

for normal host responses and therefore more likely to be eliminated on a sensitivity or stoichiastic basis, permitting a normal underlying immune system to re-emerge. But we also know that AHA and ITP are common and refractory to intervention in patients with inherited or acquired disruptions in immune regulation, for example, APLS or CVID, so more immunosuppression is not necessarily more effective.²

It is possible that the dose of anti-CD20 that impairs B-cell proliferation, antibody production, and T-cell education differs among patients and autoimmune disorders. Are even higher doses (eg, 1000 mg) used to treat rheumatoid arthritis more efficacious in the long run? What is the optimal agent(s) to combine with rituximab and in which patients? Anti-CD20 antibodies re-engineered to enhance effector functions are in development; synergy with other immunosuppressive agents has been demonstrated,⁸ and sensitization to anti-CD20⁹ has been reported in the setting of B-cell malignancy. In addition, patients with chronic lymphocytic leukemia previously treated with rituximab may show a significant response to a second type I antibody, ofatumumab, that recognizes a different epitope on CD20.¹⁰ Such combination or sequential therapy with different anti-CD20s has not been reported in AHA or ITP. Moreover, recent studies indicate that type I and type II anti-CD20 antibodies differ in their distribution in lipid rafts in the plasma membrane and in their capacity to cause complement-dependent cytotoxicity versus programmed cell death.⁷ The implications of these findings in the treatment of AHA and ITP are unknown.

Thus, we are left with many unanswered questions but also potential opportunities to

improve outcome. However, unless we can identify and track the B- and possibly T-cell clones that cause AHA and ITP, we are doomed to human trials based on empiricism rather than controlled trials based on rational principles.

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In some individuals the ingestion of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) induces AERD.² NSAIDs are a chemically heterogeneous class of drugs that act by reducing the biosynthesis of prostanooids for their inhibitory effect on cyclooxygenase (COX)-1 and COX-2 activity.³ The mechanism of NSAID sensitivity is not immunologically mediated but seems related to their ability to interfere with arachidonic acid (AA) metabolism. Because the COX product prostaglandin (PG)_{E2} has beneficial anti-inflammatory effects in the lung,⁴ numerous studies have been performed to assess whether this prostanoid was reduced in AERD. However, no in vivo study has found diminished levels of PGE₂ at baseline in aspirin-sensitive patients. Thus, it is difficult to draw any firm conclusion on this hypothesis.

In contrast, numerous studies consistently showed that AERD is associated with an excessive production of cysteinyl leukotrienes (cys-LTs).² The cys-LTs are active compounds with smooth muscle-stimulating and edema-inducing properties that are thought to contribute to several of the characteristic features of AERD.⁵ They comprise LTC₄, D₄, and E₄. LTC₄ is formed from AA through the activity of 5-lipoxygenase (5-LO) that catalyzes a 2-step reaction leading to the generation of the unstable intermediate LTA₄ that is further converted, by LTC₄ synthase (LTC₄S), to the glutathione conjugate LTC₄. The other cys-LTs are formed by hydrolytic removal of γ -Glu and Gly from LTC₄ (yielding LTD₄ and LTE₄) by the activity of enzymes present in plasma (see figure). Leukocytes express the complete enzymatic machinery necessary to generate cys-LTs.⁵ However, in some circumstances cells that do not express the complete enzymatic repertoire of eicosanoid biosynthesis can use an intermediate product generated and released from another cell type to complete the conversion into the biologically active mediator; this phenomenon is called trans-cellular biosynthesis.⁶ However, the detailed cellular and molecular determinants controlling eicosanoid transcellular generation in vivo in health and disease have not been fully elucidated. Laidlaw et al have found that the adhesion of platelets to leukocytes contributes to the higher level of cys-LTs generated in vitro by activated granulocytes isolated from subjects with AERD compared with aspirin-tolerant controls.¹ Because platelets express LTC₄S but not 5-LO,

