Factors driving the aspirin exacerbated respiratory disease phenotype

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ABSTRACT

Background: Aspirin-exacerbated respiratory disease (AERD) is explained in part by overexpression of 5-lipoxygenase and leukotriene C4 synthase (LTC4S), resulting in constitutive overproduction of cysteinyl leukotrienes (CysLTs) and driving the surge in CysLT production that occurs with aspirin ingestion. Similarly, AERD is characterized by the overexpression of CysLT receptors. 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 up-regulation of LTC4S by mast cells.

Methods: Literature review.

Results: Our previous studies demonstrated that IFN-γ, but not IL-4, drives this process in eosinophils. These published studies also extend to both IL-4 and IFN-γ the ability to up-regulate CysLT receptors. Prostaglandin E2 (PGE2) acts to prevent CysLT secretion by inhibiting mast cell and eosinophil activation. PGE2 concentrations are reduced in AERD, and our published 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 together play an important pathogenic role in generating the phenotype of AERD. Finally, induction of LTC4S and CysLT1 receptors by IL-4 reflects in part the IL-4-mediated activation of signal transducer and activator of transcription 6 (STAT6). Our previous studies demonstrated that aspirin blocks trafficking of STAT6 into the nucleus and thereby prevents IL-4-mediated induction of these transcripts, thereby suggesting a modality by which aspirin desensitization could provide therapeutic benefit for AERD patients.

Conclusion: This review will examine the evidence supporting this model.

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spirin-exacerbated respiratory disease (AERD) or Samter’s triad was originally defined by the presence of nasal polyps, aspirin sensitivity, and asthma.1 It is now recognized that this disorder is characterized by hypersensitivity not only to aspirin but also to other nonselective cyclooxygenase (COX) inhibitors.2-4 Asthma is not always present and thus the preferred terminology AERD. Other characteristics include the less common association with atopy,3,5 hyper-eosinophilia, and a tendency to develop de novo in adulthood.3,5-7 Aspirin hypersensitivity is found in as many as 10%-20% of adult asthmatics and up to 30% of asthmatics with nasal polyposis.5,8 When asthma is present in this disorder, it often becomes severe and is associated with aggressive airway remodeling.9 Similarly, the sinusitis present in this disorder is often severe and associated with complete or near complete sinus opacification.7 A central feature of AERD is its association with profound overproduction and overresponsiveness to cysteinyl leukotrienes (CysLTs)10,11 occurring concomitantly with a profound underproduction and underresponsiveness to prostaglandins.12-14 These CysLTs have important proinflammatory and profibrotic effects that contribute to the asthma severity and to the extensive hyperplastic sinusitis and nasal polyposis.7,15,16 And, conversely, the down-regulation of prostaglandin pathways reduces the constraints that would normally act to attenuate these proinflammatory pathways.17 This review will focus on the dysregulation of these respective pro- and antiinflammatory pathways and the cytokine mechanisms that underlie this dysregulation and, finally, will discuss implications of aspirin desensitization as a therapeutic intervention that acts by altering these pathways.

CYSTEINYL LEUKOTRIENE OVERPRODUCTION AND OVERRESPONSIVENESS IN AERD

AERD is characterized by the constitutive overproduction of CysLTs and a massive, potentially life-threatening, further surge in CysLT production in response to aspirin and other nonselective COX inhibitors that block COX-1.18 This includes not only nonselective nonsteroidal antiinflammatory drugs (NSAIDs) but also other inhibitors of COX-1, including alcoholic products.19,20 Overproduction of CysLTs in AERD reflects the increased expression of its primary synthesis enzymes 5-lipoxygenase and especially leukotriene C4 synthase (LTC4S). Up-regulation of these enzymes is observed in the lungs, sinuses, and nasal polyps of AERD subjects, localized in large part to the infiltrating eosinophils, and resident mast cells.12,15,21 AERD subjects also demonstrate an increased sensitivity to Cys-LTs,21 reflecting in part their up-regulation of CysLT1 receptors.24 The two originally characterized CysLT receptors were distinguished by their differing potency for the CysLTs: CysLT1 receptors primarily respond to LTD4 whereas CysLT2 receptors respond equally to LTD4 and LTE4. However, this pattern could not explain a body of literature demonstrating the capacity of LTE4 and not either of these other CysLT receptors, to drive smooth muscle contraction and profibrotic influences on both airway explicans28 and, via inhalation challenges, on the airway itself of AERD subjects.23,26,27 The relative insensitivity of either CysLT1 or CysLT2 receptors to LTE4r in contrast to the sensitivity of AERD subjects to this lipid mediator, led to the exploration for and ultimate identification of additional CysLT receptors that selectively respond to LTE4r. CysLT type 1 receptors are prominently expressed on airway smooth muscle,29 and these receptors do mediate much of the CysLT-induced bronchospasm associated with aspirin challenges or desensitizations.30-32 As evidenced by the ability of leukotriene receptor antagonists to attenuate much of the bronchospasm that occurs with these procedures. Thus, AERD is characterized by enhanced sensitivity to leukotrienes, reflecting in part the overexpression of CysLT1 receptors. The enhanced responsiveness of these subjects to LTE4r is intriguing, although as of now, the expression and function of putative LTE4 receptors in this disorder remains unstudied.

