Rapid and sustained effect of dupilumab on clinical and mechanistic outcomes in aspirin-exacerbated respiratory disease

Kathleen M. Buchheit, MD,[a,b] Aaqib Sohail, PhD,[a,b] Jonathan Hacker, BA,[b] Rie Maurer, MA,[c] Deborah Gakpo, BA,[b] Jillian C. Bensko, PA-C,[b] Faith Taliaferro, BS,[d,f,h] Jose Ordovas-Montanes, PhD,[d,e,f,g,h] and Tanya M. Laidlaw, MD[a,b]

Boston and Cambridge, Mass

Background: Dupilumab, a mAb targeting IL-4Rα, improves upper and lower airway symptoms in patients with aspirin-exacerbated respiratory disease (AERD), but the mechanisms leading to clinical improvement are not fully elucidated.
Objective: Our aim was to identify the mechanistic basis of clinical improvement in patients with AERD treated with dupilumab.
Methods: A total of 22 patients with AERD were treated with dupilumab for 3 months for severe asthma and/or chronic rhinosinusitis with nasal polyps. Clinical outcomes were assessed at baseline and at 1 and 3 months after initiation of dupilumab. Nasal fluid, urine, blood, and inferior turbinate scrapings were collected at the 3 time points for determination of mediator levels, cellular assays, and RNA sequencing.
Results: Participants had rapid improvement in clinical measures, including sense of smell, sinonasal symptoms, and lung function after 1 month of treatment with dupilumab; the improvements were sustained after 3 months of dupilumab. Baseline severity of smell loss was correlated with lower nasal prostaglandin E_2 levels. Dupilumab increased nasal prostaglandin E_2 level and decreased levels of nasal albumin, nasal and urinary leukotriene E_4, and serum and nasal IgE. Transcripts related to epithelial dysfunction and leukocyte activation and migration were downregulated in inferior turbinate tissue after treatment with dupilumab. There were no dupilumab-induced changes in nasal eosinophilia.
Conclusion: Inhibition of IL-4Rα in AERD led to rapid improvement in respiratory symptoms and smell, with a concomitant improvement in epithelial barrier function, a decrease in inflammatory eicosanoid levels, and an increase in the anti-inflammatory eicosanoid prostaglandin E_2 level. The therapeutic effects of dupilumab are likely due to decreased IL-4Rα signaling on respiratory tissue granulocytes, epithelial cells, and B cells. (J Allergy Clin Immunol 2022;■■■:■■■-■■■.)

Key words: *Aspirin-exacerbated respiratory disease, AERD, IL-4Rα, IL-4, IL-13, nasal polyp, dupilumab, anosmia, leukotriene E_4, prostaglandin E_2*

The advent of targeted respiratory biologic therapies for the treatment of asthma and chronic rhinosinusitis with nasal polyps (CRSwNP) has yielded significant improvement in control of these diseases and in patient quality of life.[1] In aspirin-exacerbated respiratory disease (AERD), the triad of asthma, CRSwNP, and respiratory reactions to COX-1 inhibitors, the upper and lower airway inflammation is often difficult to treat, with many patients failing first-line therapies.[2,3]

Dupilumab, a fully human mAb targeting IL-4Rα, inhibits signaling of both IL-4 and IL-13. It is approved for both moderate-to-severe eosinophilic asthma and inadequately controlled CRSwNP,[4-7] as well as for atopic dermatitis, and it is also under investigation for other type 2 inflammatory diseases.[8,9] Prior studies have shown that within 16 weeks of treatment, dupilumab leads to robust improvements in upper and lower respiratory tract symptoms in patients with AERD, including increased sense of smell, reduction in nasal polyp size, and improvements in lung function.[10,11] In our experience, we have noted an even more rapid onset of clinical improvement with dupilumab, often within the first month of initiating treatment.

Inhibiting the type 2 cytokines IL-4 and IL-13 has broad impacts on chronic inflammation, including IgE class switching, T_H2 lymphocyte differentiation, mast cell activation, mucus secretion, and airway smooth muscle proliferation.[12-14] Indeed, IL-4Rα is expressed on multiple cell types that are potentially relevant to AERD, including mast cells, eosinophils, B cells, and epithelial cells, and it is not yet known which of these cells are causative effectors of the chronic disease in AERD or which of them are most affected by dupilumab and underlie the mechanism of benefit of IL-4Rα inhibition.[15] Dupilumab is known to

From [a]the Department of Medicine and [e]the Program in Immunology, Harvard Medical School, Boston; [b]the Division of Allergy and Clinical Immunology and [c]the Center for Clinical Investigation, Brigham and Women's Hospital, Boston; [d]the Division of Gastroenterology, Hepatology, and Nutrition, Boston Children's Hospital; [f]the Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge; [g]the Harvard Stem Cell Institute, Cambridge; and [h]the Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard, 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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Abbreviations used
AERD: Aspirin-exacerbated respiratory disease
CRSwNP: Chronic rhinosinusitis with nasal polyps
CRTH2: Chemoattractant receptor-homologous molecule expressed on T_H2 cells
CysLT: Cysteinyl leukotriene
ECP: Eosinophilic cationic protein
FVC: Forced vital capacity
5-LO: 5-Lipoxygenase
LTE_4: Leukotriene E_4
PGD_2: Prostaglandin D_2
PGE_2: Prostaglandin E_2
RNA-seq: RNA sequencing
UPSIT: University of Pennsylvania Smell Identification Test

induce a decrease in both serum IgE[6] and urinary leukotriene E_4 (LTE_4) levels,[16] but medications that target only IgE (omalizumab) or only leukotriene production (zileuton) may be less efficacious than dupilumab in many patients with AERD, suggesting that there are additional mechanisms by which IL-4Rα inhibition affords therapeutic benefit.

Elucidating which dupilumab-induced cellular changes lead to the drug's dramatic clinical benefit in AERD will allow for increased understanding of the underlying pathobiology of the disease and may help teach us which effector cell(s) cause the chronic inflammation in AERD. With this study we sought to determine the impact of IL-4Rα blockade in AERD, with parallel examination of early treatment time points (1 month and 3 months) for both clinical and mechanistic outcomes.

METHODS

Patient characterization

Participants between the ages of 18 and 75 years were recruited from the Brigham and Women's Hospital (Boston, Mass) Allergy and Immunology Clinics from 2018 to 2020 (Table I). The Mass General Brigham Institutional Review Board approved the study, and all participants provided written informed consent. The patients with AERD all had asthma, CRSwNP, and a diagnosis of AERD previously confirmed via a physician-observed oral challenge to aspirin, which induced objectively defined upper and/or lower respiratory symptoms, including nasal congestion, rhinorrhea, sneezing, wheezing, dyspnea, and/or fall in FEV_1.

Patients with AERD who met criteria for the US Food and Drug Administration–approved use of every-other-week dupilumab (300 mg subcutaneously) for the treatment of moderate-to-severe eosinophilic asthma or CRSwNP were prescribed dupilumab by their pulmonologist or allergist/immunologist as part of their usual clinical care. Patients were recruited before initiation of their first dose of dupilumab. Participants were followed for this observational study at 3 visits: a baseline visit just before their first dose of dupilumab (baseline), after 1 month (2 doses) of taking dupilumab (month 1), and again after 3 months of taking dupilumab (month 3). Participants were excluded if they had received any other biologic therapy within 6 months of enrollment.

Clinical procedures and specimen procurement

At each of the 3 study visits, clinical parameters were collected, including sense of smell (using the University of Pennsylvania Smell Identification Test [UPSIT]), peak nasal inspiratory flow, nasal polyp size, as assessed with an otoscope (a unilateral polyp score of 0 if not visible, 1 if visible above the middle turbinate, and 2 if visible below the middle turbinate, for a total maximum bilateral score of 4), spirometry (FEV_1 and forced vital capacity [FVC]), and patient-reported outcome measures (including 22-Item Sinonasal Outcome Test and Asthma Control Questionnaire-6 scores). Biologic specimens, including urine, blood, nasal fluid, and nasal cells from the inferior turbinate, were collected at the same time points. All measurements for a single participant were done together in the same batch. For full details, see the Supplemental Methods section in this article's Online Repository (at www.jacionline.org).

