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Lipid-mediated innate lymphoid cell recruitment and activation in AERD

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Abstract

Objective: To synthesize investigations into the role of lipid-mediated recruitment and activation of ILC2s in AERD.

Data Sources: A comprehensive literature review of reports pertaining to AERD cellular mechanisms, cytokine and lipid mediators in AERD, and ILC2 activation and recruitment was performed using PubMed and Google Scholar.

Study Selections: Selections of studies were based on reports of lipid mediators in AERD, cytokine mediators in AERD, type two effector cells in AERD, platelets in AERD, AERD treatment, ILC2s in allergic airway disease, and ILC2 activation, inhibition, and trafficking.

Results: The precise mechanisms of AERD pathogenesis are not well understood. Greater levels of proinflammatory lipid mediators and type two cytokines are found in tissues derived from AERD patients relative to controls. Following pathognomonic COX-1 inhibitor reactions, proinflammatory mediator concentrations (prostaglandin D2 and cysteinyl leukotrienes) are rapidly increased, as are ILC2 levels in the nasal mucosa. ILC2s, which potently generate type 2 cytokines in response to lipid mediator stimulation, may play a key role in AERD pathogenesis.

Conclusion: While the literature suggests that lipid-mediated ILC2 activation may occur in AERD, there is a dearth of definitive evidence. Future investigations leveraging novel next-generation single cell sequencing approaches along with recently developed AERD murine models will better define lipid mediator-induced ILC2 trafficking in patients with AERD.

Introduction

Allergic airway disease is a major global health problem. As of 2018, the CDC has reported that over 24 million people in the US (7.7%) are living with asthma and greater than 28 million people (11.6%) are afflicted with chronic rhinosinusitis (CRS). 20% of CRS patients will develop nasal polyps (CRSwNP), noncancerous growths of the sinus cavities. A subset

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of asthmatics (9%) are diagnosed with aspirin-exacerbated respiratory disease (AERD). The prevalence of AERD in asthmatics increases from 9% to 30% in those with comorbid CRSwNP.¹ AERD was previously known as Samter's Triad (also aspirin-intolerant-asthma and non-steroidal anti-inflammatory drug exacerbated respiratory disease) as it was first described by Max Samter in 1968 and is characterized by three clinical hallmarks, which typically develop in sequence in the 3rd–4th decade of life: CRSwNP, moderate-to-severe asthma, and pathognomonic respiratory reactions to COX-1-inhibition. The underlying cause of AERD is unknown; however, it is clear that AERD is a type 2 immune-mediated respiratory disease, like asthma and CRS, but with a particularly marked proinflammatory lipid signature involving cysteinyl leukotrienes (CysLTs) and prostaglandin D2 (PGD2).

Innate lymphoid cells (ILCs) are recently discovered innate counterparts to adaptive helper T (Th) cells. The family of ILCs includes ILC1s, ILC2s, and ILC3s, which correspond to Th1, Th2, and Th17/22 cells, respectively.² ILCs mirror Th cell expression of transcription factors and cytokines. For example, ILC2s and Th2 cells are defined by expression of the key transcriptional regulator GATA-3 and produce IL-4, IL-5, and IL-13. However, unlike Th cells, ILCs lack T cell receptors and antigen specificity. Instead, ILCs respond rapidly and robustly to the local cytokine (IL-33, IL-25, TSLP) and lipid mediators (CysLTs, PGD2). Largely residential to mucosal surfaces, ILCs are positioned as immune sentinels, and ILC2s are the primary ILC found in the airways. Various environmental stressors, including allergens, viruses, and parasites, initiate ILC2 responses. More recently, ILC2s were shown to be recruited to the airway in AERD patients. Here, we review the current understanding of mediators that regulate and recruit ILC2s and how ILC2s might contribute to AERD pathogenesis.

Lipid mediators in AERD that regulate ILC2s

Cysteinyl leukotrienes

Long before ILC2s were discovered and shown to be activated by CysLTs^{3–5}, Ferreri et al. provided the first insight into the role of CysLTs in AERD pathogenesis.⁶ Following aspirin challenge, the levels of nasal leukotriene (LT) C4 were greater in aspirin intolerant patients compared to tolerant controls. LTC4 is a member of the cysteinyl leukotriene (CysLT) family of bioactive lipid molecules, which are products of the 5-lipoxygenase (5-LO) branch of arachidonic acid (AA) metabolism.^{7,2} 5-LO converts AA to leukotriene A4 (LTA4), which is subsequently metabolized into leukotriene B4 (LTB4) or LTC4. The former is a terminal metabolite, whereas the latter rapidly converts to leukotriene D4 (LTD4), and finally leukotriene E4 (LTE4). CysLTs include LTC4, LTD4, and LTE4 and are mediators of bronchoconstriction, mucus hypersecretion, and eosinophilia. Since Ferreri's initial discovery, a wealth of evidence supporting LT involvement in AERD has been reported. Baseline urinary levels of LTE4 are increased in AERD patients relative to healthy controls, and concentrations are further increased following aspirin challenge.⁸ While elevated levels of urinary LTE4 are also found in patients with asthma and CRSwNP, the LTE4 concentrations in urine from AERD patients are increased above those with asthma and CRSwNP. These observations were later extended to LTB4, though the concentrations reported were lower than those of LTE4.⁹ In support of these findings, 5-LO was found to be

