Proton pump inhibitors decrease eotaxin-3/CCL26 expression in patients with chronic rhinosinusitis with nasal polyps: Possible role of the nongastric H,K-ATPase

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Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is often characterized by tissue eosinophilia that is associated with poor prognosis. Recent findings that proton pump inhibitors (PPIs) directly modulate the expression of eotaxin-3, an eosinophil chemoattractant, in patients with eosinophilic diseases suggest therapeutic potential for PPIs in those with CRSwNP.
Objective: We assessed the effect of type 2 mediators, particularly IL-13 and eotaxin-3, on tissue eosinophilia and disease severity in patients with chronic rhinosinusitis (CRS). Further investigation focused on PPI suppression of eotaxin-3 expression *in vivo* and *in vitro*, with exploration of underlying mechanisms.
Methods: Type 2 mediator levels in nasal tissues and secretions were measured by using a multiplex immunoassay. Eotaxin-3 and other chemokines expressed in IL-13–stimulated human sinonasal epithelial cells (HNECs) and BEAS-2B cells with or without PPIs were assessed by using ELISA, Western blotting, real-time PCR, and intracellular pH imaging.
Results: Nasal tissues and secretions from patients with CRSwNP had increased IL-13, eotaxin-2, and eotaxin-3 levels, and these were positively correlated with tissue eosinophil cationic protein levels and radiographic scores in patients with CRS ($P < .05$). IL-13 stimulation of HNECs and BEAS-2B cells dominantly induced eotaxin-3 expression, which was significantly inhibited by PPIs ($P < .05$). Patients with CRS taking PPIs also showed lower *in vivo* eotaxin-3 levels compared with those without PPIs ($P < .05$). Using intracellular pH imaging and altering extracellular K^+, we found that IL-13 enhanced H^+,K^+-exchange, which was blocked by PPIs and the mechanistically unrelated H,K-ATPase inhibitor, SCH-28080. Furthermore, knockdown of *ATP12A* (gene for the nongastric H,K-ATPase) significantly attenuated IL-13–induced eotaxin-3 expression in HNECs. PPIs also had effects on accelerating IL-13–induced eotaxin-3 mRNA decay.
Conclusion: Our results demonstrated that PPIs reduce IL-13–induced eotaxin-3 expression by airway epithelial cells. Furthermore, mechanistic studies suggest that the nongastric H,K-ATPase is necessary for IL-13–mediated epithelial responses, and its inhibitors, including PPIs, might be of therapeutic value in patients with CRSwNP by reducing epithelial production of eotaxin-3. (J Allergy Clin Immunol 2017;139:130-41.)

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Supported by National Institutes of Health (NIH) grant K23DC012067 (to B.K.T.); Chronic Rhinosinusitis Integrative Studies Program (CRISP) U19 AI106683 (to B.K.T., R.C.K., K.E.H, A.K., and R.P.S.); R01 HL078860, R01 HL068546, R01 AI072570, and R01 AI104733; the Triological Society/American College of Surgeons; (to B.K.T.) and the Ernest S. Bazley Foundation.

Disclosure of potential conflict of interest: J.-Y. Min, C. J. Ocampo, W. W. Stevens, C. P. E. Price, C. F. Thompson, T. Homma, J. H. Huang, J. E. Norton, L. A. Suh, K. L. Pothoven, D. B. Conley, K. C. Welch, A. T. Peters, K. E. Harris, R. C. Kern, and B. K. Tan's institution has received grants K23 DC012067 and U19 AI106683 from the National Institutes of Health (NIH). S. Shintani-Smith's institution has received grants K23 DC012067 and U19 AI106683 from the NIH, grant K12HS023011 from the Agency for Healthcare Research and Quality (AHRQ), and UL1TR001422 from NIH. L. C. Grammer's institution has received grants and support for travel from the NIH and other forms of support from the Bazley Foundation grant; money for consultancy from Astellas Pharmaceuticals, employment from Northwestern University and Northwestern Medical Faculty Foundation; grants/grants pending from the NIH and Food Allergy Network; payment for lectures from the American Academy of Allergy, Asthma & Immunology and Mount Sinai; and royalties from Lippincott, UpToDate, BMJ, Elsevier, Kluwers Wolter, and other from Bazley Foundation grant. K. E. Hulse's institution has received grants K23 DC012067, U19 AI106683, and K12 HD 055884 from the NIH. A. Kato's institution has received grants U19 AI106683 and R01 AI104733 from the NIH, a research grant from Janssen Research, and payment for lectures from Janssen Research. R. P. Schleimer has received grants from the NIH; consultancy fees from Intersect ENT, GlaxoSmithKline, Allakos, Aurasense, Merck, BioMarck, Sanofi, AstraZeneca/Medimmune, Genentech, and Exicure; and stock options from Allakos, Aurasense, BioMarck, and Exicure. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 3, 2016; revised May 31, 2016; accepted for publication July 14, 2016.
Available online October 4, 2016.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.07.020

