type II anti-CD20 in the HexAb might be more effective. CD74 is a good choice for the HexAb because it binds a critical molecule for B-cell survival and proliferation.[10] Nonetheless, the emergence of this novel genetically engineered bispecific antibody should be a welcome new product for the treatment of mantle zone lymphoma, chronic lymphocytic leukemia, and other difficult-to-treat B-cell tumors expressing both CD20 and CD74 antigens. The ingenuity and creativity displayed in this study demonstrates further the ever-increasing role of genetic engineering in the development of promising new antibody reagents for cancer immunotherapy.

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● ● ● CLINICAL TRIALS

Comment on Barcellini et al, page 3691

Avoiding intuxication

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Why primary autoimmune hemolytic anemia (AHA) is more refractory to standard "frontline" treatment than immune thrombocytopenia (ITP) remains unclear. The response of AHA to steroids, splenectomy, and especially intravenous immunoglobulin is inferior to that seen in patients with ITP, making reliance on immunosuppression more important.

This observation is especially intriguing in patients with "warm" immunoglobulin G (IgG)–mediated AHA that is not primarily complement-mediated. Almost all patients with "warm" IgG-mediated AHA also show a brisk reticulocytosis, unlike ITP in which ineffective platelet production contributes to thrombocytopenia. Perhaps the size and rigidity of the antibody, complement in the case of "cold," IgM-coated red blood cells, and the location and developmental expression of antigens on red cell precursors and on mature red cell surface permits more efficient clearance by tissue macrophages than comparably opsonized platelets. Clinical disease might develop at lower antibody density more rapidly after autoantibodies form, when they might be more susceptible to immunosuppression.[1] Lower rates of spontaneous or treatment-derived responses may also relate to the site of the underlying immune defect as AHA or Evans syndrome is more common in patients with constitutive defects, such as the autoimmune lymphoproliferative syndrome.[2]

This conundrum brings us to the observations of Barcellini et al in their article in this issue of *Blood*.[3] They report that each of 14 patients with primary warm AHA and 5 of 9 patients with primary cold AHA responded to 4 weekly intravenous injections of low-dose rituximab (100 mg/wk × 4), which they substituted for conventional dosing (375 mg/m^2/wk × 4). The choice of a lower dose was based on the reasoning that less drug would be needed to ablate a smaller number of autoreactive B cells in AHA than is required to induce remission in B-cell lymphoma. Similar findings of efficacy in ITP have been reported in single-arm studies, but only 20% of patients appear to maintain a complete response more than 3 years after low or conventional doses of rituximab.[4] The results of Barcellini et al compare very favorably to other series of adults with warm AHA treated with rituximab using conventional dosing.[4] However, more time will need to elapse to determine whether patients with AHA treated with "low-dose" rituximab relapse at the same rate as those with ITP. Likewise, it will be important to see whether the reduced dosage leads to better preservation of pretreatment B- and T-cell repertoires and preserves response to vaccination[5] and lowers risk of infection. In addition, paraproteins (if present) may serve as useful surrogate markers of efficacy and mechanism of effect.

Why does ITP or AHA recur despite prolonged and virtually complete B-cell depletion in the peripheral blood? Potential reasons include down-regulation, internalization or proteolytic shedding of CD20, failure to ablate "CD20 low"–expressing early B-cell progenitors, plasma cells and long-lived memory cells, and cell sanctuaries in the bone marrow, spleen, and lymphoid organs where the effect on B cell–T cell cooperation and induction of T-regulatory suppressor cells may be incomplete.[6] The mechanism of action of rituximab is complex, incompletely understood, and may even involve induction of an idiotype-specific T-cell response that sustains remission.[7] It is possible that low-dose rituximab invokes an immunomodulatory response that does not require complete B-cell depletion.

The article by Barcellini et al also reminds us that we know frightfully little as to how red cell or platelet autoantibodies arise, how rituximab works, or how it should be administered and monitored. The premise underlying immunosuppression for AHA and ITP is that the responsible autoreactive B and T cells are more susceptible to eradication or are simply less abundant in the immune repertoire than those responsible

for normal host responses and therefore more likely to be eliminated on a sensitivity or stoichastic 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-CD20[9] 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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● ● ● 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]

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 prostanoids 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 prostaglanding (PG)E_2 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 PGE_2 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 LTC_4, D_4, and E_4. LTC_4 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 LTA_4 that is further converted, by LTC_4 synthase (LTC_4S), to the glutathione conjugate LTC_4. The other cys-LTs are formed by hydrolytic removal of γ-Glu and Gly from LTC_4 (yielding LTD_4 and LTE_4) 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 LTC_4S but not 5-LO,