

Innate immune cell dysregulation drives inflammation and disease in aspirin-exacerbated respiratory disease



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Aspirin-exacerbated respiratory disease (AERD) is a complex inflammatory disorder that is not generally viewed as a disease involving the adaptive immune system but instead one largely driven by the innate immune system. This article focuses on the cellular dysregulation involving 4 central cell types: eosinophils, basophils, mast cells, and innate lymphoid type 2 cells. AERD can be envisioned as involving a self-perpetuating vicious circle in which mediators produced by a differentiated activated epithelial layer, such as IL-25, IL-33, and thymic stromal lymphopoietin, engage and activate each of these innate immune cells. The activation of these innate immune cells with their production of additional cytokine/chemokine and lipid mediators leads to further recruitment and activation of these innate immune cells. More importantly, numerous mediators produced by these innate immune cells provoke the epithelium to induce further inflammation. This self-perpetuating cycle of inflammation partially explains both current interventions suggested to ameliorate AERD (eg, aspirin desensitization, leukotriene modifiers, anti-IL-5/IL-5 receptor, anti-IL-4 receptor, and anti-IgE) and invites exploration of novel targets as specific therapies for this condition (prostaglandin D₂ antagonists or cytokine antagonists [IL-25, IL-33, thymic stromal lymphopoietin]). Several of these interventions

currently show promise in small retrospective analyses but now require definite clinical trials. (J Allergy Clin Immunol 2021;148:309-18.)

Key words: Aspirin-exacerbated respiratory disease, mast cells, basophils, eosinophils, innate lymphoid cells, type 2 inflammation, IgE

Aspirin-exacerbated respiratory disease (AERD) is a complex inflammatory disorder involving numerous cellular components of the immune system. Although a discussion of all the myriad of immune cells and cellular mechanisms by which these cells become dysregulated is beyond the scope of this review, in this article we will focus on cellular dysregulation within the submucosal compartment and, specifically, the overexpression and dysregulation of 4 central cell types: eosinophils, basophils, mast cells (MCs), and innate lymphoid type 2 (ILC2) cells (summarized in Table I).

EOSINOPHILS

Eosinophils are potent type 2 inflammatory granulocytes associated with various atopic and nonatopic conditions. In AERD, eosinophils are significantly elevated in the hyperplastic sinuses, nasal polyps (NPs), and lower airway when compared with healthy airway tissue.^{1,2} Likewise, levels of the eosinophil granule protein, eosinophil cationic protein (ECP), are also significantly elevated in NPs and lungs when compared with healthy controls.^{3,4} When assessing patients with NPs, studies are conflicting as to whether eosinophil numbers and ECP levels are similar^{2,3} or significantly elevated^{1,4} in AERD compared with aspirin-tolerant chronic rhinosinusitis (CRS) with NPs (CRSwNP). This is mirrored by studies that differ as to whether mediators important in eosinophil survival and *chemotaxis* (eg, IL-5 and CCL11 [eotaxin-1]) are similar in both conditions^{4,5} or are significantly elevated in AERD.^{3,6,7}

The reported differences in eosinophils likely reflect the underlying heterogeneous *endotypes* observed among patient populations. CRSwNP is *predominantly* a type 2 inflammatory process in the United States and Europe, but many patients can have a type 1, type 3, or mixed inflammatory endotype.^{8,9} Similarly, some patients with AERD have a non-type 2 inflammatory profile with enhanced numbers of neutrophils and reduced eosinophils.^{7,10,11} Higher levels of IFN- γ have also been reported in NPs of a subset of patients with AERD.^{7,12} Why certain patients develop a type 2 versus a non-type 2 inflammatory endotype remains unknown.

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Terms in boldface and italics are detailed in the glossary on page 310.

Abbreviations used

AERD: Aspirin-exacerbated respiratory disease
 CCS: Corticosteroid
 COX: Cyclooxygenase
 CRS: Chronic rhinosinusitis
 CRSwNP: Chronic rhinosinusitis with NPs
 CysLT: Cysteinyl leukotriene
 ECP: Eosinophil cationic protein
 ILC2: Innate lymphoid type 2 cell
 LTC₄: Leukotriene C₄
 MC: Mast cell
 NP: Nasal polyp
 NSAID: Nonsteroidal anti-inflammatory drug
 PGD₂: Prostaglandin D₂
 PGE₂: Prostaglandin E₂
 TSLP: Thymic stromal lymphopoietin