Flow cytometry

Flow cytometry measurement of peripheral blood eosinophils, basophils, neutrophils, and surface chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) expression was performed as previously described.[17] The presence of platelet-adherent leukocytes was assessed as previously described.[18] For full details, see the Supplementary Methods section and Table E1 in this article's Online Repository (at www.jacionline.org).

Mediator quantification

Nasal fluid and serum were measured for levels of total IgG (Invitrogen, Waltham, Mass), IgA (Invitrogen), IgE (Invitrogen for serum level and Abcam, Waltham, Mass, for nasal secretions) and IgG4 (eBioscience, San Diego, Calif) by ELISA, and for total tryptase level (at Virginia Commonwealth University). Nasal secretions were further analyzed for albumin (Abcam) and eosinophilic cationic protein (ECP) by ELISA (Lifespan Biosciences, Seattle, Wash). Urinary eicosanoids (Vanderbilt University, Nashville, Tenn) and nasal eicosanoids (University of California San Diego) were measured by mass spectrometry.[19]

Inferior nasal turbinate epithelial cell bulk RNA-seq

For bulk RNA sequencing (RNA-seq), cells were scraped from the inferior turbinate and immediately preserved in RNAprotect (Qiagen, Germantown, Md). Sequencing libraries were generated by using a modified low-input bulk RNA-seq pipeline, as described.[17] Differential expression analysis was conducted with DESeq2.[20] The WebGestalt online tool was used to identify the distribution of preranked DEGs involved in specific signaling pathways in the Gene Ontology Biological Processes database. The R package ggplot2 was used to plot pathways with a false discovery rate less than 0.01 in either of the comparisons. For full details, see the Methods section in this article's Online Repository.

Statistical analysis

Mixed effect analysis was used to assess change over time for numeric clinical outcomes. The model included a fixed categoric effect of visit as month and random intercept. An unstructured covariance structure was used to model the within-participant errors. *P* values were reported for overall time effect, month 1 compared with baseline, and month 3 compared with baseline. The *P* values for 2 pairwise comparisons (month 1 compared with baseline and month 3 compared with baseline) were Tukey-Kramer–adjusted. Categorical clinical outcomes were analyzed by using the generalized estimating equations model. Wilcoxon signed rank tests were used for biomarker analysis. A reduced *P* value of 2.5% was used to test significance level for biomarker analyses because of analysis of multiple biomarkers. Correlation between biomarkers was assessed by using the Pearson and/or Spearman correlation coefficient. Analysis was performed by using SAS, version 9.4 (SAS Institute, Cary, NC), and figures were prepared in GraphPad Prism, version 9.2.0 (GraphPad, La Jolla, Calif).

RESULTS

Study population and demographics

A total of 22 patients with physician-diagnosed AERD who initiated treatment with dupilumab as an add-on asthma or

TABLE I. Patient characteristics (N = 22)

Characteristic	Value
Age (y), mean ± SD	52.6 ± 13.4
Female sex, no. (%)	12 (54.5%)
White race, no. (%)	19 (86.4%)
Lifetime endoscopic sinus surgeries, mean ± SD	2.4 ± 1.1
Prior use of a respiratory biologic, no. (%)*	3 (13.6%)
Current use of daily aspirin therapy at study entry, no. (%)†	8 (36.3%)
Current use of montelukast, no. (%)	11 (50%)
FEV_1 % predicted, mean ± SD	75.7 ± 19.6
Baseline SNOT-22, mean ± SD	48.7 ± 22.3
Baseline ACQ-6, mean ± SD	1.6 ± 1.3
Baseline UPSIT score, mean ± SD	15.5 ± 9.6

ACQ-6, Asthma Control Questionnaire-6; *SNOT-22*, 22-Item Sino-Nasal Outcome Test.
*Participants previously treated with a respiratory biologic had a minimum of a 6-month washout period.
†Aspirin therapy was administered in a dose of either 650 mg daily or 1300 mg daily.

CRSwNP therapy participated in this observational study (Table I). No patients were taking systemic corticosteroids, 11 of 22 patients were taking montelukast, and 15 of 22 patients used nasal budesonide irrigations, with the remaining patients using over-the-counter intranasal steroid sprays for at least 1 month before entering the study. Of the 22 participants, 8 were taking daily aspirin therapy after desensitization for 6 months or more before starting dupilumab, but they did not achieve sufficient control of asthma or CRSwNP with aspirin therapy and thus elected to pursue treatment with dupilumab. There were no baseline differences in number of endoscopic sinus surgeries, patient-reported rate of polyp regrowth, or FEV_1 % predicted between participants who were taking high-dose daily aspirin and those who were not. All participants had previously undergone endoscopic sinus surgery. All baseline medications were kept consistent through the study period.

Dupilumab-induced changes in clinical outcomes

UPSIT score was significantly improved after 1 and 3 months of dupilumab treatment (Fig 1, *A*; mean change of 11.3 and 11.9, respectively; $P < .0001$ at both time points). On the basis of their UPSIT scores, 16 of 22 participants were classified as anosmic (UPSIT score <19) at baseline, but after 1 month of treatment only 4 participants were still anosmic (Fig 1, *B*; odds ratio = 0.12; 95% CI = 0.04-0.36; $P = .0002$), and this effect was sustained after 3 months of dupilumab treatment (odds ratio = 0.08; 95% CI = 0.02-0.28; $P < .0001$). Improvement in 22-Item Sino-Nasal Outcome Test score beyond the minimal clinically important difference of 8.9 points[21] was observed after 1 month of dupilumab therapy (mean change –34.4; $P < .0001$) and was sustained at the 3-month time point (mean change –34.5; $P < .0001$) (Fig 1, *C*). The peak nasal inspiratory flow was increased after 1 and 3 months of dupilumab (Fig 1, *D*; mean change 31.4 mL ($P = .0023$) and 36.5 mL ($P = .0007$), respectively). Nasal polyp scores also improved with dupilumab, with significant reduction in nasal polyp burden identified after just 1 month of treatment (Fig 1, *E*; $P < .0001$).

Lower airway assessments also showed improvements in lung function and asthma control. The FEV_1% predicted values improved by a mean of 12.6% ($P = .0002$) and 12.1% ($P = .0015$) at months 1 and 3, respectively (Fig 2, *B*), and there were also significant improvements in the total liters of FEV_1 and FVC values at both visits (Fig 2, *A* and *C*). Patient-reported asthma control, assessed by Asthma Control Questionnaire-6, improved significantly after 1 month of dupilumab treatment (mean change –1.3; $P < .0001$), which was sustained after 3 months of treatment (Fig 2, *D*).

Effects of dupilumab on nasal and urinary eicosanoid levels

Urinary levels of LTE_4 decreased significantly after 1 and 3 months of dupilumab treatment (Fig 3, *A*; $P < .0001$ and $P = .0095$, respectively), and nasal LTE_4 level decreased by more than 7-fold from baseline to month 1 ($P = .0256$) and even further at month 3 ($P = .0020$) (Fig 3, *D*). There were no significant changes in urinary levels of the prostaglandin D_2 (PGD_2) metabolite tetranor PGD-M, although there was a trend toward a decrease at month 1 (Fig 3, *B*), and there were no dupilumab-induced changes in nasal levels of the PGD_2 metabolite $DHKPGD_2$ (Fig 3, *E*). Urinary PGE-M levels did not change with dupilumab (Fig 3, *C*). However, there was a significant dupilumab-induced increase in level of nasal fluid prostaglandin E_2 (PGE_2), with a nearly 2-fold increase after 1 and 3 months of treatment (Fig 3, *F*; $P = .0103$ and .0063, respectively). There was no significant difference in baseline levels of urinary LTE_4, urinary PGE-M, nasal $DHKPGD_2$, or nasal PGE_2 in the participants taking or not taking high-dose daily aspirin (data not shown). However, patients taking high-dose aspirin at the start of the study had lower baseline urinary PGD-M levels than did those patients not taking high-dose aspirin (mean 1.1 ng/mL vs 3.1 ng/mL; $P < .01$).

