Group 2 innate lymphoid cells are recruited to the nasal mucosa in patients with aspirin-exacerbated respiratory disease

Jacqueline J. Eastman, MD,[a] Kellen J. Cavagnero, BS,[a] Adam S. Deconde, MD,[b] Alex S. Kim, MD,[a] Maya R. Karta, PhD,[a] David H. Broide, MB, ChB,[a] Bruce L. Zuraw, MD,[a] Andrew A. White, MD,[c] Sandra C. Christiansen, MD,[a] and Taylor A. Doherty, MD[a] *San Diego and La Jolla, Calif*

Background: Aspirin-exacerbated respiratory disease (AERD) is characterized by tissue eosinophilia and mast cell activation, including abundant production of prostaglandin D_2 (PGD_2). Group 2 innate lymphoid cells (ILC2s), which promote tissue eosinophilia and mast cell responses, undergo chemotaxis and cytokine production in response to PGD_2, but it is unknown whether ILC2s are active in patients with AERD.

Objective: We sought to determine whether ILC2 numbers change in peripheral blood and the nasal mucosa during COX-1 inhibitor–induced reactions in patients with AERD.

Methods: Blood and nasal scrapings were collected at baseline, during reactions, and after completion of ketorolac/aspirin challenge/desensitization in 12 patients with AERD. ILC2s and eosinophils were quantitated by means of flow cytometry. Urine was also collected, and quantification of PGD_2 metabolite and leukotriene E_4 levels was done by using ELISA. Baseline and nonsteroidal anti-inflammatory drug reaction clinical data were correlated with cell changes.

Results: ILC2 numbers significantly increased in nasal mucosal samples and decreased in blood at the time of COX-1 inhibitor reactions in 12 patients with AERD. These changes were not observed in 2 patients without AERD. Furthermore, eosinophil numbers decreased in blood concurrently with significant increases in urinary PGD_2 metabolite and leukotriene E_4 levels. The magnitude of increases in nasal mucosal ILC2 numbers positively correlated with maximum symptom scores during challenges. Furthermore, blood ILC2 numbers during the reaction correlated with time for the reaction to resolve, possibly reflecting reaction severity.

Conclusions: ILC2s are recruited to the nasal mucosa during COX-1 inhibitor–induced reactions in patients with AERD, correlating with enhanced production of prostaglandins and leukotrienes. (J Allergy Clin Immunol 2017;140:101-8.)

Key words: *Group 2 innate lymphoid cells, aspirin-exacerbated respiratory disease*

Aspirin-exacerbated respiratory disease (AERD) is clinically characterized by a relentless progression of nasal congestion, anosmia, formation of nasal polyps, and, typically, asthma. Naso-ocular and lower respiratory tract symptoms after ingestion of COX-1 inhibitors are pathognomonic for AERD. AERD is present in about 7% of adult asthmatic patients and 14% of patients with severe asthma[1] and can develop in patients without a prior history of atopy, asthma, or rhinitis.[2] Nasal polyps of patients with AERD are intensely eosinophilic and contain activated mast cells.[3,4] The disease is characterized by release of large amounts of cysteinyl leukotrienes (CysLTs) and mast cell mediators, particularly prostaglandin D_2 (PGD_2), after COX-1 inhibitor ingestion.[5,6] Recently, thymic stromal lymphopoietin (TSLP) and IL-33 have also been implicated in AERD pathogenesis.[7,8] These 2 cytokines, in addition to PGD_2 and CysLTs, are known to promote group 2 innate lymphoid cell (ILC2) activation,[9-12] and their presence suggests that ILC2s might be active in patients with AERD.

ILC2s are a recently described lymphocyte population that lack antigen specificity but are capable of producing large amounts of type 2 cytokines (IL-4, IL-5, IL-6, IL-9, and IL-13) and have been shown to promote type 2 inflammation.[13] In human subjects ILC2 numbers are increased in affected tissues from patients with atopic dermatitis, allergic asthma, eosinophilic esophagitis, and allergic rhinitis.[14-18] Importantly, ILC2 numbers are increased in nasal polyps and levels correlate with the degree of eosinophilia.[19] ILC2s are identified as lymphocytes that are lineage negative (ie, lacking the markers of B, T, or natural killer cells) and express chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) and CD161.[3] CRTH2 is a G protein–coupled receptor that binds to PGD_2, and PGD_2 has been shown to promote ILC2 chemotaxis and cytokine production *in vitro*.[10,20] Thus these suggest a potential role for ILC2s in patients with AERD.