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Presented at the North American Rhinology & Allergy Conference, January 23, 2014, Puerto Rico
This work was supported by National Institutes of Health RO1 AI47737 and PO1 AI50989
The authors have no conflicts of interest to declare pertaining to this article
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PROSTAGLANDIN E<sub>2</sub> (PGE<sub>2</sub>) AND PGE<sub>2</sub> RECEPTOR DYSREGULATION IN AERD

PGE<sub>2</sub> displays both pro- and antiinflammatory functions reflecting its ability to interact with four distinct receptors (EP1–EP4), each having various activating or inhibitory functions. However, it is the role of PGE<sub>2</sub> acting through antiinflammatory EP2 receptors to block eosinophil and mast cell degranulation that is central to the pathogenesis of AERD. AERD patients constitutively display low levels of PGE<sub>2</sub>, attenuating the antiinflammatory constraints provided by this lipid. The further reduction of tissue PGE<sub>2</sub> 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 an infusion of PGE<sub>2</sub> to protect against these reactions.37,38

Several studies have investigated the mechanism behind the reduced levels of PGE<sub>2</sub> in AERD and, perhaps not surprisingly, have correlated this with a decrease in the responsible upstream metabolic enzymes. The production of PGE<sub>2</sub> from arachidonic acid involves the sequential synthesis of PGG<sub>2</sub>/PGH<sub>2</sub> by the two COX enzymes (COX-1 and COX-2) followed by the synthesis of PGE<sub>2</sub> by the microsomal PGE<sub>2</sub> synthase. It is mPGES-1 that is most relevant to PGE<sub>2</sub> production in inflammatory disorders such as AERD, because it is the enzyme primarily functionally coupled to COX-2.39 COX-2 mRNA and protein expression are markedly diminished in AERD.12,13,40 Our studies have confirmed this diminished expression of COX-2.41 We found no significant change in COX-1 and a trend toward diminished mPGES-1 expression.

Diminished COX-2 expression and the reduced capacity to synthesize PGE<sub>2</sub> contributes to the severity of inflammation observed in AERD and accentuates the sensitivity of these individuals to the inhibition of PGE<sub>2</sub> synthesis associated with aspirin and other NSAIDs. With this relative absence of COX-2, AERD subjects become dependent upon COX-1 for the PGE<sub>2</sub> that is necessary to restrain mast cell and eosinophil activation. Most AERD patients tolerate selective COX-2 inhibitors, supporting this concept regarding the unique importance of COX-1-derived PGE<sub>2</sub>. In summary, AERD represents a state characterized by constitutive overexpression and overresponsiveness to CysLT<sub>1</sub>s occurring concomitantly with diminished expression and underresponsiveness to PGE<sub>2</sub>. This dysfunctional state reflects the cytokine milieu of AERD (Fig. 1).