We then assessed for a relationship between PGE_2 and LTE_4 levels and sense of smell and identified an association between baseline nasal PGE_2 level and UPSIT score, but not between LTE_4 level and UPSIT score. At baseline, 6 of 22 participants had an UPSIT score of 19 or higher and were classified as normosmic or hyposmic, whereas 16 of 22 participants had an UPSIT score less than 19 and were classified as anosmic. The anosmic participants had significantly lower nasal PGE_2 levels at baseline than did the hyposmic and normosmic participants, with a mean nasal PGE_2 level of 1.60 ng/mL versus 5.11 ng/mL ($P = .0168$) (Fig 4, *A*). Further, we observed a positive correlation between baseline UPSIT score and nasal PGE_2 levels ($r = 0.503$; $P = .0171$) and a trend toward a positive correlation between the dupilumab-induced change in nasal PGE_2 level between baseline and month 1, and the corresponding change in UPSIT score (Fig 4, *B* and *C*).

Effects of dupilumab on nasal albumin, ECP, and nasal and serum tryptase levels

Nasal albumin levels fell significantly after 1 month of dupilumab treatment (Fig 5, *A*; $P = .0149$), which was sustained at the 3-month time point ($P = .0160$). There was a slight trend toward decreased nasal ECP levels after 1 and 3 months of dupilumab treatment (Fig 5, *B*), and there was no significant dupilumab-induced change in serum or nasal tryptase level (data not shown).

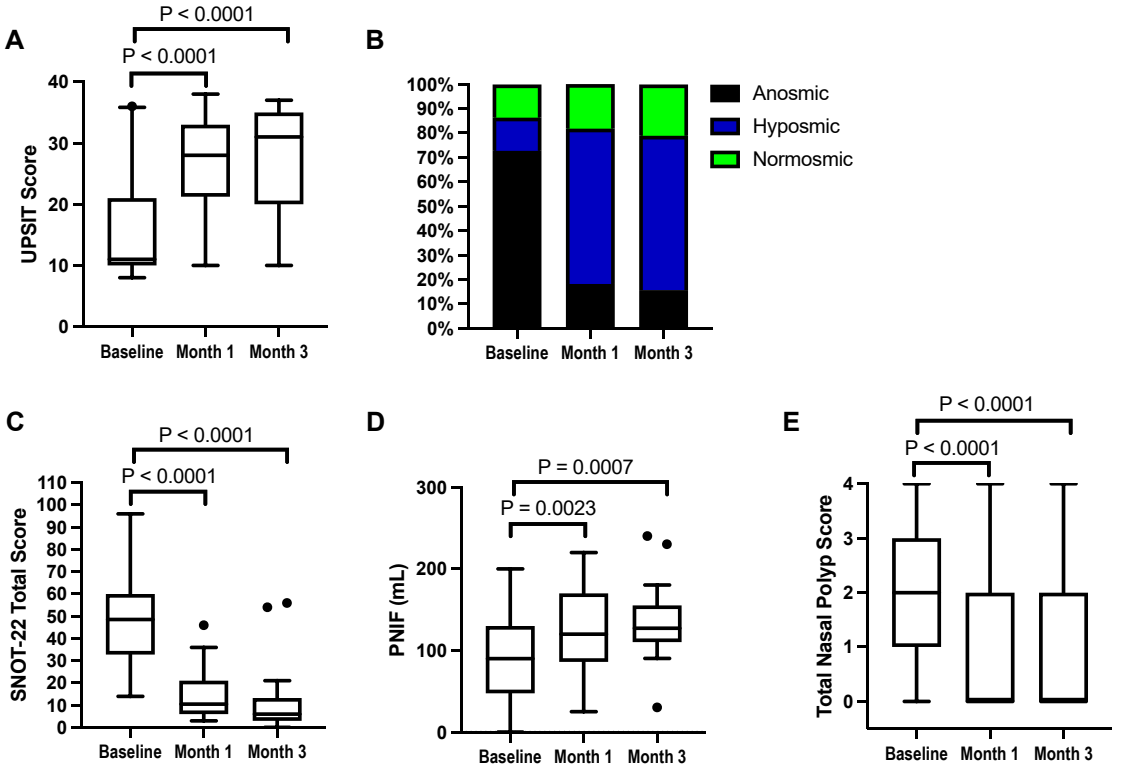

FIG 1. Dupilumab-induced changes in clinical upper respiratory outcomes. Smell identification level as measured by UPSIT is shown as raw scores (**A**) or as summarized levels of anosmia, hyposmia, and normosmia (**B**) at the pre-dupilumab baseline, and after 1 and 3 months of treatment with dupilumab. Patients' 22-Item Sino-Nasal Outcome Test (SNOT-22) scores (**C**), peak nasal inspiratory flow (PNIF) (**D**), and otoscopically scored bilateral nasal polyp scores (**E**) are shown for the same time points. Data in (**A**) and (**C-E**) are shown as Tukey box-and-whisker plots (N = 22 participants).

Effects of dupilumab on nasal and serum antibody levels

Both serum and nasal IgE levels decreased after 1 month of dupilumab treatment ($P < .0001$ and $P = .0003$, respectively), with the decreases sustained after 3 months (Fig 6, *A* and *B*; $P < .0001$). There were no significant changes in nasal IgA, total nasal IgG, or nasal or serum IgG4 levels measured at these time points (data not shown).

Blood granulocyte levels, their CRTH2 expression, and the levels of leukocyte-platelet aggregates are unchanged by dupilumab

Dupilumab did not significantly change the peripheral blood levels of eosinophils, basophils, or neutrophils measured as a percentage of total $CD45^+$ cells (see Fig E1, *A-C* in the Online Repository at www.jacionline.org), and it did not change the percentages of any platelet-adherent leukocyte subsets (see Fig E1, *D-G*). Dupilumab did not significantly change the surface expression of CRTH2 on either blood eosinophils or basophils, calculated as a median fluorescence intensity (see Fig E2 in the Online Repository at www.jacionline.org).

Inferior turbinate RNA-seq

Analysis of the inferior turbinate scraping RNA-seq samples revealed that after 1 month of dupilumab treatment there were 32 upregulated and 25 downregulated transcripts, and after 3 months there were 34 upregulated and 86 downregulated transcripts that passed the false discovery rate with an adjusted *P* value less than .05 (Fig 7, *A* and *B* and see Table E2 in the Online Repository at www.jacionline.org) when long noncoding RNA was included. Two transcripts related to mucus overproduction and epithelial dysfunction (*MUC5B*[22-24] and *PTHLH*[25]) were significantly downregulated after the first month of dupilumab treatment. On the basis of prior single-cell RNA-seq, we recover mostly basal, secretory, and ciliated epithelial cells, but we also capture some lymphocytes, granulocytes, and myeloid cells from inferior turbinate scrapings.[15] Gene Ontogeny analyses of the data suggest downregulation of 24 pathways and upregulation of 4 pathways after 1 or 3 months of treatment with dupilumab (Fig 7, *C*). Among other pathways, there was dupilumab-induced downregulation of Gene Ontogeny pathways involved in leukocyte activation and migration, differentiation, and cell-cell adhesion (see Table E3 in the Online Repository at www.jacionline.org). Notably, there were no differences in epithelial transcripts known to be upregulated or downregulated by topical corticosteroid use[26] in patients who used intranasal budesonide irrigations versus fluticasone nasal spray.