increased in AERD nasal mucosa, and LTC₄-synthase shown to be increased in both the lungs and nasal mucosa.

Increased PGD₂

In addition to 5-LO, AA is also metabolized by the two cyclo-oxygenase (COX) enzymes, COX-1 and COX-2. This process yields the prostaglandin (PG) family of lipid mediators that regulate inflammatory and anti-inflammatory responses in allergic airway disease. High levels of PGD₂, a known bronchoconstrictor, are also associated with severe asthma.^{2,10,11} COX products are of particular interest as AERD is characterized by COX-1 inhibitor reactions. Significantly higher concentrations of PGD₂ are found in nasal polyps from aspirin intolerant patients compared to aspirin tolerant patients.^{12,13} Further, baseline urinary PGD₂ metabolites (PGD₂M) levels are greater in AERD patients compared to controls, and PGD₂M concentrations negatively correlate with tolerance to aspirin desensitization.¹³ Importantly, human ILC2s are identified by expression of CRTH2², one of the receptors for PGD₂, and therefore the reported effects of PGD₂ on ILC2 recruitment and activation are particularly relevant to AERD (Figure 1).^{14,15}

Reduced PGE₂

Prostaglandin E₂ (PGE₂) is also formed by through COX processing of AA. Unlike PGD₂, PGE₂ plays a protective role in allergic airway disease by attenuating airway inflammation and supporting normal lung function.¹⁶ Studies have demonstrated that PGE₂ reduces both 5-LO activity and allergen-induced IL-33 release may explain the former mechanism.^{17,18} Importantly, defects in PGE₂ responses have been linked to AERD as expression of PGE₂ is lower in nasal polyps from aspirin intolerant patients compared to controls.^{12,19,20} Additionally, the PGE₂ receptor is downregulated in nasal and lung tissue derived from aspirin intolerant patients.^{21,22} Further, a recent report demonstrated that suppression of CysLT production by PGE₂ was diminished in AERD patients thus highlighting complex interplay between these eicosanoids.²⁰ In addition to activation by CysLTs and PGD₂, human ILC2 responses are inhibited by PGE₂.²³ Together, these studies suggest that tissue ILC2s may be more pro-inflammatory in patients with AERD. Overall, AERD pathophysiology driven by high levels of pro-inflammatory mediators may be compounded by concomitant impairment of PGE₂ signaling.

ILC2-activating cytokine mediators in AERD

Epithelial-cell derived cytokines

In response to injury, airway epithelial cells release proinflammatory “alarmin” cytokines IL-25, IL-33, and TSLP which activate many type 2 cytokine producing cell types including ILC2s.² These mediators have been linked to asthma, CRS, nasal polyposis, and, more recently, AERD. While early investigation into AERD pathogenesis focused on bioactive lipid mediators, recent studies have elucidated the role of these epithelial-cell derived cytokines. Increased expression of IL-33 and TSLP were found in nasal polyps from AERD patients relative to those derived from aspirin tolerant individuals.^{24,25} Another study reported higher baseline plasma levels of IL-25 in AERD patients relative to aspirin tolerant controls.²⁶ Importantly, alarmin induced type 2 cytokines drive proliferation of PGD₂- and

CysLT-producing eosinophils and mast cells. Taken together, these reports suggest a revised model of AERD pathogenesis wherein epithelial injury triggers alarmin release and subsequent PGD₂ and CysLT production.

Pro-inflammatory type 1 and 2 cytokines

Epithelial cell derived cytokines drive immune cells (primarily ILC2s and Th2 cells) to produce the type 2 cytokines IL-4, IL-5, IL-13, and IL-9, which have been inextricably linked to allergic airway disease. Recent studies of nasal polyposis have demonstrated type 2 cytokine involvement in AERD. AERD polyps contain greater IL-4 transcripts relative to chronic hyperplastic eosinophilic sinusitis polyps, and surprisingly, higher levels of the type 1 cytokine IFN- γ from eosinophils.²⁷ Levels of IL-5 are also greater in nasal polyps from allergic individuals with type 2 disease compared to non-allergic individuals, and nasal secretions from patients with CRSwNP contain more IL-5 than those from patients with CRSsNP.^{28–30} Further, IL-9, GATA-3, IL-5, and IL-13 expression is increased in tissue from patients with CRSwNP compared to CRSsNP and non-diseased controls.^{31,32} Overall, nasal polyp tissue from AERD patients contains a high type 2 cytokine signature consistent with the presence of ILC2s, though the type 1 cytokine IFN- γ may also contribute to pathogenesis.

