

Comment on Ohlfest et al, page 2691

## An ounce of prevention potentiates a pound of cure for hemophilia A

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Ohlfest and colleagues demonstrate that injection of human factor VIII (hFVIII) protein into hemophilia A mice shortly after birth induces long-lasting tolerance to hFVIII and allows gene therapy to be performed during adulthood with a Sleeping Beauty transposon without inhibitor formation.

**H**emophilia A has an incidence of 1 in 5000 males and is due to deficiency of hFVIII. The mainstay of treatment is intravenous injection of hFVIII protein prophylactically or during bleeding, which is expensive and inconvenient. Identification of alternative treatments would be a very important advance. Gene therapy for hemophilia A involves transfer of a FVIII gene into cells that can secrete functional FVIII into the blood. Gene therapy has been successful with retroviral, adenoviral, adeno-associated viral (AAV), and plasmid-based vectors after delivery to the liver or other organs.<sup>1</sup> However, inhibitory antibodies that block the coagulation function of hFVIII have developed.<sup>2</sup>

Identifying a way to prevent inhibitor formation would be an important advance for gene therapy for hemophilia. It was reported previously that injection of hFVIII protein shortly after birth into hemophilia A mice induced tolerance to subsequent infusion of hFVIII protein during adulthood.<sup>3</sup> Ohlfest and colleagues extend these studies here by showing that injection of hFVIII protein shortly after birth induces tolerance to subsequent gene therapy in 82% of adults that had the Sleeping Beauty transposase system delivered to their livers, allowing them to achieve stable expression at 16% of normal hFVIII levels. In contrast, all mice that received the same gene therapy procedure without neonatal tolerization developed inhibitors and lost expression in plasma.

There are 2 major implications of this study. First, neonatal tolerance might be used to prepare a patient for gene or protein therapy at an older age. Indeed, 58% of hemophilia B dogs that began to receive human factor IX (hFIX) treatment shortly after birth were tolerant to infusion of hFIX at an older age, whereas 0% of those that initi-

ated hFIX treatment at an older age were tolerant.<sup>4</sup> However, is not clear if neonatal tolerance will be effective in humans, as their immune system is much more mature at birth than that of mice.<sup>5</sup> Trials in which humans who are at high risk for inhibitor formation are initiated on immune tolerance induction shortly after birth might be indicated. The second important finding of this study is that therapeutic levels of hFVIII were achieved in plasma in mice after rapid

high-pressure intravenous injection of the Sleeping Beauty transposon system to the liver. However, a method for delivering plasmids that does not involve a rapid high-pressure injection, and demonstration that the risk of cancer is very low with this vector that integrates randomly into the chromosome, will be necessary before this approach should be considered for the use in humans with hemophilia A. ■

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Comment on Butenas et al, page 2764

## Functional tissue factor in blood?

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Several lines of evidence suggest that blood-borne tissue factor can promote thrombus growth. This study demonstrates that the amount of functional tissue factor in the blood of healthy individuals under nonflow conditions is vanishingly small.

**A** report by Giesen et al<sup>1</sup> in 1999 led to a resurgence of interest in the hypothesis that blood-borne tissue factor plays an important role in thrombogenesis, thereby challenging the traditional view that the dominant tissue factor source is the vascular wall. Some of the tissue factor molecules in blood are truncated or result from alternative splicing of tissue factor mRNA<sup>2</sup> and lack the transmembrane domain. Several studies have demonstrated that tissue factor antigen is present in plasma and the levels can be elevated in a number of disease states associated with increased coagulation activation. Plasma tissue factor antigen levels have varied considerably between studies, and levels in young healthy individuals are reported to range from undetectable up to the picomolar range. There has however been controversy as to whether

blood-borne tissue factor is functionally active under physiologic conditions.

In live mice, it has been demonstrated that blood-borne tissue factor accumulates in newly formed thrombi via monocyte-derived microparticles in a process dependent upon P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1).<sup>3</sup> To assess the functional significance of blood-borne tissue factor relative to vascular wall tissue factor under physiologic conditions, 2 groups have studied thrombus formation in bone marrow transplant chimeras of low-tissue factor mice and wild-type mice. Using intravital microscopy to study thrombus formation following laser-induced arterial injury, Chou et al<sup>4</sup> concluded that tissue factor on hematopoietic cell-associated microparticles contributes significantly to thrombus propagation. However, Day et al<sup>5</sup>