DISCUSSION

Consistent with previous studies,[10,16] our open-label observational trial of dupilumab in 22 adult patients with AERD showed

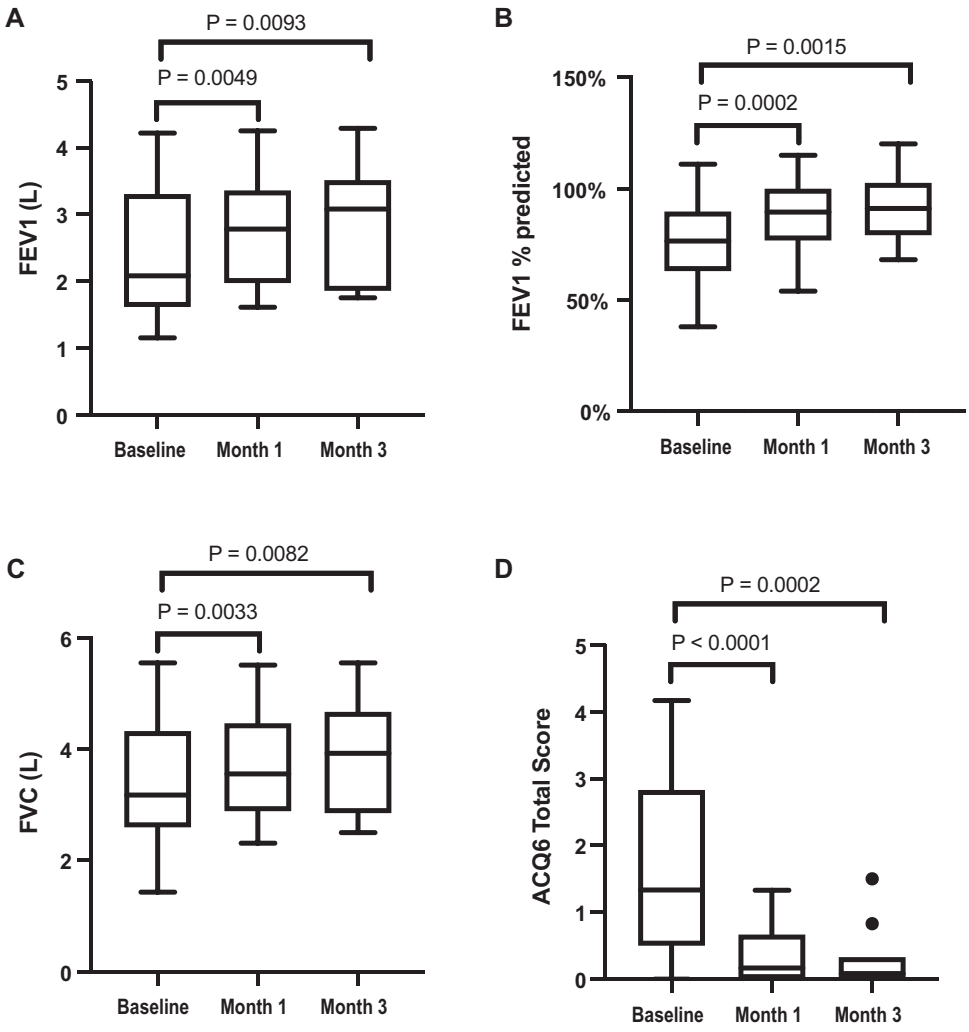

FIG 2. Dupilumab-induced changes in pulmonary outcomes. Pulmonary function and asthma control as measured by liters of FEV_1 (**A**), FEV1 % predicted (**B**), liters of FVC (**C**), and Asthma Control Questionnaire-6 (ACQ-6) score (**D**) are shown at the pre-dupilumab baseline and after 1 and 3 months of treatment with dupilumab. Data are shown as Tukey box-and-whisker plots (N = 22 participants).

that inhibition of IL-4Rα led to significant improvement in sense of smell, nasal congestion, nasal polyp size, asthma symptoms, and lung function. Our nasal polyp scoring system was limited in that it was done by otoscopic examination rather than by nasal endoscopy, but we identified significant changes in polyp size nonetheless. We saw near-universal improvement in our well phenotyped AERD cohort, whereas studies of severe asthma and CRSwNP show more variability in response, likely reflecting the heterogeneity of those patient populations and non–type 2 mechanisms of disease. Ours is the first study to show that the majority of the clinical benefit afforded by dupilumab in AERD, including the striking olfactory improvement, actually occurs within the first month of treatment (Figs 1 and 2) and is sustained at 3 months, suggesting that 3 months is an adequate time for trial of dupilumab in patients with AERD. Given the widespread expression of IL-4Rα on multiple potentially relevant cell types, we suspect that the rapid clinical improvement noted in this study is likely due to several concomitant mechanistic changes, including a reduction in cysteinyl leukotriene levels (cysLTs), an increase in local nasal PGE_2 levels, an improvement in airway epithelial barrier integrity, and transcriptional changes in epithelial cell dysfunction and leukocyte differentiation and proliferation pathways within the sinus tissue.

Changes in eicosanoids, specifically, the increases in nasal PGE_2 level and decreases in LTE_4 level (Fig 3) may underlie a major portion of the clinical benefit seen with dupilumab treatment in AERD. PGE_2, which is generated dominantly by macrophages, epithelial cells, and other stromal cells through the COX-2 pathway,[27] with some contribution from COX-1, plays a critical protective role in AERD and is considered to function largely as an anti-inflammatory mediator in the airway. COX-2 mRNA expression is diminished in nasal polyp epithelial cells from patients with AERD, and subsequent PGE_2 production is also reduced.[28] Of note, high levels of IL-4 within the tissues may be a direct cause of the reduction in COX-2 and PGE_2 levels in AERD, as IL-4 can inhibit PGE_2 production through selective inhibition of COX-2 mRNA,[29,30] although the specific enzymatic pathways that were affected by dupilumab and led to the increase in PGE_2 level are not yet clear. Deficient levels of PGE_2 likely have a major role in AERD disease pathogenesis, as PGE_2

