Potential Role of Abnormal Ion Transport in the Pathogenesis of Chronic Sinusitis

Kenji Dejima, MD; Scott H. Randell, PhD; M. Jackson Stutts, PhD; Brent A. Senior, MD; Richard C. Boucher, MD

**Objectives:** To create well-differentiated cultures of normal and chronic sinusitis paranasal sinus epithelial cells and to compare their electrophysiologic properties.

**Design:** In vitro investigation using primary sinus epithelial cells, initially cultured on plastic tissue culture dishes. Cells were characterized by means of immunocytochemical analysis and then passaged to air-liquid interface culture conditions. The morphologic features of air-liquid interface cultures were assessed using light and electron microscopy. Epithelial Na\(^+\) channel, Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter, cystic fibrosis transmembrane conductance regulator, and Ca\(^{2+}\)-activated Cl\(^-\) channel function were investigated in Ussing chambers.

**Subjects:** Specimens were obtained from 15 patients undergoing transsphenoidal pituitary procedures, tumor removal, or trauma repair and from 9 patients with chronic sinusitis.

**Results:** After culture at an air-liquid interface for 21 days, the epithelium was pseudostratified and contained basal, mucous secretory, and ciliated cells. There were no detectable morphologic differences between normal and chronic sinusitis cells. In cultures of normal cells, median basal short circuit current was 4.7 µA/cm\(^2\), and Na\(^+\) transport, defined as the amiloride hydrochloride-sensitive component, was approximately 20% of the total. Basal and amiloride-sensitive short circuit currents were greater in cultures of chronic sinusitis cells. Basal short circuit currents in both types of cultures were insensitive to the Ci\(^-\) transport inhibitor bumetanide, but all responded to forskolin or uridine triphosphate. After amiloride pretreatment, forskolin and uridine triphosphate responses were greater in chronic sinusitis cells.

**Conclusions:** We established methods for well-differentiated sinus epithelial cultures. The cells exhibited Na\(^+\) absorption and Cl\(^-\) secretion, and elevated rates of ion transport may be pathophysiologically relevant in chronic sinusitis.

pecially compared with large nasal turbinate and polyp specimens. Furthermore, normal sinus mucosa was rarely available. However, in recent years, endoscopic transphenoidal sinus surgery has been applied to the treatment of pituitary disease, increasing access to normal sinus mucosa. Furthermore, advances in cell culture techniques permit differentiation of cells from very small specimens, such as cytologic brushes and murine tracheal epithelium. Improved passaging of airway epithelial cells has also allowed expansion of cell numbers.

In view of the few studies of sinus epithelial cell cultures, the greater tissue availability, and the improved methods, our goal was to produce and characterize ALI cultures of human paranasal sinus epithelia. First we established conditions that produce mucociliary differentiation of normal paranasal sinus epithelial (NPSE) cells, and then we measured their ion transport properties, including basal rates of Na⁺ absorption and Cl⁻ secretion and pharmacologic regulation of Cl⁻ transport. These data were compared with those from epithelial cell cultures derived from patients with chronic sinusitis (CSE cells) to test the hypothesis that altered epithelial ion transport may contribute to the pathogenesis of sinusitis.

### METHODS

#### PRIMARY CULTURE

Normal paranasal sinus tissue specimens (n=15) were obtained from patients treated with endonasal surgery for pituitary tumors (n=10), benign sinonasal tumors (n=3), or facial trauma (n=2). Specimens were also obtained from patients with chronic sinusitis (n=9), excluding cystic fibrosis, bronchial asthma, allergic fungal sinusitis, and primary ciliary dyskinesia. The University of North Carolina Committee for the Rights of Human Subjects approved the use of excess surgical pathology tissue. Discrete tissue samples were obtained under endoscopic guidance in the operating room with the patient under general anesthesia. Cutting forceps were used to remove the tissue, and freshly removed tissue was placed in isotonic sodium chloride solution before transfer to the laboratory.

Procedures for sinus epithelial cell culture were adapted from methods previously described for nasal turbinate and polyp tissues and tracheobronchial specimens. Freshly excised sinus tissues were collected as small fragments and were incubated at 4°C for 3 to 8 hours in Joklik minimum essential medium containing 0.1% protease type XIV (Sigma-Aldrich Corp, St Louis, Mo), 0.1 mg/mL of deoxyribonuclease (Sigma-Aldrich Corp), and penicillin G sodium, streptomycin sulfate, and primary ciliary dyskinesia. The University of North Carolina Committee for the Rights of Human Subjects approved the use of excess surgical pathology tissue. Discrete tissue samples were obtained under endoscopic guidance in the operating room with the patient under general anesthesia. Cutting forceps were used to remove the tissue, and freshly removed tissue was placed in isotonic sodium chloride solution before transfer to the laboratory.