Eosinophils and lipid mediators

Another outstanding question in AERD is how (or even if) eosinophils contribute to disease pathogenesis. Eosinophils are a source of prostaglandin D₂ (PGD₂),¹³ and higher levels of the enzyme responsible for PGD₂ synthesis, PGD₂ synthase (PGDS), were reported in peripheral blood and NP eosinophils from patients with AERD.¹⁴ Eosinophils are also capable of producing cysteinyl **leukotrienes** (CysLTs), and the key enzyme needed for CysLT production, leukotriene C₄ (LTC₄) synthase, was expressed in a significantly greater proportion of eosinophils in bronchial biopsies of patients with AERD compared with aspirin-tolerant patients with asthma.¹⁵ Furthermore, eosinophils can produce even higher levels of LTC₄ when primed with IL-5 or CCL11 (eotaxin), cytokines known to be elevated in AERD compared with healthy sinonasal tissue.¹⁶ Steinke et al¹² additionally found that CD34⁺-derived eosinophils primed with IFN- γ release significantly higher levels of CysLTs following stimulation with **phorbol 12-myristate 13-acetate**/ionomycin than those differentiated in media alone. Recent studies have also explored a relationship between platelets and eosinophils and found an increase in numbers of platelet-adherent eosinophils in patients with AERD compared with aspirin-tolerant controls.^{17,18} These adherent platelets can contribute to an

overproduction of CysLTs through an augmented transcellular conversion of leukotrienes.^{17,18} Together, these findings suggest that eosinophils may be an important source of various lipid mediators known to be enhanced in AERD and thus could play a key role in driving disease pathogenesis.^{19,20}

Eosinophils and fibrin formation

In addition to their production of inflammatory mediators, eosinophils may play a role in fibrin formation. Fibrin is the end product following activation of the coagulation cascade and is elevated in NPs, serving to trap plasma proteins and promote mucosal edema.²¹ Takabayashi et al²² found that eosinophils in AERD NPs expressed higher levels of L-plastin than in CRSwNP. L-plastin assists with the translocation of **tissue factor** to the surface of eosinophils. Elevated levels of L-plastin in AERD could in turn promote subsequent downstream activation of the coagulation cascade and lead to enhanced fibrin formation. However, more work is needed investigating the associations between L-plastin, coagulation factors, and fibrin in AERD.

Eosinophils and inflammation

There are several other *in vitro* and *ex vivo* studies investigating eosinophils in CRSwNP that, although not performed in AERD specifically, provide insights into disease pathogenesis. For example, in patients with eosinophilic CRS, eosinophils were more activated in NPs than in blood as measured by intensity of CD69 surface expression.²³ Activated eosinophils can then release granule proteins such as ECP and eosinophil-derived neurotoxin, which in turn have cytotoxic activity. Eosinophil-derived neurotoxin also upregulates matrix metalloproteinase-9 expression in nasal epithelial cells, which could in turn contribute to tissue remodeling.²⁴ Finally, eosinophils are a major source of Charcot-Leyden crystal protein/galectin-10, which is elevated in NPs and can contribute to both innate and adaptive inflammatory responses.^{25,26} *In vitro* treatment of epithelial cells with Charcot-Leyden crystal induced neutrophil chemotaxis as well as an upregulation of several proinflammatory cytokines.²⁵ Taken together, eosinophils are activated within NPs and their products likely contribute to epithelial barrier dysfunction as well as the

GLOSSARY

ALARMINs: Molecules, such as cytokines, that are released from damaged or necrotic cells to induce immune responses.

AUTOCRINE SIGNALING: A form of cell signaling in which a cell releases a hormone or other soluble mediator that binds to receptors on the same cell.

CHEMOTAXIS: The movement of a cell in response to a concentration gradient of a particular substance.

EICOSANOIDS: Proinflammatory cell signaling lipid molecules that are derived from arachidonic acid. Examples of eicosanoids include prostaglandins, leukotrienes, and thromboxanes.

ENDOTYPES: A subtype of a disease condition that is caused by a distinct functional or pathophysiological mechanism.

Fc ϵ RI: The high-affinity receptor for the Fc region of IgE. Cross-linking of Fc ϵ RI with IgE leads to degranulation and inflammatory mediator release from mast cells and basophils.

LEUKOTRIENES: Soluble lipid mediators that are generated from arachidonic acid by lipoxygenase enzymes and serve as important inflammatory mediators.

METAPLASTIC TRANSFORMATION: The transformation from one differentiated cell type to another differentiated cell type.

PROSTAGLANDINS: Soluble lipid mediators that are generated from arachidonic acid by cyclooxygenase enzymes and help generate inflammatory responses.

SUPERANTIGENS: A class of antigens that cause nonspecific polyclonal T-cell activation and massive cytokine release, leading to excess adaptive immunity.

TISSUE FACTOR: A cell surface glycoprotein that initiates blood coagulation cascades by inducing the formation of thrombin.