Type 2 effector cells in AERD

Mast cells and eosinophils

IL-4, IL-5, IL-13, and IL-9 are known to drive accumulation of eosinophils and mast cells, which are primary sources of PGD₂ and CysLTs and implicated in AERD. In the same study that first linked LTE₄ to AERD, it was also demonstrated that nasal histamine levels were greater in aspirin intolerant patients compared to tolerant controls following aspirin challenge which suggested mast cell involvement in AERD.⁶ Consistent with this, another 1991 study reported that systemic mast cell activation occurred in a subset of aspirin intolerant patients after aspirin challenge.³³ More recently, Cahill et al. provided evidence bolstering these early reports.³⁴ AERD patients with high levels of mast cells exhibited increased symptom scores and reduced forced expiratory volume in one second (FEV₁) following aspirin challenge. This subset of patients also had greater levels of the inflammatory mediators and metabolites, PGD₂M and LTE₄. Tissue eosinophil infiltration, a hallmark of type 2 allergic responses, are enriched in lung and nasal polyp tissue from aspirin intolerant compared to aspirin tolerant individuals and are also an important source of PGD₂.^{35–37} Peripheral eosinophilia can also be found in AERD, but is reduced during aspirin challenge, indicating possible tissue migration from blood. Eosinophils derived from aspirin intolerant patients also produce more ECP, indicating an enhanced activation state.³⁵ Thus, studies using human samples have revealed enhanced production of critical lipid mediators and accumulation and activation of mast cells and eosinophils in AERD patient tissue.

Platelets in AERD

Interestingly, platelets have also been implicated in AERD pathogenesis. Unlike mast cells and eosinophils, platelets do not possess the full machinery required to produce PGD₂ and CysLTs. However, platelets express high levels LTC₄-synthase and can form aggregates with 5-LO expressing white blood cells, a process that is required for pathogenesis in murine asthma models.³⁸ Enriched levels of granulocyte-adherent platelets levels were detected in AERD polyp and blood samples and contributed significantly to LTC₄ overproduction.³⁸ Highlighting the importance of platelets in type 2 inflammation, platelet depletion in a murine asthma models significantly attenuated airway eosinophilia as well as ILC2 activation.^{39,40} More recently, platelets were also found to express functional IL-33 supporting a role for both lipid and alarmin contributions to inflammation in AERD.⁴¹

Activation and recruitment of ILC2s

Innate type 2 allergic airway inflammation

Elevated levels of epithelial cell derived cytokines and type 2 effector cells, coupled with an antigen-independent pathognomonic COX-1 inhibitor reactions, suggest that AERD pathogenesis may be orchestrated by an innate type 2 cytokine producing cell. ILC2s highly express the PGD₂ receptor (CRTH2), are responsive to IL-33 and PGD₂, and are potent producers of the type 2 cytokines IL-4, IL-5, IL-9, and IL-13.⁴²⁻⁴⁵ In mice, allergens induce release of ILC2-activating epithelial cell derived cytokines IL-33, IL-25, and TSLP (Figure 2). Upon stimulation, lung ILC2s rapidly drive eosinophilia, airway hyperresponsiveness, mucus hypersecretion, and mast cell accumulation independent of T and B cells. ILC2s are also enriched in nasal polyps from patients with the eosinophilic endotype, which is uniformly present in AERD.^{46,47} Further, the number of ILC2s is significantly greater in tissues from patients with allergic airway diseases compared to healthy controls and correlates with more severe disease.^{48,49} ILC2s from allergic individuals also have greater cytokine producing and chemotactic potential compared to non-diseased controls.^{48,50}

Eicosanoid regulation of ILC2s

In 2013, ILC2s were first shown to be activated by bioactive lipids.^{4,51} Our group demonstrated that murine ILC2s highly express CysLT1R and that CysLTs, particularly LTD₄, potently induced ILC2 Th2 cytokine production and calcium flux.⁴ In a CysLT1R-dependent fashion, LTD₄ elicited IL-5 and IL-13 production on par with IL-33, but also promoted IL-4 production unlike IL-33. These findings were later translated to human ILC2s, which also express CysLT1R.⁵² However, in humans LTE₄, rather than LTD₄, more potently promotes ILC2 survival and cytokine production. While original studies found ILC2 induction by CysLTs to be CysLT1R-dependent, a more recent study indicated that LTC₄ activates ILC2s through a CysLT2R-dependent mechanism.⁵³ The authors also reported that ILC2s were indirectly activated through a CysLT2R through LTC₄ stimulation of IL-33 by alveolar type 2 cells. Overall, CysLTs which are highly expressed in AERD also potently activate ILC2s, suggesting a link with ILC2s in AERD pathogenesis.