Key words: *Chronic rhinosinusitis, eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26, omeprazole, proton pump inhibitors, IL-13, epithelial cells, H(+)-K(+)-exchanging ATPase, eosinophils*

Chronic rhinosinusitis (CRS) is characterized by local inflammation of the sinonasal mucosa, with symptoms persisting for at least 12 weeks.[1] It is further classified into 2 clinical phenotypes: chronic rhinosinusitis with nasal polyps (CRSwNP) and CRS without nasal polyps.[1-3] In Western populations, CRSwNP is frequently associated with type 2 inflammation and tissue eosinophilia.[4] Because tissue eosinophilia has been implicated in increased postsurgical recurrence rates[5,6] and decreased improvement in quality-of-life outcomes,[7] strategies for blocking eosinophil recruitment could improve treatment for CRSwNP.

Eosinophil recruitment is generally regulated by type 2 cytokines (eg, IL-4, IL-5, and IL-13) and CC chemokines (eg, eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26, and monocyte chemoattractant protein 4/CCL13).[8-10] Among the cytokines, IL-13 appears to drive epithelial responses, including barrier dysfunction, mucus overproduction, and production of chemokines in type 2 inflammatory airway diseases.[11-14] The induced eotaxins are ligands for CCR3, which is highly expressed on eosinophils.[9,15-19] Of the eotaxins expressed by human subjects, recent studies increasingly emphasize a critical role for eotaxin-3 in patients with eosinophilic diseases, showing greater and more sustained eosinophil recruitment in asthma[8,20] and strong associations with susceptibility to eosinophilic esophagitis (EoE).[21]

In patients with CRSwNP, increased levels of type 2 mediators and type 2 cytokine–producing cells, such as T_H2 cells and group 2 innate lymphoid cells, are found in nasal mucosa,[22-25] supporting a crucial role of tissue eosinophilia in CRSwNP pathogenesis. However, whether eotaxin-3 is associated with eosinophilic responses in patients with CRSwNP is yet to be established.

Although novel mAbs, such as mepolizumab (anti–IL-5) and dupilumab (anti–IL-4 receptor α), have shown promise for CRSwNP treatment,[26,27] cost, parenteral administration, and lack of clinical approval for a CRSwNP indication will foreseeably limit access to these agents.[28] Interestingly, recent studies have shown that proton pump inhibitors (PPIs), which are used traditionally for treating gastroesophageal reflux disease (GERD), suppress IL-13–induced eotaxin-3 production in esophageal squamous cells[29-31] and have clinically relevant antieosinophil effects in patients with EoE, even in those without coexisting GERD.[31,32] Because PPIs are not used for treating CRSwNP, mechanistic evidence that PPIs can also directly suppress IL-13 responses in the upper airway might open new avenues for treating this common chronic inflammatory condition.

Given the biological parallels between EoE and CRSwNP,[33] we characterized the relationship of type 2 mediators, particularly IL-13 and eotaxin-3, with tissue eosinophilia and disease severity in patients with CRSwNP. We further evaluated the relative production of eotaxins by IL-13–stimulated airway cells *in vitro* and explored the efficacy of PPIs on inhibiting eotaxin-3 expression *in vivo* and *in vitro*. Finally, we investigated potential mechanisms through which PPIs suppressed IL-13–induced eotaxin-3 expression in airway epithelial cells.

