Increased expression of the epithelial anion transporter pendrin/SLC26A4 in nasal polyps of patients with chronic rhinosinusitis

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Background: Chronic rhinosinusitis (CRS) is a multifactorial disease of unknown cause characterized by sinonasal inflammation, increased mucus production, and defective mucociliary clearance. Expression of Pendrin, an epithelial anion transporter, is increased in asthma and chronic obstructive pulmonary disease. Pendrin increases mucus production and regulates mucociliary clearance.

Objectives: We sought to investigate the expression of pendrin and the mucus-related protein Muc5AC in sinonasal tissues of control subjects and patients with CRS and to evaluate the regulation of pendrin expression in nasal epithelial cells (NECs) *in vitro*.

Methods: The expression and distribution of pendrin in sinonasal tissues was analyzed by using real-time PCR, immunoblot analysis, and immunohistochemistry. Differentiated NECs were used to study the regulation of pendrin expression.

Results: Increased pendrin expression was observed in nasal polyp (NP) tissue of patients with CRS. Immunohistochemistry analysis revealed that pendrin was largely restricted to the epithelial layer. Pendrin expression significantly correlated with inflammatory cell markers, suggesting that the factors made by these cells might induce pendrin expression. Furthermore, both pendrin and periostin levels (a biomarker in asthma) correlated with IL-13 levels, suggesting that pendrin can be induced by this cytokine in sinonasal tissues. Expression of the mucus component protein Muc5AC correlated weakly with pendrin expression, indicating that pendrin might modulate mucus production in NPs. In cultured NECs pendrin expression was induced by T_H2 cytokines and induced synergistically when T_H2 cytokines were combined with IL-17A. Interestingly, human rhinovirus had a potentiating effect on IL-13–induced pendrin expression. Dexamethasone suppressed pendrin expression, suggesting that the therapeutic benefit of dexamethasone in asthmatic patients and those with CRS might involve regulation of pendrin expression.

Conclusions: T_H2-mediated pendrin expression is increased in NPs of patients with CRS and might lead to increased inflammation, mucus production, and decreased mucociliary clearance. (J Allergy Clin Immunol 2015;136:1548-58.)

Key words: *Pendrin, SLC26A4, periostin, Muc5AC, chronic rhinosinusitis, nasal polyp, mucus, mucociliary clearance, nasal epithelial cells*

Chronic rhinosinusitis (CRS) is a common disease affecting 10% of the population in developed countries.[1] Patients with CRS have a poor quality of life comparable with that of patients with other chronic conditions, such as congestive heart failure, chronic obstructive pulmonary disease, angina, and back pain, yet have few therapeutic options.[2,3] Most patients do not respond to medical interventions and often undergo surgery in an attempt to alleviate symptoms, which results in a significant cost burden.[4]

CRS is characterized by persistent inflammation of the nasal and paranasal sinus mucosa that lasts longer than 12 weeks. Diagnosis requires endoscopic confirmation of inflammation,

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Abbreviations used
ALI: Air-liquid interface
CRS: Chronic rhinosinusitis
CRSsNP: Chronic rhinosinusitis without nasal polyps
CRSwNP: Chronic rhinosinusitis with nasal polyps
NEC: Nasal epithelial cell
NP: Nasal polyp
Poly(I:C): Polyinosinic:polycytidylic acid
UT: Uncinate tissue

purulent discharge, or edema in the middle meatus or ethmoid region; the presence of nasal polyposis; or radiographic evidence of paranasal sinus inflammation in the setting of rhinosinusitis symptoms (eg, purulent discharge, nasal obstruction, facial pain/pressure/fullness, and hyposmia).[5]

CRS can be further divided into 2 major subtypes: chronic rhinosinusitis without nasal polyps (CRSsNP) and chronic rhinosinusitis with nasal polyps (CRSwNP). Although the cytokines and cells driving CRSsNP are not clear, CRSwNP is often associated with an increased T_H2 cytokine profile and generally eosinophilic inflammation.[6] Recent studies have proposed the existence of multiple endotypes of the disease, highlighting the complex nature of CRS.[7] Although prior efforts have deepened our understanding of the inflammatory profiles and cells associated with CRS, the cause and pathogenesis of CRS remain largely unclear.[8,9] One potential contributing factor highlighted in previous studies is an imbalance in mucociliary clearance and mucus production.[10] Additional work has also demonstrated abnormalities in ion transport in the setting of CRS.[11,12]