Procedures for sinus epithelial cell culture were adapted from methods previously described for nasal turbinate and polyp tissues and tracheobronchial specimens. Freshly excised sinus tissues were collected as small fragments and were incubated at 4°C for 3 to 8 hours in Joklik minimum essential medium containing 0.1% protease type XIV (Sigma-Aldrich Corp, St Louis, Mo), 0.1 mg/mL of deoxyribonuclease (Sigma-Aldrich Corp), and penicillin G sodium, streptomycin sulfate, and amphotericin B in the concentrations given later in this paragraph. Ten percent fetal bovine serum was added to test the hypothesis that altered epithelial ion transport may contribute to the pathogenesis of sinusitis.

Procedures for sinus epithelial cell culture were adapted from methods previously described for nasal turbinate and polyp tissues and tracheobronchial specimens. Freshly excised sinus tissues were collected as small fragments and were incubated at 4°C for 3 to 8 hours in Joklik minimum essential medium containing 0.1% protease type XIV (Sigma-Aldrich Corp, St Louis, Mo), 0.1 mg/mL of deoxyribonuclease (Sigma-Aldrich Corp), and penicillin G sodium, streptomycin sulfate, and amphotericin B in the concentrations given later in this paragraph. Ten percent fetal bovine serum was added to test the hypothesis that altered epithelial ion transport may contribute to the pathogenesis of sinusitis.

### ALI CULTURE

Passage 1 cells (0.1 to 0.3×10⁶) were plated in 0.2 mL of ALI culture medium on 12-mm, 0.4-µm-pore culture inserts (Transwell-Col or Snapwell; Costar Corp, Cambridge, Mass). The Snapwells were coated with human placental collagen type IV (product No. C7521; Sigma-Aldrich Corp) as described previously and were used for Ussing chamber experiments. The ALI medium is similar to bronchial epithelial growth medium except that a 50:50 mixture of LHC basal and Dulbecco modified Eagle medium-H was used as the base, amphotericin B and gentamicin were omitted, and the epidermal growth factor concentration was reduced to 0.5 ng/mL. On reaching confluence, the apical surface was rinsed with phosphate-buffered saline, and ALI medium was replaced only in the bottom compartment of the culture. Cultures were maintained at 37°C in an atmosphere of 5% carbon dioxide in air. Culture medium was changed every other day until the cultures reached approximately 75% confluence, at which time they were dissociated with trypsin-EDTA solution. Passage 1 cells were subcultured as described in the following subsection.

### IMMUNOHISTOCHEMICAL ANALYSIS

Cytospin slides were made with 5 to 7.5×10⁴ cells per slide for primary dissociations and passage 1 NPSE cells and were...
stored without fixation at –80°C until staining. Slides were stained with 1 of 4 primary antibodies, followed by biotinylated secondary antibodies, streptavidin–horseradish peroxidase, diaminobenzidine color development, and light green counterstaining. We used the following antibodies: mouse monoclonal anti–human pan cytokeratin (1:200 dilution), mouse monoclonal anti–human epithelial membrane antigen (1:100 dilution), mouse monoclonal anti–human vimentin (1:1600 dilution), and mouse monoclonal anti-CD45 (1:10 dilution) (all from Sigma-Aldrich Corp) and appropriate isotype controls. The number of dark brown cells in approximately 300 cells on each slide was scored to determine the mean percentage of positively stained cells.

HISTOLOGIC ANALYSIS

After 10 or 21 days, ALI cultures were fixed with 10% neutral buffered formalin and embedded in paraffin. Five-micrometer-thick sections were cut and stained with hematoxylin-eosin or Alcian blue–periodic acid–Schiff (AB-PAS). These sections were used to measure epithelial thickness, the number of cell layers, the number of ciliated cells, and the number of cells containing AB-PAS–positive granules (defined as mucous secretary cells). Five to 10 consecutive high-power fields per section were counted.

TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy was performed on cultured NPSE cells using a special fixation procedure designed to preserve the structure of airway surface liquid. Briefly, cultures were fixed with perfluorocarbon–osmium tetroxide and embedded in epon-araldite resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope.

