Eosinophils and Mast Cells in Aspirin-Exacerbated Respiratory Disease

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Abstract

Aspirin-exacerbated respiratory disease (AERD) is explained in part by over-expression of pro-inflammatory mediators including 5-lipoxygenase and leukotriene C_4 synthase (LTC_4S) that results in constitutive over-production of cysteinyl leukotrienes (CysLTs). Mast cells and eosinophils are two cell types that have important roles in mediating many of the effects observed in this disease. Increased levels of both interleukin (IL-4) and interferon (IFN)-γ are present in the tissue of AERD subjects. Previous studies demonstrated that IL-4 is primarily responsible for the upregulation of LTC_4S by mast cells. Our studies demonstrate that IFN-γ, but not IL-4 drives this process in eosinophils. We also extend to both IL-4 and IFN-γ the ability to upregulate CysLT receptors. Prostaglandin E_2 (PGE_2) acts to prevent CysLT secretion by inhibiting mast cell and eosinophil activation. PGE_2 concentrations are reduced in AERD and studies confirm that this reflects diminished expression of cyclooxygenase (COX)-2, a process again that is driven by IL-4. Thus, IL-4 and IFN-γ acting on eosinophils and mast cells together play an important pathogenic role in generating the phenotype of AERD. This review will examine the overall role that eosinophils and mast cells contribute to the pathophysiology of AERD.

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Introduction

In 1968, the term Samter's triad was coined and was defined by the presence of nasal polyps, aspirin sensitivity, and asthma [1], however this disease is now referred to as aspirin-exacerbated respiratory disease (AERD) as asthma is not always present despite reactions to aspirin. AERD comprises as many as 7% of adult-onset asthmatics and up to 12–14% of adult asthmatics with severe asthma [2, 3]. This disorder is characterized by the unique intolerance to aspirin and other non-selective cyclooxygenase (COX) inhibitors [4–6]. Other characteristics include hypereosinophilia, both in circulation and in the tissue, a tendency to develop *de novo* in adulthood [5, 7–9] and often an absence of identifiable atopy [5, 7]. Sinusitis is present in this disorder, the degree of which is often severe and associated with complete or near complete opacification of the sinus cavity [9]. Though not a requirement, when asthma is present it often progresses in severity and is associated with aggressive airway remodeling [10].

During aspirin reactions, many mediators are released including cysteinyl leukotrienes (CysLT), tryptase, eosinophil cationic protein (ECP) and prostaglandin D_2 (PGD_2) suggesting both mast cell and eosinophil activation [11–13]. Recently, aspirin was shown to directly activate both of these cell types *ex vivo* potentiating mediator release [14]. A predominant physiological feature of AERD is the robust over-production and over-responsiveness to CysLTs (inflammatory) [12, 15] while at the same time there is under-production and under-responsiveness to the anti-inflammatory lipid mediator PGE_2 [16–18]. These CysLTs have important pro-inflammatory and pro-fibrotic effects that contribute to the asthma severity and to the extensive hyperplastic sinusitis and nasal polyposis [9, 19, 20]. And, conversely, the down-regulation of PGE_2 pathways reduces the constraints that would normally act to attenuate these pro-inflammatory pathways [21]. This review will focus on the role that eosinophils and mast cells play in contributing to these cardinal features of AERD.

Eosinophil and mast cell numbers in AERD

Chronic sinusitis is now recognized as a collection of disorders that result from inflammation of the sinuses and in many cases can be separated into different types based on the cellular infiltrate. One distinguishing feature in the nasal polyps that often form in association with chronic sinusitis is the presence or absence of eosinophils and amongst eosinophilic polyps a distinction can be made between AERD, allergic fungal sinusitis (AFS) and chronic hyperplastic eosinophilic sinusitis (CHES) [22, 23]. Within the eosinophilic polyps, AERD has more than twice the number of eosinophils in the polyp tissue than AFS or CHES, implicating them as important cells in the disease process [23]. Examination of bronchial biopsies from AERD subjects also revealed highly elevated eosinophil numbers in comparison to aspirin-tolerant asthmatics and non-asthmatics [24]. These eosinophils were in an activated state as evidenced by the presence of secretory ECP [24].