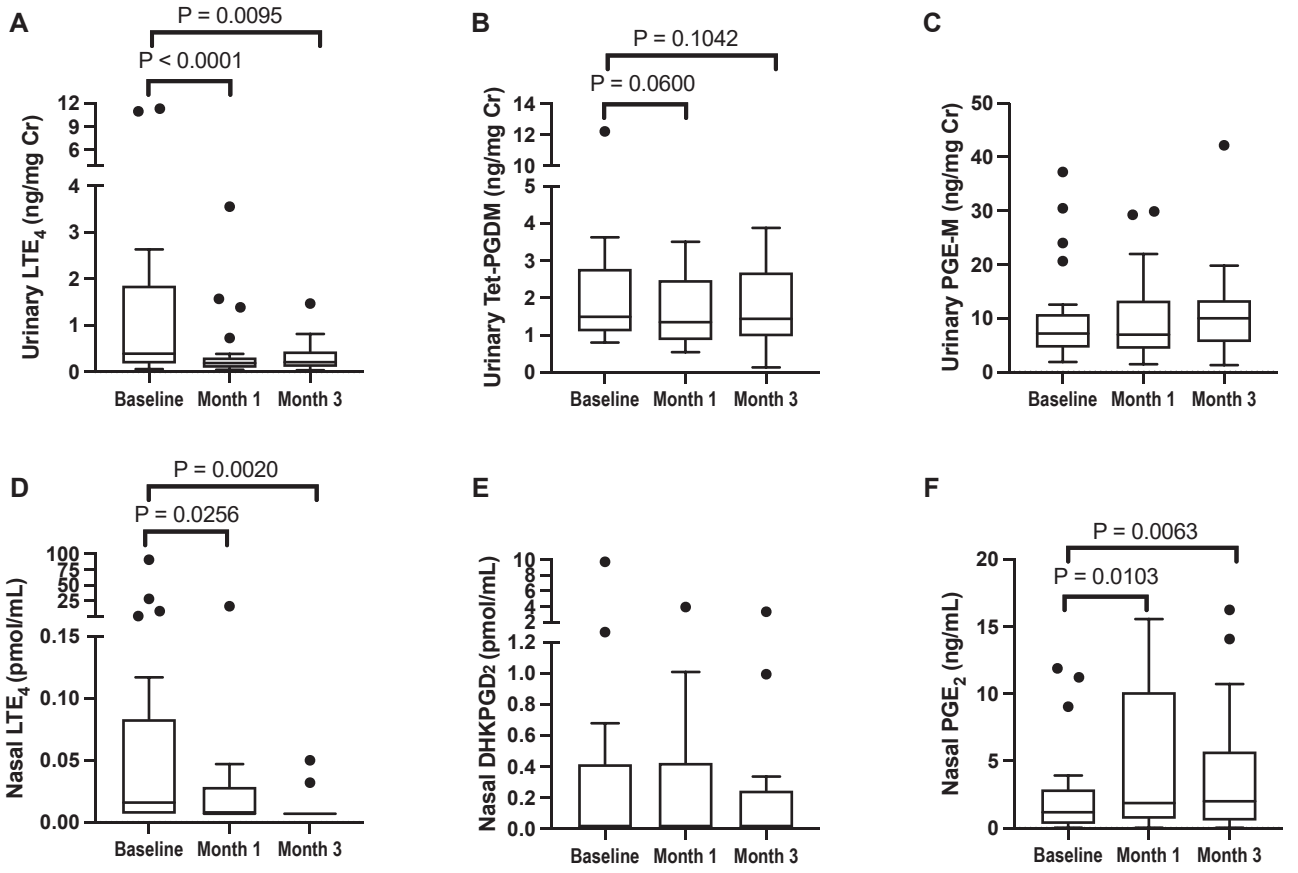

FIG 3. Dupilumab-induced changes in nasal and urinary eicosanoids. Urinary levels of LTE_4, tetranor prostaglandin D-M (PGD-M), and prostaglandin E-M (PGE-M) (**A-C**) and nasal fluid levels of LTE_4, $DHKPGD_2$, and PGE_2 (**D-E**) are shown at the pre-dupilumab baseline and after 1 and 3 months of treatment with dupilumab. Data are shown as Tukey box-and-whisker plots. Analysis with Wilcoxon signed rank test (N = 22 participants).

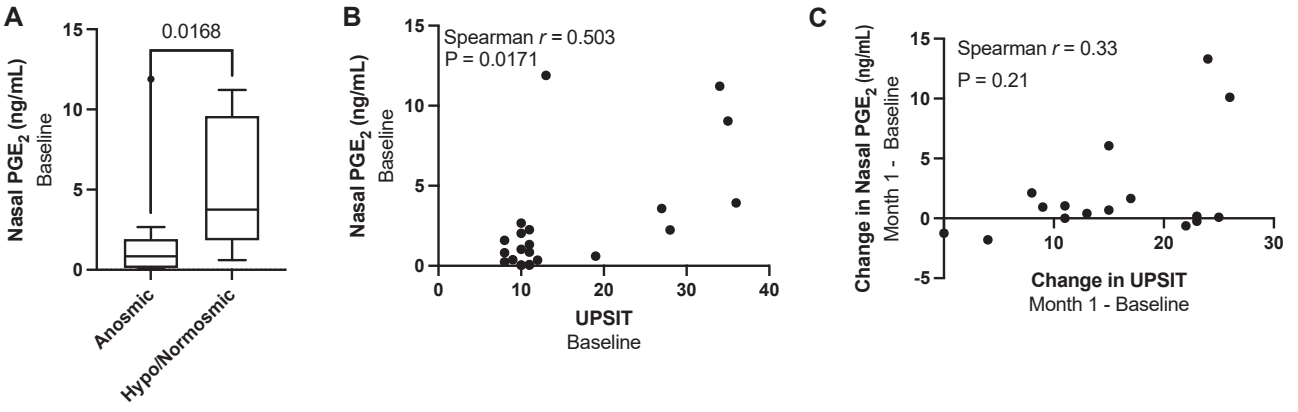

FIG 4. Relationship between nasal PGE_2 and sense of smell. Nasal fluid PGE_2 levels of patients who were anosmic (could identify <19 items on the UPSIT) or hyposmic or normosmic (could identify ≥19 items on the UPSIT) at baseline are compared (**A**). Correlations between baseline nasal PGE_2 levels and baseline UPSIT score (**B**) and between the dupilumab-induced change from baseline to month 1 in nasal PGE_2 level and UPSIT score (**C**) are shown with Spearman correlation coefficients.

signaling inhibits 5-lipoxygenase (5-LO) function, preventing the synthesis of cysLTs,[31] and PGE_2 also has direct protective effects on mast cells.[32] Thus, the rise in nasal PGE_2 levels and subsequent restoration of respiratory tissue levels of PGE_2 that we observed with dupilumab treatment could contribute to the significant decrease seen in nasal and urinary LTE_4 through suppression of mast cell activation and through 5-LO inhibition of cysLT-producing granulocytes (Fig 3, *A* and *D*). However, IL-4 also

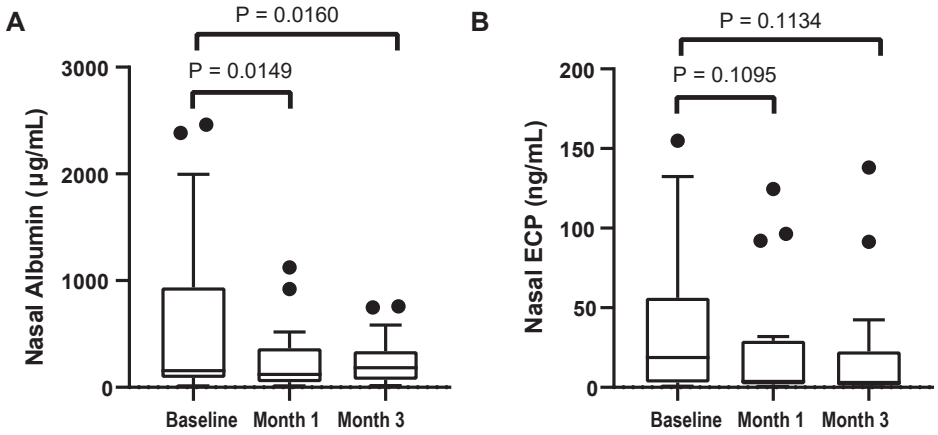

FIG 5. Dupilumab-induced decrease in nasal albumin and ECP levels. Nasal fluid levels of albumin (**A**) and ECP (**B**) are shown at the pre-dupilumab baseline and after 1 and 3 months of treatment with dupilumab. Data are shown as Tukey box-and-whisker plots.

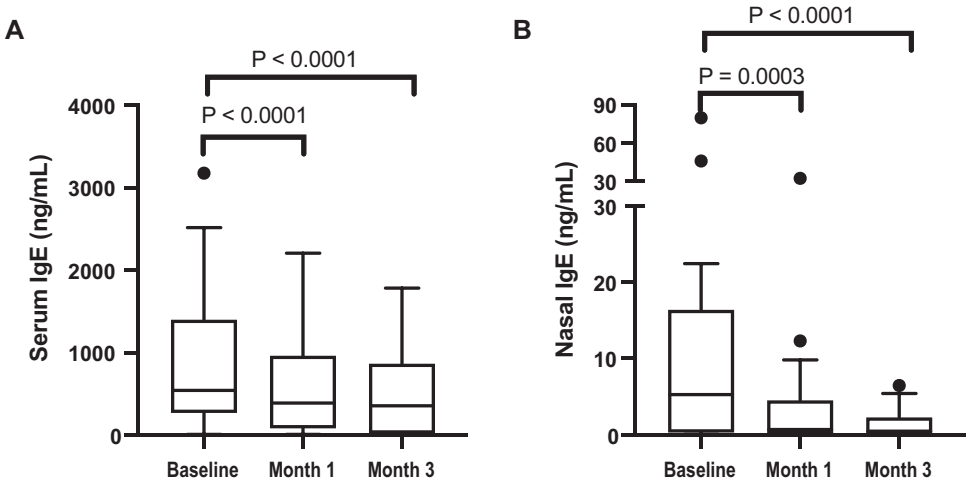

FIG 6. Dupilumab-induced decrease in IgE. Serum IgE (**A**) and nasal fluid IgE (**B**) levels are shown at the pre-dupilumab baseline and after 1 and 3 months of treatment with dupilumab. Data are shown as Tukey box-and-whisker plots.