Our aim for this study was to determine ILC2 changes in the blood and nasal mucosa of patients with AERD during COX-1 inhibitor challenges. To our knowledge, there are no reports focused on a potential role for ILC2s in AERD-related nonsteroidal anti-inflammatory drug (NSAID)–induced reactions. Importantly, we found that ILC2 numbers were significantly increased in nasal scrapings and decreased in peripheral blood

From [a]the Department of Medicine, University of California, San Diego; [b]the Department of Surgery, University of California–San Diego, La Jolla; and [c]the Division of Allergy, Asthma and Immunology, Scripps Clinic, La Jolla.

Supported by National Institutes of Health grants AI114585 and AI70535 (to T.A.D.), NIH grant T32 AI007469 (to J.J.E.), NIH grants AI107779, AI38425, AI70535, and AI72115 (to D.H.B.), and divisional support from the Division of Rheumatology, Allergy and Immunology at University of California, San Diego.

Disclosure of potential conflict of interest: J. J. Eastman has received a grant from the National Institutes of Health (NIH; T32). A. S. DeConde has consultant arrangements with IntersectENT and Stryker Endoscopy. D. H. Broide has received grants from the NIH and Janssen. A. A. White is employed by the Scripps Clinic Medical Group. T. A. Doherty has received a grant from the NIH. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 1, 2016; revised October 26, 2016; accepted for publication November 22, 2016.

Available online March 6, 2017.

Corresponding author: Taylor A. Doherty, MD, Department of Medicine, University of California–San Diego, Biomedical Sciences Building, Room 5090, 9500 Gilman Dr, La Jolla, CA 92093-0635. E-mail: tdoherty@ucsd.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.11.023

Abbreviations used
AERD: Aspirin-exacerbated respiratory disease
CRTH2: Chemoattractant receptor-homologous molecule expressed on T_H2 cells
CysLT: Cysteinyl leukotriene
ILC2: Group 2 innate lymphoid cell
LTE_4: Leukotriene E_4
NSAID: Nonsteroidal anti-inflammatory drug
PGD_2: Prostaglandin D_2
11β-PGF2α: 11β Prostaglandin F2α
SNOT-22: Sino-Nasal Outcome Test 22
TSLP: Thymic stromal lymphopoietin

during the ketorolac-induced reactions in patients with AERD. These patients also had a corresponding decrease in blood eosinophil numbers, indicating that these processes happen simultaneously. Concomitantly, patients showed an increase in levels of 11β prostaglandin F2α (11β-PGF2α; a stable metabolite of PGD_2),[21] as well as the terminal CysLT leukotriene E_4 (LTE_4). Overall, this study implicates ILC2s as an additional cell type apart from mast cells and eosinophils that can contribute to AERD pathogenesis.

METHODS

Study subjects with AERD

Patients (n = 14) undergoing aspirin desensitization for AERD at the Allergy Divisions of the University of California, San Diego and Scripps Clinic were recruited after institutional review board approval at both institutions. Informed consent was obtained from all patients before starting the study. All patients were suspected of having a diagnosis of AERD based on a history of nasal polyps and asthma and a compelling history of reactions to aspirin/NSAIDs within 2 to 3 hours. Demographic and clinical data were collected from retrospective chart review and included age, sex, ethnicity, asthma, and rhinoconjunctivitis characteristics; nasal polyp surgical details and clinical response; aspirin/NSAID reaction characteristics; current medications; and laboratory data. Symptom scores and spirometry were assessed at baseline. The modified AERD symptom scoring system included 8 symptoms (nasal congestion, runny nose, sneezing, itchy nose, itchy/watery eyes, tight throat, wheezing, and chest tightness), which the patients rated on a scale of 0 to 3 (range of possible scores, 0-24; see Fig E1 in this article's Online Repository at www.jacionline.org). Sino-Nasal Outcome Test 22 (SNOT-22) scores were recorded either that day or from a recent clinic visit.

Intranasal ketorolac and aspirin challenge/desensitizations

Intranasal ketorolac and aspirin challenge/desensitizations were done per the standard protocol at each institution (see Fig E2 in this article's Online Repository at www.jacionline.org, which is adapted from Lee et al[22]). Reactions were identified as any upper and/or lower respiratory tract signs or symptoms consistent with conjunctivitis, rhinitis, laryngospasm, and bronchospasm or decrease in FEV_1.

