Aspirin-Exacerbated Respiratory Disease Involves a Cysteinyl Leukotriene–Driven IL-33–Mediated Mast Cell Activation Pathway

Tao Liu, Yoshihide Kanaoka, Nora A. Barrett, Chunli Feng, Denise Garofalo, Juying Lai, Kathleen Buchheit, Neil Bhattacharya, Tanya M. Laidlaw, Howard R. Katz and Joshua A. Boyce

*Published online.*

Supplementary Material http://www.jimmunol.org/content/suppl/2015/09/04/jimmunol.1500905.DCSupplemental

Why The JJ?

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

*Print ISSN: 0022-1767 Online ISSN: 1550-6606. Copyright © 2015 by The American Association of Immunologists, Inc. All rights reserved.*
Aspirin-Exacerbated Respiratory Disease Involves a Cysteinyl Leukotriene–Driven IL-33–Mediated Mast Cell Activation Pathway

Tao Liu,*†‡ Yoshihide Kanaoka,*†‡ Nora A. Barrett,*†‡ Chuni Feng,*†‡ Denise Garofalo,†‡ Juying Lai,†‡ Kathleen Buchheit,*†‡ Neil Bhattacharya,‡ Tanya M. Laidlaw,*†‡ Howard R. Katz,*†‡ and Joshua A. Boyce*†‡

Aspirin-exacerbated respiratory disease (AERD), a severe eosinophilic inflammatory disorder of the airways, involves overproduction of cysteinyl leukotrienes (cysLTs), activation of airway mast cells (MCs), and bronchoconstriction in response to nonselective cyclooxygenase inhibitors that deplete homeostatic PGE2. The mechanistic basis for MC activation in this disorder is unknown. We now demonstrate that patients with AERD have markedly increased epithelial expression of the alarmin-like cytokine IL-33 in nasal polyps, as compared with polyps from aspirin-tolerant control subjects. The murine model of AERD, generated by dust mite priming of mice lacking microsomal PGE2 synthase (ptges<sup>−/−</sup> mice), shows a similar upregulation of IL-33 protein in the airway epithelium, along with marked eosinophilic bronchovascular inflammation. Deletion of leukotriene C<sub>4</sub> synthase, the terminal enzyme needed to generate cysLTs, eliminates the increased IL-33 content of the ptges<sup>−/−</sup> lungs and sharply reduces pulmonary eosinophilia and basal secretion of MC products. Challenges of dust mite–primed ptges<sup>−/−</sup> mice with lysine aspirin induce IL-33–dependent MC activation and bronchoconstriction. Thus, IL-33 is a component of a cysLT-driven innate type 2 immune response that drives pathogenic MC activation and contributes substantially to AERD pathogenesis. The Journal of Immunology, 2015, 195: 000–000.

Aspirin-exacerbated respiratory disease (AERD) is a distinctive, idiopathic syndrome that affects 7–10% of all asthmatics and ~15% of severe asthmatics (1). It is characterized by severe eosinophilic respiratory mucosal inflammation, refractory nasal polyposis, and idiosyncratic respiratory reactions to aspirin and other nonselective cyclooxygenase (COX) inhibitors (2). Dysregulated activity of the 5-lipoxygenase (5-LO)/leukotriene (LT) C<sub>4</sub> synthase (LTCS<sub>S</sub>) pathway in AERD patients results in increased synthesis of LTC<sub>4</sub>, the parent of the cysteinyl LTs [cysLTs; LTC<sub>4</sub>, LT D<sub>2</sub> (LTD<sub>2</sub>), and the stable metabolite LT E<sub>4</sub> (LTE<sub>4</sub>)] from arachidonic acid (2). As a consequence of this dysregulation, the levels of LTE<sub>4</sub> in urine and respiratory secretions from subjects with AERD are elevated compared with those in aspirin-tolerant (AT) asthmatic (ATA) control subjects and increase further (by several fold) in response to provocative challenges with aspirin (3, 4). Curiously, although subjects with AERD and ATA control subjects are equivalently sensitive to the bronchoconstricting effects of inhaled LTC<sub>4</sub> (5) and LTD<sub>2</sub> (6), both of which are powerful but short-lived contractile agonists, subjects with AERD develop airflow obstruction in response to challenges with LTE<sub>4</sub>, the weakest constrictor of the three cysLTs, at doses >1 log lower than those that induce bronchoconstriction in ATA control subjects (5, 7). Thus, overproduction of cysLTs and hyperresponsiveness to LTE<sub>4</sub> may each contribute to the persistent respiratory tract inflammation and to the pathognomonic clinical reactions associated with AERD. Although high levels of LTC<sub>S</sub> expression by tissue eosinophils (8) and aberrant transcellular cysLT generation by granulocyte–platelet complexes (9) contribute to cysLT overproduction, the basis for LTE<sub>4</sub> hyperresponsiveness is unknown.

