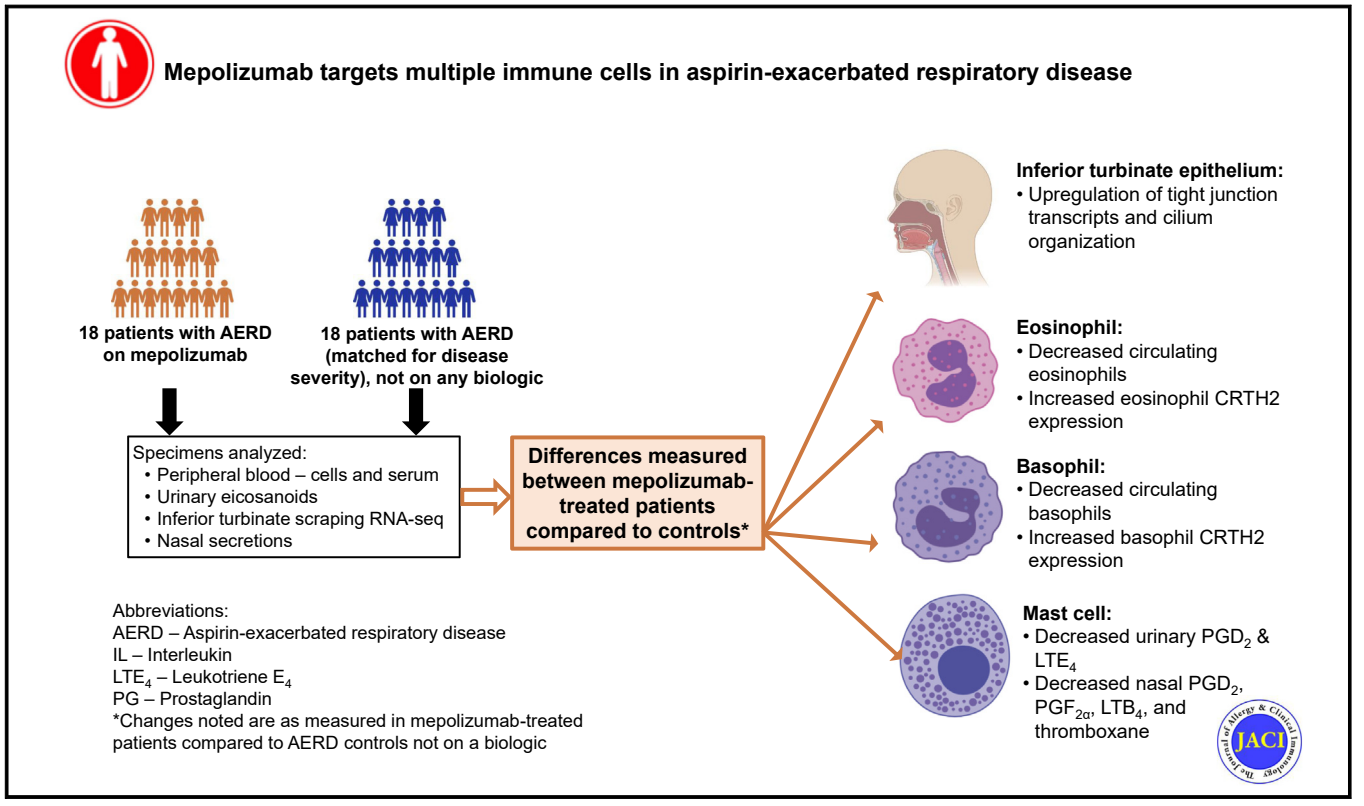
Mepolizumab targets multiple immune cells in aspirin-exacerbated respiratory disease

Kathleen M. Buchheit, MD,[a] Erin Lewis, BA,[b] Deborah Gakpo, BA,[b] Jonathan Hacker, BA,[b] Aaqib Sohail, PhD,[a] Faith Taliaferro, BS,[c,d] Evans Berreondo Giron,[c] Chelsea Asare,[c] Marko Vukovic, BS,[d,e,f] Jillian C. Bensko, PA-C,[b] Daniel F. Dwyer, PhD,[a] Alex K. Shalek, PhD,[d,e,f,g,h] Jose Ordovas-Montanes, PhD,[c,d,g,i] and Tanya M. Laidlaw, MD[a]

Boston and Cambridge, Mass

GRAPHICAL ABSTRACT



From [a]the Department of Medicine, Harvard Medical School, the Division of Allergy and Clinical Immunology, and [b]the Division of Allergy and Clinical Immunology, Brigham and Women's Hospital, Boston; [c]the Division of Gastroenterology, Boston Children's Hospital; [d]the Broad Institute of MIT and Harvard, Cambridge; [e]the Ragon Institute of Massachusetts General Hospital, MIT and Harvard, Cambridge; [f]the Institute for Medical Engineering and Science, Department of Chemistry, and Koch Institute for Integrative Cancer Research, MIT, Cambridge; [g]the Program in Immunology, Harvard Medical School, Boston; [h]the Harvard-MIT Division of Health Sciences & Technology, Cambridge; and [i]the Harvard Stem Cell Institute, Cambridge.

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Corresponding author: Tanya M. Laidlaw, MD, Brigham and Women's Hospital, 60 Fenwood Rd, Building of Transformative Medicine, Rm 5002M, Boston, MA 02115. E-mail: tlaidlaw@bwh.harvard.edu.

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Background: Eosinophilic asthma and nasal polyposis are hallmarks of aspirin-exacerbated respiratory disease (AERD), and IL-5 inhibition has been shown to provide therapeutic benefit. However, IL-5Rα is expressed on many cells in addition to eosinophils, and the mechanisms by which IL-5 inhibition leads to clinical benefit in eosinophilic asthma and nasal polyposis are unlikely to be due exclusively to antieosinophil effects.
Objective: We sought to identify the mechanisms by which anti–IL-5 treatment with mepolizumab improves respiratory inflammation in AERD.
Methods: The clinical characteristics, circulating granulocytes, nasal scraping transcripts, eosinophilic cationic protein, tryptase, and antibody levels, and urinary and nasal eicosanoid levels were measured for 18 subjects with AERD who were taking mepolizumab and compared with those of 18 matched subjects with AERD who were not taking mepolizumab.
Results: Subjects taking mepolizumab had significantly fewer peripheral blood eosinophils and basophils, and those cells that remained had higher surface CRTH2 expression than did the cells from subjects not taking mepolizumab. Nasal prostaglandin $F_{2\alpha}$, prostaglandin D_2 metabolites, leukotriene B_4, and thromboxane levels were lower in subjects taking mepolizumab, as were urinary levels of tetranor–prostaglandin D_2 and leukotriene E_4. The nasal epithelial cell transcripts that were overexpressed among subjects with AERD who were taking mepolizumab were enriched for genes involved in tight junction formation and cilium organization. Nasal and urinary prostaglandin E_2, tryptase, and antibody levels were not different between the 2 groups.
Conclusion: IL-5 inhibition in AERD decreases production of inflammatory eicosanoids and upregulates tight junction–associated nasal epithelial cell transcripts, likely due to decreased IL-5 signaling on tissue mast cells, eosinophils, and epithelial cells. These direct effects on multiple relevant immune cells contribute to the mechanism of benefit afforded by mepolizumab. (J Allergy Clin Immunol 2021;148:574-84.)

