CLINICAL IMPLICATIONS OF BASIC RESEARCH

The Clinical Implications of Basic Research *series has focused on highlighting laboratory research that could lead to advances in clinical therapeutics. However, the path between the laboratory and the bedside runs both ways: clinical observations often pose new questions for laboratory investigations that then lead back to the clinic. One of a series of occasional articles drawing attention to the bedside-to-bench flow of information is presented here, under the* Basic Implications of Clinical Observations *rubric. We hope our readers will enjoy these stories of discovery, and we invite them to submit their own examples of clinical findings that have led to insights in basic science.*

BASIC IMPLICATIONS OF CLINICAL OBSERVATIONS

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Aspirin-Exacerbated Respiratory Disease — New Prime Suspects

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Aspirin-exacerbated respiratory disease (AERD) is characterized by asthma, chronic rhinosinusitis with nasal polyposis, and pathognomonic respiratory reactions to aspirin (Samter's triad).[1] It has been estimated that this syndrome affects 7% of adults with asthma and 14% of those who have severe asthma.[2] Pathologically, AERD is characterized by marked eosinophilic inflammation and ongoing mast-cell activation in the respiratory mucosa. The frequent recurrence of nasal polyps after surgery, as well as the requirement for high-dose glucocorticoids to manage the asthma, reflect the aggressive, persistent nature of the disease. The typical onset is in adulthood, with or without preexisting asthma, rhinitis, or atopy.[3] An absence of familial clustering argues against a strong genetic basis, and the identification of variants of candidate genes in small studies has not been replicated.[4]

All nonsteroidal antiinflammatory drugs (NSAIDs) that inhibit both cyclooxygenase (COX)-1 and COX-2 may provoke the pathognomonic reactions in AERD; these reactions are accompanied by idiosyncratic activation of respiratory tract mast cells. In contrast, patients with AERD can usually be treated with COX-2–selective drugs without having these reactions.[5] The fact that structurally diverse NSAIDs that block COX-1 all provoke reactions reflects an enigmatic requirement for COX-1–derived prostaglandins to maintain a tenuous homeostasis. Curiously, the reactions also induce a refractory state in which NSAIDs can be used with diminished or no sequelae (desensitization); in fact, after desensitization, high-dose aspirin has therapeutic benefits.[6] Insights into the mechanisms responsible for the pathogenesis of AERD or its treatment have been limited.

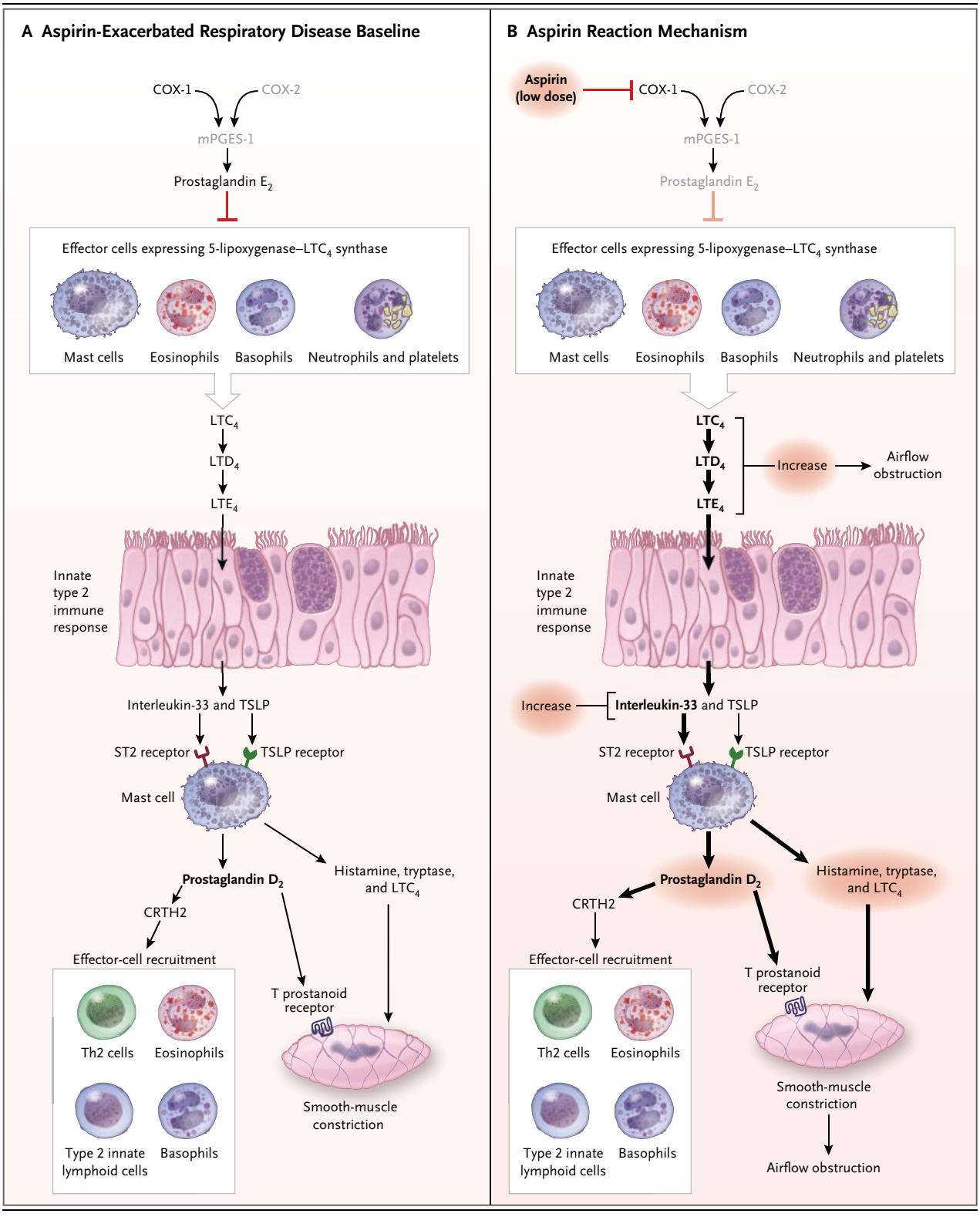
Dysregulated function of the 5-lipoxygenase–leukotriene C_4 (LTC_4) synthase pathway, which converts arachidonic acid to the cysteinyl leukotrienes, is a hallmark of AERD. Cysteinyl leukotrienes potently induce bronchoconstriction, vascular leak, and mucous secretion; they also amplify eosinophilic inflammation. The levels of leukotriene E_4 (LTE_4), the stable end metabolite of the cysteinyl leukotrienes, in urine from patients with AERD exceed those in urine from controls with asthma who are able to take aspirin, and they increase markedly during NSAID-induced reactions.[7] Both zileuton, which blocks 5-lipoxygenase activity, and drugs that selectively block the type 1 receptor for the cysteinyl leukotrienes attenuate the bronchoconstriction induced by aspirin challenges[8,9] and can improve sinonasal function and the control of asthma symptoms.[10,11] NSAID-induced increases in LTE_4 during clinical reactions are paralleled by increases in the products of activated mast cells (histamine, tryptase, and prostaglandin D_2 [PGD_2]) and are blocked by the administration of mast-cell–stabilizing drugs.[12] Thus, mast-cell activation contributes substantially to cysteinyl leukotriene formation when COX-1 is inhibited in AERD. Additional cells probably contribute to the baseline production of cysteinyl leukotrienes. One study reported strong eosinophil-specific overexpression of LTC_4 synthase in bronchial biopsy