USING CHAMBER STUDIES

The electrophysiologic properties of ALI cultures of NPSE and CSE cells were measured as previously described. Briefly, Snap-well cultures were mounted in Ussing chambers (Physiologic Instruments, La Jolla, Calif). Each bath contained warmed (37°C) Krebs bicarbonate Ringer solution (containing 140mM Na+, 120mM Cl−, 5.2mM K+, 1.2mM Ca²⁺, 1.2mM Mg²⁺, 2.4mM HPO₄²⁻, 0.4mM H₂PO₄⁻, 25mM HCO₃⁻, and 5mM glucose), circuated by gas lift with 95% oxygen and 5% carbon dioxide. Bioelectric properties were digitally recorded from the output of voltage clamps (Physiologic Instruments) using a software program (ACQUIRE; Physiologic Instruments). The transepithelial voltage was clamped to 0 mV, and the short circuit current (Iₛ) was continuously recorded. To measure basal bioelectric properties and rates of Na⁺ absorption and Cl⁻ secretion, tissues were mounted and Iₛ and potential difference were recorded after Iₛ stabilized (mean±SD, 14.8±1.0 minutes). Transepithelial resistance (Rₑ) was calculated using Ohm’s law. Low-resistance tissues (Rₑ<300 Ω·cm²) were excluded from the study. In one protocol, an epithelial Na⁺ channel inhibitor, amiloride hydrochloride (100µM) (Sigma-Aldrich Corp), was added to the apical bath to measure basal Na⁺ transport rates. In a second protocol, an inhibitor of the Na⁺/K⁺–2Cl⁻ channel, bumetanide (100µM) (Sigma-Aldrich Corp), was added to the basolateral bath to measure the Na⁺/K⁺–2Cl⁻ cotransport system–mediated current, that is, basal Cl⁻ transport. In this protocol, after 20 minutes, amiloride was added to measure Na⁺ transport, and a purinergic receptor agonist, uridine triphosphate (UTP), 100µM (Sigma-Aldrich Corp), which ultimately raises intracellular Ca²⁺ levels, was added to the apical bath to assess Ca²⁺–activated Cl⁻ secretion. Two additional protocols were designed to also explore regulation of Cl⁻ transport. First, the cyclic adenosine monophosphate–stimulating agent forskolin (10µM) (Sigma-Aldrich Corp) was added bilaterally to activate cystic fibrosis transmembrane conductance regulator, followed by 100µM UTP and, finally, 100µM bumetanide. Second, after pretreatment with 100µM amiloride, 100µM UTP was added to the apical bath, and then 10µM forskolin was added bilaterally. Separate culture preparations for each patient were included in each protocol.

STATISTICAL ANALYSIS

The data were subjected to initial testing for normal distribution and equal variance using Kolmogorov-Smirnov and Bartlett tests, respectively (SigmaStat; Systat Software Inc, Richmond, Calif). Several of the data sets did not meet assumptions for parametric testing, and nonparametric testing was consistently applied when statistical comparisons were sought. Medians and 25th and 75th percentiles are presented in the tables, and the actual values are given in the figures. The Wilcoxon test for paired data, the Kruskal-Wallis test, and Dunn’s multiple comparison test or the Mann-Whitney rank sum test were applied as indicated. Because nonparametric testing has less power to detect differences when they actually exist, we illustrate when a 1-tailed P=.06 is present to indicate a trend, but a 1-tailed P<.05 was considered statistically significant.

RESULTS

CHARACTERIZATION OF PRIMARY AND PASSAGE 1 CELLS

Endosinus tissues obtained from patients without chronic sinusitis exhibited no obvious inflammatory changes, whereas tissues obtained from patients with chronic sinusitis appeared grossly erythematous and edematous, with variable amounts and character of associated mucus. A small portion of every normal sinus tissue specimen and a representative sample of chronic sinusitis tissues (6 specimens) were examined histologically (Figure 2). All normal sinus epithelia consisted of ciliated, basal, and scattered goblet cells, and scattered inflammatory cells were observed in some specimens. Edema, severe inflammatory cell infiltration, fibrosis, goblet cell hyperplasia, and squamous cell metaplasia were not observed in normal tissues, consistent with normal paranasal sinus mucosa. In contrast, chronic sinusitis specimens displayed variable abnormalities, including general epithelial hyperplasia, goblet cell hyperplasia, a thickened basement membrane, intraepithelial and submucosal mixed cell infiltrates, and subjacent fibrotic and granulation tissue with reactive glands.