Cytokine Expression in AERD

Numerous studies have addressed the cytokine milieu of eosinophilic sinusitis and, to a lesser extent, AERD. Not surprisingly, most of these studies have demonstrated a prominent T helper (Th2)-like profile, as would be expected in any eosinophilic process.42–44 However, there are numerous observations that suggest that in contrast to asthmatic or eosinophilic sinusitis patients who tolerate aspirin, AERD seems much more to be a mixed Th2- and Th1-like milieu with prominent expression of interferon (IFN)-γ. This was first suggested in studies demonstrating enhanced IFN-γ expression in a group labeled “nonallergic” sinusitis patients. One characteristic of this group was a higher prevalence of AERD in comparison with the other sinusitis groups.49 Other investigators have also described high IFN-γ levels in “chronic sinusitis” although, again, did not specify the extent to which this was driven by inclusion of AERD subjects.50 More recently, a group of adult-onset severe asthmatics with hypereosinophilic and absence of allergy, a cohort in whom AERD subjects were known to be particularly overexpressed, were distinctly characterized by their expression of a high IFN-γ gene signature (Sally Wenzel, AAAAI National Meeting, San Diego, CA; March 2014). The only other study that evaluated IFN-γ expression in AERD demonstrated enhanced levels of IFN-γ in circulating CD8<sup>+</sup> cells.51

The concept that AERD might reflect a prominent Th1-like component was confirmed in our recently published studies, in which nasal polyp tissue derived from AERD subjects was contrasted from those obtained from aspirin tolerant and control subjects by their overexpression of IFN-γ mRNA transcripts (Fig. 2) and protein.52 Surprisingly, we subsequently also demonstrated that eosinophils themselves were the most important source for this cytokine consistent with recognition that IFN-γ can be expressed by eosinophils in substantial amounts.53–55 Eosinophils secrete numerous cytokines and chemokines,54 and unlike lymphocytes, eosinophils store these cytokines preformed within granules that can be instantly released with activation.55,56 The robust expression of interleukin (IL-4) in AERD did not extend to IL-5 (Fig. 2), likely representing the physiology of eosinophils, specifically that IL-4 but not IL-5 is a prominent eosinophil-derived cytokine. Given the capacity of IFN-γ to block IgE class switch recombination, it is intriguing to speculate that this coexpress-
sion of IFN-γ contributes to the frequent absence of allergy in AERD previously noted, despite the observed expression of IL-4. The recognition of AERD as a combined Th2-/Th1-like disease prompted us to query the role of this mixed cytokine milieu in driving the AERD phenotype.

HYPEREOSINOPHILIA IN AERD

AERD sinonasal and lung tissue are characterized by dramatic (−10-fold) up-regulation in the number of infiltrating eosinophils. An IFN-γ-induced transcription factor (interferon consensus sequence binding protein) can drive the differentiation of eosinophils, leading us to speculate on its role in contributing to this eosinophilia. Consistent with this, we were struck by the capacity of IFN-γ, acting in synergy with IL-5, to promote both the survival and differentiation of mature bleded, CC chemokine receptor 3+ and sialic acid-binding, immunoglobulin-like lectin 8-expressing eosinophils from CD34+ hematopoietic progenitors, confirming earlier studies regarding the influence of this cytokine on eosinophil-mediated inflammation. However, for the remainder of this review, we will primarily focus on influences of cytokines on CysLT and PG pathways.

CYTOKINE DYSREGULATION OF LTC4S AND CysLT RECEPTORS

 Mast cells typically express modest levels of LTC4S and its up-regulation can be mediated by IL-4 (but not by IL-5 or IL-13). Interestingly, a role for IFN-γ in up-regulating LTC4S expression in umbilical cord-derived mast cell progenitors was also observed (J. Boyce, Harvard Medical School, 2014, personal communications). However, as noted, studies investigating the source of CysLTs in AERD have suggested that eosinophils are likely the more important cell type overexpressing LTC4S. In our studies, we were unable to demonstrate an ability of numerous innate or adaptive cytokines, including IL-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor, IL-1, tumor necrosis factor-α, or even IFN-γ to modulate LTC4S expression in circulating eosinophils, presumably reflecting their terminal differentiation state. And our studies also failed to demonstrate an influence of IL-4 on LTC4S expression by eosinophils during their terminal differentiation state. In our studies, IL-4 also increased the expression of CysLT1 on T cells and eosinophils. Interestingly, our studies also demonstrated robust up-regulation of CysLT1 receptor in response to IL-4 on T cells and eosinophils. This was an effect that, at the time of publication, we did not appreciate in regard to its likely relevance to AERD. More recently, we have also extended this capacity of IFN-γ to up-regulate CysLT1 receptors to eosinophils newly differentiated from CD34+ progenitors.