Human ILC2s were first identified by their high expression of CRTH2, one of the receptors for PGD2.⁴² Three after the initial discovery that human CRTH2⁺ ILC2s, PGD2 was shown to induce migration and activation of ILC2s through CRTH2.^{54,55} Similar to LTs, PGD2 rapidly and potently induces IL-4, IL-5, and IL-13 production.⁴ In contrast, other members of the PG family can negatively regulate ILC2s. In a 2013 report, Barnig et al. demonstrated that Lipoxin A4 (LXA4) inhibits human peripheral blood ILC2 IL-13 production.⁵¹ While LXA4 concentrations are suppressed in some individuals with severe asthma, it remains unclear whether levels are further reduced in severe asthmatics with AERD. More recent studies have shown that PGI2 and PGE2 also suppress both human and mouse ILC2s, and PGE2.⁵⁶⁻⁵⁸ While PGE2 resistance has been described in AERD, whether this occurs specifically in ILC2s requires additional investigation. Importantly, two very recent studies showed that COX inhibition potentiates IL-33-induced murine ILC2 activation suggesting that the sum of prostaglandin effects are to inhibit ILC2 responses.^{17,59}

Lipid enhancement of alarmin-induced ILC2 activation

In 2017, our group and others reported that lipid mediators could potentiate IL-33-induced activation of ILC2s suggesting non-redundant roles for lipids in ILC2 responses.^{60,61} Specifically, CysLTs enhanced IL-33-induced activation through an NFAT- and CysLT1R-dependent mechanism. These findings were later translated to human ILC2s as LTE4 synergized with epithelial derived cytokines (IL-33, IL-25, and TSLP) to further activate ILC2s.⁵² ILC2s achieved the highest level of activation when stimulated with the combination of PGD2, LTE4, and epithelial derived cytokines which are all implicated in AERD. IL-25 and IL-33 receptor transcript levels were also upregulated in the combined group, which may have contributed to the potentiation. In another study, LTC4 was found to both directly and indirectly activate ILC2s, the latter being through enhanced IL-33 release.⁵³ A more recent report demonstrated that ILC2s may utilize lipid and cytokine synergistic activation to promote homeostasis.⁶² Interestingly, in this study ILC2s were shown to generate and respond to PGD2, which was necessary for activation by epithelial derived cytokines as CRTH2 inhibitors attenuated responses in isolated human ILC2s. Thus, lipids and cytokines important in AERD pathogenesis regulate ILC2 activation in multiple ways.

Tissue trafficking of ILC2s

In addition to the role AERD-related lipid and cytokine mediators play in ILC2 survival and activation, several of these mediators have also been shown to induce ILC2 migration *in vitro*. PGD2 induces ILC2 chemotaxis through CRTH2 in a dose-dependent fashion, and ILC2s from asthmatics and allergic individuals are more chemotactic both at baseline and after PGD2 treatment.^{4,54,55,63} IL-33 and IL-25 induce migration of human ILC2s, albeit more modestly than PGD2.^{54,63} CysLTs, particularly LTE4, promote ILC2 chemotaxis as well and LTE4 potentiates PGD2 induced migration, as it does for cytokine production.⁵² Other mediators have also recently been described to induce ILC2 migration. TGF- β increases the distance and speed of ILC2 travel and CCL8 was reported to drive ILC2 migration, in a dose- and CCR8-dependent fashion.^{63,64}

While ILC2s are largely considered to be tissue resident cells that locally proliferate, the aforementioned chemotaxis studies have led researchers to investigate whether ILC2

migration plays a role in allergic airway disease pathogenesis. Initial studies investigating ILC2 migration *in vivo* demonstrated that intranasal PGD2 challenge induced peripheral ILC2 trafficking to the lungs in a CRTH2-dependent manner and revealed that CRTH2 expression was downregulated once ILC2s reached the lungs.⁶⁵ More recently, systemic IL-33 treatment was also found to promote ILC2 migration to the lungs in a PGD2-dependent fashion.⁶⁶

