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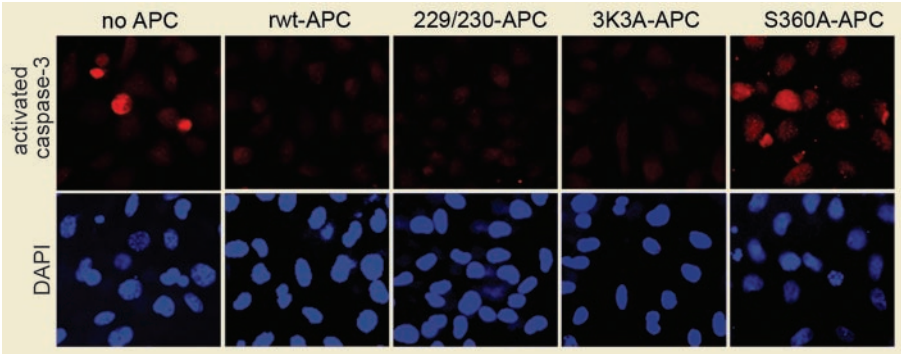
APC has anticoagulant and cytoprotective functions. For the first time, these are selectively modulated by targeted APC exosite mutation. This approach offers the prospect of improved treatment for sepsis.

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.

inactivating factor Va and factor VIIIa. The importance of its cleavage of factor Va at residue Arg506 is illustrated by the role of the factor V Leiden mutation (Arg506Gln) as a risk factor for venous thrombosis. It has been shown recently that cleavage inactivation of factor Va depends not only on the active site of APC but also on basic residues including Lys191, Lys192, Lys193, Arg229, and Arg230.[1,2] These basic residues lie in loops forming a positively charged surface, or exosite, on APC, which helps position the active site over the factor Va residue Arg506.

APC also participates in a nonanticoagulant reaction, cleavage of protease-activated receptor-1 (PAR-1) on the surface of endothelial cells.[3] This reaction requires the endothelial cell protein C receptor (EPCR) and results in an antiapoptotic/neuroprotective cell phenotype.[4] In this issue of *Blood*, Mosnier and colleagues demonstrate that while mutation of the above basic residues of APC dramatically reduces anticoagulant activity, it does not alter proteolysis of PAR-1 and consequent cytoprotective functions. Proximity of APC to its PAR-1 cleavage site therefore is not facilitated by its exosite but solely by its engagement with EPCR.

What is the importance of these findings? First, they illustrate how proteinases can use different mechanisms to achieve specificity. They show that substrate specificity of APC can be exquisitely controlled by fine-tuning its molecular recognition mechanisms. The mutation of the basic residues in surface loops of APC only alters interaction with factor Va and does not alter its active site and those substrates that do not require broader contact for their proteolysis. Second, they have potentially important implications for the future treatment of severe sepsis. While numerous diverse approaches to therapy of severe sepsis, including those based on bacterial toxins, cytokine function, and cell signaling pathways, have produced encouraging results in animal models, all have failed to be clinically effective.[5] In contrast, intervention in the downstream consequences of sepsis using recombinant anticoagulant proteins has been more promising in humans. Of the 3 natural anticoagulants, antithrombin, tissue factor pathway inhibitor, and activated protein C, however, only the latter has been sufficiently decisive in terms of mortality in large clinical trials to obtain regulatory approval for routine clinical use. This has focused interest on the unique aspects of APC function, their cytoprotective effects, and their possible role in protection against sepsis. This interest has been heightened because, despite APC's beneficial effect upon mortality, bleeding remains a concern in APC therapy and may be a factor in slow uptake of this therapy. The paper by Mosnier et al provides a rationale for preparation and evaluation of restricted-specificity APC preparations with enhanced cytoprotective–to–anticoagulant activity ratios. Such products will have to undergo extensive efficacy and safety testing but offer the potential of an improved therapy based upon a solitary success in this difficult area. ■

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IMMUNOBIOLOGY

Comment on Schlecht et al, page 1808

Plasmacytoid dendritic cell: vive le dilettante!

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Whether plasmacytoid dendritic cells (pDCs) are effective in presenting antigens has been a matter of debate. Schlecht and colleagues provide evidence that pDCs can indeed prime CD8 T cells specific for viral antigens.

Plasmacytoid dendritic cells (pDCs) are a rare population morphologically similar to plasma cells that express major histocompatibility complex (MHC) class II and costimulatory molecules, thereby triggering T-cell proliferation and differentiation. Moreover, they secrete large amounts of type I interferon (IFN; IFN-α and IFN-β) when exposed to DNA and RNA viruses. The ability of pDCs to stimulate T cells and secrete type I IFN has raised 3 fundamental questions. (1) Do pDCs present antigens as effectively as classical DCs? While it is generally agreed that pDCs are less efficient than DCs, whether pDCs can prime naive T cells, as DCs do, or just expand memory T cells, as B cells do, remains a matter of debate. (2) Does activation of pDCs affect the quality of antigen-specific T-cell responses? While activated pDCs up-regulate T-cell stimulatory molecules, it has been proposed that immature pDCs may in fact induce regulatory T cells that actively suppress antigen-specific T-cell responses. (3) Does pDC secretion of type I IFN contribute directly or indirectly to antigen presentation?

In this issue of *Blood*, Schlecht and colleagues address these issues using an original and clever approach that involves activating pDCs with different stimuli in vivo, recovering activated pDCs, loading them with antigen in vitro, and adoptively transferring them into mice. Remarkably, pDCs injected intravenously home efficiently to the spleen, making it possible to study their ability to stimulate or inhibit naive and memory T-cell responses. The results of these experiments demonstrate that the antigen-presenting capacity of pDCs is critically dependent on their state of activation and, in particular, the stimuli used to activate them in vivo. Two types of stimuli were evaluated: a cytosine-guanine (CpG) oligonucleotide, which activates pDCs through toll-like receptor (TLR) 9, and heat-inactivated influenza virus, which stimulates pDCs through TLR7. The pDCs activated with CpG can stimulate memory CD8 T cells but not naive CD8 T cells. In contrast, pDCs activated with influenza virus can prime naive CD8 T cells, although less efficiently than DCs. Moreover, T-cell responses primed by pDCs can be