistry with the anti-CD20 antibody revealed significantly higher B-cell numbers in the sinus mucosa of ACS subjects (mean count = 5.5 ± 0.8 positive cells/field) compared with NCS patients (mean count = 3.0 ± 0.5 positive cells/field, $P < 0.05$) and control subjects (mean count = 1.8 ± 0.4 positive cells/field, $P < 0.01$). CD20 immunoreactivity did not differ significantly between the NCS and the normal control subjects.

Detection of $C\epsilon$ and $I\epsilon$ transcripts by

In Situ Hybridization

The density of cells expressing $C\epsilon$ transcripts was significantly higher in biopsy sections from ACS subjects (mean count = 5.9 ± 0.4 positive cells/field) than in NCS patients (mean count = 2.4 ± 0.4 positive cells/field, $P < 0.001$) and normal control subjects (mean count = 0.5 ± 0.2 positive cells/field, $P < 0.001$; Figure 2A). $C\epsilon$ transcript-

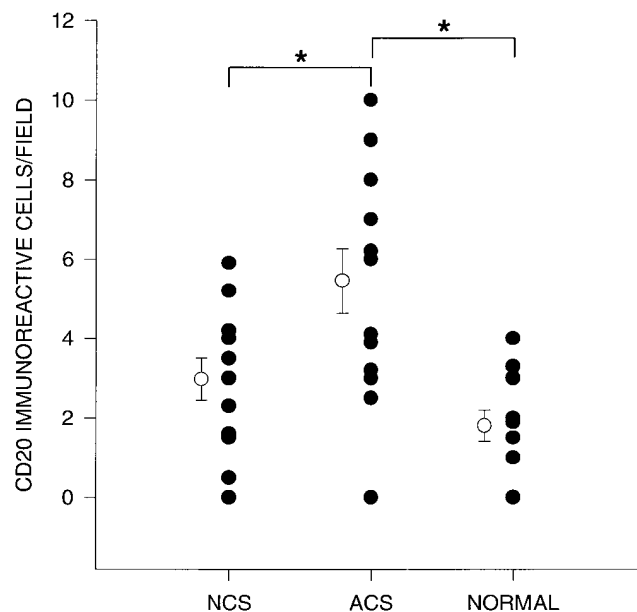


Figure 1. CD20 immunoreactivity assessed by the APAAP technique using anti-CD20 monoclonal antibodies in mucosal sinus biopsy sections from NCS, ACS, and normal control subjects (filled circles). Open circles with error bars indicate mean values $\pm$ SEM. $*P < 0.05$.

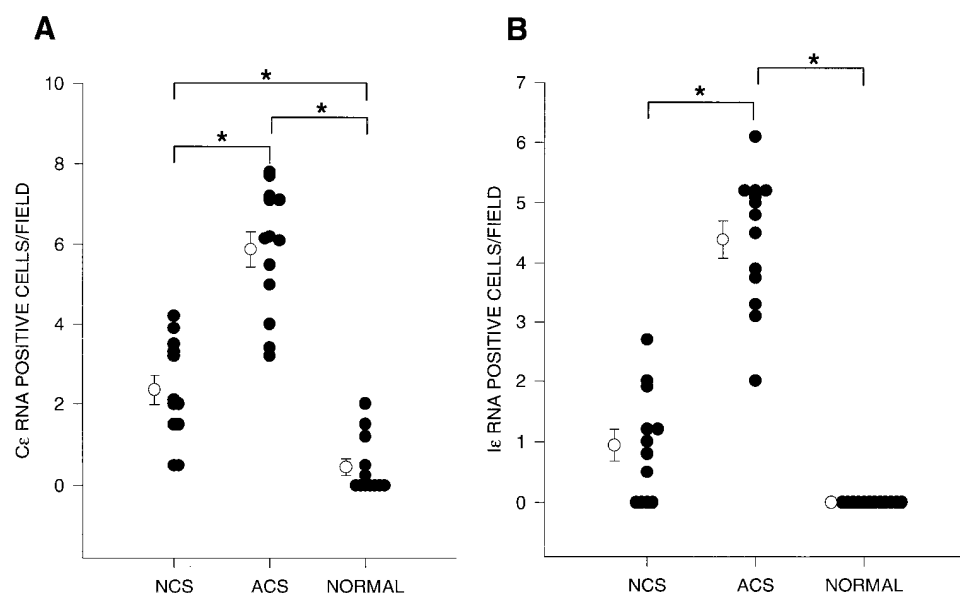


Figure 2. Results of *in situ* hybridization of sinus biopsy sections from NCS, ACS, and normal control subjects with antisense, radio-labeled riboprobes complementary to (A) Cε RNA and (B) Iε RNA. Filled circles represent individual subjects, and open circles with error bars denote mean values $\pm$ SEM. * $P < 0.05$.