We have reported lower numbers of mast cells in nasal polyps from eosinophilic sinus disease as compared to healthy tissue by both toluidine blue and chloroacetate (chymase) staining, and there was no difference between aspirin tolerant and AERD groups [23]. This contrasts somewhat with a previous report that found no difference in mast cell numbers in

nasal polyps from AERD groups when compared to allergic or non-allergic subjects via tryptase staining [25]. The differences in the results of these studies may reflect the use of different markers of mast cells (chymase vs. tryptase) or the stratification of the groups, the latter study not taking into account eosinophilic infiltration into the polyp tissue. Regardless, there do not appear to be more mast cells in nasal polyps in subjects with AERD. However, this result may be erroneous – given the high expression of mast cell-derived mediators – and perhaps reflects the inability to stain for activated, granule-depleted mast cells (so-called "phantom" mast cells). Similarly, when the lungs have been examined, as with NPs, fewer numbers of mast cells have been found in AERD subjects compared to non-asthmatic controls using immunohistochemistry to stain for tryptase positive cells [24]. Another study examining the bronchial mucosa found increased numbers of tryptase-positive mast cells only in subjects with non-aspirin sensitive asthma: again, AERD and healthy controls paradoxically had similar numbers [26].

Development of Eosinophils and Mast Cells

Eosinophils develop from pluripotent hematopoietic stem cells in bone marrow that initially differentiate into eosinophil/basophil progenitors or colony forming units (Eo/B CFU) (Figure 1). Eo/B CFU are mononuclear cells that express CD34, CD35, and interleukin (IL)-5 receptors (CD125) that are capable of responding to appropriate cytokine signals allowing differentiation into mature basophils and eosinophils [27, 28]. Eo/B CFU are increased in numbers in both the blood and bone marrow of allergic patients and further increases in their number are observed following allergen exposure [28]. These progenitors are also observed in nasal polyp tissue [29]. Several transcription factors including GATA-1, PU.1 and C/EBP are induced in response to appropriate cytokine signals and become involved in the development of the eosinophil lineage and eosinophil-associated genes [30–32]. *In vitro* eosinophil differentiation experiments have demonstrated that GATA-1 is the primary transcription factor responsible for this eosinophil lineage specification [33].

Three cytokines, IL-3, IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF) play the most important roles in the regulation of eosinophil development (Figure 1). The function of IL-3 is the broadest as it leads to the expansion of a variety of cell types including monocytes, megakaryocytes, erythrocytes, basophils, neutrophils and eosinophils [27]. GM-CSF acts in a similar fashion, albeit with more mature precursor cells, inducing the formation of macrophages, neutrophils and eosinophils [34]. IL-5 is the cytokine responsible for selective terminal differentiation of eosinophils [35] and stimulates the release of eosinophils from the bone marrow into peripheral circulation [36]. GM-CSF, IL-3 and the chemokines CCL11, CCL24, and CCL26 (eotaxins) are also involved in eosinophil homeostasis and play an important role upon arrival of the eosinophil at a tissue location. In addition, an IFN-γ-induced transcription factor ICSBP can also drive the differentiation of eosinophils [37]. As IFN-γ is routinely present in allergic inflammation and, in our studies, was particularly upregulated in AERD [38], this led us to speculate on the role of IFN-γ being able to contribute to eosinophilia. Using an *in vitro* model with CD34+ hematopoietic progenitors, we demonstrated the capacity of IFN-γ, acting in synergy with IL-5, to promote the survival and differentiation of mature bi-lobed, CCR3- and Siglec-8-expressing

eosinophils [38] confirming prior studies [39] regarding the influence of this cytokine on eosinophil-mediated inflammation.

As with eosinophils, mast cells are derived from pluripotent hematopoietic CD34+ cells from the bone marrow [40], however mast cells do not fully mature until they reach their final tissue destination with the exception of mast cell leukemia. IL-3 and IL-6 increase early CD34+ progenitor cell numbers and begin the differentiation process, however it is binding of stem cell factor (SCF) to its receptor c-Kit (CD117) that is the master growth and differentiation factor for human mast cells (Figure 1) [41]. SCF is produced primarily by stromal cells [42] and it can either be released as a soluble growth factor or expressed on the cell surface of these cells. It is the expression and binding of SCF at tissue sites that cause the CD34+ precursor cells to arrest and terminally differentiate into mast cells.