The surge in cysLT production characteristically elicited by challenges of AERD subjects with aspirin is accompanied by mast cell (MC) activation, as evidenced by increases in the levels of histamine, tryptase, and PGD<sub>2</sub> metabolites in nasal lavage fluids, plasma, and urine (4, 10). The administration of the MC-stabilizing drugs cromolyn or nedocromil before challenge prevents aspirin-induced bronchoconstriction (11, 12), supporting the physiologic importance of MCs in the pathognomonic reactions. Although the casse of mediators released by MCs in response to aspirin challenges in AERD is identical to that released in response to allergen challenges in sensitized individuals (13–15), there is no evidence that AERD involves classical allergen-driven immunopathology (16, 17). The potency of COX-1 inhibition, rather than drug antigenicity or structure, is the major determinant of clinical reactivity to...
a given agent (18). Thus, the mechanism of MC activation in AERD likely involves disease-specific nonclassical immune pathways that are braked by COX-1–derived PGs. Curiously, the administration of the 5-LO inhibitor zileuton to subjects with AERD not only prevents symptoms of reactions to intranasal challenge with lysine aspirin (Lys-ASA), but also blocks the release of MC products, suggesting that one or more endogenous 5-LO products (likely cysteine leukotrienes) are necessary for MC activation in AERD (19). However, the precise identity of the products and the causative mechanism(s) by which they induce MC activation are unclear, and there is no explanation for why this mechanism is unique to AERD.

PGE2 stabilizes MCs (20) and prevents 5-LO activation (21) by acting at the E prostanooid 2 receptor and activating protein kinase A. Deficient respiratory tract production of PGE2 (22), reduced expression of E prostanooid 2 receptors by MCs and other tissue leukocytes (6, 23), and diminished function of protein kinase A (24) are all reported in association with AERD. Mice that are selectively PGE2 deficient because of targeted deletion of microsomal PGE synthase (ptges−/− mice) develop a phenotype strikingly similar to AERD when challenged with Lys-ASA after a period of exposure to a Dermatophagoides farinae extract (Df) to elicit airway inflammation (25). Lys-ASA elicits bronchoconstriction, pulmonary MC activation, and release of cysteine leukotrienes from the lungs of ptges−/− mice, but not of PGE2-sufficient WT controls. All of these features are blocked by pretreatment with zileuton or montelukast [an inhibitor of the type 1 receptor for cysteine leukotrienes (CysLT1, R)] (25). We now demonstrate that ptges−/− mice also exhibit hyperresponsiveness to LTE4 and that exogenous LTE4, like aspirin, induces the release of MC-derived mediators. Remarkably, the CysLT-driven MC activation pathway hinges critically on the alarmin-like cytokine IL-33, which is markedly overexpressed in the lung epithelial cells of ptges−/− mice, as well as in nasal polyp epithelial cells from patients with AERD. Deletion of LTC4S, which eliminates CysLT synthesis in the ptges−/− mice, protects mice from pulmonary eosinophilic inflammation and markedly attenuates the overexpression of IL-33 in the lung. Thus, cysteine leukotriene-amplified innate type 2 immunity contributes prominently to AERD, and IL-33 mediates a novel LTE4-induced pathway for MC activation.