Abbreviations used

CRS:	Chronic rhinosinusitis
CRSwNP:	Chronic rhinosinusitis with nasal polyps
ECP:	Eosinophil cationic protein
EoE:	Eosinophilic esophagitis
GERD:	Gastroesophageal reflux disease
gH,K-ATPase:	Gastric H,K-ATPase
HBEC:	Human bronchial epithelial cell
HNEC:	Human sinonasal epithelial cell
IT:	Inferior turbinate
$[K^+]_e$:	Extracellular K^+ concentration
ngH,K-ATPase:	Nongastric H,K-ATPase
NP:	Nasal polyp
pH_i:	Intracellular pH
PPI:	Proton pump inhibitor
siRNA:	Small interfering RNA
STAT6:	Signal transducer and activator of transcription 6
UT:	Uncinate tissue

METHODS

Subjects and sample collection

Healthy control subjects and patients with CRS[2,34] were recruited from the Otolaryngology and Allergy-Immunology Clinics at Northwestern Medicine. Computed tomographic scans were graded according to the methods defined by Okushi et al,[35] and the history of taking PPIs listed in preoperative anesthesia records on the day of sinus surgery was obtained. Subjects' characteristics are included in Table E1 in this article's Online Repository at www.jacionline.org. All subjects provided informed consent. The Institutional Review Board of Northwestern University–Feinberg School of Medicine approved this study. Tissue specimens, including uncinate tissue (UT) and nasal polyps (NPs); nasal lavage fluid; and epithelial scrapings from the inferior turbinate (IT) and NPs were obtained from subjects and prepared, as previously described.[36,37] Further details are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Measurement of cytokines, eotaxins, and eosinophil cationic protein levels in specimens

We measured IL-4, IL-13, eotaxin-1, eotaxin-2, and eotaxin-3 levels by using the Milliplex Map Kit (EMD Millipore, Billerica, Mass) with a Luminex 200 instrument (Life Technologies, Gaithersburg, Md). We measured eosinophil cationic protein (ECP) levels using the Mesacup ECP Test (MBL International, Woburn, Mass). Tissue concentrations of these mediators were normalized to the total protein concentration measured by using the Bicinchoninic Acid Protein Assay (Thermo Fisher Scientific, Waltham, Mass).

Cell culture

BEAS-2B, a human bronchial epithelial cell (HBEC) line transformed with a hybrid adenovirus 12–simian virus 40, was obtained from ATCC (Manassas, Va). Primary human sinonasal epithelial cells (HNECs) were collected by means of epithelial scraping of IT and NPs and cultured. For cytokine (PeproTech, Rocky Hill, NJ) stimulation, submerged cultured cells were treated with 1 to 100 ng/mL IL-13, 10 ng/mL IFN-γ, 100 ng/mL TNF, or 50 ng/mL IL-17 for 6 or 48 hours. Cells were pretreated for 2 hours with acid-activated omeprazole (0.1-50 μmol/L) or other PPIs (lansoprazole, rabeprazole, pantoprazole, and esomeprazole [1-50 μmol/L]) before stimulation with 5 ng/mL IL-13 to study the effects of PPIs (Sigma-Aldrich, St Louis, Mo) on cytokine-induced chemokines. Additionally, SCH-28080 (1-50 μmol/L, Sigma-Aldrich) was used with the same protocol. In experiments altering extracellular K^+ concentration ($[K^+]_e$), modified Ringer solution that contained different contents of K^+ (0-11.2 mmol/L KCl, see Table E2 in this article's Online Repository at www.jacionline.org) was used as culture medium. For mRNA stability assessment, actinomycin D (3 μg/mL, Sigma-Aldrich) was used, and eotaxin-3 mRNA was measured by using real-time PCR. Supernatants, whole-cell lysates, and total RNAs were harvested for further analysis. Further detailed methods are described in the Methods section in this article's Online Repository.