Gilles and colleagues (page 2617) present here the results of a pilot study in which an anti-idiotype antibody response was generated against an inhibitory monoclonal antibody in a mouse model system. Their elegant study is an outgrowth of earlier investigations using the patient-derived, inhibitory monoclonal antibody BO2C11. This antibody is specific for the C2 domain of FVIII, and a crystal structure of the Fab fragment from BO2C11 bound to the C2 domain showed that the epitope comprised several loops at one end of the molecule. The epitope was essentially identical to the region that had been proposed to be the membrane-binding site on the C2 surface. BO2C11 is clearly representative of a large fraction of the inhibitory antibodies found in patients, many of which interfere with membrane binding. In the present study, mice were immunized with BO2C11, and one of the resulting anti-idiotypic monoclonal antibodies, mAb14C12, blocked the inhibitory properties of BO2C11 in hemophilic mice that had received therapeutic infusions of FVIII. It also neutralized inhibitory antibodies from patient plasma. These results support the concept of therapeutic infusions of FVIII. It also neutralized inhibitory antibodies from patient plasma.

The distinctive hydrophobic projection from the C2 domain showed that the epitope comprises several loops at each end of the molecule. The distinctive hydrophobic projection from the C2 domain showed that the epitope comprises several loops at each end of the molecule. In addition to its medical relevance, this study is very intriguing from a structure/function standpoint, as these 2 similar yet distinct molecules mediate specific, high-affinity binding to a series of antibody inhibitors. The distinctive hydrophobic projection from the C2 domain showed that the epitope comprises several loops at each end of the molecule. In addition to its medical relevance, this study is very intriguing from a structure/function standpoint, as these 2 similar yet distinct molecules mediate specific, high-affinity binding to a series of antibody inhibitors.

Both the C2 domain and antibody VH regions are beta-sandwich structures, and each consists of a relatively rigid scaffold that presents loops at each end of the molecule. When the conformation of mAb14C12 was modeled and compared with the C2 structure using algorithms allowing for different connectivities between beta strands, a significant structural homology was found. The distinctive hydrophobic projection from the C2 loop, consisting of residues Leu2251-Leu2252, was duplicated in the antibody model structure, and additional residues were similar to those at analogous positions in the C2 domain structure. In addition to its medical relevance, this study is very intriguing from a structure/function standpoint, as these 2 similar yet distinct molecular surfaces mediate specific, high-affinity binding to a series of antibody inhibitors.

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1. Jacquemin MG, Desqueper BG, Benhida A, et al. Mechanisms and kinetics of factor VIII inactivation: study with an IgG monoclonal antibody derived from a hemophilia A patient with inhibitor. Blood. 1998;92:496-501.
2. Spiegel PC, Jacquemin M, Saint-Remy JM, Stoddard BL, Pratt KP. Structure of a FVIII C2 domain-immunoglobulin G kappa Fab complex: identification of an inhibitory antibody epitope on the surface of factor VIII. Blood. 2001;98:13-19.
3. Pratt KP, Shen BW, Takeshima K, Davie EW, Fujikawa K, Stoddard BL. Structure of the C2 domain of human factor VIII at 1.5 A resolution. Nature.1999;402:439-442.

Wobbling with warfarin

All physicians who have used warfarin as an oral anticoagulant—and that includes most of us—know of the great variability in the prothrombin time measured in a patient given a specific dose of warfarin. We have interpreted this as due to the variation of the counterbalancing vitamin K in the patient’s diet, variations in metabolism of warfarin in different people, and wobble in accuracy of the prothrombin time measurement due to variations in sample handling, anticoagulant to whole blood ratios, or instrument errors. Given the narrow therapeutic index for warfarin, we titrate the warfarin dose against the prothrombin time, as standardized using the International Normalized Ratio (INR). In the report by Shikata and colleagues (page 2630), we finally begin to get to the molecular basis of warfarin sensitivity. Warfarin is a racemic mixture of (R)-warfarin and (S)-warfarin; (S)-warfarin is more potent in inhibiting vitamin K–dependent carboxylation. Warfarin sensitivity is correlated in part to the presence of a relatively infrequent allele of cytochrome P450 known as CYP2C9*.3. In the presence of this allele, (S)-warfarin clearance was reduced and the INR increased for a given plasma concentration of warfarin. In addition, polymorphisms in the genes encoding the vitamin K–dependent proteins and the vitamin K–dependent carboxylase are analyzed. As one might predict, any mutation/polymerorphism that decreased functional activities of proteins within the pathway monitored by the prothrombin time would lead to a decrease in the INR for a given plasma warfarin concentration—and vice versa. There were 4 independent functional polymorphisms identified, including the Thr165Met in prothrombin and −402G>A (37-bp repeat) and −746T>C in factor VII. Multiple regression analysis identified CYP2C9*3 in cytochrome 450, and Thr165Met and −402G>A in prothrombin and (CAA repeat), in the vitamin K–dependent carboxylase as associated with warfarin sensitivity. These results suggest that genotyping of the genes encoding the carboxylase, cytochrome P450, and some of the vitamin K–dependent protein can predict warfarin response.