Key words: *Aspirin-exacerbated respiratory disease, IL-5, nasal polyp, mepolizumab, prostaglandin $F_{2\alpha}$, prostaglandin D_2, CRTH2, chronic rhinosinusitis, leukotriene*

Abbreviations used
AERD: Aspirin-exacerbated respiratory disease
CRSwNP: Chronic rhinosinusitis with nasal polyps
CRTH2: Chemoattractant receptor–homologous molecule expressed on T_H2 cells
ECP: Eosinophilic cationic protein
GO: Gene Ontology
ILC2: Type 2 innate lymphoid cells
LTB_4: Leukotriene B_4
LTE_4: Leukotriene E_4
PGD_2: Prostaglandin D_2
$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$
TXB_2: Thromboxane B_2

Aspirin-exacerbated respiratory disease (AERD) is characterized by chronic eosinophilic type 2 inflammation of the upper and lower airways and marked by chronic rhinosinusitis with nasal polyps (CRSwNP), difficult-to-control asthma, and pathognomonic respiratory reactions to medications that inhibit cyclooxygenase-1. Nasal polyps are associated with nasal obstruction and anosmia, significant impairment in quality of life, and substantial medical resource consumption,[1,2] and they are remarkably severe and recalcitrant in patients with AERD.[3]

The mechanisms underlying the severe nasal polyposis and difficult-to-control asthma in patients with AERD are complex. Tissue eosinophilia is more pronounced in patients with AERD than in patients with aspirin-tolerant CRSwNP,[4] but the role of eosinophils in disease pathogenesis is unclear. A recent study of dexpramipexole, an experimental drug that nearly completely depletes all eosinophils from within the blood and nasal polyp tissue, failed to show any significant improvement of symptoms or reduction in nasal polyp size in patients with CRSwNP.[5] This "negative" study suggests that although the tissue eosinophilia in most patients with CRSwNP and AERD is substantial, eosinophils are not the main effector cells that drive ongoing inflammation in the disease.

Mast cell–derived mediators, epithelial barrier dysfunction, and locally produced antibodies are also thought to contribute to tissue inflammation in CRSwNP. Activated tissue mast cells play an important role in AERD pathophysiology, with ongoing release of inflammatory mediators, including cysteinyl leukotrienes and prostaglandin D_2 (PGD_2).[6-8] PGD_2 can then amplify respiratory inflammation by binding the chemoattractant receptor–homologous molecule expressed on T_H2 cells (CRTH2) receptor, which is expressed on eosinophils, basophils, type 2 innate lymphoid cells, and T_H2 cells. Defects in epithelial barrier integrity and epithelial tight junction expression have been noted in CRSwNP and AERD, leading to increased permeability and compromised host defense responses within the upper airway.[9,10] Further, elevated levels of several antibody classes have been noted within nasal polyp tissue.[11] We recently found elevated levels of local IgE within the nasal polyps in patients with AERD and described a role for local nasal tissue IgE in relation to the rapidity of nasal polyp regrowth. This study also identified elevated IL-5Rα transcript and surface expression in plasma cells from subjects with AERD, and it found that IL-5 signaling on plasma cells may play a role in facilitating their survival.[12] A more complete understanding of the role of IL-5/IL-5Rα signaling in inflammatory disorders has both mechanistic and therapeutic implications.

Humanized mAbs against IL-5 or IL-5Rα demonstrate efficacy in the treatment of eosinophilic asthma, and studies suggest improvement in some patients with nasal polyposis.[13,14] A phase 2 trial of IL-5 inhibition with mepolizumab in patients with CRSwNP showed that 60% of the subjects in the mepolizumab treatment arm experienced a therapeutic response with a corresponding decrease in total polyp score,[15] and we reported that mepolizumab can improve both upper and lower airway symptoms in some subjects with AERD.[16] Although the mechanism of response in the subset of patients for whom mepolizumab is efficacious has been attributed to the effects of inhibition of IL-5 on eosinophils, IL-5Rα is expressed on many relevant cells, including mast cells, basophils, B cells, plasma cells, some T cells, and ciliated epithelial cells.[17-20] Further, it was recently shown that human airway epithelial cells express functional IL-5Rα and that IL-5 signaling leads to downregulation of adhesion molecules, suggesting that IL-5 may reduce the strength of the epithelial barrier through weakening of cell-to-cell adhesions.[19]

Given our recent findings that IL-5Rα expression is increased in nasal polyp cells from subjects with AERD[12] and the negative results of the clinical trial of eosinophil depletion with dexpramipexole in CRSwNP, we suspect that targeting of IL-5 or IL-5Rα may work through multiple cellular mechanisms, in addition to inhibition of eosinophils. In this study, we sought to explore the effect of treatment with mepolizumab, a mAb targeting IL-5, on mast cell activation, antibody and eicosanoid production, and nasal epithelial transcript expression in subjects with AERD.

METHODS

Patient characterization

Subjects between the ages of 18 and 75 years were recruited from the Brigham and Women's Hospital (Boston, Mass) Allergy and Immunology Clinics between October 2018 and October 2019 (Table I). The Mass General Brigham Institutional Review Board approved the study, and all subjects provided written informed consent. The patients with AERD all had asthma, nasal polyposis, and a diagnosis of AERD confirmed via a physician-observed graded oral challenge to aspirin, which induced objectively defined upper and/or lower respiratory symptoms, including nasal congestion, rhinorrhea, sneezing, ocular pruritus, conjunctival injection, wheezing, dyspnea, and/or fall in FEV_1 value.

Subjects with AERD who met the clinical criteria for the US Food and Drug Administration–approved use of monthly mepolizumab (100 mg subcutaneously) for treatment of severe, uncontrolled, eosinophilic asthma and who had been receiving treatment with mepolizumab for at least 3 months were recruited. Subjects had been started on mepolizumab by their pulmonologist or allergist/immunologist as part of their usual clinical care. Subjects taking mepolizumab were compared with an age-, sex-, and disease severity–matched control population of patients with AERD who elected not to use mepolizumab either because of insurance rejection or because of a desire to avoid use of a biologic agent. Subjects were excluded if they were taking any other biologic therapy within 6 months of enrollment.