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TABLE I. Innate immune cells in AERD

Cell types	Select mechanisms of activation in AERD	Select inflammatory products in AERD
Eosinophils	IL-5, IL-33 Chemokines: eotaxins (CCL11/24/26), RANTES (CCL5)	PGD ₂ , CysLTs EDN, ECP, MBP, EPX CLC/Galectin 10 IFN- γ L-plastin (fibrinogenesis)
MCs	IL-25, IL-33, TSLP Aspirin/NSAIDs IgE? CysLTs (LTE ₄)	PGD ₂ , CysLTs Tryptase Histamine
ILC2s	IL-25, IL-33, TSLP PGD ₂ , CysLTs	IL-4, IL-5, IL-13

CLC, Charcot-Leyden crystal; EDN, eosinophil-derived neurotoxin; EPX, eosinophil peroxidase; MBP, major basic protein.

generation of mixed inflammatory responses. However, future studies are needed to specifically reconfirm these observations in AERD.

Targeting eosinophils in AERD

Daily aspirin maintenance therapy following an aspirin desensitization is an effective treatment option for patients with AERD.²⁷ Cahill et al²⁸ found that eosinophils were significantly higher in the peripheral blood following 8 weeks of daily aspirin therapy than before aspirin desensitization. This leads to the hypothesis that aspirin therapy may improve sinonasal disease by preventing the recruitment of circulating eosinophils to NPs. However, a separate study found that patients with worsening clinical symptoms despite 4 weeks of daily aspirin therapy following desensitization had a significantly greater increase in peripheral blood eosinophils on therapy from baseline (210%) compared with patients who reported symptom improvement after 4 weeks of daily aspirin therapy (37%).²⁹ The mechanisms by which eosinophils increase in the circulation following aspirin therapy and the correlation with disease improvement (or worsening) over time warrants further investigation.

Corticosteroids (CCSs) remain the first-line medical treatment for patients with CRSwNP (and AERD) and are known to reduce eosinophils.^{30,31} However, it is unlikely that the clinical benefits of CCSs are predominantly mediated solely through the reduction of eosinophils. Instead, benefits are likely mediated through a combination of this and their other anti-inflammatory effects including reduced inflammatory mediator release, decreased mucus secretion, reduced microvascular leak, and decreased epithelial remodeling.

Further support for eosinophils contributing to clinical disease comes from clinical trials of mepolizumab in CRSwNP. Mepolizumab, which binds soluble IL-5, significantly reduced both peripheral eosinophils and NP size compared with placebo-treated controls.³² Although 17% of enrolled patients in this study had AERD, all received mepolizumab, so a *post hoc* analysis specifically examining patients with AERD could not be performed. Separately, a small retrospective single-site study of patients with AERD found that mepolizumab treatment was associated with improvement in nasal symptoms.³³ Interestingly, mepolizumab did not prevent clinical symptoms from developing during aspirin

desensitization.³⁴ These findings suggest that eosinophils play a role in overall disease pathogenesis but potentially not during acute reactions to aspirin.

However, there is also evidence arguing against eosinophils contributing to NP pathogenesis. In patients with CRSwNP, treatment with dexamipexole, a drug that depletes eosinophils by an unknown mechanism, led to a significant reduction in peripheral and NP eosinophils but produced no significant decrease in NP size or sinonasal symptoms.³⁵ In this study, only 3 patients (17%) had AERD, so effects of dexamipexole specifically in AERD could not be discerned. In a small case series examining 4 patients with AERD who had severe corticosteroid-dependent asthma, treatment with mepolizumab was associated with poor asthma control albeit the authors did not evaluate sinonasal symptoms in these same patients.³⁶ It is thus possible that targeting eosinophils may provide differential effects in the nose and lung, might not be effective in all patients with AERD, and that disease heterogeneity may exist among the population.

Finally, dupilumab is an mAb that targets the IL-4 receptor α -chain and thereby blocks the downstream signaling of IL-4 and IL-13, which is effective in patients with AERD and CRSwNP.^{37,38} Although not specifically directed toward eosinophils, dupilumab was associated with a reduction in ECP, CCL26 (eotaxin-3), and IL-5 levels in nasal lavage fluid after 24 weeks of treatment.³⁸ An increase in peripheral blood eosinophilia was noted with dupilumab, presumably reflecting the ability of dupilumab to inhibit the recruitment of eosinophils from the peripheral blood into NPs. However, patients with higher peripheral blood eosinophil counts (>300 cells/ μ L) did not have significantly better responses to dupilumab than patients with lower eosinophil counts (<300 cells/ μ L). It has therefore been argued that other type 2 inflammatory cells and pathways may serve as the main targets of dupilumab.

In summary, eosinophils are elevated in patients with AERD and, in general, serve as a biomarker predictive of more severe sinonasal disease.³⁹ *In vitro* studies have identified several mechanisms by which eosinophils could be contributing to pathogenesis, but *in vivo* studies have yet to conclusively confirm a specific role. In addition, most studies to date have focused on patients with CRSwNP, only a subset of which had AERD. As a result, it remains unclear whether eosinophils play a distinct role in AERD in comparison to CRSwNP or, instead, have similar roles in all forms of (eosinophilic) CRS.