CYTOKINE DYSREGULATION OF PGE2 SYNTHESIS AND EP RECEPTORS

We investigated the molecular mechanism underlying inhibition of PGE2 synthesis pathways in AERD. For these studies we focused on influences of IL-4, reflecting again, its prominent expression in AERD, its previously described influences on the prostaglandin metabolic pathways, and its involvement in the other facets of arachidonate dysregulation previously discussed. IFN-γ is unlikely to be involved in this aspect of the AERD phenotype, given its established role in up-regulating inducible COX-2 (COX-2). Our studies were performed on nasal polyp-derived fibroblasts and mononuclear phagocytic cells. Monocytes were used both as representative inflammatory cells, but also because PGE2 is their dominant prostaglandin product. Significant inhibition of COX-2 and mPGES-1 (but not COX-1) mRNA and protein expression was observed in response to IL-4. This appears to be a generalized effect of IL-4 insofar as similar inhibition was also observed in fibroblasts. Inhibition of COX-2 and mPGES-1 synergize to result in dramatically less stimulated PGE2 secretion by monocytes. Thus, in addition to up-regulating CysLT pathways, IL-4 contributes to the AERD phenotype by inhibiting their PG pathways. However, it is necessary to remark that more than just loss of the tempering influences of PGE2, underlies these reactions, otherwise all asthmatics and, indeed, even healthy subjects would react to aspirin/NSAID ingestion with activation of their mast cells and eosinophils. We therefore questioned the capacity of aspirin (and other NSAIDs) to directly drive the activation of eosinophils and mast cells.

DIRECT ACTIVATION OF EOSINOPHILS AND MAST CELLS BY ASPIRIN

How aspirin triggers these non-IgE-mediated reactions has been an enigma. We evaluated the capacity of aspirin and other NSAIDs to directly activate eosinophils and mast cells. For these studies, we used the water-soluble aspirin-like compound lysine aspirin (LysASA). Both eosinophils and mast cells displayed Ca2+ fluxes after stimulation with LysASA, and similar results were observed with eosinophil eosinophil-derived neurotoxin secretion. Similar results were obtained with ketorolac but not sodium salicylates. To our surprise, when eosinophils from control, aspirin tolerant, and aspirin intolerant subjects were compared, no differences were observed. We suspect the explanation as to why hypersensitivity reactions to aspirin/NSAID are not observed in these control cohorts reflects the relative lack of reactivity in the control cohorts is that a large component of the anaphylactoid response to aspirin is driven by CysLTs (as shown as previously mentioned by the ability of LT modifiers to greatly attenuate their severity). Interestingly, we never observed LysASA or ketorolac-mediated CysLT secretion from circulating eosinophils, even when obtained from AERD donors. AERD sinonasal and lung tissue is characterized by high numbers of eosinophils in the hematopoietic progenitor (CD34+IL-5Rα+) cells, and CD34+IL-5Rα+ cells. This will mature in the presence of IFN-γ and, as previously discussed, acquire the ability to produce CysLTs. This would suggest that the robust CysLT production would be limited to airway, and not circulating, eosinophils. 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. Consistent with their increased LTC4S expression, CysLT secretion was subsequently detected upon LysASA activation (Fig. 3).