Clinical procedures

All subjects had a single study visit at Brigham and Women's Hospital. Biologic specimens, including urine, blood, nasal fluid, and nasal cells from the inferior turbinate, were collected, as were clinical parameters, FEV_1 value, and patient-reported outcome measures, including 22-Item Sino-Nasal Outcome Test and Asthma Control Questionnaire-6 scores.

Specimen procurement

Peripheral blood was drawn into heparinized tubes and processed or assayed within 1 hour of collection. Serum was obtained from the top layer after a 15-minute centrifugation at 1500 *g* at 4°C. Nasal secretions were sampled separately from both nostrils by using Nasosorption FX-R (Hunt Developments Ltd, Midhurst, United Kingdom), which is a noninvasive upper airway sampling method that uses a synthetic absorptive matrix to collect nasal mucosal lining fluid directly from the nasal mucosal surface. Nasal secretions were placed in either 300 μL of 0.5% BSA (2 samples from 1 nostril) or 300 μL of 100% methanol (from the other nostril) and were then stored in 75-μL, 150-μL, or 200-μL aliquots at –80°C until analysis.[21] Nasal epithelial tissue was collected from the inferior turbinate by using the Rhino-Pro Curette, a sterile, disposable mucosal collection device, as described.[22] One sample was taken from the right or left mid-inferior portion of the inferior turbinate by using a gentle scraping motion and was then placed directly in RNAprotect Tissue Reagent (Qiagen, Germantown, Md). Urine was collected and stored at –80°C until further analysis.

Flow cytometry

Peripheral blood was kept at room temperature, and 50 μL of whole blood was used per staining condition. Red blood cells were lysed within 30 minutes of staining, and cells were fixed in 1% paraformaldehyde. Eosinophils were identified as $CD45^+/CCR3^+$ cells within the granulocyte gate on forward scatter (FSC)/side scatter (SSC), basophils were identified as $CD45^{low}/CCR3^+$ cells within the SSC^{low}/lymphocyte gate, and neutrophils were identified as $CD45^+/CD16^+$ cells within the SSC^{high}/FSC^{high} granulocyte gate (for gating strategy, see Fig E1 in the Online Repository at www.jacionline.org). Surface CRTH2 expression was measured on eosinophils and basophils as compared to an isotype control. All antibodies were commercially available from either BioLegend (San Diego, Calif) or BD Biosciences (San Jose, Calif); the specific details regarding vendor, clone, and fluorophore are included in Table E1 (available in this article's Online Repository at www.jacionline.org). As cells were stained immediately after collection, no live-dead stain was used.

Mediator quantification

Nasal secretions and serum were measured for levels of total IgG (Invitrogen, Waltham Mass), IgA (Invitrogen), IgE (Invitrogen for serum and Abcam, Cambridge, Mass, for nasal secretions) and IgG4 (eBioscience, San Diego, Calif) by ELISA, according to the manufacturer's instructions. Serum was further analyzed for IL-5 by ELISA (R&D Systems, Minneapolis, Minn) and for total tryptase at Virginia Commonwealth University. Nasal secretions were further analyzed for eosinophilic cationic protein (ECP) by ELISA (Lifespan Biosciences, Seattle, Wash). Urinary eicosanoids (Vanderbilt University, Nashville, Tenn) and nasal eicosanoids (University of California San Diego, San Diego, Calif) were measured by using gas chromatography–mass spectrometry.[23]

Inferior nasal turbinate epithelial cell bulk RNA sequencing

For bulk RNA sequencing, epithelial cells were sampled from the inferior turbinate via nasal curettage as described earlier in this article. RNA was normalized to 10 ng as the input amount for a 2.2X SPRI ratio cleanup using Agencourt RNAClean XP beads (Beckman Coulter, catalog no. A63987). After oligo-dT priming, Maxima H Minus Reverse Transcriptase (ThermoFisher, catalog no. EP0753) was used to synthesize cDNA with an elongation step at 52°C before PCR amplification (15 cycles) using KAPA HiFi PCR Mastermix (Kapa Biosystems KK2602). Sequencing libraries were prepared using the Nextera XT DNA tagmentation kit (Illumina FC-131-1096) with 250-pg input for each sample. Libraries were pooled after processing with the Nextera kit and cleaned using Agencourt AMPure SPRI beads with successive 0.7× and 0.8× ratio SPRIs and sequenced with an Illumina 75 Cycle NextSeq500/550v2.5 kit (Illumina FC-404-2005) with a loading density at 2.2 pM, with a paired-end 35-cycle read structure. Samples were sequenced at an average read depth of 9.7 million reads per sample. Samples were aligned to the Hg19 genome and transcriptome by using STAR and RSEM.[24,25] Samples with an alignment rate less than 20% were not analyzed further. After concatenation of read counts for technical replicates, differential expression analysis was conducted by using the DESeq2 package for R, taking patient origin into account.[26] Genes with Benjamini-Hochberg–adjusted *P* values corresponding to a false discovery rate less than 0.05 were regarded as differentially expressed. Preranked genes with an unadjusted *P* value of .05 or less were used for enrichment analysis based on Gene Ontology (GO) (the GO Biological Process) and Kyoto Encyclopedia of Genes and Genomes pathways, using GEne SeT AnaLysis Toolkit.[27] Expression levels of genes enriched for tight junction pathway and hierarchic clustering analysis was performed with the R tool pheatmap (version 1.0.12).

See the Methods section of this article's Online Repository for additional details regarding single-cell RNA sequencing analysis of surgically excised sinus tissue to identify sinus tissue cells expressing *IL5RA*.

Statistical analysis

Data are expressed as means ± SEMs unless otherwise noted. The 2-sided unpaired Student *t* test, Mann-Whitney test, and Fisher exact test were used to assess differences between patients with AERD who were taking

TABLE 1. Patient characteristics

Characteristic	Mepolizumab (n = 18)	Control (n = 18)	*P* value
Age (y), mean ± SEM	53.9 ± 3.1	47.2 ± 2.7	.11*
Sex (% female)	50%	61%	.74†
FEV_1 % predicted, mean ± SEM	74.8 ± 3.9	83.7 ± 3.5	.10*
SNOT-22 score, mean ± SEM	34.7 ± 5.1	38.3 ± 6.3	.66*
ACQ-6, mean ± SEM	1.0 ± 0.2	1.1 ± 0.3	.70*
Daily aspirin use (% of patients taking aspirin)	33%	22%	.71†
Zileuton (% of patients taking zileuton)	28%	11%	.40†
Budesonide sinonasal irrigation use (% of patients taking undergoing budesonide irrigations)	89%	83%	>.99†
Duration of mepolizumab use (mo), mean (range)	15.9 (5-32)	N/A	

ACQ-6, Asthma Control Questionnaire-6; *N/A*, not applicable; *SNOT22*, 22-Item Sino-Nasal Outcome Test.
*Student *t* test.
†Fisher exact test.

mepolizumab and the controls. Correlation between biomarkers was assessed by using the Pearson correlation coefficient. Analysis was performed using GraphPad Prism software, version 7.0d (GraphPad, La Jolla, Calif).