Mean±SD NPSE and CSE primary cell yields after protease exposure were 3.2±0.6 (n=15) and 6.7±1.5 x10⁶ (n=9) cells, respectively. Aliquots of the NPSE cells were used to make cytospin slides, and NPSE and CSE cells were plated on plastic dishes in bronchial epithelial growth medium. In primary culture, a variable proportion of the cells attached and increased in number, gradually reaching confluence in 5 to 14 days. Mean±SD NPSE and CSE passage 1 cell yields after primary culture were 2.3±0.5 (n=15) and 1.7±0.2 x10⁶ (n=9) cells, respectively. Cy-
tosspin slides of freshly dissociated primary cells and passage 1 NPSE cells were immunostained to confirm the nature of seeded cells. Photomicrographs are shown in Figure 3, and mean data are given in Figure 4. Most cells in both populations were positive for cytokeratin, and passage 1 cells were 96.4% cytokeratin positive. Approximately half of all cells were positive for epithelial membrane antigen. The vimentin-positive fraction was lower in passage 1 cells. Eighteen percent of the primary NPSE cells were positive for the leukocyte marker CD45, whereas this value was reduced to less than 1% in passage 1 cells. These results are consistent with inflammatory and, potentially, fibroblastic cell contamination of the primary cell harvests. However, the serum-free culture conditions do not support the survival of nonepithelial cells, which were eliminated by the time of passage. Thus, the passage 1 cells that were seeded on porous supports for ALI culture were almost exclusively epithelial cells.

MORPHOLOGIC FEATURES OF ALI CULTURES FROM PASSAGE 1 NPSE AND CSE CELLS

When grown on plastic, primary sinon epithelial cells appeared flat and cobblestone (Figure 5A). These were dissociated into passage 1 cells and were seeded on Transwell-Col or Snapwell culture inserts and grown submerged in ALI culture medium until confluence, 2 to 7 days after seeding. At this point, the apical surface was rinsed with phosphate-buffered saline, and media was replaced only in the basolateral compartment. The cells opposed a hydrostatic pressure gradient, maintaining a "dry" apical surface, and cellular differentiation continued under ALI conditions. Representative wells of NPSE cells were fixed on day 10 and examined histologically. The epithelial cells were flat or cuboidal, and no cilia were observed at the apical surface on day 10, but a few cells contained AB-PAS–positive mucous granules (Figure 5B and C). On day 21, the epithelium from NPSE (Figure 5D and E) and CSE (Figure 5F and G) cells was well differentiated, stratified with mature ciliated and AB-PAS–positive goblet cells visible across the membrane. The quantitative morphologic properties of the cells are summarized in Table 1. There was a trend for the number of cell layers, epithelial thickness, and the number of goblet cells to increase in NPSE cells between days 10 and 21, but they were not statistically significantly increased. However, the number of ciliated cells significantly increased during this interval. There were no statistically significant morphologic differences between NPSE and CSE cells on day 21.

Day 21 ALI NPSE cell cultures were fixed with perfluorocarbon–osmium tetroxide to preserve airway surface liquid and were examined by means of electron microscopy (Figure 6). Mature cilia and microvilli were present in an electron-lucent layer close to the cell surface. A thin, more electron-dense layer, likely mucous, was observed above the clear periciliary layer (Figure 6A). In addition, images were captured presumably representing mucous granule–filled membrane extensions from mucous secretory cells into the airway surface liquid (Figure 6B).

ELECTROPHYSIOLOGIC PROPERTIES OF ALI CULTURES

To investigate transepithelial ion transport by NPSE and CSE cells, we mounted day 21 ALI Snapwell culture inserts in Ussing chambers and recorded \( I_{sc} \) continuously. Under baseline conditions, sinus epithelium exhibited relatively high \( R_{t} \) and relatively low \( I_{sc} \) (Table 2) compared with reported properties of primary nasal surface epithelial cells seeded at high density and studied shortly after confluence.\(^{11-13} \) The \( R_{t} \) was similar to that of passage 2 tracheobronchial epithelial cells grown under identical conditions for 21 days, but again, \( I_{sc} \), and potential difference were somewhat lower.\(^{14} \) The \( I_{sc} \) and potential difference were significantly higher in CSE cells than in NPSE cells, and there was no difference in basal \( R_{t} \) (Table 2).

We next tested the effects of the epithelial \( \text{Na}^{+} \) channel blocker amiloride on NPSE and CSE cells (Figure 7). Amiloride inhibited approximately 20% of the basal \( I_{sc} \) in NPSE cells (\( n=6; P=.03 \)) and induced an even larger decrease in \( I_{sc} \) in CSE cells, an approximately 60% reduction (\( n=7; P=.02 \)).