BASOPHILS

Basophils represent another potent granulocyte subset that are important in various type 2 respiratory disorders. Basophils were recently shown to be elevated in both NPs and peripheral blood of patients with AERD when compared with patients with CRSwNP.⁴⁰ Interestingly, in this study, basophils in both AERD and CRSwNP were similarly activated, as noted by the intensity of their CD63 surface expression. However, NP basophils demonstrated more extensive degranulation in AERD than in CRSwNP as measured by their loss in intracellular 2D7⁺ granules. There was a significant correlation between the loss of 2D7⁺ expression in basophils and overall sinonasal disease severity. Furthermore, analysis of microparticles (submicron-sized shed membrane vesicles released from activated or injured cells) from nasal lavage

samples in patients with AERD demonstrated that basophils, MCs, and platelets were more highly activated in patients with AERD than in patients with CRS. These findings together suggest that basophils may be another important contributor to AERD pathogenesis.⁴¹

MCs

Numerous observations suggest that MCs play a central role in the pathogenesis of AERD. The pathology of AERD is characterized by an increased concentration of MCs in NP and bronchial tissue compared with aspirin-tolerant controls.⁴² Although some older studies failed to directly demonstrate this increase in MCs using immunohistochemistry, even these studies did reliably demonstrate an increase in MC-derived mediators—a phenomenon attributed to the inability to stain for activated, granule-depleted MCs (so-called phantom MCs).⁴³ This limitation of immunohistochemistry was effectively overcome using both flow cytometry and targeted single-cell RNA sequencing, approaches that definitively identified increased MC numbers in AERD NP tissue relative to tissue samples obtained from aspirin-tolerant patients with asthma and normal controls.⁴² These techniques have also been used to characterize distinct populations of MCs within NP tissue. Although a population of MCs unique to AERD was not found, local NP epithelial and subepithelial MC hyperplasia was more prominent in AERD NP when compared with non-AERD NP.⁴⁴

This increased MC burden occurs concomitantly with local accumulation of MC mediators, most notably CysLTs and *prostaglandins*, lipid mediators that, as previously noted, are critical to the pathogenesis of AERD. The constitutive overproduction of CysLTs in the resting state and the surge in CysLT production in response to nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) is pathognomonic for AERD.^{43,45} These elevated levels of CysLT in AERD reflect the increased expression of 5-lipoxygenase and leukotriene C₄ synthase in inflammatory cells including MCs, with, as previously noted, additional contributions from eosinophils and basophils.⁴⁶ Although these enzymes are expressed in several cell types, the elevation of other MC activation products, including tryptase, histamine, and PGD₂, as well as the surge of PGD₂ and tryptase on NSAID challenge, suggests an important role for MCs.⁴⁷ Attenuation of the bronchoconstriction associated with NSAID reactions by MC stabilizers further supports a central causative role for MC activation.⁴⁷

IgE and MC activation in AERD

The mechanism of MC activation in AERD, however, is complex and is likely mediated by both IgE-dependent and -independent mechanisms. As is discussed in more detail elsewhere in this issue, levels of IgE in NPs of patients with AERD are higher than in aspirin-tolerant individuals, and this includes both *FcεRI*-bound IgE (on MCs, basophils, and others) and also evidence for IgE being newly synthesized in the AERD tissue.⁴⁸ The driving factors and targets of local IgE production are unclear but include IgE directed against the *Staphylococcus aureus* enterotoxins that are prevalent in the CRS tissue. As many as 87% of patients with AERD are colonized with *S aureus*, and levels of *Staph* superantigen-specific IgE are increased in AERD. Reflecting their role as both antigens (targeted by the IgE) and also as *superantigens* that drive nonspecific T- and B-

lymphocyte activation, *S aureus*-derived superantigens induce proliferation of polyclonal IgE within the polyp tissue, leading to chronic MC activation. There is a direct relationship between the level of IgE in polyp tissue and the speed of NP recurrence after surgery, further supporting the concept that local IgE contributes to the pathogenesis of AERD.^{49,50} What is more controversial is the role of aeroallergens in AERD and, more specifically, aeroallergen-targeted IgE in driving MC activation. Although AERD is associated with a high prevalence of sensitization to aeroallergens, it is unclear whether this is just the coincidental occurrence of allergic rhinitis in this condition or whether these aeroallergens play a direct pathogenic role. A role for inhaled aeroallergens seems implausible given the incapacity of these agents to access the sinonasal mucosa, reflecting the usual severity of the extensive sinus hyperplastic tissue, occlusion of the sinus ostia, and severe nasal polyposis that categorize this condition. And although treatment of the allergic rhinitis as part of a comprehensive therapeutic approach to patients with AERD is recommended, further evidence against a meaningful causative role for aeroallergens is derived from the simple observation that an identical phenotype can develop in nonatopic individuals.