Materials and Methods
Patient characterization
Nonsmoking patients (18–70 y old) were recruited at Brigham and Women’s Hospital (Boston, MA). Nasal polyp and sinus tissue were collected during surgical excision from subjects with AERD or from AT control subjects with chronic hyperplastic sinusitis. AERD was suspected based on asthma, nasal polyposis, and a history of respiratory reaction upon ingestion of a COX inhibitor, and confirmed with a graded oral challenge to aspirin that resulted in characteristic sinonasal symptoms and/or a decrease in forced expiratory volume in 1 s of at least 15%. AT control subjects had taken aspirin or a nonsteroidal anti-inflammatory drug within the previous 6 mo without an adverse reaction. All subjects had been treated with oral prednisone (20 mg daily) for the week leading up to their sinus surgery. The Institutional Review Board approved the study, and all subjects provided written consent.

Polyp procurement and preparation
Portions of excised sinonasal tissue were transferred into Celllytic M Cell Lysis Reagent (Sigma-Aldrich, St. Louis, MO) with 2% protease inhibitor (Roche, Indianapolis, IN) for protein extraction. Tissue was homogenized using a gentleMACS DISSOCIATOR (Miltenyi Biotec, San Diego, CA). The supernatants were stored at −80°C until sectioning. Sections of 10-μm thickness were freshly cut, thaw-mounted onto slides, and stored at −80°C until stained. For some patients, a tissue segment was also placed in media containing 5% FBS and stored with a straight razor blade and then digested with 400 U/ml type IV collagenase (Worthington Biochemical Corporation) and 200 μg/ml DNase (Sigma-Aldrich). The resulting suspension was passed through a 70-μM filter to retrieve a single-cell suspension for flow-cytometric sorting. These cells were stained with mAbs against CD45, CD59, epithelial cell adhesion molecule (EpCAM), and CD31 (BD Biosciences) and were sorted into purified cell populations with a BD FACSARia Fusion Cell-Sorter to collect tissue fibroblasts (CD45+/EpCAM+/CD31+/CD90−), epithelial cells (CD45+/EpCAM+/CD31+/CD90−), and endothelial cells (CD45−/EpCAM+/CD31+/CD90−) separately.

FIGURE 1. Expression of IL-33 protein in nasal polyps. (A) Western blotting of nasal polyp proteins from four subjects with AERD and four CHES controls showing full-length (30 kDa) and processed (25, 18–21 kDa) forms of IL-33. (B) Quantitative densitometry of IL-33 18- to 21-kDa IL-33 signals, corrected for GAPDH. The densitometry results are from eight samples per clinical group, including the four from each group depicted in (A). (C) Tyramide-amplified anti–IL-33 immunofluorescent staining of nasal polyps from subjects with AERD and with AT chronic hyperplastic eosinophilic sinusitis. Specific immunofluorescence is shown in cells localizing to the basal layer of the epithelium (short arrows). Nonspecific staining with tyramide (larger arrows) appears in both specimens. Original magnification ×400. *p < 0.05, ***p < 0.001.
Western blot analysis

Western blots were prepared as previously described (26) and probed with primary anti-IL-33 (R&D Systems, Minneapolis, MN) or anti-GAPDH (Cell Signaling Technology, Danvers, MA) Abs, washed, and then incubated with HRP-conjugated anti-rabbit IgG (Sigma-Aldrich) and visualized by ECL (GE Healthcare). The densities of the bands corresponding to full-length and proteolytically cleaved IL-33 (∼30 and ∼18 kDa, respectively) were measured in each lane using Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA). The densities were divided by the densities of GAPDH for the same lane. Results were presented as the corrected expression for at least 10 mice/group.

ELISA

Lungs were homogenized 24 h after the final dose of PBS or Df. Total IL-33 content was measured with a commercial ELISA (R&D Systems, Minneapolis, MN) and corrected for the protein content of each sample.

Quantitative PCR

RNA was extracted from the nasal tissue specimens or from the sorted cell populations with Tri Reagent (Sigma-Aldrich) and converted to cDNA using the RT² First Strand Kit (Qiagen, Valencia, CA). The expression of IL-33 was examined using RT² SybrGreen quantitative PCR (qPCR) Master Mix (Qiagen). Expression levels of transcripts were normalized to the expression of GAPDH (all primers from Qiagen).

Mice

C57BL/6 mice lacking mPGES-1 (ptges²/²) mice were from Dr. Shizuo Akira (Osaka University, Osaka, Japan) (27). The mice were intercrossed with ltc4s²/² mice (28) to generate double knockouts. All of the mice and wild type C57BL/6 controls were housed at Charles River (Wilmington, MA). Six- to 8-wk-old male mice were used. All animal studies were approved by the Animal Care and Use Committee of the Dana-Farber Cancer Institute (protocol 03-042). Airway inflammation was induced as described elsewhere (25). Mice were studied 24 h after the last of six intranasal treatments with Df (5 μg).