Cysteinyl leukotriene over-production and over-responsiveness in AERD

Arguably, the best-characterized molecules associated with AERD are the CysLTs. A unique characteristic of the disease is the over-production of CysLTs in the resting state and a tremendous surge in CysLT production in response to aspirin and other non-selective cyclooxygenase inhibitors that target COX-1 [43]. Included in this list are the non-selective non-steroidal anti-inflammatory drugs (NSAIDs) as well as other inhibitors of COX-1 [44, 45]. The high CysLT levels in AERD reflect increased expression of the primary synthesis enzymes 5-lipoxygenase (5-LO) and more importantly leukotriene C_4 synthase (LTC_4S) (Figure 2). Increased expression of these enzymes is observed in the lungs, sinuses and nasal polyps of AERD subjects with eosinophils and resident mast cells being the primary cells expressing the enzymes [16, 19, 24, 46].

Not only do AERD subjects produce more CysLTs, but they also demonstrate an increased sensitivity to CysLTs [47]. Initially, two CysLT receptors were identified and were distinguished from each other by their differing potency for the CysLTs: CysLT1 receptors primarily respond to LTD_4 whereas CysLT2 receptors respond equally to LTD_4 and LTC_4. Neither receptor responds well to LTE_4. Acting through CysLT1 and inhibited by the CysLT2 receptor, CysLTs induce mast cell proliferation through activation of c-kit and extracellular signal-regulated kinase [48]. In AERD sinus tissue, high levels of CysLT1 were found in comparison to healthy tissue and following aspirin desensitization, the CysLT1 levels returned to normal [49]. CysLT1 receptors are also prominently expressed on airway smooth muscle [50] and these receptors mediate a portion of the CysLT-induced bronchospasm associated with aspirin challenges or desensitizations [51–54] as demonstrated by the ability of leukotriene receptor antagonists to attenuate much of the bronchospasm that occurs during these procedures.

While these findings made it appear that CysLT1 was the only important leukotriene receptor in AERD, several observations suggested they were only partially involved in the pathogenesis of disease. The most abundant leukotriene found in the circulation and airway is LTE_4 with C_4 and D_4 being rapidly converted to E_4, thus limiting their duration of action *in vivo*. Inhalation of LTE_4 by asthmatic and AERD subjects potentiates airway hyperresponsiveness to subsequent challenges with histamine, the effect of which could be

blocked with indomethacin [47, 55, 56]. In the bronchial mucosa of asthmatics, inhalation of LTE_4, but not LTD_4, causes recruitment of eosinophils, basophils and mast cells into the tissue [57, 58]. Mice in which both the CysLT1 and CysLT2 receptors have been deleted show enhanced skin swelling in response to intracutaneous LTE_4 in comparison to mice with these genes intact [59]. These studies led to the exploration for and ultimate identification of additional CysLT receptors that selectively respond to LTE_4 (GPR99 and P2Y12) [59–62]. The functional role of these LTE_4 receptors in AERD and the utility in targeting them as a therapeutic option are areas of active research, although diminished synthesis of CysLTs and, by extension, of LTE_4, could explain the superior efficacy of 5-lipoxygenase inhibitors in treating this disorder [63].

Underappreciated role for PGD_2 in AERD

PGD_2 and its metabolites have been found in blood and urine following aspirin challenge [11, 13, 64] and the conventional thought is that it is primarily mast cell derived, however reports demonstrate that eosinophils are also a source [65, 66]. Synthesis of PGD_2 is regulated by hematopoietic prostaglandin D_2 synthase (hPGDS) (Figure 2) and when secreted it binds two receptors CRTH2 (DP2) and DP1 that are expressed on numerous cell types. Activities of PGD_2 binding include stimulation of cell migration (including eosinophils and innate lymphoid type 2 (ILC2) cells), bronchoconstriction, vasodilation (flushing), and cellular activation and differentiation [67–70]. A role for PGD_2 in the pathogenesis of AERD is supported by studies in chronic sinusitis. Expression of hPGDS has been observed in polyps, specifically in eosinophils [66, 71, 72]. The degree of hPGDS expression correlated with eosinophil number and severity of disease. These studies did not examine AERD subjects, but given the high levels of eosinophilic infiltrate in AERD tissue, it is likely that hPGDS levels would have correlated as well. Recent work from our lab demonstrated that amongst CRS syndromes, in AERD the highest levels of hPGDS transcripts and proteins expression were observed and with eosinophils being the predominant cell type where expression was localized [66]. As discussed in other chapters, aspirin desensitization followed by high-dose aspirin therapy is often used to treat AERD, however not all patients tolerate the desensitization protocol. It has recently been shown that those subjects who cannot be desensitized express higher basal levels of PGD_2 (and thromboxane) in their serum and urine [64]. During the desensitization procedure, these patients had a surge of PGD_2 release but not thromboxane, whereas those who were successfully desensitized had decreased thromboxane and unchanged PGD_2 levels [64]. In this study, the correlation between PGD_2 and eosinophil number in the polyp tissue was not performed. Baseline PGD_2 levels may serve as a marker to identify those who will successfully undergo desensitization.