specimens from patients with AERD, as compared with aspirin-tolerant controls.[13] Both the blood and sinonasal tissues of patients with AERD contain aberrantly large numbers of platelet-adherent neutrophils, monocytes, and eosinophils. Platelets express LTC_4 synthase, and this study estimated that they contribute approximately 70% of cysteinyl leukotriene formation by adhering to 5-lipoxygenase–expressing granulocytes and driving LTC_4 synthesis through transcellular transfer of metabolic intermediates.[14] The numbers of platelet-adherent granulocytes in the blood were strongly correlated with basal levels of LTE_4 in urine. Depletion of platelet-adherent granulocytes in a recent study of a mouse model of AERD was found to markedly attenuate cysteinyl leukotriene formation and NSAID-induced changes in lung function.[15] Collectively, these studies suggest that the mechanism or mechanisms accounting for dysregulation of the 5-lipoxygenase–LTC_4 synthase pathway is fundamental to AERD pathogenesis and that this dysregulation is manifested across several effector-cell types.

What accounts for the dependency on COX-1 for the maintenance of homeostasis over cysteinyl leukotriene synthesis and mast-cell activation in AERD? Several lines of evidence suggest that prostaglandin E_2 (PGE_2), a ubiquitous COX pathway product, maintains a critical "check" against 5-lipoxygenase activity and mast-cell activation. PGE_2 synthesis characteristically increases with inflammation, as a result of up-regulated expression of COX-2 and microsomal PGE_2 synthase 1 in several cell types.[16] Studies of nasal polyp fibroblasts suggest that this up-regulation may be locally impaired in the respiratory tissues of patients with AERD.[17] A potential consequence is that ongoing production of PGE_2 in AERD may depend disproportionately on COX-1 and thus be especially susceptible to depletion by nonselective NSAIDs. PGE_2 signaling through E prostanoid 2 receptors prevents mast-cell activation,[18,19] inhibits 5-lipoxygenase function,[20] and inhibits platelet activation.[21] Inhalation of aerosols of PGE_2 by persons with AERD blocks aspirin-induced changes in lung function and in urinary LTE_4 levels.[22] Expression of E prostanoid 2 receptor by mast cells, eosinophils, neutrophils, and T cells infiltrating the respiratory mucosa of persons with AERD is diminished as compared with that in controls.[23] Collectively, these studies suggest that a spectrum of abnormalities in inducible COX-2–dependent PGE_2 production and E prostanoid 2 receptor expression, potentially due to subtle genetic or epigenetic functional perturbations,[24] could compromise homeostasis to permit exaggerated cysteinyl leukotriene production, with activation of platelets and mast cells in patients with aspirin sensitivity. This is supported by the observation that an AERD-like phenotype develops when pulmonary inflammation is induced experimentally in mice that are selectively deficient in microsomal PGE_2 synthase 1 or E prostanoid 2 receptors.[15]