Reagents

Mice were treated with saline or Df obtained from Greer Laboratories (XPB81D3A25; Lenoir, NC). Montelukast was obtained from Brigham and Women’s Hospital Pharmacy. The mouse MC protease 1 (mMCP-1) enzyme immunoassay kit was purchased from eBioscience (San Diego, CA). Histamine, PGE₂, thromboxane B₂, and cysLT enzyme immunoassay kits were from Cayman Chemical (Ann Arbor, MI).

Measurement of lung resistance

Lung resistance (Rₐ) in response to Lys-ASA was assessed with an Invasive Pulmonary Function Device (Buxco, Sharon, CT). In brief, mice were anesthetized 24 h after the last Df challenge, and a tracheotomy was performed. After allowing for Rₐ to reach a stable baseline, Lys-ASA (12 μl of 100 mg/ml) was delivered to the lung via nebulizer, and Rₐ was recorded for 45 min. In some experiments, mice were challenged with escalating doses of LTC₄, LTD₄, or LTE₄. Because each cysLT elicited its peak response at 0.1 nmol/mouse, this dose was chosen for subsequent experiments. The results were expressed as percentage change of Rₐ from baseline. Some mice were treated with montelukast (6.7 μg/ml in drinking water by mouth 24 h before Lys-ASA) or SQ29.548 (50 mg/mouse i.p. 24 h before Lys-ASA). Goat anti-mouse IL-33 Ab (3.6 μg/mouse; R&D Systems, Minneapolis, MN) or recombinant mouse ST2-Fc fusion protein (5 μg/mouse; R&D Systems) (29) were given i.p. at 24 h before Lys-ASA or LTE₄ challenge. The same amount of normal goat IgG (R&D Systems) and recombinant human IgG1 Fc (R&D Systems) were used as controls.

Immunohistologic analysis

Frozen sections of human nasal polyps were stained with rabbit IgG or rabbit anti-human IL-33 (HPA 024426; Sigma) and developed with HRP-goat anti-rabbit IgG and Alexa Fluor 594 tyramide (T-20925; Life Technologies). Immunohistologic staining for IL-33 protein (left panel) or isotype control (right panel) in the lungs of Df-treated ptges²/² mice. Epithelial staining is indicated by arrows. Original magnification ×100. Results are representative of at least 10 mice in two separate experiments. *p < 0.05, **p < 0.01.
Technologies). Other sections were incubated in Alexa Fluor 594 tyramide alone to control for nonspecific binding of tyramide. For mouse tissue, the left lungs were fixed in 10% neutral-buffered formalin for 24 h and embedded in paraffin blocks. Tissue sections were deparaffinized and rehydrated. Endogenous peroxidase was inhibited by incubation with freshly prepared 3% H$_2$O$_2$ with 0.1% sodium azide. Rat anti-mouse IL-33 mAb (MAB3626; R&D Systems) was applied to the sections, and incubations were carried out for 1 h at room temperature. Ab binding was detected with rat-on-mouse HRP micropolymer detection system (Biocare Medical) and visualized with DAB chromogen. Slides were counterstained with Gill’s No. 2 hematoxylin, dehydrated, and mounted.

Statistical analysis

Data are expressed as ±SEM from at least 10 mice from at least two experiments, except where otherwise indicated. Analyses were performed with Prism software (GraphPad). Differences between two treatment groups were assessed using Student t test, and differences among multiple groups were assessed using one-way ANOVA and Bonferroni post hoc test. A p value <0.05 was considered statistically significant.