directly induces expression of leukotriene C_4 synthase by human mast cells and primes them for cysLT generation[33]; therefore, direct inhibition of mast cell IL-4Rα could account for the dupilumab-induced decrease in LTE_4 level. Whether the decreases in LTE_4 are due to direct (inhibition of IL-4Rα on 5-LO– and leukotriene C_4 synthase–expressing granulocytes) or indirect (inhibition of IL-4Rα on respiratory epithelial cells, allowing for increased PGE_2 production and 5-LO inhibition) effects of dupilumab cannot be determined by this study but warrant further investigation with single-cell analysis techniques. Although there was a trend toward a dupilumab-induced decrease in level of PGD_2 metabolites (Fig 3, *B*), this was not statistically significant. Whether IL-4 or IL-13 signaling on mast cells directly regulates their production of PGD_2 is not known, and there are tissue-specific mast cell differences (mucosal mast cells vs connective tissue–type mast cells) in their metabolism of arachidonic acid to leukotrienes or prostaglandins.[34] Nonetheless, PGD_2 is the primary eicosanoid product of mast cells, and considering the dramatic dupilumab-induced decrease in LTE_4 level but lack of change in tryptase or PGD_2 level, we suspect that dupilumab led either to selective changes in mast cell phenotype or activation or to changes in other cysLT-producing granulocyte activation levels. CysLTs are known to mediate much of the chronic inflammation and the acute aspirin-induced reactions in AERD,[35,36] and PGE_2 has an additional role in limiting eosinophil migration and enhancing smooth muscle relaxation through EP2 receptor signaling,[37-39] both of which have important pathogenic implications in AERD. Therefore, the changes that we noted in this study in both PGE_2 and LTE_4 levels likely underlie much of the clinical benefit afforded by dupilumab, including the improvements in lung function and asthma control (Fig 2).

Chronic upper airway symptoms and loss of smell contribute deeply to quality of life impairment in AERD.[40] Patients with AERD have even more severe anosmia than aspirin-tolerant patients with CRSwNP do,[41] but the underlying etiology of the abnormality in olfaction is not known. We found that the baseline nasal fluid PGE_2 levels of participants who were anosmic were more than 4-fold lower than those of participants who were

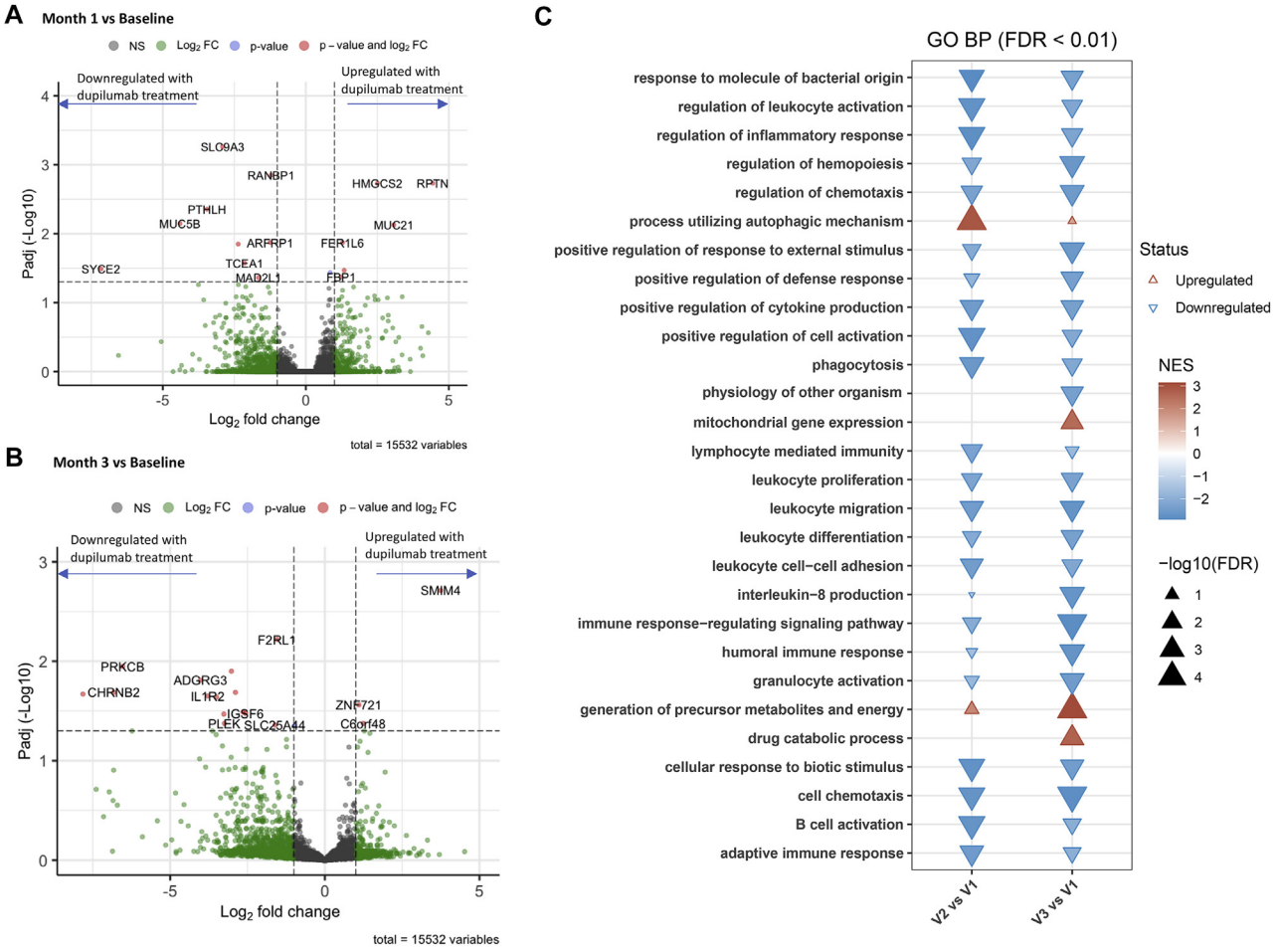

FIG 7. Dupilumab-induced differential gene expression and gene enrichment analyses. Volcano plots showing dupilumab-induced differential gene expression at month 1 compared with baseline (**A**) and month 3 compared with baseline (**B**). Gene Ontogeny (GO) analysis reveals upregulation and downregulation of specific pathways by dupilumab treatment after 1 and 3 months of treatment (**C**). *BP,* Biologic process; *FC,* fold change; *FDR,* false discovery rate; *NES,* normalized enrichment score; *NS,* not significant; P_{adj}, adjusted *P* value.

normosmic or only hyposmic (Fig 4, *A*) and that the baseline nasal fluid PGE_2 level was positively correlated with the number of scents that each participant could identify (Fig 4, *B*). In our study, sense of smell improved dramatically for most patients after just 1 month of dupilumab treatment (Fig 1, *A* and *B*), with the improvement sustained at the 3-month visit. There were 4 patients who remained anosmic after treatment with dupilumab, possibility due to permanent damage to olfactory neurons or pathways. Nasal PGE_2 levels also increased during this time frame (Fig 3, *F*), with a trend toward a positive correlation with improvement in sense of smell (Fig 4, *C*). To our knowledge, there is no known direct link between PGE_2 and either olfaction or the function of olfactory receptor neurons or sustentacular supporting cells, and olfactory neurons have not been shown to express any PGE_2 receptors. Further, administration of high-dose aspirin may reduce PGE_2 production, although paradoxically it is a treatment that has been shown to improve sense of smell in some patients with AERD. Therefore, our findings could be correlative but not causative, as perhaps a dupilumab-induced restoration of epithelial-derived PGE_2 is a reflection of overall improvement in the epithelial health within the sinuses, an improvement that may extend to the olfactory epithelium.