expressing cells were also increased in NCS compared with normal, non-atopic subjects ($P < 0.05$).

Similarly, the numbers of cells expressing Iε transcripts were markedly increased in the sinus mucosa of ACS patients (mean count = 4.4 ± 0.3 positive cells/field) compared with NCS patients (mean count = 0.9 ± 0.3 positive cells/field, $P < 0.001$) and normal subjects (mean count = 0 ± 0 positive cells/field, $P < 0.001$; Figure 2B). There was no significant difference in the density of cells expressing Iε transcripts between NCS patients and normal control subjects, however.

In contrast to Cε and Iε RNA, the numbers of cells expressing Cγ transcripts were not significantly different between ACS, NCS, and normal control subjects ($P > 0.05$). Combined *in situ* hybridization-immunocytochemistry studies were performed on sinus biopsy sections from three ACS patients to confirm that B cells were the prominent source of Cε and Iε RNA. Over 90% of the Cε RNA positive cells (92%, 95%, 100%) were CD20-positive. In all cases, 100% of the Iε transcripts co-localized to CD20-positive cells.

Discussion

The possibility of local IgE production in chronic sinusitis has been suggested before. For example, elevated specific IgE in polyp fluid (15, 16) and tissue (17) were demonstrated in the absence of increased serum-specific IgE. Huggins and Brostoff (18) detected specific IgE in nasal secretions of patients clinically sensitive to *D. pteronyssinus*. These patients exhibited negative skin-test reactions but positive nasal provocation. It was concluded that local IgE production may occur in allergic disease and could be important in eliciting symptoms without being detected systemically in either serum or skin. The work of Nakajima and colleagues (19) examined IgE in the nasal washings of allergic subjects. Unlike IgA, the ratio of IgE to total protein in nasal washings was lower than in the serum.

However, compared with the nonsecretory protein albumin, IgE proportions in nasal secretions were greater than predicted by passive diffusion from the blood. These authors suggested that IgE is produced locally and, rather than accumulating in nasal secretions, most of this antibody diffuses into the blood to elevate serum levels.

From the perspective of local IgE production, these and other early studies may have been limited partly by the presence of inflammatory cells bearing IgE receptors (FcεRI, CD23/FcεRII, Gal3/Mac2) (20–22). In immunohistochemical analyses, IgE receptor-positive cells make it difficult to differentiate IgE-producing cells from cells expressing immunoreactivity for IgE due to receptor-bound antibody. In studies examining polyp fluid and secretions, IgE concentrations could be affected by inflammatory cells sequestering IgE in the tissue via Fc receptors. Our study is the first to examine IgE heavy chain expression at the RNA level in sinus tissue. This approach provides a means for identifying putative IgE-switching and -producing cells in the absence of background from IgE receptor-positive cells.

In the present report, we have shown an increased density of B lymphocytes, Iε RNA-positive cells, and cells expressing Cε transcripts in mucosal sinus biopsies of ACS patients compared with NCS and normal control subjects. In contrast, Cγ RNA expression did not differ between the groups. The latter indicates selectivity for IgE in ACS and confirms that increases in Cε and Iε in these patients were not simply due to higher B-cell numbers. Together, our results support a role for local IgE switch recombination and production in the pathogenesis of ACS.

The enhanced expression of IL-4 and IL-13 has been shown in ACS, whereas only IL-13 was elevated in NCS (3). The increased expression of Iε RNA observed in ACS is in agreement with evidence that IL-4 and IL-13 induce the production of ε germline gene transcripts and stimulate immunoglobulin isotype switching to IgE *in vitro*. In contrast, interferon-γ (IFN-γ) has an inhibitory effect on

IgE class switching through the repression of transcription from I ϵ (4). Consistent with the prominent expression of IFN- γ in NCS, the density of I ϵ RNA-positive cells did not differ significantly between NCS and normal control subjects (23).