ASPIRIN DESENSITIZATION IN AERD, TARGETING OF IL-4

Aspirin desensitization is an effective treatment for AERD and has been associated with the diminished need for nasal endoscopic sur-
matured from CD34
CysLT1R and LTC4S promoters. Both by electrophoretic mobility shift
assays, we confirmed the presence of STAT6-binding sites within the
electrophoretic mobility shift, oligomer competition, and supershift
mediated transcription, and similarly, a putative STAT site has been
A STAT6 site in the CysLT1 receptor promoter is central to its IL-4-
recognition that engagement of the IL-4 receptor by IL-4 induces
by which aspirin may induce these effects. However, we focused on
STAT6,75 suggesting to us that aspirin may produce its clinical utility
necessity for oral corticosteroids, and less severe asthma.2,71,72 The molec-
gery, improved sense of smell, fewer bouts of acute sinusitis, reduced
need for oral corticosteroids, and less severe asthma.2,71,72 The molec-
ular mechanisms driving the beneficial effects of aspirin have not
been determined, but we believe it is due in part to the ability of this
compound to inhibit the biologic activities of IL-4 (and perhaps, by
extension, IFN-γ). Consistent with this concept are the observations
that successful aspirin desensitization is associated with reversal of
many of the IL-4-modulated features of AERD discussed above, in-
cluding the ability of desensitization to down regulate CysLT synthe-
sis and responsiveness pathways.21,24,25 There are many mechanisms
by which aspirin may induce these effects. However, we focused on
recognition that engagement of the IL-4 receptor by IL-4 induces
activation of signal transducer and activator of transcription (STAT).
A STAT6 site in the CysLT1 receptor promoter is central to its IL-4-
mediated transcription, and similarly, a putative STAT site has been
identified in the LTC4S gene.74 Aspirin inhibits the activation of
STAT6,75 suggesting to us that aspirin may produce its clinical utility
in AERD through direct inhibition of the IL-4-activated STAT6 path-
way. Our studies investigated the inhibition by aspirin and other
NSAIDs of the STAT6-mediated regulation of the CysLT1 receptor
and LTC4S genes.41 In a dose-dependent fashion, aspirin inhibited
transcription of IL-4-induced CysLT1 receptors. Subsequently, via
electrophoretic mobility shift, oligomer competition, and supershift
assays, we confirmed the presence of STAT6-binding sites within the
CysLT1R and LTC4S promoters. Both by electrophoretic mobility shift
and Western hybridization assays, our data demonstrated the absence
of phosphoSTAT6 protein within the nuclei of aspirin-treated cells
(Fig. 4). These results were extended to other NSAIDs, including
ketorolac, but not sodium salicylate. The mechanism by which aspirin
blocks pSTAT6 nuclear expression is not known but has been sug-
gested to involve nuclear trafficking and recycling of transcription
factors.76 These data suggest that aspirin desensitization may provide
effective therapy for AERD, in part through mitigation of STAT6
activation, leading to down-regulation of the leukotriene pathways.
That similar mechanisms could be involved in blocking IFN-γ-acti-
vated pathways (e.g., STAT1 trafficking) would be an intriguing area
for future research.

**SUMMARY**

**Toward a Generalized Model for the Induction of the AERD Phenotype**

Although the exact mechanisms driving AERD are not fully under-
stood, part of the explanation is the marked overexpression of the
5-lipoxygenase and LTC4S genes, resulting in constitutive overpro-
duction of CysLTs, and the decrease in PGE2 expression that would
normally act to constrain mast cell and eosinophil activation. We
present a series of studies that strongly suggest that this AERD
phenotype is derived, in large part, from the increased expression of
both IL-4 and IFN-γ and that, as such, this disease reflects a mixed
Th1/Th2 process. The increased expression of proinflammatory me-
diators and loss of protective PGE2 leads to uncontrolled release of
mediators, and especially CysLTs, when eosinophils and mast cells
are directly triggered by aspirin in AERD subjects. This increased
understanding of the cellular reactions provides an opportunity to
develop new therapeutic approaches aimed at dampening the severe
impacts of this disease.

**REFERENCES**

1. Samter M, and Beers RF Jr. Intolerance to aspirin. Clinical studies and
consideration of its pathogenesis. Ann Intern Med 68:973–983, 1968.
2. Szczeklik A, and Stevenson DD. Aspirin-induced asthma: advances
in pathogenesis and management. J Allergy Clin Immunol 104:5–13,
1999.
3. Szczeklik A, and Nizankowska E. Clinical features and diagnosis of
aspirin induced asthma. Thorax 55:S42–SS44, 2000.
4. Berges-Gimeno MP, Simon RA, and Stevenson DD. The natural his-
tory and clinical characteristics of aspirin-exacerbated respiratory
disease. Ann Allergy Asthma Immunol 89:474–478, 2002.
5. Vally H, Taylor ML, and Thompson PJ. The prevalence of aspirin
intolerant asthma (AIA) in Australian asthmatic patients. Thorax
57:569–574, 2002.
6. Payne SC, Early SB, Huyett P, et al. Evidence for distinct histologic
profile of nasal polyps with and without eosinophilia. Laryngoscope
121:2262–2267, 2011.
7. Mascia K, Borish L, Patrice J, et al. Chronic hyperplastic eosinophilic
sinusitis as a predictor of aspirin-exacerbated respiratory disease.
Ann Allergy Asthma Immunol 94:652–657, 2005.
8. Szczeklik A, and Sanak M. Molecular mechanisms in aspirin-induced
asthma. ACI International 12/4:171–176, 2000.
9. Mascia K, Haselkorn T, Deniz YM, et al. Aspirin sensitivity and severity of asthma: evidence for irreversible airway obstruction in patients with severe or difficult-to-treat asthma. J Allergy Clin Immunol 2011;128:87–95.