If PGE_2 is critical to homeostasis, what accounts for the idiosyncratic activation of mast cells and the severe, recalcitrant eosinophilic inflammation of AERD? Interleukin-33 and thymic stromal lymphopoietin (TSLP) are cytokines derived from structural cells that provide a first response to environmental proteases, helminths, and viruses.[25] In experimental models, administration of interleukin-33 and TSLP can induce eosinophil-rich pathologic changes (innate type 2 immunity) by acting directly on lymphoid and myeloid effector cells, including mast cells. Recent studies demonstrate that both interleukin-33 and TSLP are expressed strongly by nasal polyp tissue from persons with AERD.[26,27] Nasal polyp TSLP messenger RNA (mRNA) expression in AERD correlates with the expression of mast-cell markers in the same samples. In a mouse model of AERD, blockade of interleukin-33 or its receptor completely prevents aspirin-induced changes in lung function and release of mast-cell mediators.[26] In this model, deletion of *Ltc4s*, the gene encoding the pivotal enzyme in cysteinyl leukotriene synthesis, attenuates interleukin-33 expression and eosinophilic inflammation. These studies suggest that mast-cell activation and eosinophilic inflammation in AERD may reflect persistent cysteinyl leukotriene–mediated amplification of the innate type 2 immune system (Fig. 1). It is possible that mast cells convey responses from the upstream cytokines (interleukin-33 and TSLP) to downstream effector cells through elaboration of soluble mediators in AERD.

One such mediator, PGD_2, is generated at particularly high levels in persons with AERD at baseline and (especially) during reactions. PGD_2 is a potent chemotactic factor and activating ligand for eosinophils, basophils, and lymphoid helper cells acting through chemoattractant receptor homologue expressed by type 2 helper T cells (CRTH2),[28] and it elicits broncho-



constriction through T prostanoid receptors.[19] Urinary PGD_2 levels correlate with nasal polyp TSLP mRNA expression in patients with AERD[27] and increase during clinical reactions, reflecting mast-cell activation. These increases correlate with sharp reductions in blood eosinophil counts,

Figure 1 (facing page). Putative Mechanisms of Aspirin-Exacerbated Respiratory Disease Driven by a Lipid Mediator Hierarchy.

In Panel A, insufficient function of the inducible cyclooxygenase (COX)-2–microsomal prostaglandin E_2 synthase 1 (mPGES-1)–dependent system of prostaglandin E_2 production facilitates persistent synthesis of leukotriene C_4 (LTC_4) by effector cells expressing 5-lipoxygenase–LTC_4 synthase (including LTC_4 synthase–expressing platelets adherent to 5-lipoxygenase–expressing granulocytes). The structural cell–derived cytokines interleukin-33 and thymic stromal lymphopoietin (TSLP) promote ongoing inflammation and release of mast-cell products, including prostaglandin D_2. In Panel B, the administration of aspirin or other nonselective nonsteroidal antiinflammatory drugs depletes the residual prostaglandin E_2, permitting strong 5-lipoxygenase activation and further generation of LTC_4. Cysteinyl leukotrienes induce the release of interleukin-33 and consequent mast-cell activation, with bronchoconstriction occurring as a result of the direct effects of cysteinyl leukotrienes, prostaglandin D_2, and other mast cell–derived products. Prostaglandin D_2 recruits effector cells expressing chemoattractant receptor homologue expressed by type 2 helper T (Th2) cells (CRTH2) to the respiratory tissue and induces bronchoconstriction through T prostanoid receptors. LTD_4 denotes leukotriene D_4, LTE_4 leukotriene E_4, and ST2 suppression of tumorigenicity 2.

which probably reflects their recruitment to the tissue.[27] It is notable that the clinical benefits of high-dose aspirin occur concomitantly with sharply reduced levels of PGD_2 metabolites in urine and increases in blood eosinophils, potentially reflecting a loss of the PGD_2 chemotactic gradient.[29]

The AERD puzzle is not solved. The inciting cause or causes are yet to be determined, and the contributions of potential epigenetic or environmental factors to disease pathogenesis are largely unknown. Nonetheless, the evolving knowledge base suggests potential for therapeutic strategies. Clinical trials are under way to determine the therapeutic efficacy of prasugrel, an antiplatelet drug, in blocking transcellular cysteinyl leukotriene synthesis, as well as that of ifetroban, a T prostanoid receptor antagonist, in the treatment of patients with AERD (ClinicalTrials.gov numbers NCT01597375 and NCT02216357, respectively). Future therapeutic targets could include antagonists of CRTH2, blockers of interleukin-33 and TSLP, monoclonal antibodies against interleukin-5 or interleukin-5 receptor that deplete tissue eosinophils, and stable mimics of protective eicosanoids (PGE_2 and lipoxins[30]). The results of trials with such agents will also undoubtedly reveal additional insights into the mechanisms underlying this disease.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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