We found little evidence of basal bumetanide–inhibitable, \( \text{Na}^{+}-\text{K}^{+}-2\text{Cl}^{-} \) channel–mediated \( \text{Cl}^{-} \) secretion in NPSE and CSE cells (Figure 8A and B). The effect of bumetanide on basal \( I_{sc} \) was variable and inconsistent. In ALI cultures of 1 tissue donor, basal \( I_{sc} \) was decreased by 9.36 µA/cm² (approximately 70%), but in cul-
tures from other patients it was increased or not changed after the addition of bumetanide. Amiloride addition, more than 20 minutes after bumetanide exposure, showed a trend toward decreased Isc in NPSE and CSE cells (Figure 8C and D). A large residual Isc was observed after bumetanide and amiloride exposure, approximately 60% and 45% of basal Isc in NPSE and CSE cells, respectively.

A comparison of basal Na⁺ transport rates between NPSE and CSE cells is shown in Figure 9. The CSE cells exhibited relatively large Isc responses to amiloride, whether amiloride was added alone or after bumetanide, suggesting increased Na⁺ transport rates in CSE cells.

Regarding regulation of Cl⁻ transport, forskolin, which stimulates cyclic adenosine monophosphate formation and activates cystic fibrosis transmembrane conductance regulator, induced a trend toward increased Isc in NPSE and CSE cells. Uridine triphosphate, which raises intracellular Ca²⁺ levels and activates Ca²⁺-activated Cl⁻ channel, when added after forskolin, significantly increased Isc in NPSE cells and induced a trend toward increased Isc in CSE cells (Figure 10A). In the stable state after forskolin and UTP administration, bumetanide showed a trend toward inhibiting Isc in NPSE and CSE cells. There were no differences in response to drug addition between NPSE and CSE cells in this series of experiments.

We also investigated the regulation of Cl⁻ secretion by UTP and forskolin in the presence of amiloride (Figure 10B). Amiloride in other respiratory epithelia increases the electrochemical driving force for Cl⁻ secre-
tion and increases the magnitude of the response to Cl− secretagogues. Under these conditions, UTP increased Isc in NPSE cells and induced an even larger increase in CSE cells that was significantly greater than that in NPSE cells. Subsequent addition of forskolin also increased Isc in NPSE cells and again produced a significantly larger response in CSE cells.

COMMENT

To our knowledge, this is the first study reporting ALI culture of human paranasal sinus epithelial cells. The methods are modified from the standard ALI culture procedures for human nasal turbinate and polyp and tracheobronchial specimens.8 The initial incubation with 0.1% protease for cell detachment was reduced from the 24 to 48 hours typically used with other tissues to much shorter intervals. This reduction is consistent with studies of other very small resected specimens. For example, murine tracheal epithelium was incubated in 0.1% protease for only 60 minutes,6 and Black et al3 did not use protease to isolate cells from nasal brush samples. Thus, the duration of protease exposure is probably directly related to specimen size. We found that 3 to 8 hours was optimal for the typical small and thin tissue fragments obtained from sinus mucosa.
Because identification of the mucosal side of the specimen to selectively remove epithelial cells by scraping was difficult, we used immunohistochemical analysis of cytospin slides to determine the phenotype of proteolytically released primary and passage 1 NPSE cells. Before primary culture, approximately 20% of the freshly harvested cells were pan-cytokeratin negative and CD45 or vimentin positive and were presumably hematopoietic cells or fibroblasts. However, the contaminating CD45-positive cells did not persist in culture and were absent in suspensions of passage 1 cells. Vimentin-positive cells were observed in the passage 1 population, but these were not likely fibroblasts and instead were respiratory epithelial cells exhibiting pan-cytokeratin and vimentin double staining as previously reported. Epithelial membrane antigen staining was similar (approximately 43%) in primary and passage 1 cells. Although this marker is variably expressed, we speculate that this result indicates minimal terminal squamous differentiation during culture on plastic. Thus, primary culture of sinus cells on plastic in serum-free media eliminated inflammatory cells and fibroblasts and resulted in an enriched population of passage 1 respiratory epithelial cells able to undergo mucociliary differentiation typical of the sinus epithelium in vivo.

The morphologic features of ALI cultures of NPSE cells were examined on days 10 and 21. The cells progressed from flat and poorly differentiated cells on day 10 to polarized, columnar ciliated and AB-PAS–positive mucous secretory cells on day 21. Previous studies of nasal epithelial cells in ALI culture on day 21 showed approximately 30 ciliated cells per 1 mm and few secretory cells. We found a similar degree of ciliogenesis (Figure 5). Usui and colleagues found that approximately half of the cells in ALI nasal surface cultures were AB-PAS positive. Although we found fewer AB-PAS–positive cells, it is generally accepted that the paranasal sinus contains fewer goblet cells than the nasal surface, and the present results may reflect this in vivo difference. Thus, ALI cultures on day 21 resembled, but did not precisely recapitulate, the morphologic features of the native epithelium. The cultured cells were generally more stratified than pseudostratified, ciliated cells were not as abundant, and cilia seemed to be shorter than in vivo. Nevertheless, the overall morphologic similarity to the paranasal sinus respiratory epithelium suggests that this system will be useful for modeling the function of this tissue. We found no histologic differences between NPSE and CSE cell cultures on day 21, suggesting that the in vivo effect of injury and inflammation had become minimized, at least at the morphologic level.