10. Antczak A, Montuschi P, Khairitoron S, et al. Increased exhaled cysteinyl-leukotrienes and 8-isoprostan in aspirin-induced asthma. Am J Respir Crit Care Med 166:301–308, 2002.

11. Daflem PJ, Mullenburg D, Hugli TE, and Stevenson DD. Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses. J Allergy Clin Immunol 104:539–544, 1999.

12. Perez-Novo CA, Waterb EB, Claey C, et al. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. J Allergy Clin Immunol 2005; 115:1189–1196, 2005.

13. Picado C, Fernandez-Morata JC, Juan M, et al. Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. Am J Respir Crit Care Med 160:291–296, 1999.

14. Ying S, Meng Q, Scadding G, et al. Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells. J Allergy Clin Immunol 117:312–318, 2006.

15. Steineke JW, Bradley D, Arango P, et al. Cysteinyl leukotriene expression in chronic hyperplastic sinusitis-nasal polyposis: importance to eosiophilia and asthma. J Allergy Clin Immunol 111:342–349, 2003.

16. Belvisi MG, Friend DS, Mokawar A, et al. Cysteinyl leukotriene 1 receptor controls the severity of chronic pulmonary inflammation and fibrosis. Proc Natl Acad Sci USA 101:3047–3052, 2004.

17. Steineke JW. Editorial: Yin-Yang of EP receptor expression. J Leuk Biol 92:1129–1131, 2012.

18. Christie PE, Tagari P, Ford-Hutchinson AW, et al. Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Respir Dis 143:1025–1029, 1991.

19. Cowburn AS, White AA, Barrett NA, et al. Alcohol-induced respiratory symptoms are common in patients with aspirin exacerbated respiratory disease. J Allergy Clin Immunol. In practice 2:208–213, 2014.

20. Payne SC. Re: Alcohol-induced respiratory symptoms are common in patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol Pract 2:644, 2014.

21. Sampson AP, Cowburn AS, and Sladek K. Profound overexpression of leukotriene C4 synthase in bronchial biopsies from aspirin-intolerant asthmatic patients. Int Archives Allergy Immunol 113:355–357, 1997.

22. Cowburn AS, Sladek K, Soja J, et al. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest 101:834–846, 1998.

23. Arm JP, O'Hickey SP, Spur BW, and Lee TH. Airway responsiveness to histamine and leukotriene E4 in subjects with aspirin-intolerant asthma. Am Rev Respir Dis 140:1486–1491, 1989.

24. Sousa AR, Parikh A, Scadding G, et al. Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells. J Allergy Clin Immunol 117:312–318, 2006.

25. Lee TH, Austen KF, Corey EJ, and Drazen JM. Leukotriene E4–induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. Proc Natl Acad Sci USA 81:4922–4925, 1984.

26. Steinke JW. Re: Alcohol-induced respiratory symptoms are common in patients with aspirin exacerbated respiratory disease. J Allergy Clin Immunol Pract 2:644, 2014.

27. Laitinen LA, Laitinen A, Haahtela T, et al. Leukotriene E4 and granulocyte infiltration into asthmatic airways. Lancet 341:989–996, 1993.

28. Nomura Y, Hiramoto T, and Fujita N. Identification of endogenous surrogate ligands for human P2Y12 receptors by in-silico and in vitro methods. Biochem Biophys Res Commun 337:281–288, 2005.

29. Paruchuri S, Tashimo H, Feng C, et al. Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. J Exp Med 206:2543–2555, 2009.

30. Kanaoka Y, Maekawa A, and Austen KF. Identification of GP199 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. J Biol Chem 288:10967–10972, 2013.

31. Lynch KR, O'Neill GP, Liu Q, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature 399:789–793, 1999.
55. Spencer LA, Szela CT, Perez SA, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. J Leukoc Biol 85:117–123, 2009.