Evidence of ILC2 recruitment to the lung is not limited to artificial exogenous mediator-induced trafficking, but also includes migration invoked by clinically relevant allergens in animal models. *Alternaria* was shown to induce ILC2 progenitor egress from the bone marrow and ILC2 accumulation in the lung in an IL-33R-dependent fashion.⁶⁷ Additionally, our group demonstrated that inhibition of the β 2 integrin adhesion receptor inhibited mouse ILC2 lung accumulation after *Alternaria* challenge without affecting ILC2 proliferation or apoptosis.⁶⁸

More recently, Huang et al. published a seminal report detailing several novel aspects of ILC2 migration *in vivo*. The authors reported that ILC2s traffic through lymphatics and blood to distal tissues to participate in immune responses.⁶⁹ Specifically, ILC2s were shown to traverse the gut to the lung. *In vivo* ILC2 migration was S1P-dependent as the S1P-antagonist FTY720 inhibited migration. This process may be relevant to AERD as gastrointestinal manifestations occur in a subset of patients. To support this idea, a study found that, compared to aspirin tolerant controls, AERD patients have greater levels of serum S1P, the levels of which positively correlate with the change in FEV1 following aspirin challenge.⁷⁰ Thus, there are multiple possible mechanisms by which ILC2 trafficking into tissues may occur in AERD.

ILC2 accumulation in allergic rhinitis

Investigations into allergic airway diseases more broadly have provided insight into the potential role of ILC2s in AERD. At baseline, levels of blood ILC2s are greater in patients with allergic rhinitis (AR) compared to controls.^{48, 71} Moreover, ILC2s are further enriched in AR patient blood during allergy season.^{72,73} In 2014, we reported that nasal cat allergen challenge in sensitized patients elicited ILC2 expansion in PBMCs relative to control challenge.⁷³ A more recent study with a similar design showed that nasal allergen challenge led to nasal tissue enrichment of ILC2s, the level of which positively correlated with symptom severity and markers of type 2 inflammation.⁷⁴ Conversely, immunotherapy decreases ILC2 levels and symptom severity.^{72,75} While the kinetics of ILC2 mobilization to the nasal mucosa remain unclear, it is evident that ILC2s are recruited from distal tissues and accumulate at the site of environmental insult.

ILC2s in AERD

Since the discovery of ILC2s, numerous groups have found elevated levels of ILC2s in nasal polyps.^{42,46,76,77} On average, ILC2s are more enriched in nasal polyps compared to any other tissue. Until recently, no studies had specifically assessed changes in ILCs in AERD patients. In 2017, we reported that ILC2 recruitment occurs during AERD desensitization reactions.⁷⁸ We assessed levels of ILC2s in blood and nasal mucosa samples from AERD

patients before, during, and after COX-1 inhibitor challenges. ILC2s were significantly enriched in the mucosa and depleted in the blood during reactions compared to baseline, while control patient ILC2 levels were unaltered. Further, ILC2 levels positively correlated with urinary LTE4 and PGD2 concentrations as well as symptom severity. This study suggests that ILC2s traffic from the blood to the nose during aspirin challenge and may contribute to disease exacerbation.

Platelet aggregates and ILC2 activation

Nearly 20 years ago, mouse platelets were demonstrated to form aggregates with leukocytes.⁷⁹ Without platelets, leukocyte migration into the lung after allergen exposure was severely attenuated. Recently, our group showed that platelet attachment to ILC2s occurs in mice.⁴⁰ Depletion of platelets suppressed ILC2 cytokine production, indicating that platelets interact with ILC2s in order to support ILC2 homeostasis and function. Thus, platelets might promote ILC2 responses in the lung and nasal mucosa in AERD.

Pharmacological intervention of ILC2 pathways in AERD

Both conventional and potential novel treatments for AERD mirror those for asthma and CRSwNP. More traditional drugs include beta agonists and corticosteroids. In addition to their direct effects on airway physiology, beta agonists may also work through ILC2-intrinsic mechanisms (Figure 2). Human and mouse lung ILC2s were recently found to express the beta 2 adrenergic receptor. In a model of *Alternaria*-induced allergic airway inflammation, a beta agonist reduced ILC2 numbers and cytokine production.⁸⁰ Corticosteroids are often the first line of therapy for patients with allergic airway diseases, yet some patients do not respond adequately.⁸¹ Importantly, a recent landmark study demonstrated that TSLP renders human ILC2s steroid resistant.⁸² Further, the level of TSLP positively correlated with the magnitude of steroid resistance in BAL ILC2s. Finally, the authors showed that steroid resistance could be reversed through MEK and STAT5 inhibition.