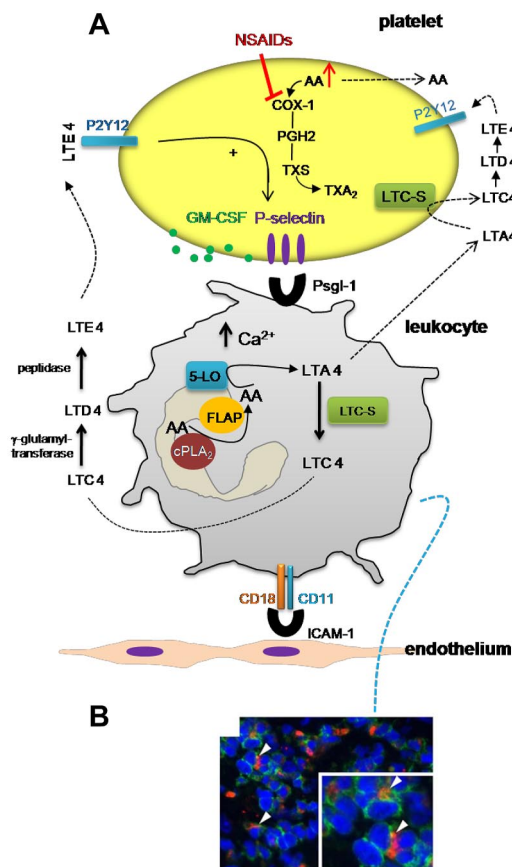
● ● ● PLATELETS & THROMBOPOIESIS

Comment on Laidlaw et al, page 3790

Inside platelet-leukocyte cross-talk

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In this issue of *Blood*, Laidlaw and colleagues show that in subjects with aspirin exacerbated respiratory disease (AERD), dysregulated platelet-leukocyte cross-talk may be partly responsible for the respiratory tissue inflammation and overproduction of cysteinyl leukotrienes, providing new clues for the treatment of the disease.¹



Inside platelet-leukocyte cross-talk in aspirin exacerbated respiratory disease. (A) In AERD, enhanced propensity of platelets to adhere to leukocytes translates into increased biosynthesis and release of LTC₄ that is metabolized to LTE₄ by the activity of enzymes present in plasma. LTE₄ may interact with platelet P2Y₁₂ receptor, thus inducing platelet P-selectin expression and facilitating the formation of platelet-leukocyte aggregates. Other platelet products, such as GM-CSF, can be released and may activate leukocyte 5-LO and induce a pro-adhesion phenotype and prolong the survival of eosinophils. As shown in panel B (from Figure 1 in the article by Laidlaw et al beginning on page 3790¹), nasal polyps from subjects with AERD contained many extravascular platelets that co-localized with leukocytes. In this scenario, NSAID treatment may increase the biosynthesis of cys-LTs. NSAIDs inhibit platelet COX-1 that can cause the accumulation and release of free AA that can be taken up by leukocytes thus inducing 5-LO translocation and enhancing cys-LT generation. PGH₂ indicates prostaglandin-H₂; and TXS, thromboxane synthase.

it is plausible that enhanced LTC₄ biosynthesis occurred by transcellular metabolism of AA through platelet-leukocyte cross-talk.⁷ In addition to this mechanism, activated platelets can induce LTC₄ biosynthesis in leukocytes through the release of soluble products, such as free AA and granulocyte-macrophage colony stimulating factor (GM-CSF), which can activate 5-LO enzyme.⁸

In AERD, the systemic biosynthesis of LTC₄ in vivo, as assessed by measuring urinary levels of LTE₄, was also increased and correlated strongly with percentages of circulating platelet-adherent granulocytes.¹ Interestingly, it was found that platelet-adherent subsets of leukocytes had higher expression of several adhesion markers, such as CD11b/CD18 (MAC-1), than did platelet nonadherent subsets.¹ Altogether, these findings suggest that AERD is associated with enhanced

adhesion of circulating platelets to leukocytes and this event may trigger enhanced cys-LT biosynthesis and induce a pro-adhesive phenotype in leukocytes thus facilitating respiratory tissue inflammation (see figure).

Enhanced circulating LTE₄ levels can mediate pulmonary inflammation (mucosal eosinophilia and airway hyperresponsiveness),² but this effect is not dependent on the activation of the type 1 and 2 receptors for cys-LTs (CysLT1R and CysLT2R). In contrast, the activation of the purinergic (P2Y₁₂) receptor (the target of the thienopyridine antiplatelet drugs) by LTE₄ seems to play a role.⁹ The finding of Laidlaw et al that steady-state urinary excretion of LTE₄ correlates with the frequencies of platelet-adherent leukocytes in the peripheral blood may suggest a possible contribution of LTE₄ in the formation of platelet-leukocyte aggregates through the acti-

vation of platelet P2Y₁₂ and P-selectin expression.¹ This hypothesis might be addressed by testing the effects of thienopyridines on the formation of circulating platelet-leukocyte aggregates and correlating it with the clinical outcomes in this setting. Further studies are required to identify the mechanisms involved in enhanced propensity of platelets of AERD to adhere to leukocytes compared with those of aspirin-tolerant individuals.

The administration of NSAIDs may cause the accumulation of free AA, in platelets, for their inhibitory action on COX-1 activity. The presence of dysregulated platelet-leukocyte cross-talk may facilitate the transfer of platelet AA to leukocytes thus inducing 5-LO translocation and increasing cys-LT biosynthesis⁸ (see figure panel A). Increased circulating levels of LTE₄ can then contribute to enhanced formation of platelet-leukocyte aggregates possibly through the activation of platelet P2Y₁₂. Activated platelets may cause eosinophil accumulation in respiratory tissue by inducing a pro-adhesive phenotype of eosinophils and prolonging their survival, possibly through the release of GM-CSF¹⁰ (see figure panel B).

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