56. Spencer LA, Melo RC, Perez SA, et al. Cytokine receptor-mediated trafficking of preformed IL-4 in eosinophils identifies an innate immune mechanism of cytokine secretion. Proc Natl Acad Sci USA 103:3333–3338, 2006.

57. Milanovic M, Terszowski G, Struck D, et al. IFN consensus sequence binding protein (Icsbp) is critical for eosinophil development. J Immunol 181:5045–5053, 2008.

58. de Bruin AM, Buitenhuys M, van der Sluijs KF, et al. Eosinophil differentiation in the bone marrow is inhibited by T cell-derived IFN-γ. Blood 116:2559–2569, 2010.

59. Hsieh FH, Lam BK, Penrose JF, et al. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. J Exp Med 193:123–133, 2001.

60. Mellor EA, AustenKF, and Boyce JA. Cysteinyl leukotrienes and uridine diphosphate induce cytokine generation by human mast cells through an interleukin 4-regulated pathway that is inhibited by leukotriene receptor antagonists. J Exp Med 195:583–592, 2002.

61. Mellor EA, Frank N, Soler D, et al. Expression of the type 2 receptor for cysteinyl leukotrienes (CysLT2R) by human mast cells: functional distinction from CysLT1R. Proc Natl Acad Sci USA 100:11589–11593, 2003.

62. Thivierge M, Stanková J, and Rola-Pleszczynski M. IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages. J Immunol 167:2855–2860, 2001.

63. Early SB, Barekzi E, Negri J, et al. Concordant modulation of cysteinyl leukotriene receptor expression by IL-4 and IFN-γ on peripheral immune cells. Am J Respir Cell Mol Biol 36:715–720, 2007.

64. Yano T, Hopkins HA, Hempel SL, et al. Interleukin-4 inhibits lipopolysaccharide-induced expression of prostaglandin H synthase-2 in human alveolar macrophages. J Cell Physiol 165:77–82, 1995.

65. Dworski R, and Sheller JR. Differential sensitivities of human blood monocytes and alveolar macrophages to the inhibition of prostaglandin endoperoxide synthase-2 by interleukin-4. Prostaglandins 53:237–251, 1997.

66. Steinke JW, Negri J, Liu L, et al. Aspirin activation of eosinophils and mast cells: implications in the pathogenesis of aspirin-exacerbated respiratory disease. J Immunol 193:41–47, 2014.

67. Kowalski ML, Pawliczak R, Wozniak J, et al. Differential metabolism of arachidonic acid in nasal poly epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. Am J Respir Crit Care Med 161:391–398, 2000.

68. Liu T, Laidlaw TM, Katz HR, and Boyce JA. Prostaglandin E2 deficiency causes a phenotype of aspirin sensitivity that depends on platelets and cysteinyl leukotrienes. Proc Natl Acad Sci USA 110:16987–16992, 2013.

69. Kim YK, Uno M, Hamilos DL, et al. Immunolocalization of CD34 in nasal polyposis. Effect of topical corticosteroids. Am J Respir Cell Mol Biol 20:388–397, 1999.

70. Denburg JA. Haemopoietic mechanisms in nasal polyposis and asthma. Thorax 55:524–525, 2000.

71. Sweet JM, Stevenson DD, Simon RA, and Mathison DA. Long-term effects of aspirin desensitization-treatment for aspirin-sensitive rhinosinusitis-asthma. J Allergy Clin Immunol 85:69–65, 1990.

72. Stevenson DD, and Simon RA. Selection of patients for aspirin desensitization treatment. J Allergy Clin Immunol 118:801–804, 2006.

73. Juergens UR, Christiansen SC, Stevenson DD, and Zuraw BL. Inhibition of monocyte leukotriene B4 production after aspirin desensitization. J Allergy Clin Immunol 96:148–156, 1995.

74. Woszczek G, Pawliczak R, Qi HY, et al. Functional characterization of human cysteinyl leukotriene 1 receptor gene structure. J Immunol 175:5152–5159, 2005.

75. Perez-G M, Melo M, Keegan AD, and Zamorano J. Aspirin and salicylates inhibit the IL-4- and IL-13-induced activation of STAT6. J Immunol 168:1428–1434, 2002.

76. Tegeder I, Heilschüter J, and Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. FASEB J 15:2057–2072, 2001.