PGE_2 and PGE_2 receptor dysregulation in AERD

PGE_2 displays both pro- and anti-inflammatory functions reflecting its ability to interact with 4 distinct receptors (EP1-4) each having various activating or inhibitory functions. However, it is the role of PGE_2 acting through anti-inflammatory EP2 receptors to block eosinophil and mast cell degranulation that is central to the pathogenesis of AERD. AERD patients constitutively produce low levels of PGE_2 [16, 73] attenuating the anti-inflammatory

constraints provided by this lipid in this basal state. The further reduction of tissue PGE_2 concentrations by aspirin and other NSAIDs through COX-1 inhibition precipitates the activation of eosinophils and mast cells in AERD, as demonstrated by the ability of inhaled PGE_2 to protect against these reactions [74, 75]. This sensitivity of AERD patients to low tissue PGE_2 concentrations is amplified by their reduced expression of the anti-inflammatory EP2 receptor [18]. Serra-Pages and colleagues demonstrated that the ratio of EP2 to EP3 receptors on the surface of a mast cell influences the activation potential of these cells when the high affinity IgE receptor (FcεRI) is stimulated in a PGE_2-containing milieu [76]. Through examination of various mast cell lines, the authors found that those with high levels of EP2 could suppress FcεRI activation in the presence of PGE_2, but when EP3 levels were high FcεRI activation of mast cells was enhanced. It is likely that the low EP2/EP3 ratio on mast cells and possibly eosinophils in AERD contributes to this disease as any PGE_2 that is available would preferentially signal through the EP3 receptor and activate these cells, thus contributing to the pro-inflammatory cascade.

Several studies have investigated the mechanism behind the reduced levels of PGE_2 in AERD and, perhaps not surprisingly, have correlated this with a decrease in the relevant upstream metabolic enzymes. The production of PGE_2 from arachidonic acid (Figure 2) involves the sequential synthesis of PGG_2/PGH_2 by the two cyclooxygenase enzymes (COX-1 and COX-2) followed by the synthesis of PGE_2 by the microsomal PGE_2 synthases (mPGES-1, mPGES-2) and cytosolic PGE_2 synthase (cPGES). It is mPGES-1 that is most relevant to PGE_2 production in inflammatory disorders such as AERD as it is the enzyme primarily functionally coupled to COX-2 [77]. COX-2 mRNA and protein expression are markedly diminished in AERD [16, 17, 78]. Our studies have confirmed this diminished expression of COX-2 [79]. We found no significant change in COX-1 and a trend towards diminished mPGES-1 expression and that this is driven in part by IL-4. Diminished COX-2 expression and the reduced capacity to synthesize PGE_2 contributes to the severity of inflammation observed in AERD and accentuates the sensitivity of these individuals to the further inhibition of PGE_2 synthesis associated with aspirin and other NSAIDs. With this relative absence of COX-2, AERD subjects become dependent upon COX-1 for the PGE_2 that is necessary to restrain mast cell and eosinophil activation. Thus, most AERD patients tolerate selective COX-2 inhibitors [80], supporting this concept regarding the unique importance of COX-1-derived PGE_2.

Cytokine expression in AERD

Numerous studies have examined the cytokine milieu found in the tissue of eosinophilic sinusitis and AERD. Many of these studies have reported expression of a Th2-like immune profile (IL-4, IL-13 and IL-5), similar to other allergic diseases with eosinophilic infiltrate, though few have specifically focused on AERD [81–88]. However, numerous observations suggest that in contrast to eosinophilic sinusitis patients who tolerate aspirin, AERD appears to have a mixed Th2- and Th1-like cytokine milieu characterized by prominent expression of IFN-γ. The first study to suggest this examined non-allergic sinusitis patients and demonstrated enhanced IFN-γ expression in this cohort: a group in which AERD is likely to be over-expressed [89]. Since this initial report, high levels of IFN-γ in chronic sinusitis have been reported by other investigators, although AERD subjects were not specifically