Nasal scrapings, blood draws, and urine samples

Nasal scraping was done with a Rhino-probe (Arlington Scientific, Springville, Utah) and involved 1 to 3 passes over the inferior turbinate on one side. Nasal scrapings were transported in RPMI for processing for flow cytometry to detect ILC2s. Blood was collected in BD vacutainers (BD Biosciences, San Diego, Calif) to quantitate ILC2 and eosinophil numbers. Samples, including nasal scrapings, blood, and urine, were collected at baseline. At the time of the first clinician-confirmed reaction, blood and a nasal scraping were again collected, and urine was collected 1 hour later. A third set of samples was collected at the end of the desensitization before patient discharge. Some patients were not able to provide all 3 samples. Symptom scores (per the system noted above), FEV_1, and medications required were recorded at each time point.

Identification of ILC2s and eosinophils in peripheral blood and nasal scrapings

PMBCs and granulocytes were separated with Histopaque 1119 and Histopaque 1077 (Sigma-Aldrich, St Louis, Mo) per the manufacturer's protocol. Nasal scrapings were processed through a strainer and washed before staining. Cells were first incubated with Fc Receptor Blocking Reagent (Miltenyi Biotec, San Diego, Calif). Granulocytes were then stained with peridinin-chlorophyll-protein complex–conjugated CD45, fluorescein isothiocyanate–conjugated FcεRIα, and allophycocyanin-conjugated CCR3 (BioLegend, San Diego, Calif). Eosinophils were defined as the percentage of $CD45^+$ granulocytes that were $CCR3^+FcεRIα^-$. PBMCs and nasal scrapings were stained with peridinin-chlorophyll-protein complex–conjugated CD45 (BioLegend), fluorescein isothiocyanate–conjugated lineage markers (CD235a, FcεRIα, T-cell receptor γ/δ, and CD4 [BioLegend] and BD Lineage Cocktail 1 [CD3, CD14, CD16, CD19, CD20, and CD56]) and phycoerythrin-conjugated CRTH2 (Miltenyi Biotec). PBMCs were also stained for allophycocyanin-conjugated CD161 (BioLegend). Blood ILC2s were defined as percent $CD45^+Lin^-CD161^+CRTH2^+$ lymphocytes of all cells. Percentages of ILC2s are reported as percentages of enriched PBMCs. Nasal scraping ILC2s were defined as $CD45^+Lin^-CRTH2^+$ lymphocytes. Percentages of ILC2s are reported as percentages of total cells in nasal scraping samples. Flow cytometry was performed with a NovoCyte Cytometer (Acea Biosciences, San Diego, Calif). Data were further analyzed with FlowJo software (FlowJo, Ashland, Ore).

Urine 11β-$PGF_2α$ and LTE_4

Urine samples were stored at −80°C. PGD_2 is metabolized to 11β-$PGF_2α$, a stable active metabolite that is measurable in urine.[21] 11β-$PGF_2α$ levels were analyzed by means of ELISA, according to the manufacturer's protocol (Cayman Chemical, Ann Arbor, Mich). Urinary LTE_4 levels were analyzed by means of ELISA, according to the manufacturer's protocol (Cayman Chemical). Values were corrected for specific gravity, as previously described.[23]

Nasal polyps

Nasal polyps were collected from 2 subjects undergoing endoscopic sinus surgery at the University of California, San Diego who ultimately underwent desensitization for AERD and are included in that analysis. Tissue samples were transported in RPMI and processed for flow cytometry the same day. Nasal polyp tissue was digested with Collagenase Type 4 (Worthington, Lakewood, NJ) and DNase I (200 μg/mL; Roche, Indianapolis, Ind) and then filtered into a single-cell suspension. ILC2s were identified per above. GATA-3^+ cells were identified as $CD45^+Lin^-GATA\text{-}3^+$ lymphocytes. The cells were initially stained for cell-surface CD45 and lineage markers per above. The cells then underwent fixation and permeabilization (eBioscience, San Diego, Calif) and were stained for phycoerythrin-conjugated GATA-3 (eBioscience). Flow cytometry and analysis were done as indicated above.

Statistical analyses

Statistical analysis was performed with GraphPad Prism software (GraphPad Software, La Jolla, Calif). The paired *t* test or Pearson correlation test were used, where indicated. A *P* value of less than .05 was considered statistically significant.

RESULTS

Demographics of patients with AERD and NSAID reaction characteristics

A total of 14 suspected patients with AERD were enrolled in the study. Baseline characteristics of patients are summarized in