Results

IL-33 is strongly expressed in respiratory tissues from subjects with AERD and ptges$^{-/-}$ mice

To determine whether IL-33 might contribute to tissue pathology in AERD, we examined surgically excised nasal polyps obtained from carefully phenotyped subjects with and without AERD for evidence of IL-33 expression. Western blots of whole polyp lysates from subjects with AERD displayed strong expression of IL-33 protein. Both the ~30 kDa unprocessed form and processed forms of 18–21 and ~25 kDa were detected (Fig. 1A). Both the full-length form and the 18- to 21-kDa forms were more abundant in polyps from subjects with AERD than from control subjects (Fig. 1B). IL-33 protein was present in the basal layer of epithelial cells in the nasal polyps from patients with AERD, but not from AT control subjects with nasal polyposis (Fig. 1C). The staining appeared primarily cytosolic. qPCR analysis of cells sorted from nasal polyps confirmed the presence of IL-33 transcript in the EpCAM$^+$ epithelial cells from five of five samples tested, and in four of five samples of CD90$^+$ fibroblasts and four of five samples of CD31$^+$ endothelial cells sorted from the same polyps (data not shown).

To determine whether IL-33 was also overexpressed in the lungs of AERD-like ptges$^{-/-}$ mice, we performed ELISA for total IL-33 protein on lysates of whole lungs of naive and Df-treated WT and ptges$^{-/-}$ mice. We also performed Western blots to detect the presence of the full-length and cleaved IL-33 isoforms. Low levels of IL-33 protein were detected in the lungs of the naive mice. These levels increased significantly in response to Df and were markedly greater in the lungs of Df-treated ptges$^{-/-}$ mice than in WT controls (Fig. 2A). Both the

![Figure 3](http://www.jimmunol.org/DownloadedFrom/411822183.png)

**FIGURE 3.** Effect of LTC4S deletion on IL-33 expression and attendant physiologic effects. (A) BAL fluid total cell counts and eosinophil counts in the lungs of ptges$^{-/-}$ and ptges$^{-/-}$/ltc4s$^{-/-}$ mice measured 24 h after the last of six doses of PBS or Df. (B) ELISA of total IL-33 protein levels in lung lysates from the indicated strains. (C) Western blotting of lung proteins from the indicated strains showing full-length and cleaved forms of IL-33. (D) Quantitative densitometry showing effect of ltc4s deletion from ptges$^{-/-}$ mice on lung IL-33 protein levels. (E) BAL fluid levels of MC granule-derived mediators from the indicated groups. Results in (A), (B), (D), and (E) are mean ± SEM from at least 10 mice/group in two separate experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
full-length protein and proteolytically processed fragments (including the active 18-kDa fragment) were detected by Western blot and were more abundant in the lungs of the Df-treated ptges−/− mice than controls (Fig. 2B, 2C). Immunohistochemistry revealed prominent cytosolic staining of bronchial epithelial cells for IL-33 protein in the lungs of ptges−/− mice (Fig. 2C, arrows).

*CysLT-deficient mice are protected from upregulation of IL-33 and basal MC activation*

Persistent high-level cysLT generation is a hallmark of AERD that distinguishes it from AT asthma (3). This feature is recapitulated by Df-treated ptges−/− mice (25). To determine whether endogenous cysLTs might drive IL-33 overexpression, we intercrossed ptges−/− mice with mice that lack LTC4S (ltc4s−/− mice) (28) to generate double-knockout ptges−/−/ltc4s−/− mice. The resultant ptges−/−/ltc4s−/− mice and ptges−/− controls were treated with Df on six occasions. Compared with the lungs of the Df-treated ptges−/− mice, the ptges−/−/ltc4s−/− mice displayed reduced levels of bronchoalveolar lavage (BAL) fluid total cells and eosinophils 24 h after the final exposure to Df (Fig. 3A). The lungs of the ptges−/−/ltc4s−/− mice also contained substantially less IL-33 protein than did the lungs of the ptges−/− controls (Fig. 3B), with reductions in both the 30- and 18-kDa bands (Fig. 3C, 3D). Df treatment of the ptges−/− mice modestly increased the BAL fluid levels of histamine and mMCP-1 compared with PBS-treated controls. These increases were absent in the BAL fluids of Df-treated ptges−/−/ltc4s−/− mice (Fig. 3E).

**FIGURE 4.** Role of IL-33 in Lys-ASA–induced changes in lung function and MC activation. (A) Effect of blocking IL-33 or ST2 on pulmonary responses to Lys-ASA challenge. Ptges−/− mice were challenged with Lys-ASA by inhalation 24 h after the last of six intranasal doses of Df. Rl was monitored continuously for 45 min, and the peak change from baseline was recorded for each mouse. The indicated groups received single i.p. doses of rat anti-mouse IL-33, IgG isotype control, recombinant ST2-Fc fusion protein, or Fc control. (B) Levels of BAL fluid mMCP-1, histamine, and PGD2 in BAL fluids from the same mice. Results are from 10 mice/group in two separate experiments. *p < 0.05, **p < 0.01, ***p < 0.001.