separated or recruited for the studies [90]. One study specifically addressed IFN-γ expression in AERD and found enhanced levels of IFN-γ in circulating CD8$^+$ cells compared to aspirin-tolerant controls [91]. The concept that AERD reflects a mixed Th2- and Th1-like cytokine profile was confirmed in our recent study in which NP tissue derived from AERD subjects was contrasted with those obtained from aspirin tolerant and control subjects by their over-expression of IFN-γ mRNA transcripts and protein [38]. To our surprise, we found that eosinophils themselves were the most important source for this cytokine, which is consistent with previous studies that demonstrated IFN-γ can be expressed by eosinophils in substantial quantities [92–94]. In contrast to our findings, a recent report did not find elevated levels of IFN-γ protein in nasal polyps from AERD subjects, however this group did not perform flow cytometry, immunohistochemistry or quantitative PCR to verify their results [88].

Cytokine dysregulation of LTC$_4$S and CysLT receptors

Under non-inflammatory conditions, mast cells express moderate levels of LTC$_4$S that can be increased greatly following stimulation by IL-4 (but not by IL-5 or IL-13) [95]. It has also been observed that IFN-γ also has the capability of upregulating LTC$_4$S expression in umbilical cord-derived mast cell progenitors (Joshua Boyce, personal communication). While mast cells are capable of synthesizing CysLTs, previous studies [24] have demonstrated that eosinophils are the more important cell type over-expressing LTC$_4$S in AERD. Examining a battery of cytokines (including IL-3, IL-4, IL-5, GM-CSF, IL-1, TNF-α and IFN-γ), we were unable to demonstrate an ability of any of these cytokines to modulate LTC$_4$S expression on circulating eosinophils. This may be due to their terminal differentiation state and short life span. We have also failed to demonstrate the ability of IL-4 to increase LTC$_4$S expression on eosinophils differentiated from progenitors in the presence of IL-3 and IL-5. However, when the progenitors were incubated with IFN-γ during the differentiation stage, a significant increase in LTC$_4$S expression was observed [38]. The increase in LTC$_4$S expression translated into increased capacity of these newly differentiated eosinophils to secrete CysLTs upon activation by aspirin [14]. Another mechanism of CysLT production involves the transcellular conversion of LTA$_4$ by adherent platelets expressing LTC$_4$S [96]. The frequency of platelet-adherent eosinophils, neutrophils and monocytes are markedly elevated in the blood of AERD subjects. Any LTA$_4$ that is released into the extracellular space can be captured by the platelets and converted to CysLTs. It has been estimated that adherent-platelets contribute up to 50% of the total LTC$_4$S activity in blood and thus would represent a significant source of the CysLTs found in AERD [96].

CysLT receptor expression is regulated by numerous cytokines including IL-4 and IFN-γ, but also IL-5 and IL-13. IL-4 increases expression of CysLT1 on mast cells [97, 98] and monocytes [99]. In our studies, IL-4 also increased the expression of both CysLT1 and CysLT2 on T and B lymphocytes and eosinophils [100]. We also demonstrated robust upregulation of CysLT1 and CysLT2 receptors in response to IFN-γ on T cells and eosinophils [100]. In recent studies, examination of eosinophils derived from CD34+ progenitors have also demonstrated the ability of IFN-γ to upregulate CysLT1 and CysLT2 receptor expression [38]. There have been no reports of cytokine modulation of other CysLT receptors. In summary, in the AERD

cytokine environment, both mast cells and eosinophils are primed to produce and respond to the CysLTs that are produced during the disease process.