IgE-independent mechanisms and AERD

In addition to IgE, MC activation in AERD involves numerous IgE-independent mechanisms. One such mechanism reflects the ability of aspirin and NSAIDs themselves to directly trigger MC activation.^{51,52} In these studies, both aspirin and NSAIDs directly stimulated MCs to initiate degranulation and arachidonate metabolic pathways.

Interestingly, and suggesting a feed-forward proinflammatory cascade, in addition to their being a prominent MC product, CysLTs act in an autocrine fashion to induce further MC activation (*autocrine signaling*). Cysteinyl leukotriene receptors are expressed on MCs, and the direct effects of these CysLTs on MC activation have been demonstrated including via engagement of a recently described LTE₄-specific receptor.⁵³⁻⁵⁵

In addition, decreased PGE₂ signaling on the anti-inflammatory EP2 receptor found on MCs is important for MC activation in AERD.³ NSAIDs decrease the production and release of PGE₂ from inflammatory cells. In combination with the baseline decreased expression of EP2 observed in AERD, the decrease in PGE₂, in response to ingestion of NSAIDs, releases the constraint that PGE₂/EP2 signaling provides and drives the surge in MC (and eosinophil) activation.^{56,57} This role for PGE₂ has been verified by the ability of PGE₂ homologs to block reactions to NSAIDs in patients with AERD.⁵⁸

Lastly, innate cytokines, most notably IL-33, and thymic stromal lymphopoietin (TSLP) have been implicated in non-IgE-mediated MC activation.⁵⁹ This has been best studied with regard to IL-33, which can stimulate both eicosanoid generation and type 2-cytokine production by MCs.⁶⁰

Together, these observations regarding MC behavior can explain the paradoxical contrasting actions of aspirin in AERD. Thus, initially aspirin (and other NSAIDs) drives the anaphylactoid response via inhibition of PGE₂ expression and loss of constraints on MCs, leading to the observed surge of MC-derived PGD₂ and tryptase. But longer-term administration of high-dose aspirin after desensitization leads to decreased cyclooxygenase (COX) activity, thereby blocking both IL-33-associated COX-1-dependent MC activation and COX-2-dependent generation

of PGD₂. MC-derived PGD₂ is a central mediator in AERD through its importance in the chemotaxis and activation of eosinophils, basophils, T_H2 lymphocytes, and, in particular, ILC2s, as discussed below.¹⁹ This putative importance of PGD₂ is supported by observations that the clinical improvement in AERD after aspirin desensitization correlates with a striking decrease in expression of MC (and eosinophil)-derived PGD₂.²⁸ Although CysLT production increases after aspirin desensitization, end-organ reactivity, at least to LTE₄, is drastically attenuated after aspirin desensitization, arguing that CysLTs and PGD₂ are important in the pathophysiology of AERD.⁶¹

In addition to a putative role in targeting MCs as the mechanism of aspirin desensitization, additional arguments supporting a central role for MCs in AERD are derived from experience with biologics. Omalizumab improves sinonasal symptoms in AERD, and this occurs in association with decreases in the urinary PGD₂ metabolite and LTE₄ levels.⁶²⁻⁶⁴ This argues not only for a role for MCs in AERD but presumably also for MC- (and basophil)-associated IgE, albeit IgE that is directed against an unknown antigen. In addition, mepolizumab and dupilumab, as previously noted, have both demonstrated benefit in AERD.^{33,37} Although both agents target tissue eosinophilia, dupilumab, unlike mepolizumab, has also been shown to target local IgE-producing cells in NPs to inhibit local IgE production.^{37,65} And dupilumab has direct inhibitory effects on MCs as well. The argument that the beneficial effects of these biologics reflect their targeting of IgE-MCs rather than tissue eosinophilia in AERD includes the dexamipexole study discussed above. Interestingly, this treatment although largely eliminating tissue eosinophils was associated with increases in MC numbers.³⁵ Finally, imatinib, an MC-depleting tyrosine kinase inhibitor, was shown to improve lung function and airway reactivity in a population of patients with severe asthma, and this occurred in association with MC depletion. This observation invites investigation into the benefits of imatinib as a therapeutic option in AERD.⁶⁶

