

So why is the leukemogenic mechanism in humans so indirect? What is protecting the human *PU.1* gene from the mutation, deletion, and translocation events suffered by other tumor-suppressor genes? More importantly, will these observations have therapeutic implications? Directly stimulating *PU.1* expression could circumvent the problems associated with ATRA therapy, namely toxicity of the drug and acquired resistance of the leukemic cells to differentiation. ■

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easier to perform gene therapy outside of specialized medical centers and might reduce the risks of the procedure.

The paper from Ting-De Ravin and colleagues demonstrated that X-linked SCID could be corrected with a simple intravenous injection of a retroviral vector (RV) expressing the common  $\gamma$  chain within 3 days after birth. It was previously demonstrated that intravenous injection of an amphotropic RV shortly after birth resulted in transduction of approximately 1% blood cells, and transduced cells were maintained at stable levels for up to 4 years.<sup>3</sup> This transduction of HSCs may be related to the fact that hematopoiesis occurs in the liver in newborn dogs, and the liver has direct contact with blood. The study by Ting-De Ravin et al took advantage of this phenomenon by injecting an RD114-pseudotyped RV that expressed both the common  $\gamma$  chain and the green fluorescence protein (GFP) into newborn dogs with X-linked SCID. They found that 3 of 4 dogs achieved normal levels ( $> 750$  cells/mL) of T cells within 1.5 months after gene transfer (see the figure), and more than 90% of the T cells expressed GFP. The T cells were maintained at normal levels for up to 1.5 years and led to reconstitution of immune responses. Although this result is very exciting, it is unlikely that neonatal intravenous injection of an RV will be effective for disorders that require that more than 1% of cells be modified, unless the therapeutic gene confers a selective advantage. However, it is possible that an in vivo selection strategy could be applied transiently around the time of gene transfer to achieve a high level of transduction of HSCs, and that this level could be maintained over time.

Although X-linked SCID was the first disease for which gene therapy was clearly curative in humans,<sup>4</sup> leukemias have developed at about 3 years after transduction in 3 patients; 2 of these leukemias involved integration near the *LMO2* locus, which is a known T-cell oncogene.<sup>5</sup> In contrast, leukemias did not develop in X-linked SCID mice that received gene therapy, which may be due in part to their shorter lifespan. These RV-treated dogs should represent a very important model in which to determine how often insertional mutagenesis causes an adverse event and to test vectors that have been modified to reduce this risk. ■

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## ● ● ● GENE THERAPY

Comment on Ting-De Ravin et al, page 3091

# Gene therapy goes to the dogs

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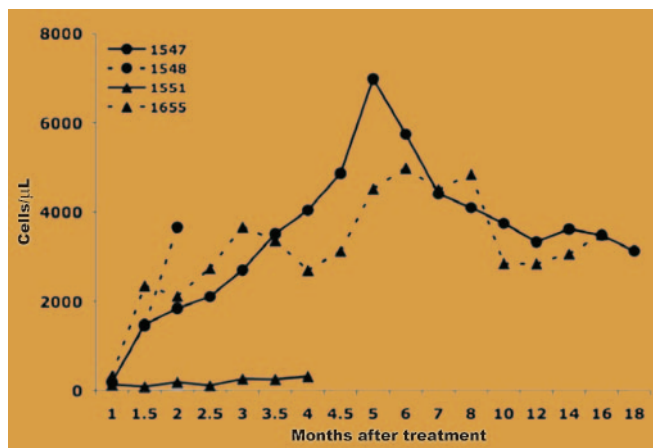
Dogs with severe combined immunodeficiency (SCID) were cured with intravenous neonatal injection of a retroviral vector expressing the common  $\gamma$  chain. This simple procedure for transducing hematopoietic stem cells in vivo might also treat other blood disorders.