In the absence of significant increases in I ϵ RNA-positive cells, we have demonstrated augmented C ϵ transcript expression in NCS compared to normal control subjects. This indicates the presence of B cells already switched to IgE production in the sinus mucosa of NCS patients. A recent meta-analysis found that 19% of patients with no systemic allergy manifested nasal mucosal allergy as determined by specific IgE (24). This raises the possibility that some patients found to be nonallergic by skin tests may actually have allergy confined to the mucosal lining of the upper respiratory tract. Such a conclusion is tentative but it may explain the overlap observed between some NCS and ACS patients with respect to CD20 immunoreactivity, C ϵ RNA expression, and expression of I ϵ transcripts. Indeed, although I ϵ RNA expression in NCS patients did not differ significantly from normal control subjects, 8 of 12 NCS biopsies exhibited positive hybridization signals whereas none of the samples from the normal controls did.

Other studies have shown that some nasal polypoid patients defined as nonallergic on the basis of allergen skin-test results may have IgE specific for bacterial antigens which are not routinely tested for (25). Alternatively, transient local expression of IgE may be a feature of resident B cells in sinus mucosal inflammation regardless of allergic status or cytokine profile. Reports examining nasal polyps (15, 26, 27), polyp fluid (17, 27, 28), and nasal secretions (27) have also detected IgE protein in both allergic and nonallergic patients. At the level of local cytokines, the significant increase of C ϵ and the trend for an increase in I ϵ RNA-positive cells could be related to the enhanced expression of IL-13 in NCS versus normal control subjects (3). Our results suggest that sinus mucosal IgE production in NCS patients is significantly less than in ACS, and that class switching to IgE does not occur to the same extent in the local microenvironment of the former.

The basis for increased B-cell numbers in ACS compared with NCS and normal control subjects is unclear. B cells express very late antigen (VLA)-4, the counterligand for vascular cell adhesion molecule (VCAM)-1 (29). In chronic hyperplastic sinusitis with nasal polypoid, we have recently shown increased expression of VCAM-1 in allergic and nonallergic patients compared with normal, healthy subjects (30). However, contrary to our present findings of increased B-cell numbers in ACS versus NCS, the intensity of VCAM-1 expression was significantly greater in nonallergic versus allergic subjects. This might be attributed to differences between polyps and sinus tissue, but it is also possible that adhesion molecules other than the VCAM-1/VLA-4 pathways are involved in the recruitment of B cells to the sinus mucosa.

The results of this study indicate that therapies directed toward decreasing local IgE switch recombination may be effective in patients with ACS. We have previously shown that topical corticosteroid treatment is associated with a decrease in the density of cells expressing IL-4 and IL-13 mRNA in ACS (3). Whether these decreases are paral-

leled by an attenuation of I ϵ and C ϵ RNA expression remains to be determined. In addition to IL-4 or IL-13, IgE class switching requires the engagement of the CD40 molecule on the surface of B cells (4), suggesting that the blockade of the CD40-CD40 ligand interaction may inhibit IgE production. Antagonists of IgE-mediated mast cell degranulation may be of limited efficacy in the treatment of established allergic diseases; recent studies have shown that another major role of local IgE may be in the processing of small concentrations of allergen for presentation to T cells by B lymphocytes which bind IgE-allergen complexes via the low-affinity IgE receptor, Fc ϵ RII (31-33).

In conclusion, this study demonstrates increased I ϵ transcripts, C ϵ RNA expression, and B-cell numbers in the sinus mucosa of ACS subjects compared with NCS and normal control subjects. The numbers of cells expressing C ϵ transcripts were also increased in NCS patients over normal control subjects. These results suggest that local IgE switch recombination in the sinus mucosal microenvironment might contribute to the pathogenesis of ACS and indicate that ACS and NCS are associated with enhanced C ϵ transcripts.

Acknowledgments: The authors thank Elsa Schotman and Zivart Yasrueel for their technical assistance. One author (O.G.) is supported by a studentship from the Canadian Cystic Fibrosis Foundation. This work was supported by M.R.C. Canada and the J.T. Costello Memorial Research Fund.