GROUP 2 INNATE LYMPHOID CELLS

ILC2s are a recently discovered subset of lymphocytes that lack T-cell receptors and represent the innate counterparts to adaptive T_H2 cells with similar transcription factors and cytokines.⁶⁷ Airway ILC2s have been implicated in AERD as they are recruited to the airway in patients with AERD and are modulated by lipids and cytokines central to AERD pathogenesis.⁶⁸ During COX-1 inhibitor challenge/desensitization in patients with AERD, accumulation of ILC2s in the nasal mucosa occurred along with concurrent reduction of ILC2s in the blood, suggesting that ILC2s migrate from the blood to the nose in the setting of COX-1 inhibition in AERD.⁶⁸ Nasal ILC2s also correlated with increased reaction severity, and similar to other studies, elevated urinary PGD₂ metabolite and LTE₄ concentrations were found at the time of reactions. Higher ILC2 levels are also detected in eosinophilic NPs compared with noneosinophilic polyps and other tissues.⁶⁹⁻⁷¹ Recently, ILC2s from the polyps of those with CRS have shown increased cytokine expression and loss of steroid responsiveness compared with blood ILC2s.⁷² Thus, ILC2s are well positioned in the airways of patients with AERD with eosinophilic nasal polyposis to propagate the type 2 inflammation that is characteristic of AERD.

ILC2s in type 2 inflammation

Similar to adaptive CD4⁺T_H2 cells, ILC2s express the transcriptional regulator GATA-3 and when stimulated, produce IL-4, IL-5, and IL-13, which leads to tissue eosinophilia, mucus production, airway hyperresponsiveness, and promotion of adaptive T_H2 and IgE responses.⁶⁷ Animal models have shown that ILC2s are critical to lung eosinophilia, mucus production, and hyperresponsiveness in response to viruses and allergens, independent of T and B cells (reviewed in Doherty⁷³). Human studies have further validated that airway ILC2s are increased in patient samples from those with eosinophilic nasal polyposis⁶⁹⁻⁷¹ and oral steroid-dependent asthma.^{74,75} Because AERD is considered an antigen-independent disorder with dysregulated eicosanoid pathways, ILC2s that are activated by cytokines and lipids (and not directly by antigens) could promote the eosinophilic CRSwNP and moderate to severe asthma in AERD.

ILC2 regulation by lipid mediators

ILC2s are activated by lipid mediators including PGD₂ and CysLTs as well as by innate cytokines known as “alarmins,” which include IL-33, IL-25, and TSLP.^{67,76} Importantly, stimulation of ILC2s with CysLTs or PGD₂ potently induces secretion of IL-4, in addition to IL-5 and IL-13, compared with IL-33, which induces minimal IL-4 production from ILC2s.⁷⁷⁻⁷⁹ Combined lipid mediator and cytokine stimulation, as is present in type 2 inflammation, further enhances ILC2 activation compared with individual mediators by themselves.^{78,80,81}

Leukotriene pathway dysfunction in AERD has been well studied, and high production of CysLTs that include LTC₄, LTD₄, and LTE₄ has been demonstrated. Patients with AERD display higher levels of nasal 5-lipoxygenase and LTC₄ and, as previously noted, have elevated baseline urinary levels of LTE₄ as well as LTB₄.^{45,82} First discovered in mice, ILC2s were shown to express CysLT1Rs and are potently activated by LTD₄ *in vivo* and *in vitro* to produce T_H2 cytokines.⁷⁷ CysLT2R has also been implicated to promote ILC2 expansion through LTC₄-driven IL-33 production by alveolar type 2 cells in AERD murine models.⁸³ In humans, the terminal and most stable CysLT, LTE₄, most potently promoted ILC2 survival and cytokine release.⁸¹ Thus, CysLTs that are central to AERD pathogenesis are also potent activators of ILC2s.

The role of PGD₂ in AERD is of exceptional focus given its downstream effects including bronchospasm and immune cell activation, as well as increased production in patients refractory to desensitization and severe asthma.^{19,84} PGD₂ potently activates and induces migration of ILC2s through binding of chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), which is often used to identify human ILC2s after lineage exclusion.^{78,85} Interestingly, a recent report showed that ILC2-endogenous production of PGD₂ promotes ILC2 cytokine production in the presence of epithelial cytokines, suggesting that the PGD₂ burden from other cell types including eosinophils and MCs may only compound the influence of endogenous ILC2 PGD₂ production.⁸⁶

In contrast to CysLTs and PGD₂, PGE₂ and PGI₂ play inhibitory roles in ILC2 responses.^{87,88} Similar to its previously noted inhibitor effects on MCs, PGE₂ suppresses human ILC2 responses through the activation of EP2 and EP4 receptors found on ILC2s.⁸⁷ In patients with AERD, PGE₂ expression is decreased and tissue resistance to PGE₂ is present, which leads to reduced