We next used perfluorocarbon–osmium tetroxide fixation and electron microscopy to examine the detailed morphologic features of the airway surface (Figure 6). We detected cilia in a clear, serous periciliary layer and an overlying, apparently mucus-rich layer. The ALI cultures of sinus epithelium may be useful for studying factors that affect the relative depth of the periciliary and mucus layers, which are presumed to strongly affect mucociliary clearance rates.

Researchers have extensively evaluated transepithelial ion transport of cultured airway epithelium in Ussing chambers. The present studies of cultured paranasal sinus epithelial cells revealed low basal I sodium and high R, compared with previous studies of primary nasal turbinate and polyp epithelial cells seeded at high density and studied after several days in culture. In human nasal surface epithelia, amiloride-sensitive Na+ absorption seems to be the dominant active ion transport system under basal physiologic conditions. In the present cultured passage 1 NPSE cells, amiloride-sensitive I sodium was surprisingly small, 20% of basal I sodium (Figure 7). These results may be due to the effects of the culture system or may indicate that Na+ transport plays a less substantial role in basal transepithelial ion transport in normal human paranasal epithelium. The reduction in basal I sodium induced by the Na+-K+–2Cl− cotransporter–specific inhibitor bumetanide was not statistically significant (Figure 8), consistent with little basal Cl− secretion. However, the expression and contribution of the Na+-K+–2Cl− cotransporter to Cl− secretory function was indicated by a trend toward inhibition of I sodium by bumetanide after forskolin and UTP treatment (Figure 10). These data suggest that there is a basal current that we have not characterized. Possibilities include amino acid or glucose-dependent Na+ transport or anion secretion reflecting HCO3− transport or Cl− transport mediated by a basolateral Cl− entry path different than the Na+-K+–2Cl− cotransporter, such as parallel Cl−–HCO3− and Na+–H+ exchangers. One clue that the residual I sodium may have reflected Cl− secretion came from the observation that Cl− secretion tended to be increased by forskolin and UTP without amiloride treatment. This response differs from nasal turbinate– and polyp–derived cells and suggests that a driving force for

**Table 1. Morphologic Properties of ALI Cultures***

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>No.</th>
<th>No. of Cell Layers</th>
<th>Thickness, µm</th>
<th>No. of Cells/300 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>5</td>
<td>4 (4-6)</td>
<td>44 (34-81)</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>21</td>
<td>7</td>
<td>6 (5-7)</td>
<td>61 (49-67)</td>
<td>7 (2-16)†</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>21</td>
<td>5</td>
<td>7 (5-9)</td>
<td>88 (70-115)</td>
<td>12 (10-32)</td>
</tr>
</tbody>
</table>

Abbreviations: AB-PAS, Alcian blue–periodic acid–Schiff; ALI, air-liquid interface.

*Data are given as median (25th-75th percentile).

†P<.05 compared with normal at day 10 (Kruskal-Wallis test followed by Dunn’s multiple comparison test). There were no significant differences between normal and sinusitis at day 21.
Cl⁻ secretion exists but that the apical Cl⁻ channels are not fully active. In contrast, in the nasal surface epithelium, a driving force for Cl⁻ secretion does not generally exist without pharmacologic blockade of epithelial Na⁺/H⁻ channel with amiloride.

The observed responses to forskolin and UTP exposure suggest the existence of cystic fibrosis transmembrane conductance regulator and Ca²⁺-activated Cl⁻ channel in the apical membrane of NPSE cells. The increase in Iᵥ, stimulated by forskolin and UTP leads us to speculate that normal paranasal sinus epithelium has a large and regulatable capacity for Cl⁻ secretion. The low rate of Na⁺ absorption and the high potential for Cl⁻ secretion are similar to the physiologic features of nasal glands, which function to secrete fluid. Thus, we suggest that the balance of ion transport in NSPE cells may favor fluid secretion.

