Comment on Jin et al, page 1733

A more clinically relevant mouse model of hemophilia B

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This paper describes hemophilia B mice in which a human factor IX (*FIX*) gene with a missense mutation replaced the normal mouse FIX gene. These mice have a reduced risk of an immune response and will be very useful for evaluating new therapies for hemophilia B.

Caused by a deficiency of factor IX (FIX), hemophilia B affects 1 in 50 000 males and results in a bleeding diathesis that can be life threatening. The mainstay of existing treatment is factor therapy, in which patients receive an intravenous injection of FIX. However, this approach is expensive and inconvenient and still carries some risk of transmitting infectious agents. Investigation of alternative new treatments such as gene therapy requires appropriate animal models of hemophilia B in which to test the treatments' efficacy. Many such studies have been initiated in mice because of cost considerations and the ability to analyze large numbers of animals for a therapeutic effect. However, existing mouse models have all involved knock-out mutations in which most or all epitopes of the protein were not expressed. Gene therapy approaches in these knock-out models have frequently resulted in the production of so-called inhibitors, which are antibodies that block the function of a coagulation factor. These immune responses complicate evaluation of the efficacy of new treatments and will probably not occur with a similar frequency in human patients, only approximately 3% of whom develop antibodies after infusion of human FIX protein. The low frequency of inhibitor formation is likely due in part to the fact that most patients have missense mutations, which are less likely to result in an antibody response than are truncations or deletions.

In this issue of *Blood*, Jin and colleagues replaced the endogenous mouse FIX gene with a human FIX gene with a missense mutation that results in loss of functional activity. These mice had little or no FIX functional activity in coagulation assays and bled profusely after injury to the tail vein. Muscle-directed gene therapy with an adeno-associated virus (AAV) vector resulted in stable expression of human FIX without inhibitor formation and in correction of the bleeding phenotype. In contrast, all hemophilia B mice with a knock-out mutation that received the same dose of AAV vector in the muscle developed inhibitors and failed to correct their bleeding diathesis. A nice feature of this model is that the knock-in human FIX gene encodes an alanine at position 148 (T148A), which is not recognized efficiently by a monoclonal antibody specific for a threonine at this position. This polymorphism made it possible to quantify the amount of the FIX with a threonine at codon 148 that was expressed from the AAV vector by using an immunoassay, greatly facilitating evaluation of long-term expression. This mouse model will allow studies to be performed to evaluate the efficacy of different gene therapy approaches for the treatment of hemophilia B and should hasten the development of a safe and efficacious treatment for humans with this genetic disease. ■

Comment on Mosnier et al, page 1740

APC stripped bare

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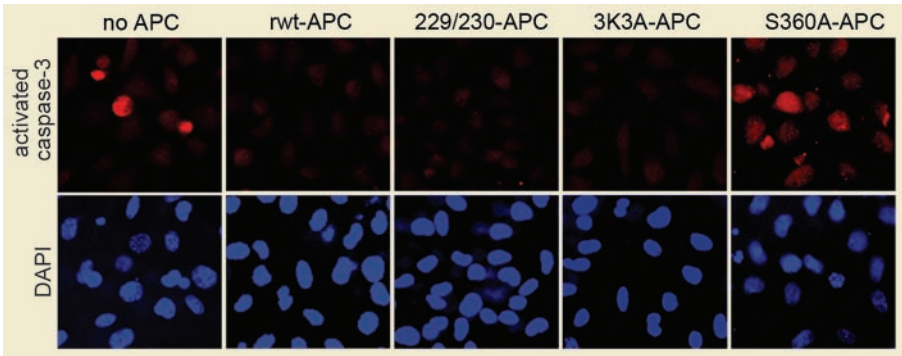
APC has anticoagulant and cytoprotective functions. For the first time, these are selectively modulated by targeted APC exosite mutation. This approach offers the prospect of improved treatment for sepsis.

Many of the proteinases generated during hemostasis have highly specific roles, each cleaving only a single protein substrate. Other proteinases have several roles involving multiple substrates. An essential requirement for proteolytic cleavage is recognition of the substrate by the proteinase. At a minimum, recognition must involve occupancy of the active site of the proteinase by the scissile bond of the substrate. But some substrates require wider interactions with the proteinase to enable the scissile bond to be positioned effectively and efficiently.

An example of a proteinase with more than 1 substrate is activated protein C (APC). APC has well-established anticoagulant activities



Antiapoptotic activity of rwt APC and anticoagulantly impaired APC variants. See the complete figure in the article beginning on page 1740.