CRSwNP tissue has abnormal expression of epithelial intracellular junction proteins, driven in part by high local levels of IL-4 and IL-13, leading to a "leaky" epithelial barrier and subsequent exudation of plasma proteins into nasal secretions.[42,43] Nasal fluid albumin levels, a marker of plasma exudation and edema, are higher in patients with CRSwNP than in patients without nasal polyps, and are reduced by corticosteroids.[44,45] *In vivo* administration of IL-4 causes nasal congestion and edema, which may be due to an induction of epithelial permeability.[46] We found that after just 1 month of treatment, dupilumab decreased nasal albumin levels (Fig 5, *A*) and downregulated transcripts related to glandular cell hyperplasia (*MUC5B,* encoding a member of the mucin family, and *PTHLH,* encoding parathyroid hormone–like hormone)[22-25] as it concurrently improved nasal airflow and decreased congestion (Fig 1, *D*), suggesting an improvement in the "leakiness" of the epithelial barrier.

The treatment-induced decrease in serum and nasal IgE levels confirms previous findings that inhibition of IL-4/IL-4Rα signaling on antibody-secreting cells reduces IgE production, possibly by inhibiting IgE class switching (Fig 6).[47] Rates of clinical atopy in AERD are on par with those in the general population, and many patients with AERD are skin test

result–negative in response to all common environmental allergens.[48] Furthermore, the nonsteroidal anti-inflammatory drug–induced reactions are not associated with drug-specific IgE. Nonetheless, IgE inhibition with omalizumab has some effect as a treatment for AERD, leading to reductions in urinary levels of both LTE_4 and PGD_2 metabolites,[49] and pretreatment with omalizumab can inhibit or lessen aspirin-induced reactions.[50] The role that the dupilumab-induced reduction in local and systemic IgE levels plays in the improvement in clinical symptoms is unclear, but given that IgE receptor crosslinking induces mast cell release of cysLTs,[51] dupilumab may help to indirectly reduce mast cell cysLT release through downregulation of IgE synthesis.

We did not identify changes in blood or nasal eosinophilia (as measured by nasal ECP levels [Fig 5, *B*]) induced by dupilumab. Given that dupilumab is efficacious in patients with CRSwNP regardless of patient eosinophilic status[52] and that near-complete depletion of eosinophils from within the blood and nasal polyp tissue does not necessarily provide symptomatic improvement or reduction in nasal polyp size in patients with CRSwNP,[53] eosinophils are likely not the main effector cells that drive inflammation in AERD. Unlike our recent finding that mepolizumab treatment in AERD leads to decreased levels of nasal and urinary PGD_2 metabolites and a subsequent increase of the surface expression of the PGD_2 receptor CRTH2,[17] we did not observe a significant dupilumab-induced decrease in PGD_2 metabolite levels or a change in granulocyte CRTH2 expression, reflecting the divergent mechanistic consequences of inhibition of IL-5 versus IL-4Rα. However, we do see transcriptional changes in the nasal tissue, showing a decrease in pathways involved in the activation and migration of leukocytes as well as B-cell activation (Fig 7), which may underlie some of the benefit afforded by dupilumab, allowing for decreased recruitment of activated inflammatory leukocytes to the respiratory tissues. The limited number of differentially expressed genes was due in part to patient heterogeneity, batch effect, and depth of sequencing reads. Highly powered studies and/or single-cell RNA-seq will help to address patient-to-patient heterogeneity. Additionally, by sampling the accessible and relatively normal inferior turbinate tissue rather than the inflamed nasal polyp tissue, we may have obscured our ability to see changes in the type 2 gene signature with dupilumab. Future studies focused specifically on the highly inflamed nasal polyp tissue will allow for greater understanding of dupilumab-induced changes.

Major limitations of our study are that it was an open-label observational study without a placebo arm. However, the efficacy of dupilumab in patients with asthma and CRSwNP is well established.[4,7] Further, the longitudinal clinical and mechanistic repeated measures allowed for each of the participants to serve as their own control, as our goal was to understand the early dupilumab-induced mechanistic changes. Dupilumab leads to rapid improvement in both upper and lower airway symptoms, sense of smell, and lung function, and to reduction in nasal polyp burden in patients with AERD. Although there are multiple potential mechanisms by which dupilumab may lead to clinical improvement in patients with AERD, we cannot yet determine which mechanistic changes are the principal drivers of disease resolution, nor which are the result of direct versus indirect effects of IL-4Rα blockade. We conclude that the mechanistic changes underlying the clinical improvements in IL-4Rα inhibition with dupilumab treatment primarily involve effects on tissue mast cells, and possibly, other granulocytes, B cells, and epithelial barrier dysfunction.

Key messages

- **In an open-label trial of 22 patients with AERD treated with dupilumab for 3 months, dramatic improvements in sense of smell, nasal congestion, nasal polyp size, lung function, asthma control, and quality of life were seen within the first month of therapy.**
- **Baseline loss of smell was correlated with lower nasal PGE_2 levels. Dupilumab treatment increased nasal PGE_2 levels; decreased inflammatory eicosanoid levels; improved the nasal epithelial barrier; and downregulated nasal transcripts involved in epithelial dysfunction, which paralleled the rapid time course of the therapeutic benefits.**
- **The therapeutic effects of dupilumab in AERD are likely due to decreased IL-4Rα signaling on local respiratory tissue granulocytes and epithelial cells.**

REFERENCES

1. Bachert C, Desrosiers MY, Hellings PW, Laidlaw TM. The role of biologics in chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol Pract 2021;9:1099-106.
2. Morales DR, Guthrie B, Lipworth BJ, Jackson C, Donnan PT, Santiago VH. NSAID-exacerbated respiratory disease: a meta-analysis evaluating prevalence, mean provocative dose of aspirin and increased asthma morbidity. Allergy 2015;70:828-35.
3. Stevens WW, Peters AT, Hirsch AG, Nordberg CM, Schwartz BS, Mercer DG, et al. Clinical characteristics of patients with chronic rhinosinusitis with nasal polyps, asthma, and aspirin-exacerbated respiratory disease. J Allergy Clin Immunol Pract 2017;5:1061-70.e3.
4. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med 2018;378:2486-96.
5. Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, et al. Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. N Engl J Med 2018;378:2475-85.
6. Bachert C, Mannent L, Naclerio RM, Mullol J, Ferguson BJ, Gevaert P, et al. Effect of subcutaneous dupilumab on nasal polyp burden in patients with chronic sinusitis and nasal polyposis: a randomized clinical trial. JAMA 2016;315:469-79.
7. Bachert C, Han JK, Desrosiers M, Hellings PW, Amin N, Lee SE, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet 2019;394:1638-50.
8. Simpson EL, Akinlade B, Ardeleanu M. Two Phase 3 Trials of dupilumab versus placebo in atopic dermatitis. N Engl J Med 2017;376:1090-1.
9. Hirano I, Dellon ES, Hamilton JD, Collins MH, Peterson K, Chehade M, et al. Efficacy of dupilumab in a phase 2 randomized trial of adults with active eosinophilic esophagitis. Gastroenterology 2020;158:111-22.e10.
10. Laidlaw TM, Mullol J, Fan C, Zhang D, Amin N, Khan A, et al. Dupilumab improves nasal polyp burden and asthma control in patients with CRSwNP and AERD. J Allergy Clin Immunol Pract 2019;7:2462-5.e1.
11. Bavaro N, Gakpo D, Mittal A, Bensko JC, Laidlaw TM, Buchheit KM. Efficacy of dupilumab in patients with aspirin-exacerbated respiratory disease and previous inadequate response to anti-IL-5 or anti-IL-5Ralpha in a real-world setting. J Allergy Clin Immunol Pract 2021;9:2910-22.e1.
12. Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res 2001;2:66-70.
13. Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC, Kaghad M, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. Nature 1993;362:248-50.
14. Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 1994;15:19-26.