Cytokine dysregulation of PGE2 Synthesis and EP2 Receptors

Cytokine regulation of the PGE_2 synthesis pathway in AERD has not been thoroughly investigated. However, in other model systems, the influence of IL-4 on COX-2 expression has been reported. Inhibition of COX-2 expression by IL-4 was noted using peripheral blood monocytes, alveolar macrophages and non-small cell lung cancer cells [101–103]. We performed studies on nasal polyp-derived fibroblasts and mononuclear phagocytic cells. Monocytes were utilized both as representative inflammatory cells, but also because PGE_2 is their dominant prostaglandin product. Similar to other findings, significant inhibition of COX-2 and also mPGES-1 (but not COX-1) mRNA and protein expression was observed following stimulation with IL-4 on both the monocytes and nasal polyp-derived fibroblasts [79]. Inhibition of COX-2 and mPGES-1 synergize to result in dramatically less stimulated PGE_2 secretion by monocytes [79]. IL-13 has been reported to have a similar effect on airway epithelial cells [104]. Thus, in addition to enhancing the CysLT pathways, IL-4 and IL-13 contribute to the AERD phenotype through inhibition of the PGE_2 pathway. The role of IFN-γ modulation of the prostaglandin pathway is unclear as its action appears to be cell-type specific. IFN-γ can induce COX-2 mRNA in most inflammatory cells [105, 106] whereas it decreases COX-2 expression in placental [107] and intestinal epithelial cancer cells [108]. Actions of IFN-γ on other parts of the PGE_2 pathway have not been studied in detail.

Activation of eosinophils and mast cells by aspirin

It was unknown in AERD how aspirin triggered the release of pro-inflammatory mediators. While, as noted, inhibition of COX releases the protective constraints provided by PGE_2, this alone does not explain the positive signaling driving cell activation. In a particularly robust murine model of AERD, aspirin sensitivity is induced by the knocking out of the mPGES-1 gene [109]. In this model, the positive – activating – signal is provided by allergic inflammation, a mechanism not likely to be relevant in AERD, at least in those AERD patients who are not atopic. We speculated that aspirin and other NSAIDs had the inherent capacity to directly activate eosinophils and mast cells. When tested, both eosinophils and mast cells generated Ca^{+2} fluxes following stimulation with water soluble lysine aspirin (LysASA) [14]. Similar results were observed with eosinophil activation measured by EDN release and eosinophil and mast cell secretion of PGD_2 [14, 66]. To our surprise, when eosinophils from control, aspirin tolerant, and AERD subjects were compared, no differences were observed in levels of mediator release. Our explanation as to why hypersensitivity reactions due to mediator release caused by aspirin/NSAIDs are not observed in these control cohorts reflects alterations in their PGE_2 sensitivity, specifically the decreased capacity to produce and respond to this anti-inflammatory mediator observed in AERD. We speculate that the higher expression of PGE_2 as well as its anti-inflammatory EP2 receptor acts to prevent the acute reactions to aspirin/NSAIDs in controls and aspirin tolerant asthmatics [21, 74]. An additional explanation for the absence of clinical symptoms in these control cohorts is that when activated with aspirin, their circulating eosinophils produced very low levels of CysLTs [14] in contrast to the robust levels found in AERD

subjects following aspirin ingestion [43]. As mentioned earlier, AERD sinonasal and lung tissue is characterized by high numbers of eosinophilic hematopoietic progenitor ($CD34^+IL-5R\alpha^+$) cells [29, 110]. We therefore investigated whether eosinophils differentiated from progenitor cells in the presence of IFN-γ would recapitulate the sensitivity to aspirin displayed by tissue eosinophils *in vivo* in AERD. After maturation with IFN-γ, the mature eosinophils displayed increased gene expression for both LTC_4S and hPGDS [14, 66]. Consistent with the increase in LTC_4S gene expression, CysLT secretion was dramatically increased upon LysASA activation [14]. In addition to increased CysLT production, these IFN-γ matured eosinophils displayed enhanced PGD_2 production when stimulated with LysASA [66].

Summary: Towards a generalized model for the induction of the AERD phenotype

While our understanding that aspirin causes reactions and that AERD is a debilitating disease have been known for many years, the exact mechanisms driving AERD and how to treat it are still largely unknown. Our work and that of others have demonstrated the importance of both eosinophils and mast cells as drivers of the disease leading to increased expression of pro-inflammatory mediators and reflecting the loss of protective PGE_2. The ultimate result is constitutive over-production of and over-responsiveness to mediators by eosinophils and mast cells in the basal state in AERD, with the uncontrolled release of mediators when these cells are directly triggered by aspirin. The increased recognition of the cellular components and mechanisms of action in AERD provides an opportunity to develop alternative targeted therapeutic approaches aimed at dampening the severe impacts of this disease.