RESULTS

Study population and demographics

In all, 18 subjects with physician-diagnosed AERD and severe, uncontrolled eosinophilic asthma who were being treated with mepolizumab as an add-on asthma maintenance therapy and 18 subjects with AERD who were not taking mepolizumab participated in the study (Table I). There were no statistically significant differences in age, sex, FEV_1 value, Asthma Control Questionnaire-6 score, or 22-Item Sino-Nasal Outcome Test score between the groups, although there was a trend toward lower FEV_1 values in the mepolizumab group. There were no differences in use of high-dose daily aspirin therapy and zileuton (a 5-lipoxygenase inhibitor) between groups; no patients were taking oral corticosteroids. In all, 16 of 18 patients in the mepolizumab group and 15 of 18 patients in the control group used nasal budesonide irrigations.

Blood eosinophil and basophil levels are reduced, and their CRTH2 expression is increased, in subjects taking mepolizumab

Peripheral blood eosinophil and basophil levels, reported as a percentage of $CD45^+$ cells, were lower in the patients with AERD who were being treated with mepolizumab than in the controls (Fig 1, *A* and *B* [$P < .0001$ and $P = .0083$, respectively]). There was no difference in the numbers of neutrophils measured as a percentage of $CD45^+$ cells (Fig 1, *C*).

The surface expression of CRTH2 on both blood eosinophils and basophils, calculated as a median fluorescence intensity, was higher in the subjects treated with mepolizumab than in the controls (Fig 1, *D* and *E* [$P = .002$ and $P = .0002$, respectively]).

Effects of mepolizumab treatment on nasal and urinary eicosanoid levels

Nasal fluid levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolites ($PGF_{2\alpha}$ + tetranor-PGFM) were significantly decreased in subjects with AERD treated with mepolizumab compared with in the controls (Fig 2, *B*). Although not statistically significant, nasal levels of the PGD_2 metabolite $DHKPGD_2$ were on average lower in subjects treated with mepolizumab; the 8 highest $DHKPGD_2$ levels were all in untreated subjects (Fig 2, *A*). Both nasal thromboxane B_2 (TXB_2) and LTB_4 levels were also decreased in the mepolizumab-treated patients (Fig 2, *C* and *D* [$P = .002$ and $P = .03$, respectively]). Urinary levels of tetranor-PGDM trended to be lower in subjects taking mepolizumab, and urinary levels of leukotriene E_4 (LTE_4) were significantly decreased (Fig 2, *E* and *F*). Further, the nasal levels of both $DHKPGD_2$ and the $PGF_{2\alpha}$ metabolites were inversely correlated with surface CRTH2 expression of both circulating eosinophils and basophils (Fig 3, *A-D*).

We did not detect differences in nasal PGE_2, nasal tetranor-PGEM, or urinary PGEM levels between subjects treated with mepolizumab and the controls (data not shown). There were no differences in nasal or urinary eicosanoid levels in the patients undergoing daily high-dose aspirin therapy versus in the patients not taking a daily aspirin in either the mepolizumab group or the control group.

Nasal eosinophilic cationic protein and serum IL-5 levels

Nasal fluid ECP levels did not differ between patients taking and not taking mepolizumab (2316 pg/mL ±729 and 3171 pg/mL ±1193, respectively [$P = .71$]; data not shown). Serum IL-5 levels were significantly higher in subjects treated with mepolizumab than in those not taking mepolizumab (34.1 pg/mL ± 33.3 and 2.3 pg/mL ±3.1, respectively [$P < .0001$]; data not shown).

Nasal and serum antibody levels and tryptase are unchanged in subjects taking mepolizumab

There was no difference in levels of IgE and IgG4 in the nasal secretions or serum of patients taking or not taking mepolizumab (Fig 4, *A-D*). Serum tryptase levels also did not differ between patients taking or not taking mepolizumab (5.6 ng/mL ± 0.6, and 6.5 ng/mL ± 0.8, respectively [$P = .26$]). We could not detect nasal tryptase levels in an adequate number of subjects in each group to compare nasal tryptase levels between groups.

Inferior turbinate RNA sequencing

We utilized a previously generated single-cell RNA sequencing data set to identify sinus tissue cells that express *IL5RA* and found the highest expression of *IL5RA* in sinus tissue plasma cells, ciliated epithelial cells, and mast cells (Fig 5, *A*). Analysis of the inferior turbinate scraping samples (14 samples from subjects treated