**IL-33 is responsible for MC activation and changes in lung function**

IL-33 can directly activate human MCs to generate cytokines in vitro (30), and it elicits systemic MC-dependent anaphylaxis and IgE synthesis in vivo when injected into naive mice (31). To determine whether IL-33 was directly involved in the airway physiology changes and MC activation occurring in response to Lys-ASA, we administered a blocking Ab against IL-33, a soluble ST-Fc fusion protein, a control IgG, or a control Fc protein to separate cohorts of Df-treated ptges−/− mice before inhalation challenges with inhaled Lys-ASA. Both anti–IL-33 and anti-ST2 completely blocked the changes in Rl occurring in response to Lys-ASA (Fig. 4A). Both blockers also prevented the increases in BAL fluid, mMCP-1, histamine, and PGD2 (Fig. 4B).

**LTE4 is responsible for IL-33–dependent MC activation and airflow obstruction**

The administration of either zileuton or montelukast to ptges−/− mice blocked Lys-ASA–induced changes in Rl in our previous studies (25), indicating that their airways were sensitive to contractile effects of endogenous cysLTs. These drugs also blocked the release of MC products, implying that the endogenous cysLTs in this model were required for MC activation. To determine which cysLT was most responsible for MC activation and consequent physiologic effects, we directly administered LTC4, LTD4, and LTE4 to anesthetized, sedated, and mechanically ventilated WT and ptges−/− mice, with or without prior...
exposure to six intranasal doses of Df to elicit airway inflammation. R₉ was monitored directly for 45 min. Df-treated ptges⁻/⁻ mice displayed significant increases in R₉ after inhalation challenges with LTE₄ and LTD₄, but not with LTC₄, when compared with WT controls (Fig. 5A). The increases in R₉ in response to LTE₄ tended to exceed those to LTD₄ and approached ~75% of the magnitude of the response to Lys-ASA, administered as a positive control. Compared with vehicle-challenged controls, BAL fluids from LTD₄- and LTE₄-challenged ptges⁻/⁻ mice displayed increased quantities of mMCP-1, PGD₂, and histamine (Fig. 5B). In contrast, cysLT challenges failed to elicit increases in MC products in the BAL fluids of WT control mice (data not shown).

To determine whether LTE₄, like Lys-ASA, caused MC activation and bronchoconstriction by inducing the release of IL-33, we treated mice with anti–IL-33 or ST2-Fc and corresponding controls 24 h before inhalation challenges with LTE₄. Treatment of the ptges⁻/⁻ mice with either anti–IL-33 or ST2-Fc blocked the increases in R₉ (Fig. 6A) and eliminated the increases in MC-derived products in the BAL fluid (Fig. 6B).

LTE₄-induced changes in R₉ and cell activation are montelukast sensitive and depend partially on MC-derived PGs

We used a pharmacologic approach to identify the receptors required for LTE₄-mediated changes in R₉ and to determine whether these responses were direct or whether they involved the contractile effects of LTE₄-elicited PGs as reported in humans (32, 33). Before inhalation of LTE₄, Df-treated ptges⁻/⁻ mice were given the CysLT₁R antagonist montelukast overnight in their drinking water. Because LTE₄ elicited the release of PGD₂, which requires thromboxane prostanoid (TP) receptor signaling to elicit smooth muscle constriction (34), we treated additional mice with a single i.p. dose of the TP receptor antagonist SQ29,548. Montelukast treatment and SQ29,548 both blocked the LTE₄-mediated increase in R₉ (Fig. 6C). Montelukast also tended to block the modest increase in R₉ induced by LTD₄, although the narrow window in these experiments precluded statistical significance (Supplemental Fig. 1).