AERD therapy also includes the CysLT1R antagonists, montelukast and zafirlukast, and the 5-LO inhibitor, zileuton. Montelukast also directly inhibits CysLT-induced ILC2 cytokine production.^{4,54} A recent study showed that a diet with a high ratio of omega-3 to omega-6 fatty acids reduced urinary LTE4 and PGD2M and improved overall symptom scores.⁸³ ILC2 suppression could contribute to this effect as a 2015 study showed that ILC2 are inhibited by the omega-3 derivative, maresin-1.⁸⁴ More recently, a clinical trial was conducted investigating whether prasugrel, an anti-platelet drug and potential ILC2-platelet aggregate inhibitor, would ameliorate aspirin induced reactions in AERD patients. While prasugrel lacked effectiveness in the total study cohort, subset analysis revealed complete abrogation of symptomology in patients with greater levels of platelet activation and less severe respiratory reactions to aspirin.⁸⁵

Newly developed biologics and small molecules targeting various aspects of ILC2 biology have recently been shown to be efficacious in treating allergic airway disease. The anti-TSLP antibody tezepelumab reduces airway inflammation and limits exacerbations in asthmatics.^{86,87} Numerous CRTH2 antagonists were previously shown to improve lung

function and reduce eosinophilia in asthmatics, though efficacy in recent trials have been disappointing.^{88–90} Dupilumab, an FDA-approved IL-4 receptor blocking antibody that inhibits both IL-13 as well as IL-4 signaling, was shown to be efficacious in asthmatics and patients with nasal polyposis.⁹¹ The anti-IL-5 antibody mepolizumab is an effective treatment for eosinophilic asthma and hypereosinophilic syndromes.^{92–95} IL-13 blockade with lebrikizumab or tralokinumab yielded limited improvements in lung function, but studies for efficacy are ongoing.^{96,97} Finally, a small clinical trial in asthmatics found that SB010, a novel DNA enzyme that inhibits GATA-3 prevented reductions in FEV1, as well as reduced plasma IL-5, sputum eosinophilia and tryptase levels.⁹⁸

Several additional compounds targeting pro-ILC2 mediators and ILC2 receptors, enzymes, cytokines are currently being developed. Antibodies that block IL-25 signaling reduce airway inflammation, hyperresponsiveness, and polyposis in animal models.^{99–101} MEDI-528, a humanized IL-9 blocking antibody is effective in murine asthma models, however its effect in humans thus far is inconclusive and requires additional investigation.¹⁰² Mouse and human ILC2s require the metabolic enzyme arginase-1 (Arg1) to function, and removal of ILC Arg1 attenuates allergic airway inflammation in mice.¹⁰³ Arginase inhibitors are effective in asthma models and are currently being trialed as cancer therapeutics.¹⁰⁴ More recently, a CRTH2-depletion antibody was shown to eliminate ILC2s, eosinophils, and Th2 cells in a humanized CRTH2 mouse model¹⁰⁵. Finally, IL-33 signaling blocking antibodies are currently in development for treatment of allergic disease.¹⁰⁶

ILC2s in mouse models of AERD

Investigation into AERD pathogenesis has been limited to human samples for decades as the disease is difficult to model in mice. While murine asthma models have shed light on the pathogenesis of type 2 allergic airway inflammation in general, AERD is unique due to the pathognomonic COX-1 inhibitor sensitivity. In 2013, the first AERD mouse model was described.¹⁰⁷ The authors identified prostaglandin E-synthase deficient mice as being AERD-like. Aspirin administration to house dust mite challenged PGE-synthase deficient mice enhanced levels of mast cell activation, CysLT production, and airway hyperresponsiveness. In a later study, AERD-like mice were found to have elevated airway epithelium IL-33 levels and increased lung eosinophilia.²⁴ More recently, ILC2s were reportedly enriched threefold in the lungs of AERD mice relative to WT mice, and that ILC2 depletion nearly abrogated the response to aspirin.⁵³ Thus, some features of AERD can be modeled in mice, and such models could help decipher mechanisms of AERD pathogenesis, including the roles of ILC2s, and test newly developed pharmacological agents.

Conclusion

In summary, AERD is a complex disease though recent studies have provided great insight into the pathogenesis. Proinflammatory bioactive lipids and type two cytokines are particularly high in tissues from AERD patients and levels are further increased during pathognomonic reactions to COX-1 inhibition. These hallmarks position ILC2s, central drivers of innate type 2 airway inflammation in animal models, as potential orchestrators of AERD pathophysiology. This model is further supported by recent findings of rapid ILC2

recruitment to the airways following COX-1 inhibitor challenge. Future studies are needed to more fully characterize ILC2s in AERD patients longitudinally across tissues, at baseline, and after COX-1 inhibitor therapy. Additionally, studies investigating mechanisms of ILC2 recruitment and activation in newly described AERD mouse models are warranted. Such research may lead to the identification of novel therapeutic targets for not only AERD, but all allergic airway diseases that involve pathogenic ILC2 responses.

