investigators have begun to understand the role of different PI3K isoforms in hematopoietic cells. For example, mice lacking the p110γ catalytic subunit of PI3K have a defect in ADP-mediated platelet aggregation (Hirsch et al, FASEB J. 2001;15:2019-2021), while mice lacking the p85α regulatory subunit of PI3K have impaired B-cell development. In this issue, Watanabe and colleagues (page 541) have studied the effects of the p85α knockout on platelet function. They found that in the absence of p85α there was a defect in signaling events initiated by the platelet collagen receptor, GPVI, whereas there was no defect in platelet activation following stimulation by other platelet agonists such as ADP or thrombin.

It is noteworthy that GPVI signaling pathways are similar to those emanating from the B-cell antigen receptor, as well as other members of the immunoglobulin supergene family of receptors. Does the work by Watanabe et al suggest that therapeutic targeting of p85α would be a useful way to disrupt platelet activation following exposure to subendothelial collagen such as occurs during coronary plaque rupture? Given the ubiquitous expression and diverse functions of PI3K, such speculation is premature.

—Charles S. Abrams
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The biology and treatment of ITP: what's next?

Immune thrombocytopenic purpura (ITP) is the result of 2 pathologic processes: loss of tolerance to self-antigen accompanied by sustained autoantibody production, and antibody effector mechanisms that destroy platelets in excess of their production. There is a considerable challenge to hematologists treating the chronic, severe, refractory subset of patients. IVIG has many putative mechanisms of action in treating ITP. These include "blockade" of phagocytic Fc receptors, anti-idiotype effects, reduction of antibody production, reduced survival of the autoantibody in the circulation (Hansen and Balthasar, Blood. 2002;100:2087-2093), and, finally, stimulation of inhibitory FcγRIIb. In this issue, Crow and colleagues (page 558) follow up on the seminal observations of Samuelsson et al (Science. 2001;291:484-486) concerning how IVIG's efficacy in ITP depends on the inhibitory FcγRIIb on macrophages. FcγRIIb counters the phagocytic signal provided by the activating Fcγ receptors (I/γ and IIIa/γ in mice; I/γ, IIIa/γ, and IIA in humans) on splenic macrophages. Crow et al confirm that IVIG's effects in vivo depend on expression of FcγRIIb. They then explore the potential downstream signals of FcγRIIb used in B cells and mast cells that would explain IVIG's role in macrophages. Using germ line knockout of SHIP1, SHP1, or BTK in their ITP mouse model, Crow and colleagues found IVIG to be effective despite the absence of these individual signaling molecules. Functional redundancy among the known phosphatase families, or an as-yet undiscovered mechanism specific to macrophages, may explain their observations. The mechanism sought by Crow et al may yield new molecular targets for therapy. Equally of interest to the field is the mechanism by which IVIG increases FcγRIIb expression in the first place. Current data favor an indirect effect, in which IVIG causes a subset of splenic cells to secrete one or more substances—perhaps cytokines—that act on the phagocyte to increase the relative proportion of inhibitory FcγRIIb versus phagocytic Fcγ receptors. Elucidation of this pathway may also yield new molecular targets of therapy.

There are unanswered questions in the development of the ITP autoantibodies and in the antibody effector mechanisms. How is tolerance broken? Do dendritic cells, B cells, macrophages, or platelets themselves present antigen? Is there an intrinsic genetic predisposition for loss of tolerance and sustained autoantibody production? Are there genetic differences, for example in the Fcγ receptor endowment, that have an impact on disease course or response to therapy? Now that Fcγ receptor crystal structures have been solved, will small molecule inhibitors of IgG ligand binding be useful? Can we block receptor signaling, as this family of activating receptors prefers lipid microdomains (rafts) in which to initiate signals via members of the src and syk protein tyrosine kinase families? Will additional ways be discovered to alter the balance of activating and inhibitory receptors to allow platelets to survive in circulation? The next step in the biology and therapy of ITP is more precise understanding of the origins of the disease and the promise of molecularly targeted therapy.

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Angiopoietin expression in multiple myeloma

Multiple myeloma demonstrates a progressive, and usually fatal, course, with current treatments generally producing only temporary remissions. Antiangiogenic therapies represent a potential new approach to treating this cancer. While it is well established that growth in solid tumors is dependent on angiogenesis, the role of this process in hematopoietic tumors is not fully appreciated. There is a strong correlation between increased angiogenesis and poor survival in myeloma patients. Furthermore, both cellular and circulating levels of vascular endothelial growth factor (VEGF) are often elevated in hematologic malignancies, including myeloma, and have been shown to predict for a poor outcome, lending additional support to the concept that angiogenic cytokines are involved in the growth and progression of these malignancies.