Discussion

Chronic severe eosinophilic respiratory tract inflammation, baseline overproduction of cysLTs, and hyperresponsiveness to LTE₄ are all features of AERD. The administration of nonselective COX inhibitors elicits explosive cysLT generation and MC activation that are both blocked by the administration of the 5-LO inhibitor zileuton (19). The fact that AERD often occurs in nonatopic hosts (16), some of whom nevertheless show significantly increased total serum IgE (17) and all of whom display marked eosinophilic tissue inflammation, suggests that the disease involves immune mechanisms that are distinct from allergen-specific adaptive type 2 immune responses. Whereas IL-33 is released as an alarmin from necrotic cells (35), it is also released in a controlled manner from epithelial cells in response to danger signals from viruses (36), fungi (37), and helminths (38). IL-33 acts at the IL-1R-like protein ST2 to activate both myeloid and lymphoid innate effector cells, resulting in the production of cytokines (IL-5, IL-13, IL-9) that drive eosinophil-rich pathology, either independently of adaptive immunity (39, 40) or in concert with conventional allergen-driven Th2 responses (41). MCs express ST2 and can be activated by IL-33 in vivo, either in response to direct systemic administration (31) or in models of fungal protease challenge (37) and cellular damage (42). Although IL-33 is strongly expressed in both refractory nasal polypsis (43) and severe asthma (44), no previous study had addressed the potential role of IL-33 in the pathogenesis of AERD. In this study, we used complementary approaches in humans and mice to determine the potential role of IL-33 in disease pathophysiology, particularly in the enigmatic, 5-LO–dependent MC activation that occurs characteristically in response to challenges with aspirin in AERD.

We first surveyed surgically excised nasal polyps for IL-33 expression. Whole nasal polyp lysates from subjects with AERD contained substantially more IL-33 full-length and cleaved IL-33.
protein than did AT controls (Fig. 1A, 1B). The lower molecular mass species identified on the Western blot (∼25 and 18–21 kDa, respectively) are consistent with the sizes of the fragments generated by cleavage of full-length IL-33 by MC- and neutrophil-derived proteases (45, 46). These fragments are ∼30-fold more active than the full-length form in vitro and in vivo. Total IL-33 protein was markedly increased in the lungs of the Df-treated ptges<sup>2/2</sup> mice relative to controls (Fig. 2A). Both full-length and the 18-kDa cleaved IL-33 protein were detected by Western blots (Fig. 2B), and both were more abundant in the lungs of AERD-like ptges<sup>−/−</sup> mice than in WT controls (Fig. 2C). In both AERD nasal polyps (Fig. 1C) and in the lungs of Df-treated ptges<sup>−/−</sup> mice, (Fig. 2C), IL-33 protein localized primarily to epithelial cells, and qPCR analysis of sorted nasal polyp cells suggested additional expression by both fibroblasts and endothelial cells. The robust expression of IL-33 (and the presence of bioactive forms) is consistent with a potential role for IL-33 in particular (and innate type 2 immunity in general) in driving the eosinophil-rich pathology that is typical of AERD.

In mice, cysLTs contribute to the induction and amplification of eosinophilic pulmonary inflammation through several mechanisms, including CysLT1R-mediated priming of DCs for Th2 responses (47) and type 2 cysLT receptor–mediated upregulation of endothelial adhesion receptor expression via platelets (48). Accordingly, mice lacking LTC<sub>4</sub>S, the terminal enzyme necessary to generate cysLTs, are protected from Th2-type sensitization and pulmonary eosinophilia induced by Df (47). LTC<sub>4</sub>S in AERD is highly expressed by eosinophils (8) and by platelets that adhere to granulocytes with high frequency in both the blood and the respiratory tissue (9). Remarkably, the deletion of LTC<sub>4</sub>S from ptges<sup>−/−</sup> mice not only abrogated cysLT generation in the lung as expected (data not shown), but attenuated Df-induced pulmonary eosinophilia (Fig. 3A), IL-33 expression (Fig. 3B–D), and the increases in basal BAL fluid levels of MC activation markers (mMCP-1, histamine) (Fig. 3E). Thus, cysLTs act upstream of IL-33 in this model to drive sustained eosinophilic inflammation and associated basal activation of MCs. The ability to amplify IL-33 expression may be an additional mechanism by which cysLTs may contribute to type 2 immunity and inflammation.