Amiloride-sensitive Iᵥ was statistically significantly greater in CSE cells than in NPSE cells. In contrast, Cl⁻ secretion inhibited by bumetanide exposure was not greater in CSE cells than in NPSE cells. However, after amiloride pretreatment to block Na⁺ transport and establish driving forces for Cl⁻ secretion, the combination of UTP (Ca²⁺) and forskolin (cyclic adenosine monophosphate) stimulated Cl⁻ transport to a greater extent in CSE cells than in NPSE cells. These results suggest that basal Na⁺ absorption and the capacity to secrete Cl⁻ were up-regulated in CSE cells. This could be due to at least 2 alternative mechanisms. First, it is possible that CSE cells

---

**Figure 6.** Ultrastructural morphologic features of cultured paranasal sinus epithelial cells and airway surface liquid (ASL). Transmission electron micrographs of air-liquid interface cultures of normal paranasal sinus epithelial cells after perfluorocarbon–osmium tetroxide fixation to preserve the ASL (uranyl acetate–lead citrate, original magnification ×2000). A, Cilia in a relatively clear periciliary layer and overlying mucus are visible. B, Protrusion of a mucous granule–filled cell into the ASL. Scale bar=2.5 µm.

**Table 2. Basal Electrophysiologic Properties of Day 21 ALI Cultures***

<table>
<thead>
<tr>
<th>Group</th>
<th>No. †</th>
<th>Iᵥ, µA/cm²</th>
<th>Resistance, Ω·cm²</th>
<th>PD, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPSE cells</td>
<td>16</td>
<td>4.7 (3.5-4.8)</td>
<td>699 (407-1038)</td>
<td>2.4 (1.8-6.0)</td>
</tr>
<tr>
<td>CSE cells</td>
<td>23</td>
<td>10.4 (5.2-13.7)</td>
<td>787 (522-1008)</td>
<td>7.2 (3.7-10.6)</td>
</tr>
</tbody>
</table>

Abbreviations: ALI, air-liquid interface; CSE, chronic sinusitis epithelial; Iᵥ, short circuit current; NPSE, normal paranasal sinus epithelial; PD, potential difference.

*Data are given as median (25th-75th percentile).
†The number of individual Ussing chambers, which were derived from 7 different NPSE and 7 different CSE cell preparations.
‡P<.05 compared with NPSE cells (Mann-Whitney rank sum test).
in culture are generally more “fit” than NPSE cells. Specifically, chronic inflammation may result in more replication-competent progenitor cells in the CSE cell population that are poised to regenerate a more functionally active epithelium in vitro. However, the morphologic features of NPSE and CSE cells in ALI cultures were quantitatively similar (Table 1), and a functional difference would have to be independent of histologic appearance. Alternatively, the inflammatory milieu in chronic sinusitis may have altered the ion transport phenotype of the epithelium in situ, which persisted in vitro. Inflammatory mediators such as histamine and bradykinin and cytokines stimulate ion transport in the human airway epithelium. Although the effects of chronic inflammation found in cystic fibrosis airways in vivo persist for at least several days in primary cultures, they seem to be lost on extended culture or in passaged cells. Further studies are necessary to resolve whether the observed differences in the electrophysiologic properties of

Figure 7. Short circuit current ($I_{sc}$) before and after inhibition of Na$^+$ transport with 100µM amiloride in air-liquid interface cultures of normal paranasal sinus epithelial (NPSE) (A) and chronic sinusitis epithelial (CSE) (B) cells. Each paired specimen is connected by a line. *$P<.05$ compared with basal $I_{sc}$, Wilcoxon test for paired data.

Figure 8. Effects of sequential bumetanide and amiloride administration on basal short circuit current ($I_{sc}$) of cultured paranasal sinus epithelial cells. The $I_{sc}$ is shown before and after exposure to an Na$^+$-K$^+$-2Cl$^-$ cotransport inhibitor (100µM bumetanide) in air-liquid interface cultures of normal paranasal sinus epithelial (NPSE) (A) and chronic sinusitis epithelial (CSE) (B) cells. Also shown is the effect of inhibiting Na$^+$ transport with 100µM amiloride in NPSE (C) and CSE (D) cells 20 minutes after bumetanide pretreatment. Each paired specimen is connected by a line. *$P<.05$ compared with basal $I_{sc}$, Wilcoxon test for paired data.

Figure 9. Amiloride and bumetanide-amiloride inhibition of short circuit current ($I_{sc}$) in cultured paranasal sinus epithelial cells. The change in basal $I_{sc}$ after inhibition of Na$^+$ transport with 100µM amiloride in normal paranasal sinus epithelial (N) and chronic sinusitis epithelial (S) cells is illustrated on the left and after sequential bumetanide and amiloride treatment is shown on the right. *A significant difference between NPSE and CSE cells, $P<.05$, Mann-Whitney rank sum test.