References

- Gliklich, R. E., and R. Metson. 1995. The health impact of chronic sinusitis in patients seeking otolaryngologic care. *Otolaryngol. Head Neck Surg.* 113:104-109.
- Harlin, S. L., D. G. Ansel, S. R. Lane, J. Myers, G. M. Kephart, and G. J. Gleich. 1988. A clinical and pathological study of chronic sinusitis: the role of the eosinophil. *J. Allergy Clin. Immunol.* 81:867-875.
- Al-Ghamdi, K., O. Ghaffar, P. Small, S. Frankiel, and Q. Hamid. 1997. IL-4 and IL-13 expression in chronic sinusitis: relationship with cellular infiltrate and effect of topical corticosteroid treatment. *J. Otolaryngol.* 26:160-166.
- De Vries, J. E., J. F. Gauchat, G. G. Aversa, J. Punnonen, H. Gascan, and H. Yssel. 1991. Regulation of IgE synthesis by cytokines. *Curr. Opin. Immunol.* 3:851-858.
- Coffman, R. L., D. A. Leberman, and P. Rothman. 1993. Mechanism and regulation of immunoglobulin isotype switching. *Adv. Immunol.* 54:229-270.
- Ichiki, T., W. Takahashi, and T. Watanabe. 1993. Regulation of the expression of human C ϵ germline transcript. *J. Immunol.* 150:5408-5417.
- Purkerson, J., and P. Isakson. 1992. A two-signal model of immunoglobulin isotype switching to IgE. *FASEB J.* 6:3245-3252.
- Delphin, S., and J. Stavnezer. 1995. Characterization of IL-4 responsive region in the immunoglobulin heavy chain germline ϵ promoter: regulation by NF-IL-4, a C/EBP family member, and NF- κ B/p50. *J. Exp. Med.* 181:181-192.
- Rothman, P., Y. Y. Chen, S. Lutzker, S. C. Li, V. Stewart, R. L. Coffman, and F. W. Alt. 1990. Structure and expression of germline immunoglobulin heavy chain ϵ transcripts: interleukin-4 plus lipopolysaccharide directed switching to C ϵ . *Mol. Cell. Biol.* 10:1672-1679.
- Stavnezer, J., G. Radcliffe, Y. C. Lin, J. Nietupski, L. Berggren, R. Sitia, and E. Severinson. 1988. Immunoglobulin heavy chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. Natl. Acad. Sci. USA* 85:7704-7708.
- Durham, S. R., H. J. Gould, C. P. Thienes, M. R. Jacobson, K. Maysuyama, S. Rak, O. Lowhagen, E. Schotman, L. Cameron, and Q. Hamid. 1997. Expression of ϵ germline gene transcripts and mRNA for the ϵ heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur. J. Immunol.* 27:2899-2906.
- Frew, A. J., and A. B. Kay. 1988. The relationship between infiltrating CD4 $^{+}$ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous response in man. *J. Immunol.* 141:4158-4164.
- Hamid, Q., J. Wharton, G. Terenghi, C. Hassall, J. Aimi, K. Taylor, H. Nakazato, J. Dixon, G. Burnstock, and J. M. Polak. 1987. Localization of atrial natriuretic peptide mRNA and immunoreactivity in rat heart and