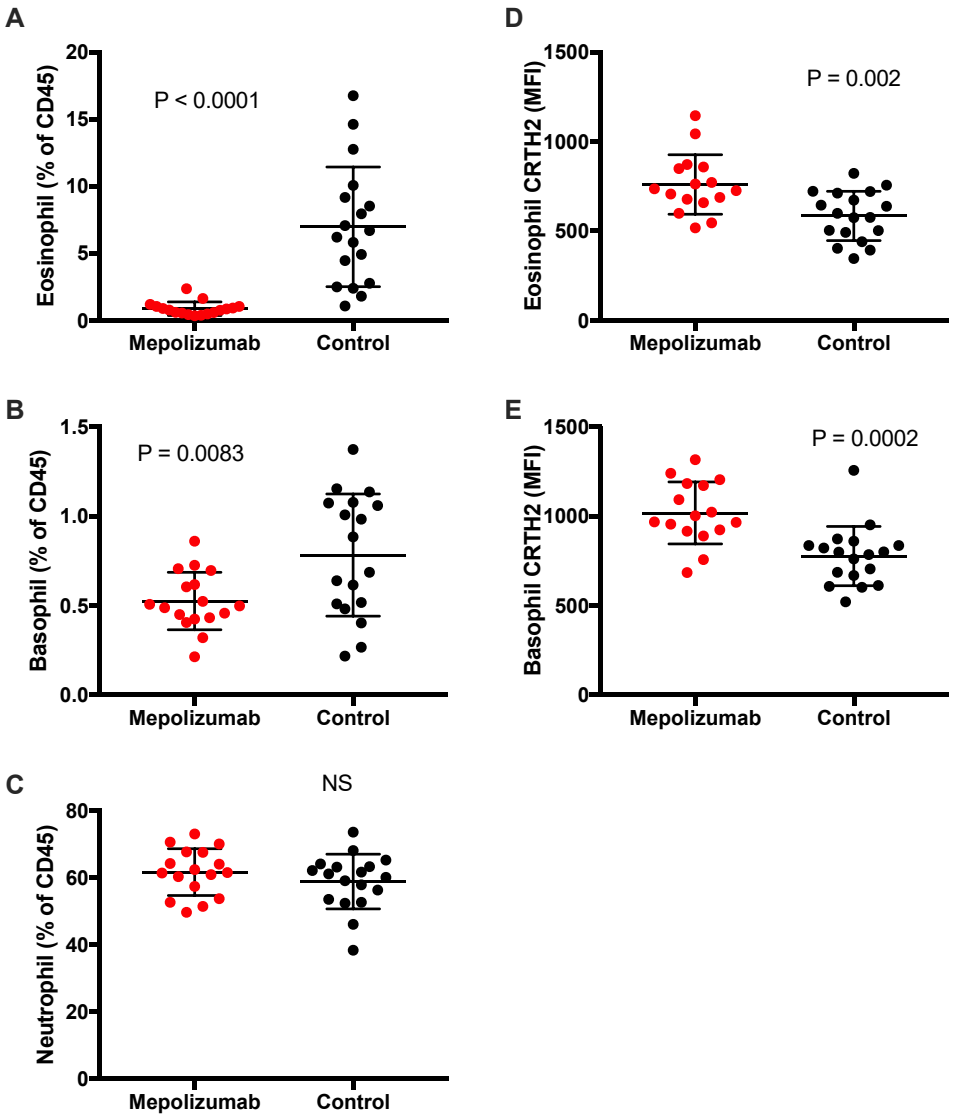

FIG 1. Peripheral blood granulocyte levels and their CRTH2 expression in subjects taking mepolizumab compared with in the controls. Circulating eosinophil **(A)**, basophil **(B),** and neutrophil (**C**) levels were measured by flow cytometry and expressed as a percentage of all $CD45^+$ cells in subjects with AERD who were being treated (with mepolizumab) or not (the controls). Surface CRTH2 expression of eosinophils **(D)** and basophils **(E)** was calculated for the same subjects and expressed as median fluorescence intensity (MFI) of CRTH2 with isotype subtracted. *NS,* Not significant.

with mepolizumab and 11 samples from patients not undergoing treatment had sufficient quality for inclusion) revealed that 242 genes were differentially regulated, including 94 upregulated genes and 148 downregulated genes in subjects treated with mepolizumab, which passed the false discovery rate with an adjusted *P* value less than .05 (see Table E2 [in the article's Online Repository at www.jacionline.org]). On the basis of prior single-cell RNA sequencing, we recovered T cells, eosinophils, mast cells, neutrophils, and myeloid cells, as well as basal, secretory, and ciliated epithelial cells from inferior turbinate scrapings. Approximately 75% of the cells recovered from the inferior turbinate scrapings were epithelial cells.[28] We enriched these differentially expressed genes in the Kyoto Encyclopedia of Genes and Genomes database and noted that the tight junction (hsa04530) pathway was enriched. GO biologic process enrichment analysis additionally revealed induction of the GO term *GO cilium organization* in the mepolizumab-treated group. Of 169 genes from the Tight Junction gene set, 19 were present in our differentially expressed genes; of these 19 genes, 15 were upregulated and 4 were downregulated. The upregulated genes included *TJP3* (tight junction protein 3),[29] *ACTN4* (actinin-4 protein, which is involved in tight junction assembly in epithelial cells),[30] and *AMOT* (angiomotin, which is part of a tight junction–associated protein complex).[31] However, several genes involved in tight junction formation, such as *CLDN17* (claudin 17),[32] were also downregulated (Fig 5, *B*).

DISCUSSION

Overall, our results comparing differences in a variety of inflammatory mediators and cellular readouts in patients with AERD show that IL-5 inhibition induces a wide array of disease-

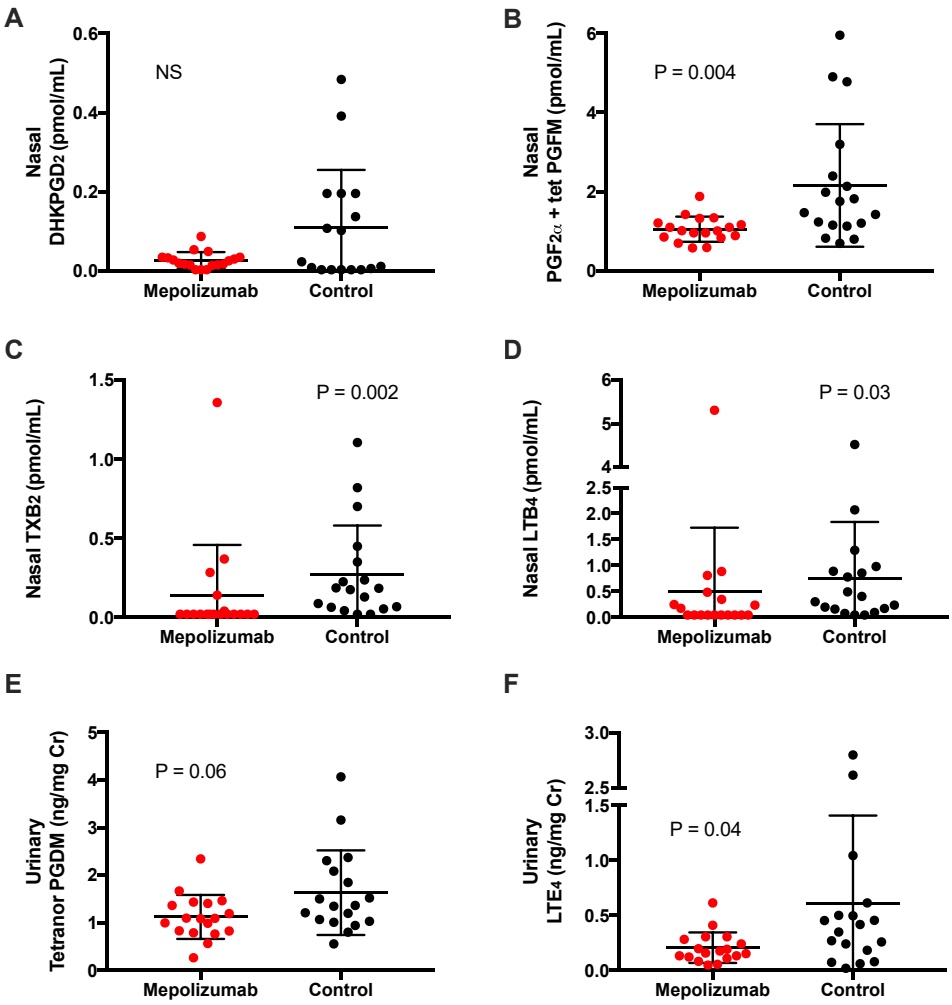

FIG 2. Nasal and urinary eicosanoid levels in subjects taking mepolizumab compared with the levels in the controls. Nasal fluid levels of $DHKPGD_2$ (**A**), $PGF_{2\alpha}$ + tetranor-PGFM (**B**), TXB_2 (**C**), and LTB_4 (**D**). Urinary levels of tetranor-PGDM (**E**) and LTE_4 (**F**). *NS*, Not significant.