15. Ordovas-Montanes J, Dwyer DF, Nyquist SK, Buchheit KM, Vukovic M, Deb C, et al. Allergic inflammatory memory in human respiratory epithelial progenitor cells. Nature 2018;560:649-54.
16. Mustafa SS, Vadamalai K, Scott B, Ramsey A. Dupilumab as add-on therapy for chronic rhinosinusitis with nasal polyposis in aspirin exacerbated respiratory disease. Am J Rhinol Allergy 2021;35:399-407.
17. Buchheit KM, Lewis E, Gakpo D, Hacker J, Sohail A, Taliaferro F, et al. Mepolizumab targets multiple immune cells in aspirin-exacerbated respiratory disease. J Allergy Clin Immunol 2021;148:574-84.
18. Laidlaw TM, Cahill KN, Cardet JC, Murphy K, Cui J, Dioneda B, et al. A trial of type 12 purinergic (P2Y12) receptor inhibition with prasugrel identifies a potentially distinct endotype of patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol 2019;143:316-24.e7.
19. Laidlaw TM, Kidder MS, Bhattacharyya N, Xing W, Shen S, Milne GL, et al. Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. Blood 2012;119:3790-8.
20. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
21. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP. Psychometric validity of the 22-Item Sinonasal Outcome Test. Clin Otolaryngol 2009;34:447-54.
22. Zhang Y, Derycke L, Holtappels G, Wang XD, Zhang L, Bachert C, et al. Th2 cytokines orchestrate the secretion of MUC5AC and MUC5B in IL-5-positive chronic rhinosinusitis with nasal polyps. Allergy 2019;74:131-40.
23. Ding GQ, Zheng CQ. The expression of MUC5AC and MUC5B mucin genes in the mucosa of chronic rhinosinusitis and nasal polyposis. Am J Rhinol 2007;21:359-66.
24. Viswanathan H, Brownlee IA, Pearson JP, Carrie S. MUC5B secretion is upregulated in sinusitis compared with controls. Am J Rhinol 2006;20:554-7.
25. Wu X, Peters-Hall JR, Ghimbovschi S, Mimms R, Rose MC, Pena MT. Glandular gene expression of sinus mucosa in chronic rhinosinusitis with and without cystic fibrosis. Am J Respir Cell Mol Biol 2011;45:525-33.
26. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A 2007;104:15858-63.
27. Brock TG, McNish RW, Peters-Golden M. Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. J Biol Chem 1999;274:11660-6.
28. Roca-Ferrer J, Garcia-Garcia FJ, Pereda J, Perez-Gonzalez M, Pujols L, Alobid I, et al. Reduced expression of COXs and production of prostaglandin E(2) in patients with nasal polyps with or without aspirin-intolerant asthma. J Allergy Clin Immunol 2011;128:66-72 e1.
29. Sugiyama E, Taki H, Kuroda A, Mino T, Yamashita N, Kobayashi M. Interleukin-4 inhibits prostaglandin E2 production by freshly prepared adherent rheumatoid synovial cells via inhibition of biosynthesis and gene expression of cyclo-oxygenase II but not of cyclo-oxygenase I. Ann Rheum Dis 1996;55:375-82.
30. Han R, Smith TJ. T helper type 1 and type 2 cytokines exert divergent influence on the induction of prostaglandin E2 and hyaluronan synthesis by interleukin-1beta in orbital fibroblasts: implications for the pathogenesis of thyroid-associated ophthalmopathy. Endocrinology 2006;147:13-9.
31. Narayanankutty A, Resendiz-Hernandez JM, Falfan-Valencia R, Teran LM. Biochemical pathogenesis of aspirin exacerbated respiratory disease (AERD). Clin Biochem 2013;46:566-78.
32. Rastogi S, Willmes DM, Nassiri M, Babina M, Worm M. PGE2 deficiency predisposes to anaphylaxis by causing mast cell hyperresponsiveness. J Allergy Clin Immunol 2020;146:1387-96.e13.
33. Jiang Y, Kanaoka Y, Feng C, Nocka K, Rao S, Boyce JA. Cutting edge: Interleukin 4-dependent mast cell proliferation requires autocrine/intracrine cysteinyl leukotriene-induced signaling. J Immunol 2006;177:2755-9.
34. Heavey DJ, Ernst PB, Stevens RL, Befus AD, Bienenstock J, Austen KF. Generation of leukotriene C4, leukotriene B4, and prostaglandin D2 by immunologically activated rat intestinal mucosa mast cells. J Immunol 1988;140:1953-7.
35. Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, et al. Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Respir Dis 1991;143:1025-9.
36. Sestini P, Armetti L, Gambaro G, Pieroni MG, Refini RM, Sala A, et al. Inhaled PGE2 prevents aspirin-induced bronchoconstriction and urinary LTE4 excretion in aspirin-sensitive asthma. Am J Respir Crit Care Med 1996;153:572-5.
37. Torres R, Picado C, de Mora F. The PGE2-EP2-mast cell axis: an antiasthma mechanism. Mol Immunol 2015;63:61-8.
38. Sastre B, del Pozo V. Role of PGE2 in asthma and nonasthmatic eosinophilic bronchitis. Mediators Inflamm 2012;2012:645383.
39. Sturm EM, Schratl P, Schuligoi R, Konya V, Sturm GJ, Lippe IT, et al. Prostaglandin E2 inhibits eosinophil trafficking through E-prostanoid 2 receptors. J Immunol 2008;181:7273-83.
40. Ta V, White AA. Survey-defined patient experiences with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol Pract 2015;3:711-8.
41. Gudziol V, Michel M, Sonnefeld C, Koschel D, Hummel T. Olfaction and sinonasal symptoms in patients with CRSwNP and AERD and without AERD: a cross-sectional and longitudinal study. Eur Arch Otorhinolaryngol 2017;274:1487-93.
42. Wise SK, Laury AM, Katz EH, Den Beste KA, Parkos CA, Nusrat A. Interleukin-4 and interleukin-13 compromise the sinonasal epithelial barrier and perturb intercellular junction protein expression. Int Forum Allergy Rhinol 2014;4:361-70.
43. Biewenga J, Stoop AE, van der Heijden HA, van der Baan S, van Kamp GJ. Albumin and immunoglobulin levels in nasal secretions of patients with nasal polyps treated with endoscopic sinus surgery and topical corticosteroids. J Allergy Clin Immunol 1995;96:334-40.
44. Rudack C, Sachse F, Alberty J. Primary role of growth-related oncogene-alpha and granulocyte chemotactic protein-2 as neutrophil chemoattractants in chronic rhinosinusitis. Clin Exp Allergy 2006;36:748-59.
45. Moloney JR, Oliver RT. HLA antigens, nasal polyps and asthma. Clin Otolaryngol Allied Sci 1980;5:183-9.
46. Emery BE, White MV, Igarashi Y, Mullol J, Berkebile C, Peden D, et al. The effect of IL-4 on human nasal mucosal responses. J Allergy Clin Immunol 1992;90:772-81.
47. Corren J, Castro M, O'Riordan T, Hanania NA, Pavord ID, Quirce S, et al. Dupilumab efficacy in patients with uncontrolled, moderate-to-severe allergic asthma. J Allergy Clin Immunol Pract 2020;8:516-26.
48. Johns CB, Laidlaw TM. Elevated total serum IgE in nonatopic patients with aspirin-exacerbated respiratory disease. Am J Rhinol Allergy 2014;28:287-9.
49. Hayashi H, Mitsui C, Nakatani E, Fukutomi Y, Kajiwara K, Watai K, et al. Omalizumab reduces cysteinyl leukotriene and 9alpha,11beta-prostaglandin F2 overproduction in aspirin-exacerbated respiratory disease. J Allergy Clin Immunol 2016;137:1585-7.e4.
50. Lang DM, Aronica MA, Maierson ES, Wang XF, Vasas DC, Hazen SL. Omalizumab can inhibit respiratory reaction during aspirin desensitization. Ann Allergy Asthma Immunol 2018;121:98-104.
51. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. J Immunol 2004;173:1503-10.
52. Fujieda S, Matsune S, Takeno S, Ohta N, Asako M, Bachert C, et al. Dupilumab efficacy in chronic rhinosinusitis with nasal polyps from SINUS-52 is unaffected by eosinophilic status. Allergy 2022;77:186-96.
53. Laidlaw TM, Prussin C, Panettieri RA, Lee S, Ferguson BJ, Adappa ND, et al. Dexpramipexole depletes blood and tissue eosinophils in nasal polyps with no change in polyp size. Laryngoscope 2019;129:E61-6.