- human atrial appendage. *Proc. Natl. Acad. Sci. USA* 84:6760–6764.
14. Ying, S., S. R. Durham, J. Barkans, K. Masuyama, M. Jacobson, S. Rak, O. Lowhagen, R. Moqbel, A. B. Kay, and Q. Hamid. 1993. T cells are the principle source of interleukin-5 mRNA in allergen-induced rhinitis. *Am. J. Respir. Cell Mol. Biol.* 9:356–360.
 15. Jones, E., S. Frankiel, P. Small, and L. Rochon. 1987. Immunopathological characteristics of nasal polyps. *J. Otolaryngol.* 16:19–22.
 16. Donovan, R., S. G. Johansson, H. Bennich, and J. F. Soothill. 1970. Immunoglobulins in nasal polyp fluid. *Int. Arch. Allergy Applied Immunol.* 32:154–166.
 17. Small, P., D. Barrett, S. Frankiel, L. Rochon, C. Cohen, and M. Black. 1985. Local specific IgE production in nasal polyps associated with negative skin tests and serum RAST. *Ann. Allergy* 55:736–739.
 18. Huggins, K. G., and J. Brostoff. 1975. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. *Lancet* 2:148–150.
 19. Nakajima, S., D. N. Gillespie, and G. J. Gleich. 1975. Differences between IgA and IgE as secretory proteins. *Clin. Exp. Immunol.* 21:306–317.
 20. Rajakulasingam, K., S. R. Durham, F. O'Brien, M. Humbert, L. T. Barata, L. Reece, A. B. Kay, and J. A. Grant. 1997. Enhanced expression of high-affinity IgE receptor (Fc-epsilon-R1) alpha chain in human allergen-induced rhinitis with colocalization to mast cells, macrophages, eosinophils, and dendritic cells. *J. Allergy Clin. Immunol.* 100:78–86.
 21. Haas, N., K. Hamann, J. Grabbe, J. Niehus, G. Kunkel, G. Kolde, and B. M. Czarnetzki. 1997. Demonstration of the high affinity IgE receptor (Fc epsilon RI) on langerhans cells of diseased nasal mucosa. *Allergy* 52:436–439.
 22. KleinJan, A., T. Godthelp, A. W. van Toornenbergen, and W. J. Fokkens. 1997. Allergen binding to specific IgE in the nasal mucosa of allergic patients. *J. Allergy Clin. Immunol.* 99:515–521.
 23. Hamilos, D. L., D. Y. M. Leung, R. Wood, L. Cunningham, D. K. Bean, Z. Yasruel, E. Schotman, and Q. Hamid. 1995. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. *J. Allergy Clin. Immunol.* 96:537–544.
 24. Shatkin, J. S., K. G. Delsupehe, R. A. Thisted, and J. P. Corey. 1994. Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. *Otolaryngol. Head Neck Surg.* 111:553–556.
 25. Calenoff, E., J. T. McMahan, G. D. Herzon, R. C. Kern, G. D. Gadge, and D. G. Hanson. 1993. Bacterial allergy in nasal polyposis. a new method for quantifying specific IgE. *Arch. Otolaryngol. Head Neck Surg.* 119:830–836.
 26. Liu, C. M., C. T. Shun, and M. M. Hsu. 1994. Lymphocyte subsets and antigen-specific IgE antibody in nasal polyps. *Ann. Allergy* 72:19–24.
 27. Perkins, J. A., D. B. Blakeslee, and P. Andrade. 1989. Nasal polyps: a manifestation of allergy? *Otolaryngol. Head Neck Surg.* 101:641–645.
 28. Frankiel, S., F. Chagnon, P. Small, L. Rochon, C. Cohen, and M. Black. 1985. The immunological basis of nasal polyp formation. *J. Otolaryngol.* 14:89–91.
 29. Koopman, G., R. M. Keehnen, E. Lindhout, W. Newman, Y. Shimizu, G. A. Van Seventer, C. de Groot, and S. T. Pals. 1994. Adhesion through the LFA-1 (CD11a/CD18)-ICAM-1 (CD54) and the VLA-4 (CD49d)-VCAM-1 (CD106) pathways prevents apoptosis of germinal center B cells. *J. Immunol.* 152:3760–3767.
 30. Hamilos, D. L., D. Y. M. Leung, R. Wood, D. K. Bean, Y. L. Song, E. Schotman, and Q. Hamid. 1996. Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF- α . *Am. J. Respir. Cell Mol. Biol.* 15:443–450.
 31. Pirron, U., T. Schlunck, J. C. Prinz, and E. P. Rieber. 1990. IgE-dependent antigen focusing by human B lymphocytes is mediated by the low affinity receptor for IgE. *Eur. J. Immunol.* 20:1547–1551.
 32. Flores-Romo, L., J. Shields, Y. Humbert, P. Graber, J. P. Aubry, J. F. Gauchat, G. Ayala, B. Allet, M. Chavez, H. Bazin, M. Capron, and J. Y. Bonnefoy. 1993. Inhibition of an in vivo antigen-specific IgE response by antibodies to CD23. *Science* 261:1038–1041.
 33. Coyle, A. J., K. Wagner, C. Bertrand, S. Tsuyuki, J. Bews, and C. Heusser. 1996. Central role of immunoglobulin E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J. Exp. Med.* 183:1303–1310.