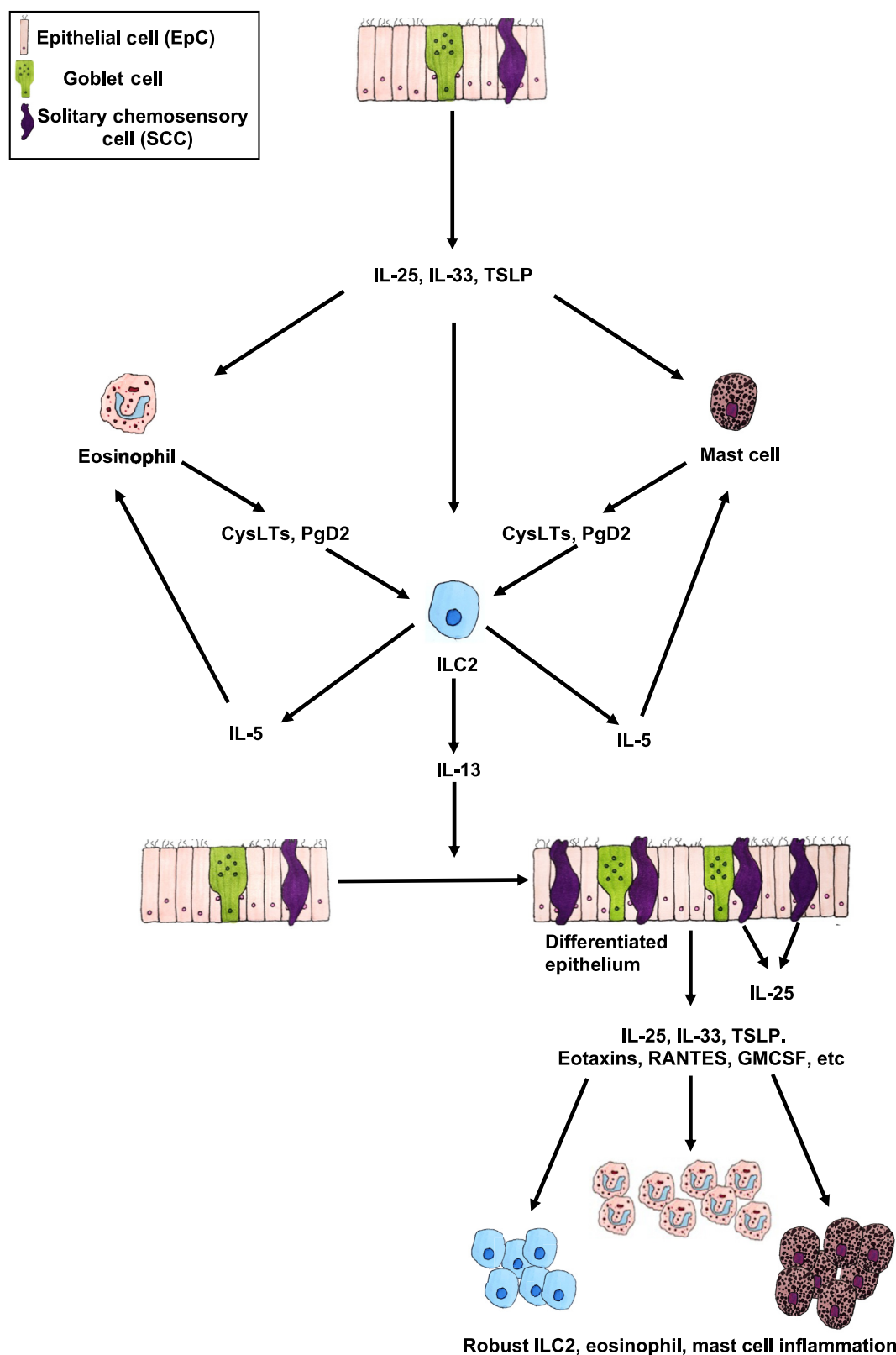


FIG 1. Mediators produced epithelial cells in AERD, and especially the cytokines IL-25, IL33, and TSLP recruit and activate innate immune cells including eosinophils, MCs, and ILC2s. Lipid mediators produced by eosinophils and MCs lead to the further activation of ILC2s. ILC2s through their secretion of IL-5 exacerbate eosinophilic inflammation. Numerous mediators released by all 3 of these cells, but, in particular, ILC2-derived IL-13, lead to the further *metaplastic transformation* of the epithelium including the expansion of goblet cells and IL-25-producing solitary chemosensory cells. Together, the epithelial and mucosal compartments of the innate immune system combine to produce a feed-forward proinflammatory vicious circle with the continuously worsening inflammation that characterizes AERD.

inhibition of leukotriene production, resulting in increased LTB₄ and CysLTs.⁸⁹⁻⁹² Similarly, PGI₂ suppresses human ILC2 production of type 2 cytokines, and administration of PGI₂ decreases lung ILC2 responses in mice.⁸⁸ Overall, **eicosanoids** relevant to AERD pathogenesis have direct effects on ILC2 recruitment, activation, and expansion, which could contribute to the pathogenetic manifestations of the disease.