Figure 10. Changes in short circuit current ($I_{sc}$) after forskolin, uridine triphosphate (UTP), and bumetanide treatment (in the absence of amiloride) (A) and after stimulation with UTP and forskolin (in the presence of amiloride) (B) in normal paranasal sinus epithelial (N) and chronic sinusitis epithelial (S) cells. *A trend toward inhibition of $I_{sc}$ ($P=.06$). *$P<.05$ compared with baseline or a significant difference between N and S cells ($P<.05$), Mann-Whitney rank sum test.
NPSE vs CSE cells were intrinsic, acquired, or somehow related to the in vitro culture system, for example, due to differences in growth and differentiation of the excised NPSE vs CSE cells. These uncertainties highlight the limitations of the in vitro approach and point to the need to ultimately verify the findings in humans in vivo.

Abnormal ion transport in CSE cells, as illustrated in Figure 11, could contribute to the pathogenesis of chronic sinusitis. With respect to the increased capacity for Cl− secretion, Passali and colleagues reported that furosemide (a bumetanide-like drug) could prevent postsurgical recurrence of sinonasal polyposis. However, bumetanide did not affect basal Isc, it tended to inhibit Isc after treatment with forskolin and UTP. If inhibition of Cl− secretion under activated conditions is important to prevent the recurrence of sinusitis, this study may partially explain the outcome of the clinical studies, although furosemide may also be active against inflammatory cells.

Ostial obstruction and subsequent bacterial infection are likely important in the pathogenesis of chronic sinusitis. Na+ absorption may become up-regulated in the paranasal sinus epithelium owing to the effects of chronic inflammation or after ostial block as a compensatory mechanism to clear residual fluid. Inflammation-induced Na+ hyperabsorption may deplete sinus surface liquid, impairing mucus transport and resulting in ostial occlusion by thickened mucus and inflammatory debris. After ostial blockage, it is possible that submucosal edema induced by up-regulated Na+ absorption, in an effort to remove accumulated materials, will further exacerbate ostial block. Thus, a vicious cycle of increased Na+ absorption may be important in the pathogenesis of chronic sinusitis.

In conclusion, we established methods to produce well-differentiated ALI cultures of normal and chronic sinusitis epithelia from small surgical specimens. The use of specific inhibitors and agonists in Ussing chambers revealed epithelial Na+ channel, Na+/K+/2Cl− cotransporter, cystic fibrosis transmembrane conductance regulator, and Ca2+-activated Cl− channel activity. Sodium absorption in normal human paranasal sinus cells was relatively low, and bumetanide-dependent chloride secretion was undetectable under basal conditions. Cultured cells derived from chronic sinusitis specimens exhibited larger Na+ absorption and a greater capacity for Cl− secretion compared with normal sinus mucosa. Each abnormal ion transport property found in vitro suggests potentially important mechanisms in the pathogenesis of chronic sinusitis, and studies in humans are needed to verify whether similar alterations are present in vivo.

Submitted for Publication: February 15, 2006; final revision received July 7, 2006; accepted August 20, 2006.

Correspondence: Scott H. Randell, PhD, Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina, CB 7248, Thurston-Bowles Building, Room 4011, Chapel Hill, NC 27599 (randell@med.unc.edu).

Author Contributions: Dr Randell had full access to all.
the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Dejima, Randell, and Boucher. Acquisition of data: Dejima, Randell, Stutts, and Senior. Analysis and interpretation of data: Dejima, Randell, Stutts, and Boucher. Drafting of the manuscript: Dejima, Randell, Senior, and Boucher. Critical revision of the manuscript for important intellectual content: Dejima, Randell, Stutts, Senior, and Boucher. Statistical analysis: Dejima and Randell. Obtained funding: Randell and Boucher. Administrative, technical, and material support: Randell and Senior. Study supervision: Randell, Stutts, and Boucher.

Financial Disclosure: None reported.

Funding/Support: This work was supported by grants from the National Institutes of Health (Drs Randell, Stutts, and Boucher).

Previous Presentation: This study was presented in abstract form at the 41st Meeting of the Japan Rhinology Society; September 27, 2002; Hiroshima City, Japan.

Acknowledgment: We thank Kim Burns, BS, HTL, and Tracy Bartolotta, BS, HTL, of the Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina Histology Core for technical assistance with histology and electron microscopy; Elizabeth Bazemore, BS, for help with the Ussing chambers; and Lisa Brown, MA, for editorial and graphics assistance.

REFERENCES