Acknowledgments

Abbreviations

5-LO	5-lipoxygenase
AERD	aspirin exacerbated respiratory disease
AFS	allergic fungal sinusitis
CHES	chronic hyperplastic eosinophilic sinusitis
COX	cyclooxygenase
CysLT	cysteinyl leukotriene
ECP	eosinophil cationic protein
Eo/B CFU	eosinophil/basophil progenitors or colony forming units
GM-CSF	granulocyte macrophage colony-stimulating factor

IFN	interferon
IL	interleukin
LT	leukotriene
LTC$_4$S	leukotriene C$_4$ synthase
NP	nasal polyposis
NSAID	non-steroidal anti-inflammatory drugs
PG	prostaglandin
PGDS	prostaglandin D$_2$ synthase
PGES	prostaglandin E$_2$ synthase
SCF	stem cell factor
STAT	signal transducer and activator of transcription

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Key Points

1. AERD is a disease of overproduction and hyper-responsiveness to lipid mediators.

2. Mast cells and eosinophils are key driver of AERD pathogenesis through production of pro-inflammatory mediators following aspirin stimulation.

3. Due to their involvement, therapies that target mast cells and eosinophils may be useful in providing clinical benefit in AERD.
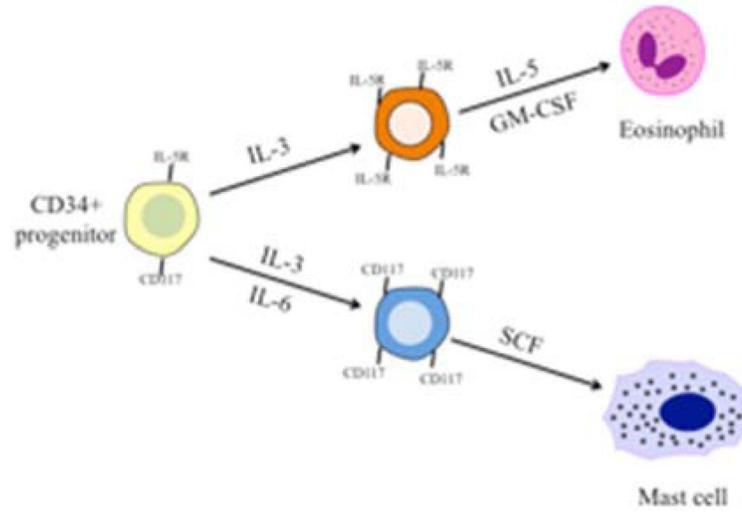
Figure 1. Eosinophil and mast cell development from CD34 progenitor cell
Through the actions of IL-3, IL-5 and GM-CSF, CD34 progenitor cells mature into eosinophils. Mast cells develop following stimulation with IL-3, IL-6 and SCF from CD34 progenitor cells.
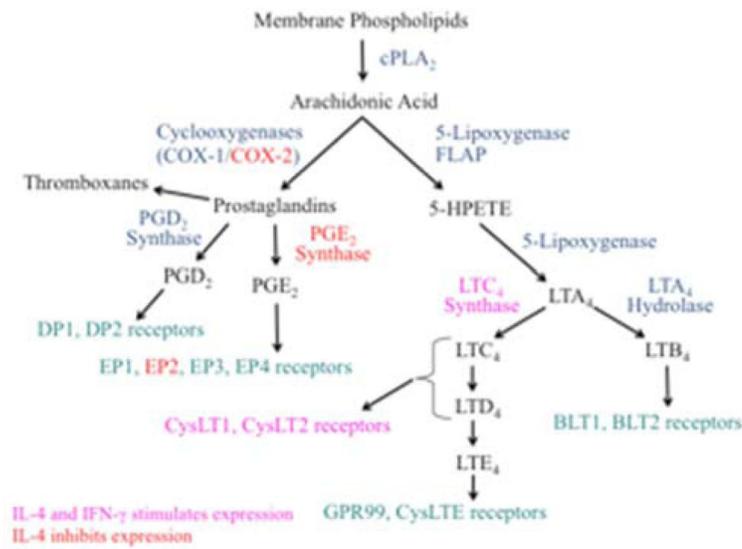
Figure 2. Pathway depicting metabolites of arachidonic acid important in AERD
Following conversion to arachidonic acid by phospholipase A2, further processing occurs via either the prostaglandin pathway mediated by COX-1/COX-2 or the leukotriene pathway mediated by 5-lipoxygenase. Red lettering shows genes inhibited by IL-4 and pink lettering show genes stimulated by IL-4 and IFN-γ.