ILC2 activation by epithelial cytokines

Epithelial-derived cytokine alarmins include IL-33, IL-25, and TSLP and were the first mediators shown to activate ILC2s (reviewed in Doherty and Broide⁶⁷ and Doherty⁷³). Human ILC2s were initially found to produce T_H2 cytokines after stimulation with IL-33 and TSLP *in vitro*, and TSLP-induced ILC2 responses were demonstrated to be dependent on the transcription factor GATA3.^{93,94} Importantly, TSLP promotes CCS resistance in ILC2s, and a recent report showed that ILC2s activated by epithelial alarmins switch from resting CD45RA⁺ ILC2s to CCS-resistant CD45RO⁺ ILC2s, which correlate with increased disease severity in patients with CRS.^{72,75} Interestingly, though AERD is largely considered a disorder of eicosanoid dysregulation, epithelial alarmins IL-33 and TSLP may contribute to pathogenesis through multiple upstream and downstream mechanisms involving MCs and lipid mediators.^{60,95,96} IL-25 levels are also higher in plasma of patients with AERD compared with controls, and IL-25 release from epithelial-associated chemosensory brush cells may specifically activate ILC2s through a CysLT pathway.^{97,98} Taken together, the abnormal lipid and epithelial cytokine pathway activation found in AERD may work in concert to promote ILC2 responses and type 2 inflammation.

Tissue trafficking of ILC2s

Although initial ILC2 paradigms largely suggested that local proliferation is the dominant mechanism of accumulation in tissues, it is clear that ILC2 trafficking contributes critically during type 2 inflammation.⁹⁹ ILC2s display increased migration during inflammatory conditions including during COX-1 inhibitor challenge in AERD.^{68,99} In particular, increased *ex vivo* chemotaxis of ILC2 cells to PGD₂ has been demonstrated in patients with asthma and allergy compared with controls and is dependent on expression of CRTH2.^{78,85,100} Furthermore, airway PGD₂ challenges in mice directly induce peripheral ILC2 trafficking to the lungs.¹⁰¹ CysLTs and less so IL-25 and IL-33 also induce migration of human ILC2s *in vitro*, which could be relevant to ILC2 trafficking in AERD.^{78,81}

Targeting of ILC2s in AERD

Many of the therapeutics used in AERD treatment mechanistically target ILC2s. β₂-Adrenergic receptors are found on lung ILC2s, and β-agonist treatment results in decreased ILC2 numbers.¹⁰² The CysLT1R antagonist montelukast also inhibits CysLT-induced ILC2 cytokine production.^{77,80} A diet rich in omega-3s may also suppress ILC2s as demonstrated in a murine study and has been studied with some efficacy in AERD.^{103,104} Newer biologics targeting ILC2 pathways include inhibition of upstream mediators including an anti-TSLP antibody,¹⁰⁵ a GATA-3 inhibitor¹⁰⁶ as well as antibodies blocking the downstream ILC2-produced cytokines IL-4, IL-5, and IL-13. These

strategies could be useful in dampening the effects of type 2 inflammation in AERD through suppression of ILC2 responses.¹⁰⁷⁻¹¹¹

CONCLUSIONS

This review has focused on the key roles for several cells of the innate immune system, specifically on the roles of eosinophils, basophils, MCs, and ILC2s in the pathogenesis of AERD. It is unlikely that any one of these cells is solely responsible for the pathogenesis of AERD. As such, a highly focused intervention targeting any single immune cell is unlikely to produce a comprehensive disease remission. It is also important to appreciate the synergistic upregulatory pathogenic interaction of all of these cells with sinonasal epithelial cells (Fig 1). It is easy to envision a self-perpetuating vicious circle emerging in which alarmins produced by activated epithelial cells, such as IL-25, IL-33, and TSLP, can engage and activate each of these innate immune cells. And in turn, activation of these cells with production of additional alarmins and lipid mediators leads to further recruitment and activation of each of these innate immune cells. More importantly, production of numerous mediators by these cells produces a feedback loop leading to the continuing and worsening inflammation being induced by epithelial cells. In particular, several mediators, but especially IL-13, are central in driving the capacity of epithelial cells to produce T2 inflammation-inducing cytokines/chemokines and lipid mediators. And, these actions on epithelial cells further drive their differentiation into goblet cells and, most importantly, IL-25-producing chemosensory cells.¹¹² Although this model does not explain the initial pathogenic “hit” initiating AERD, what is remarkable is that the perpetuation and progression of this disease can be seen as persisting even in the absence of engagement by antigen of the adaptive immune system (T or B cells). Fortunately, the models discussed throughout this article explain both current interventions either proven or suggested to specifically ameliorate AERD (eg, aspirin desensitization, anti-IL-5/IL-5R, anti-IL-4R, and anti-IgE) and invite novel targets for exploration as specific therapies for this condition (PGD₂/CRTH antagonists or cytokine antagonists [IL-25, IL-33, TSLP]). Several of these interventions already show promise in small retrospective analyses and now require definite clinical trials.

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