relevant immunologic changes. Although the effects of IL-5 inhibition on eosinophils are important, we suspect that many of the mepolizumab-induced differences found in this study are due to the consequences of decreased IL-5 signaling on other immune cells, including mast cells, basophils, and epithelial cells within the respiratory system.

Considering the role of IL-5 on both eosinophils and basophils, the mepolizumab-induced decrease in circulating eosinophils that we found is expected and the reduction in basophils follows as well.[33] However, although eosinophilic infiltration into both the upper and lower respiratory tissues is a hallmark of AERD, complete pharmacologic depletion of tissue eosinophils does not provide therapeutic benefit. Therefore, other effector cells must be playing a key role in this disease.[5] Nasal fluid ECP correlates strongly with nasal eosinophilia and thus can serve as a surrogate biomarker for local eosinophil numbers.[34] As was shown in the phase 2 study of mepolizumab in CRSwNP,[14] we too found that the level of nasal ECP was not significantly decreased in the mepolizumab-treated patients compared with in the untreated patients. The lack of a mepolizumab-induced decrease in nasal ECP level suggests that nasal eosinophil numbers may not be dramatically altered by the treatment, although most subjects with CRSwNP who are treated with mepolizumab experience a therapeutic response and a decrease in polyp burden.[14] Given this, we suspect that the mechanisms by which mepolizumab provides therapeutic improvement for the responding subset of patients with CRSwNP is largely unrelated to a decrease in nasal polyp eosinophils.

A decrease in levels of PGD_2, LTE_4, and $PGF_{2\alpha}$ (Fig 2, *A, B, E,* and *F*) may well underlie some of the mechanism of the benefit afforded by mepolizumab. These 3 eicosanoids are all known to be proinflammatory in AERD.[6,23,35] Elevated tissue levels of PGD_2 in AERD can lead to both nasal edema through vasodilation (mediated through the DP1 receptor),[36] and activation and recruitment of eosinophils, basophils, and ILC2s (mediated through the DP2/CRTH2 receptor).[37,38] LTE_4, the end product of cysteinyl leukotriene metabolism, is a major mediator of both the chronic disease in AERD and the acute aspirin-induced reactions.[39,40] $PGF_{2\alpha}$ has, been less thoroughly studied in AERD, although its levels do rise during aspirin-induced reactions,[41] and $PGF_{2\alpha}$ can induce bronchoconstriction and bronchial hyperreactivity.[42,43] Furthermore, like PGD_2, $PGF_{2\alpha}$ is a CRTH2 agonist.[44] As both

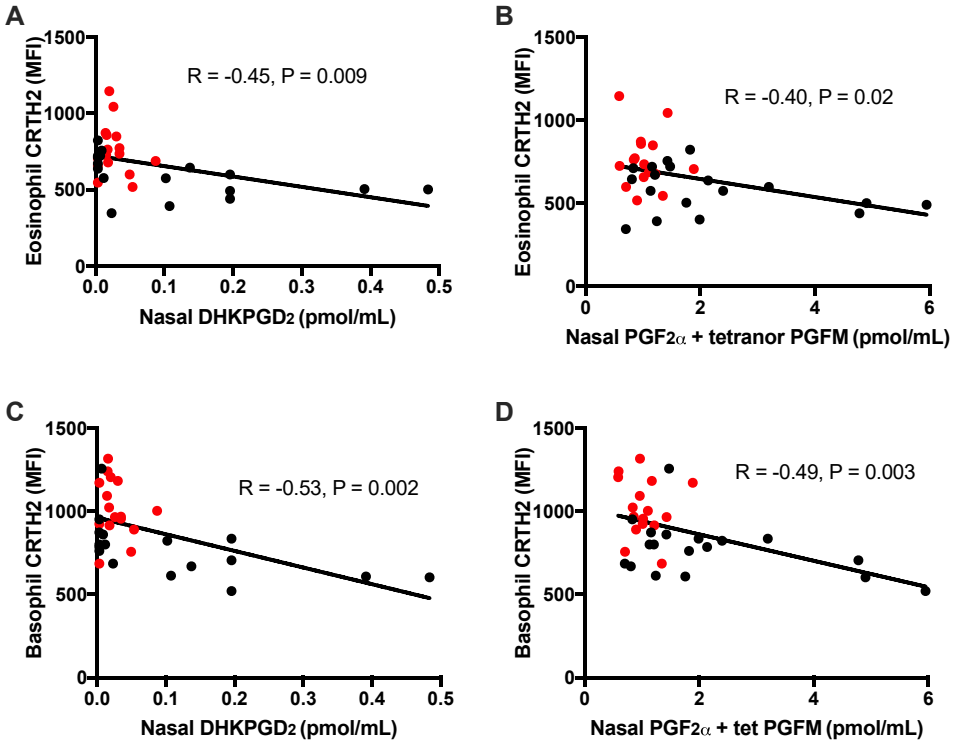

FIG 3. Relationship of nasal eicosanoids to granulocyte expression of CRTH2 in subjects taking mepolizumab and in the controls. Correlation of nasal $DHKPGD_2$ with surface CRTH2 expression of eosinophils (**A**) and basophils (**B**). Correlation of $PGF_{2\alpha}$ plus tetranor-PGFM with surface CRTH2 expression of eosinophils (**C**) and basophils (**D**). Subjects treated with mepolizumab are represented by red circles, and the controls are represented by *black circles*. *MFI*, Median fluorescence intensity.