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Key Messages:

- AERD patients have elevated levels of pro-inflammatory lipids and cytokines in nasal tissue at baseline, and levels increase further after COX-1 inhibitor challenge
- Following COX-1 inhibitor challenge, ILC2s decrease in the blood and increase in the nasal mucosa
- Lipid and cytokine mediators induce ILC2 migration *in vivo*
- A recently described mouse model recapitulating features of AERD demonstrates pathognomonic COX-1 inhibitor reactions are ILC2-dependent
- Novel therapeutics in the developmental pipeline for AERD and other allergic airway diseases target ILC2-mediated processes

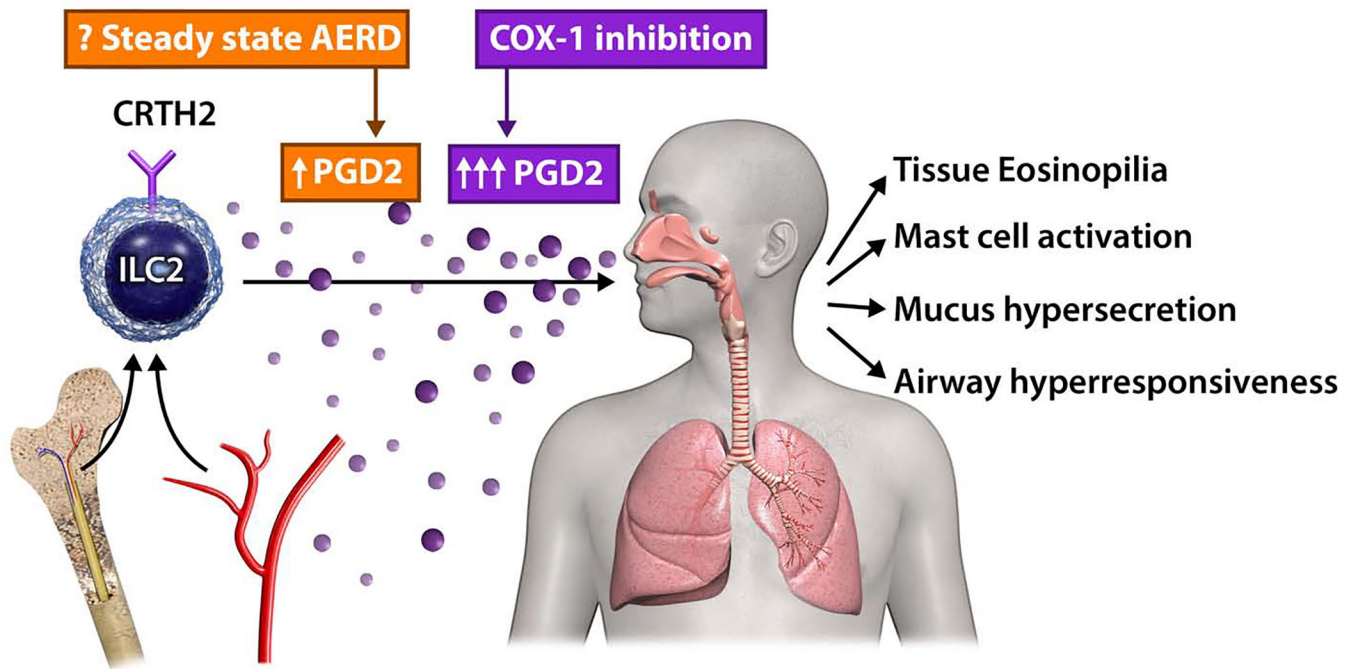


Figure 1. Recruitment of ILC2s to respiratory mucosa in AERD.

In patients with AERD challenged with COX-1 inhibitors, CRTH2+ ILC2s are reduced in the blood and accumulate in the nasal mucosa concomitantly with increased urinary prostaglandin D2 metabolites. This suggests that ILC2s that express CRTH2 (receptor for PGD2) may traffic into respiratory tissues from the bone marrow during COX-1 inhibition in AERD patients. Whether ILC2 recruitment occurs over time in the absence of COX-1 inhibitor challenge ('steady state AERD') is not known. Once in tissues, type 2 cytokine production by ILC2s and other cell types can promote tissue eosinophilia, mast cell activation, mucus hypersecretion, and airway hyperresponsiveness.