While promoting tissue eosinophilia by inducing cytokine generation from lymphoid cells (49, 50), IL-33 can also induce full activation of MCs (37), including systemic anaphylaxis (31), when released in response to environmental danger signals. The fact that endogenous cysLTs were involved in the basal expression, release, and processing of IL-33 in the lungs of ptges<sup>−/−</sup> mice (Fig. 3) led us to postulate that the increases in cysLT production might result in incremental IL-33–driven MC activation and airflow obstruction during provocative challenges with Lys-ASA. Indeed, short-term blockade of either IL-33 (with an mAb) or ST2 (with a fusion protein of soluble ST2 with Fc) markedly attenuated the Lys-ASA–induced change in R<sub>t</sub> (Fig. 4A) and prevented the increases in MC-derived products,
including PGD₂, histamine, and mMCP-1 (Fig. 4B). We verified that IL-33 was the end effector of the cysLTs released during the reaction by challenging Df-primed WT and pgts⁻/⁻ mice by inhalation of cysLTs. LTE₄ (the most biologically stable and abundant of three cysLTs), and to a lesser extent LTD₄, replicated the changes in Rₑ (Fig. 5A) and MC activation (Fig. 5B) elicited by Lys-ASA. Remarkably, the effects of LTE₄ on Rₑ and mediator release were also abrogated by blockade of IL-33 or ST2 (Fig. 6A, 6B). Thus, the cysLT-driven mechanism of airflow obstruction and MC activation in AERD (19) and pgts⁻/⁻ mice (25) may be due to incremental release and actions of IL-33 in response to endogenous LTE₄. The deficiency in PGE₂ is permissive for both cysLT overproduction and IL-33–driven hyperresponsiveness to LTE₄.

Although LTE₄ is the weakest bronchoconstrictor among the three cysLTs, it is disproportionately potent as a constrictor in the airways of subjects with asthma (51) and even more so in AERD (5). LTE₄ is also the only cysLT that can induce the accumulation of eosinophils and basophils in the bronchial mucosa when inhaled by mild asthmatics (52). It is tempting to speculate that these findings reflect the actions of LTE₄–elicited IL-33 on mCs and other components of the innate type 2 immune system. Although LTE₄ is a weak agonist at CysLT₁R in transfected cells, its effects in our study were blocked completely by the selective CysLT₁R antagonist montelukast (Fig. 6C). The administration of zafirlukast (another CysLT₁R antagonist) blocked LTE₄–induced bronchoinflammation and airway eosinophilia in AT asthmatic subjects in a previous study (52). It is possible that the magnitude of the LTE₄ effect (relative to the negligible LTC₄ effect and the modest LTD₄ effect) reflects the comparative inappropriateness of LTE₄ to elicit signaling through type 2 cysLT receptor, which inhibits CysLT₁R signaling in some contexts (53, 54).

Early studies had suggested that some actions of LTE₄ on human bronchi depended on endogenous COX products that signal through TP receptors (32). The blocking effect of the TP receptor antagonist SQ29,548 in our model (Fig. 6C) suggests that the airflow obstruction consequent to MC activation is at least partly attributable to PGD₂, which requires TP receptors to elicit smooth muscle contraction (55).

Our study suggests that IL-33 is a prominent effector of AERD that bridges the upstream production of the cysLTs to the idiosyncratic MC activation typical of the disorder. CysLTs may drive persistent tissue eosinophilia and MC activation in response to COX-1 inhibition by driving the expression and release of IL-33. High-dose aspirin, which produces substantial symptomatic improvements and reduces recurrence rates for nasal polyps in AERD, does not decrease systemic levels of cysLTs (56), but eliminates the selective hyperresponsiveness to LTE₄ (7). The latter effect could reflect the capacity of high-dose aspirin to deplete TP-active PGs (57). Our study also suggests that AERD, in which eosinophilic inflammation and MC activation are prominent despite a clear association with atopy, is largely driven by innate type 2 immunity. Drugs that block IL-33 functions may have therapeutic applications in AERD.

Disclosures

The authors have no financial conflicts of interest.

References


Downloaded from http://www.jimmunol.org/ by guest on October 28, 2017
Supplemental Figure 1. Effect of Montelukast treatment on cys-LT-mediated changes in $R_L$ in *Df*-primed *ptges*<sup>−/−</sup> mice. Results are from 3 mice/group. *P = 0.01.