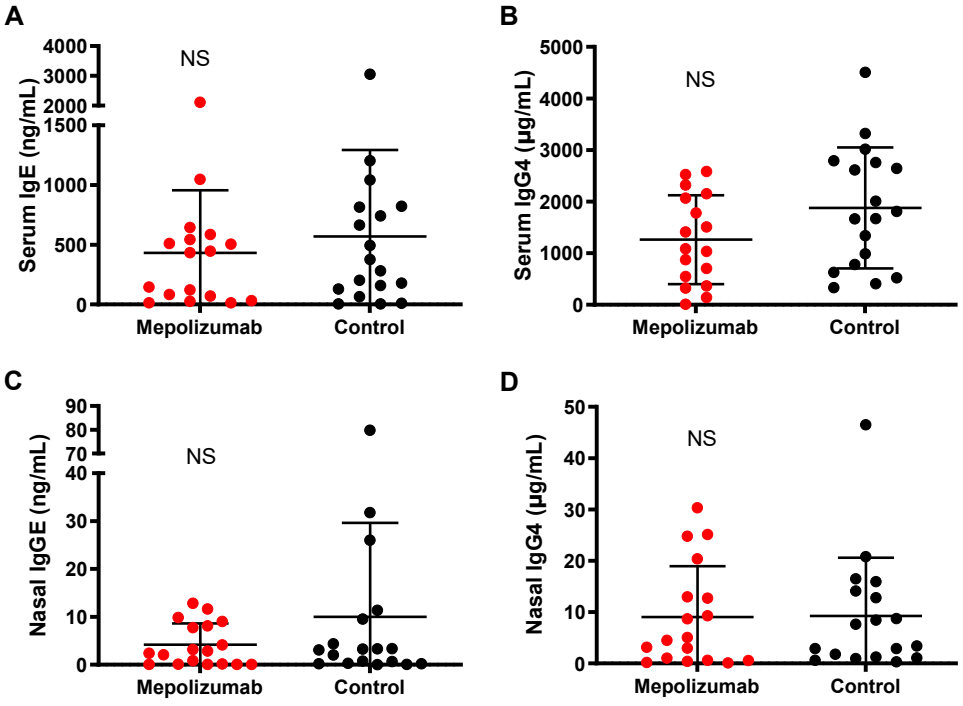

FIG 4. IgE and IgG4 levels in subjects taking mepolizumab compared with the levels in the controls. Serum (**A** and **B**) and nasal fluid (**C** and **D**) levels of IgE and IgG4 in subjects with AERD who were being treated with mepolizumab (*red circles*) or not (the control [*black circles*]). *NS*, Not significant.

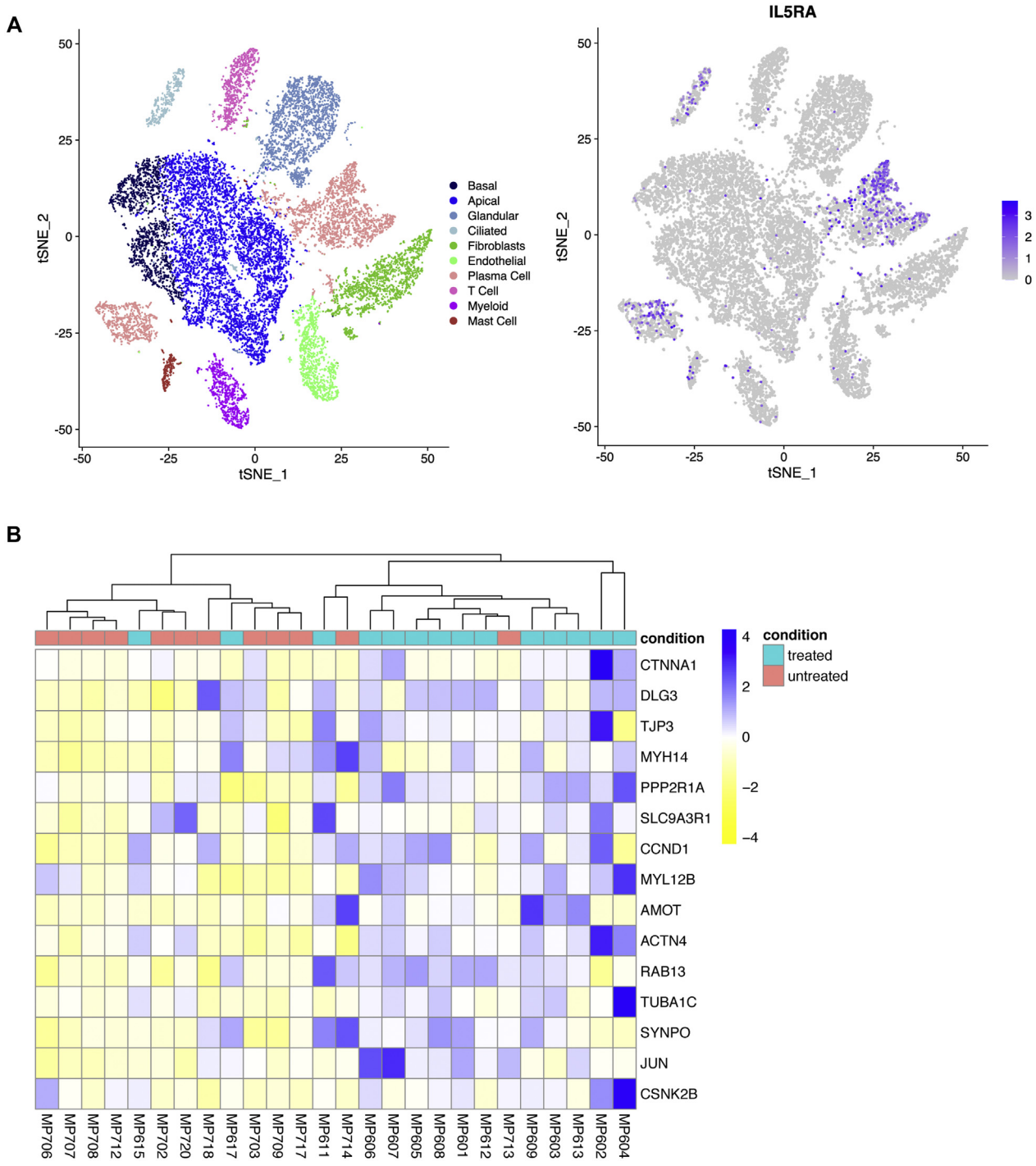

FIG 5. Sinus tissue single-cell RNA sequencing and hierarchical clustering analysis of nasal inferior turbinate scraping transcriptomic changes in mepolizumab-treated patients. **A,** T-stochastic neighbor embedding (t-SNE) plot of 18,036 surgically excised sinus tissue cells from subjects with AERD (n = 3 samples), CRSwNP (n = 3 samples), and chronic rhinosinusitis without nasal polyps (n = 5 samples) colored by cell type (*left*) and *IL5RA* expression (*right*). **B,** Unsupervised hierarchical clustering analysis of tight junction–related genes shows clustering of mepolizumab-treated subjects and untreated control subjects, with induction of tight junction–related genes in the mepolizumab-treated group (row-normalized gene expression values of
$P < .05$ for all genes).