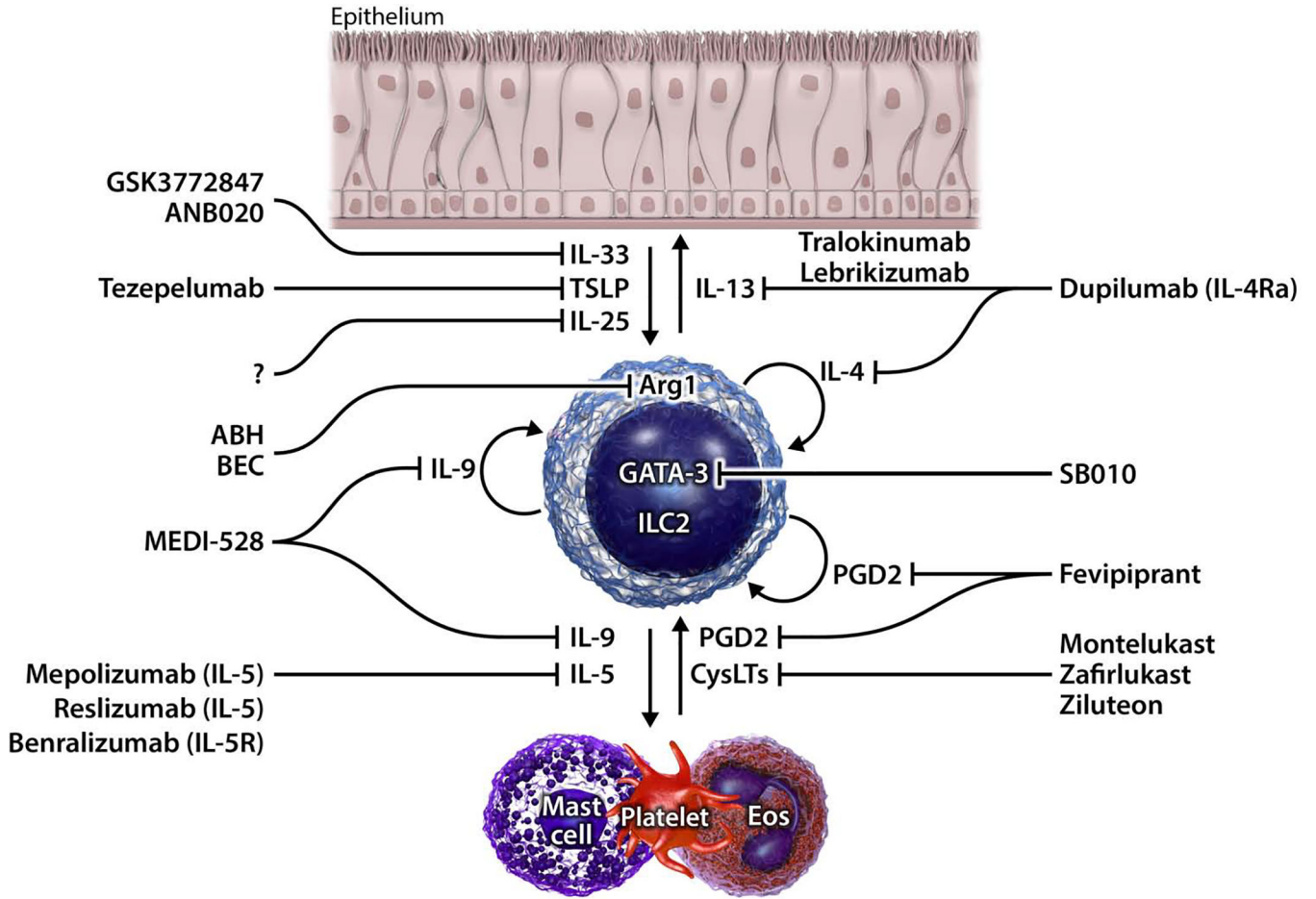


Figure 2. Proposed mechanisms and targets of ILC2-mediated inflammation in AERD.

In AERD, epithelial cytokines IL-33 and TSLP are increased in tissues and can stimulate ILC2s to produce IL-4, IL-5, IL-9, and IL-13. IL-13 promotes hyperresponsiveness and mucus production. IL-4 and IL-9 have autocrine effects on ILC2 activation in addition to IL-9 promoting mast cell accumulation. IL-5 stimulates eosinophil production and egress from bone marrow as well as contributing to recruitment and survival of eosinophils in tissues. In turn, eosinophils and mast cells produce PGD2 and CysLTs (that are elevated in AERD) which can activate ILC2s. PGD2 is also produced by ILC2s and has autocrine actions to promote cytokine production. ILC2s also highly express GATA-3 that is required for type 2 cytokine production. Approved and non-approved therapies that target various mediators in ILC2 driven inflammation are shown.