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Adults are not simply big children

During medical school clerkships and pediatric residency, trainees are constantly reminded that “children are not simply small adults.” As shown by Chiaretti and colleagues (page 2771), it seems that the converse of this statement—adults are not simply big children—is true as well, at
least with respect to T-cell acute lymphocytic leukemia (T-ALL). Chiaretti et al use the Affymetrix HGU95aV2 chip to identify gene expression profiles that are associated with either response to therapy or long-term remission in 33 adult patients with T-ALL. The authors identified an expression profile that was associated with induction failure; this profile was characterized by overexpression of a single gene (IL8), and lower expression (compared with the successful induction group) of a larger set of genes, including GFI1, BCL-6, MXI1, and CD10. Long-term remission was characterized by higher levels of CD2, BUB1B, TTK, and CENPF, while relapse was associated with overexpression of AHNAK. Although the number of evaluable patients was small, a 3-gene model based on expression of CD2, TTK, and AHNAK was more effective at predicting relapse than were traditional criteria such as white blood cell count or immunophenotype. The predictive utility of this 3-gene model was then verified by real-time polymerase chain reaction (PCR) on an independent set of 18 additional patients.

The authors also studied the expression of a set of 7 genes previously found by Yeoh et al to be relatively overexpressed in T-ALL cells from pediatric patients with a high likelihood of relapse. They found that expression levels of these genes did not correlate with remission duration in their cohort of adult T-ALL patients, and that only 1 of the 3 genes that most effectively predicted long-term remission in adult T-ALL patients was predictive in the pediatric T-ALL series. An independent series of pediatric T-ALL patients reported that high levels of HOX11 were associated with long-term remission; in contrast, only 1 of 6 adult T-ALL patients in the series by Chiaretti et al maintained a long-term remission. The observation that genes that predict long-term remission in pediatric T-ALL are not predictive for adult T-ALL is perhaps not surprising, given the documented clinical and biologic differences between pediatric and adult T-ALL.

There are several caveats that should be considered when interpreting the differences between the current adult study and the previously published pediatric series. The number of patients is relatively small, the patients came from different countries, the RNA preparation was subtly different, and the treatment regimens were different among the 3 series. Nonetheless, this type of study can be regarded as a hypothesis generator, and several lines of future investigation have been opened. Larger numbers of patients can be analyzed in parallel, using identical methods for RNA preparation and hybridization. If confirmed in larger studies, the 3-gene model could be used to identify patients at high risk of relapse, who might then benefit from more aggressive therapy. Moreover, the limited number of genes that has been identified can be studied in detail to determine whether their up- or down-regulation is causally related to the outcome of treatment.

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1. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcomes in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002; 1:133-143.
2. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T-cell acute lymphoblastic leukemia. Cancer Cell. 2002; 1:75-87.
3. Kersey JH. Fifty years of studies of the biology and therapy of childhood leukemia. Blood. 1997; 90:4243-4251.
4. Hoelzer D, Golub G. New approaches to acute lymphoblastic leukemia in adults: where do we go? Semin Oncol. 2000; 27:540-559.

A new model of plasma cell proliferation

Many antiapoptotic proteins are expressed in multiple myeloma (MM) cells. Among these proteins, the Bcl-2 family member Bcl-xL is frequently overexpressed in MM cell lines and MM patient cells and promotes tumor cell survival. The expression of both Bcl-xL, as well as that of the antiapoptotic Mcl-1, is regulated by signal transducer and activator of transcription 3 (STAT3), a downstream target of Janus kinases (JAKs). Specifically, Catlett-Falcone et al have reported that inhibition of STAT3 activation down-regulates Bcl-xL expression, thereby promoting MM cell apoptosis. To date, however, the role of genetic modulation of Bcl-xL in MM pathogenesis has not been defined.

In order to clarify the role of Bcl-xL expression in the development of normal and malignant plasma proliferation, Linden and colleagues (page 2779) used the κ immunoglobulin (Ig) gene 3′ enhancer to direct transgenic Bcl-xL expression to late-stage B cells in mice. The result was a phenotype that included increased numbers of B-lineage cells in bone marrow and extramedullary sites, increased polyclonal serum Ig, and increases in T-cell–dependent antigen-specific Ig production, consistent with a nonmalignant B-lineage proliferation. They then crossed these mice with Eμ/c-Myc transgenic mice, and the resultant phenotype included further increased numbers of B-lineage cells, including clonally related bone marrow plasma cells associated with osteolytic bone lesions. This mouse model therefore shows that the κ Ig gene 3′ enhancer can target gene expression to late-stage B cells, identifies a role for Bcl-xL in plasma cell proliferation, and demonstrates the cooperative role of Bcl-xL and c-Myc in generation of clonally related plasma cell proliferation.
Adults are not simply big children

Peter D. Aplan