PGD_2 and $PGF_{2\alpha}$ are full agonists of the CRTH2 receptor and can lead to activation, mobilization, and degranulation of eosinophils and basophils,[44-46] their reduction would allow for decreased activation of these granulocytes. Further corroborating this is our finding that the circulating eosinophils and basophils remaining in the blood of patients treated with mepolizumab had

significantly higher surface expression of CRTH2 (Fig 1, *D* and *E*). The likely explanation for this novel finding is that CRTH2 stimulation by either PGD_2 or $PGF_{2\alpha}$ leads to receptor internalization and reduced surface expression; following removal of the eicosanoid stimuli, an increase in CRTH2 expression would be expected.[46,47] Nasal polyp tissue mast cells also express CRTH2, and CRTH2 signaling on mast cells may lead to intracellular calcium mobilization and cellular migration, suggesting that decreased local levels of PGD_2 may also lead to less mast cell activation and accumulation.[48,49]

The mechanism by which mepolizumab reduces PGD_2, LTE_4, and $PGF_{2\alpha}$ is likely through direct inhibition of IL-5Rα signaling on eosinophils, basophils, and mast cells. PGD_2 and LTE_4 are produced by eosinophils, basophils, and mast cells.[50-54] $PGF_{2\alpha}$ is produced by eosinophils and mast cells[41,55,56] and possibly by basophils and respiratory epithelial cells as well.[57-59] For all 3 of the granulocytes, production of LTE_4 is upregulated following stimulation with IL-5,[50,53,54,60] suggesting that inhibition of IL-5 signaling with mepolizumab would decrease cellular release of LTE_4. Although IL-5 stimulation has not been directly linked to increased release of PGD_2, there is cross-talk between the stimulatory roles of PGD_2 and cysteinyl leukotrienes; as cysteinyl leukotrienes can stimulate PGD_2 production, mepolizumab-induced decreases in LTE_4 may in turn reduce granulocyte production of PGD_2.[41,61] IL-5 stimulation of human bronchial epithelial cells also leads to upregulation of the enzymes required to make $PGF_{2\alpha}$, suggesting that mepolizumab-induced inhibition of that pathway could decrease $PGF_{2\alpha}$ release from the respiratory epithelium.[19]

The levels of 2 additional eicosanoids, TXB_2 and LTB_4, were also lower in the nasal fluid of the patients treated with mepolizumab than in the nasal fluid of those not undergoing mepolizumab treatment, indicating a broad effect of IL-5 inhibition on eicosanoid metabolism (Fig 2, *C* and *D*). A decrease in local TXB_2 levels may be of particular therapeutic importance in AERD, as platelet activation and platelet-dependent inflammation play a role in the chronic respiratory inflammation and the acute aspirin-induced reactions.[62,63] Although the direct effect of IL-5 on TXB_2 production by immune cells is not known, both sinus tissue mast cells and eosinophils have the capacity to produce it.[64] The inflammatory role of LTB_4 in respiratory inflammation and asthma has also been well documented,[65] with high levels of LTB_4 also noted within nasal polyp tissue.[66] Neutrophils are a primary source of LTB_4, and although human lung neutrophils do have functional IL-5Rα,[67] whether IL-5 stimulation of neutrophils affects their LTB_4 release is not known. Both eosinophils and basophils can also produce LTB_4,[68] and IL-5 priming of rat basophilic leukemia-1 cells does increase their production of LTB_4,[69] suggesting a mechanism by which IL-5 inhibition could lead to decreased local LTB_4 levels in the sinuses.

Our finding of variable mepolizumab-induced differential expression of tight junction–related transcripts in the inferior turbinate scrapings is of unclear clinical significance. A number of the transcripts found to be upregulated in patients taking mepolizumab, including *ACTN4* and *AMOT*, were also noted to be downregulated in human bronchial epithelial cells stimulated *ex vivo* with IL-5.[19] Therefore, the epithelial cell transcript differences noted in our study could indeed be a result of *in vivo* inhibition of IL-5. The variability of the differences between subjects, and the finding that some tight junction–associated transcripts were actually downregulated in subjects taking mepolizumab, suggests that these changes are unlikely to be the sole driving mechanism underlying the therapeutic benefit of mepolizumab that is experienced by patients with CRSwNP. Additionally, we see that ciliated epithelial cells express IL-5Rα (Fig 5, *A*) and there is enrichment of genes related to cilium organization in the mepolizumab-treated group, suggesting that inhibition of IL-5 may also affect ciliated epithelial cells in the nasal tissue.

The treatment-induced increase in serum IL-5 found in this study is consistent with the known effects of anti–IL-5 treatment.[70] The serum IL-5 detected during treatment with mepolizumab may be part of a bound immunoglobulin complex that prolongs the half-life of IL-5, leading to detection of increased levels.[71] One major limitation of this study is that the case-control design captured clinical and mechanistic data from only a single visit, without any longitudinal data available to gauge each patient's response to mepolizumab. Therefore, the treatment-related immunologic differences seen in this study are presumed to be directly mediated by mepolizumab, but without repeat measures, this cannot be fully confirmed. Additionally, we are not able to determine the extent of clinical response to mepolizumab or relate any of the mepolizumab-related immunologic differences to a responder/nonresponder analysis. Despite these shortcomings, our findings clearly show that there are immunologic changes that occur following treatment with mepolizumab and extend beyond just the predicted effects of IL-5 inhibition on eosinophils. We conclude that IL-5 inhibition with mepolizumab in patients with AERD leads to decreased production of relevant inflammatory eicosanoids, including PGD_2, $PGF_{2\alpha}$, and cysteinyl leukotrienes, as well as to upregulation of nasal epithelial cell transcripts involved in tight junction pathways and cilium organization. These changes are likely due to the combined effects of decreased IL-5 signaling on local respiratory tissue eosinophils, basophils, mast cells, and epithelial cells, all of which have functional IL-5Rα.

Key messages

- **Compared with patients with AERD not treated with mepolizumab, subjects with AERD treated with mepolizumab had decreased production of inflammatory eicosanoids and upregulation of nasal epithelial cell transcripts involved in tight junction pathways and cilium organization.**
- **These effects of mepolizumab are likely due to decreased signaling of IL-5 on local respiratory tissue eosinophils, basophils, mast cells, and epithelial cells.**
- **The mechanism by which IL-5 inhibition provides therapeutic benefit in respiratory inflammation is not due exclusively to antieosinophil effects.**

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