Pendrin/SLC26A4 is an apically expressed ion exchanger originally identified as the causative mutation in Pendred syndrome, a condition characterized by prelingual hearing loss and iodide organification.[13] Pendrin is predominantly expressed in the inner ear, kidney, and thyroid gland but is also present in airways.[13-16] A role for pendrin in regulating inflammation and mucus production in asthmatic patients, patients with CRS, and those with chronic obstructive pulmonary disease has been previously proposed.[14-16] Pendrin has also been shown to regulate epithelial air-surface liquid levels and composition.[15,17]

In this study we determined the expression and localization of pendrin in sinonasal tissues, including uncinate tissue (UT) and nasal polyp (NP) tissue, taken from patients with CRS and control subjects. We investigated the cellular and cytokine profiles associated with pendrin expression in sinonasal tissues. Finally, we assessed the regulation of pendrin expression in differentiated nasal epithelial cells (NECs) in response to inflammatory cytokines and glucocorticoid treatment *in vitro*.

METHODS

Patients and tissue samples

Patients were recruited from the Sinus and Allergy Clinics at Northwestern Memorial Hospital by using protocols approved by the Institutional Review Board of Northwestern University. All patients with CRS (diagnosed by using task force guidelines[18,19]) and control subjects signed informed consent forms. Tissue samples (both UTs and NPs) and nasal scraping cells were collected from the patients in whom conservative medical therapy (saline irrigations, decongestants, and prolonged treatment with steroids, antibiotics, or both) had failed at the time of sinus surgery. Control UTs were collected from patients undergoing surgery for skull-based tumor excision. Control subjects did not have any history of inflammatory upper airway diseases. Patients with immunodeficiency, Churg-Strauss syndrome, or cystic fibrosis were excluded from this study. A detailed list of patients' characteristics is presented in Table I.

Cell culture

NECs were collected during surgery, expanded, and cultured under air-liquid interface (ALI) conditions.[20] A detailed protocol can be found in the Methods section in this article's Online Repository at www.jaconline.org.

Nasal protein extraction and immunoblot analysis

Proteins were isolated from tissues, as previously described.[3] A detailed protocol can be found in the Methods section in this article's Online Repository.

Real-time PCR

Tissues were lysed in QIAzol (Qiagen, Valencia, Calif), and RNA extraction and real-time PCR were performed, as described previously.[3] A detailed protocol can be found in the Methods section in this article's Online Repository.

Immunohistochemistry

The basic protocol for immunohistochemistry has been described previously.[21] A detailed protocol for immunohistochemistry is submitted in the Methods section in this article's Online Repository.

Statistical analysis

Multiple comparisons were carried out with ANOVA. The Student *t* test was used to compare *in vitro* experimental data. All analyses were performed with GraphPad Prism software (GraphPad Software, La Jolla, Calif). A P value of less than .05 was considered statistically significant.

RESULTS

Increased expression of pendrin in NPs of patients with CRS

T_H2 cytokine–mediated pendrin expression has been reported in asthmatic patients. Because nasal polyposis is often a T_H2 cytokine–dominated disease, we analyzed the expression of pendrin in 83 sinonasal tissues of control subjects (control UT, n = 18) and patients with CRS (CRSsNP UT, n = 23; CRSwNP UT, n = 22; and polyp, n = 20). Pendrin expression was profoundly increased in NPs of patients with CRSwNP (10-fold, 771.5 ± 254.5 copies/ng total RNA) and UTs of patients with CRSsNP (4-fold, 309.3 ± 74.62 copies/ng total RNA) compared with control UTs (71.68 ± 11.31 copies/ng total RNA). Although there was a trend for increased pendrin expression in UTs of patients with CRSwNP compared with control UTs, it was not statistically significant (Fig 1, *A*).

Next, we assessed whether mRNA data correlated to protein data by using immunoblot analysis in 48 sinonasal tissue samples (control UT, n = 9; CRSsNP UT, n = 12; CRSwNP UT, n = 11; and polyps, n = 16). Surprisingly, we did not detect pendrin protein at the expected molecular weight (80 kDa: native and 100-120 kDa: glycosylated pendrin). Our pendrin-specific antibody detected a cross-reacting band at a size of approximately 70 kDa. To confirm the specificity of the antibody, we performed peptide-blocking experiments. Incubation of pendrin antibody with pendrin peptide before incubating the blot completely