In this issue, Giuliani and colleagues (page 638) extend our knowledge of marrow angiogenesis with their report on the expression of angiopoietin-1 in myeloma cell lines and patient samples. Angiopoietin-1 (Ang-1) was found to be expressed in 47% of the patient samples examined. Bone marrow angiogenesis was examined and found to be elevated in 12 of 15 patients examined (80%), and there was a significant correlation between Ang-1 expression and

microvascular density (MVD), although no such correlation was present between Ang-1 and Tie2 expression. Giuliani and colleagues were also able to demonstrate that myeloma cells could up-regulate the angiopoietin receptor Tie2 in human bone marrow endothelial cells. Conditioned medium from myeloma cell lines was capable of stimulating angiogenesis, although such stimulation did not occur in the presence of an anti-Tie2 antibody. Angiopoietins, while not believed to be involved in the initial stages of angiogenesis, are known to play an essential role. Ang-1, acting through Tie2, contributes to the stabilization of newly formed vessels via recruitment of peri-endothelial supporting cells as well as endothelial cells, whereas Ang-2, also acting through Tie2, reduces these interactions, leading to vascular regression. It has also been reported that coexpression of Ang-2 and VEGF promotes new vessel sprouting and has been shown to predict a poor prognosis in myeloma and other malignancies.

The role, if any, of angiopoietins in myeloma is far from clear, however. Uneda et al have also recently reported their findings regarding angiopoietin expression in myeloma (Haematologica. 2003;88:113-115). In their study, 27 of 36 multiple myeloma patients studied showed expression of Ang-2 by reverse transcriptase–polymerase chain reaction (RT-PCR) and immunohistochemistry. Coexpression of VEGF and Ang-2 was detected in 18 of the myeloma samples. The survival rate was significantly lower in those patients expressing Ang-2. Interestingly, and in contrast to the findings by Giuliani et al, they found no evidence of Ang-1 expression.

The seemingly contradictory findings of Ang-1 and Ang-2 expression in these 2 studies should be carefully interpreted in the context of how the cells were isolated and examined. Both studies examined relatively few patients, and in neither study was the effect of the bone marrow microenvironment on expression of these molecules fully taken into account.

Several important outstanding questions remain to be addressed. First, the apparent contradiction in the results from these 2 studies must be resolved. Does expression of Ang-1 or Ang-2 have prognostic value in myeloma? The results from Uneda et al would suggest so. Does angiopoietin expression vary with the stage of the disease? Do the angiopoietins represent valid therapeutic targets? Even if the angiopoietins are not the major driving factors in marrow angiogenesis, the results of Giuliani et al suggest that they may represent a valid target. Although it is too early to answer these questions, the preliminary evidence is tantalizing.

—William T. Bellamy
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Use of FLT3 inhibitors in leukemia: a wrench in the activation loop

Tyrosine kinases may be constitutively activated in leukemias as fusion genes by intragenic juxtamembrane deletion or in frame insertions, or by "activation loop" mutations in the catalytic domain. In this issue, Grundler and colleagues (page 646) demonstrate that there is varying sensitivity of 3 activation loop mutations in *FLT3* (Asp835Try, Ile836del, and Ile836Met+Arg) to the tyrosine kinase inhibitors AG1296, PKC412, and SU5614, respectively. Most small molecule tyrosine kinase inhibitors are ATP analogs that bind with high affinity to the ATP binding site and preclude access of ATP and substrate. It might therefore be predicted that certain activation loop mutations would confer relative resistance to small molecule inhibitors. Indeed, Shah et al have shown that a collection of mutations in *BCR-ABL* may not only enhance kinase activation and confer a proliferative advantage but may simultaneously confer primary resistance to imatinib (Shah et al, Cancer Cell. 2002;2:117-125).

What are the implications of these findings? From a structural perspective, the observation of variable sensitivity of activation loop mutations to small molecules of known structure should provide valuable insights into the structure-function relationships of tyrosine kinases.

There are also important clinical implications of these findings with regard to inclusion criteria for trials of FLT3 inhibitors in acute myeloid leukemia (AML). Most phase 2 trial designs have required genotypic evidence of either juxtamembrane or activation loop mutations for inclusion in clinical trials. The authors tested sensitivity of only 3 of the dozen or so reported *FLT3* activation loop mutations. But it seems clear from their data that each of the known *FLT3* activation loop mutations should be tested for sensitivity to any tyrosine kinase inhibitor being considered for clinical trials, and that inclusion criteria should reflect sensitivity of the activation loop mutation to the given FLT3 inhibitor. For example, it may not be appropriate to treat AML patients with *FLT3* activation loop mutations that are known to be resistant to a given inhibitor. In this context it should also be noted that rare patients have both juxtamembrane and activation loop mutations on the same *FLT3* allele, indicating that all patients should have sequence analysis of both domains prior to inclusion in clinical trials.

These considerations add complexity to clinical trial design, including the apparent need to stratify patients based on the genotype of the *FLT3* juxtamembrane domain and the activation loop.

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Hematopoietic cells for targeting iatrogenic immunomodulation

Precise targeting of effective therapeutic agents represents a holy grail of medicine, as effective therapy necessitates proper tissue distribution. Antibiotics that fail to enter the central nervous system will not successfully treat meningitis, regardless of the intrinsic susceptibility of the causative organism, yet the widespread tissue distribution of potent agents produces unintentional untoward consequences on normal cells.