Gene therapy could treat a variety of disorders that involve blood cells. Most hematopoietic stem cell (HSC)-directed gene therapy has involved an ex vivo approach in which HSCs obtained from blood, bone marrow (BM), or cord blood are transduced in culture, and modified cells are returned to the body after BM ablation. BM ablation is not

necessary for diseases such as X-linked severe combined immunodeficiency (SCID) due to a deficiency of the common  $\gamma$  chain for cytokine receptors, where the modified cells have a selective advantage. Although ex vivo HSC-directed gene therapy has resulted in correction of hematopoietic disorders in animals and humans, there are several disadvantages: (1)

obtaining HSCs from BM or blood requires a medical procedure; (2) the isolation of stem cells from blood or marrow and the in vitro culture have the potential for contamination with infectious agents; and (3) the in vitro culture with cytokines may select for insertions that promote immortalization<sup>1</sup> or may alter the potential for cells to engraft.<sup>2</sup>

Identification of an in vivo gene therapy approach would make it



Longitudinal analysis of T-cell development and persistence of gene marking in different blood cell lineages. See the complete figure in the article beginning on page 3091.

primary bone marrow progenitor cells. *Blood*. 2005;106:3932-3939.

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## ● ● ● HEMOSTASIS

Comment on Sood et al, page 3173

# Trophoblast cells sense maternal hemostasis

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When trophoblast stem cells differentiate, they acquire an endothelial cell–like thromboregulatory gene expression program and sense, via the expression of protease-activated receptors, the presence of activated coagulation factors.

**P**regnancy complications are one of the leading problems in women's health issues: for instance, 9% to 13% of women in the reproductive age group experience 1 clinically recognized pregnancy loss, 5% experience 2 or more losses, and 1% to 2% suffer 3 or more losses. Although several medical causes have been established, up to 50% of cases still remain unexplained.

Clinical studies have shown that maternal inherited and acquired hypercoagulable disorders that promote thrombosis, termed thrombophilia, may increase susceptibility to fetal loss. Among them, the factor V Leiden and prothrombin 20210G>A polymorphisms are associated with an increased risk of spontaneous abortion, if clinical signs occur from the 10th week of the first intended pregnancy.<sup>1</sup> However, available data show variable degrees of the association between maternal thrombophilia and adverse pregnancy outcomes, indicating the existence of as yet uncharacterized cofactors acting as risk modifiers.

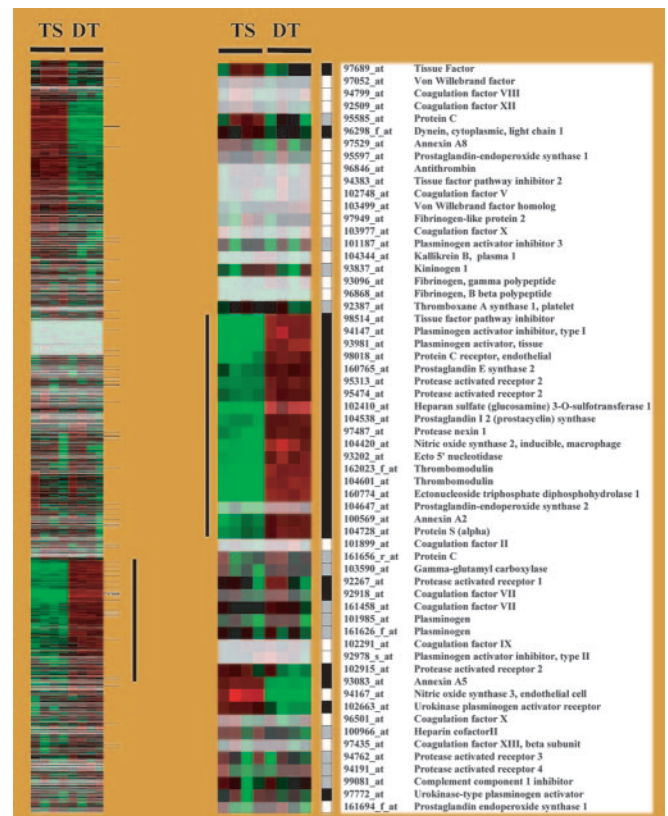
Humans and mice share a hemochorial placenta model, with zygote-derived trophoblast cells uptaking nutrients from circulating maternal blood. Mouse knock-out models have increased our understanding of the effect of inherited thrombophilia on pregnancy outcome, showing the link with trophoblastic physiology. Thrombomodulin (TM) and endothelial protein C receptor (EPCR) play a crucial role during development—mice lacking either of these molecules die during midgestation.<sup>2,3</sup> Restoration of

TM expression in the placenta leads TM-null embryos to develop normally during mid-gestation, and the crucial site wherein EPCR expression is required during development is the placenta. The growth defects of both TM and EPCR knock-out embryos suggested that the protein C system plays a role in regulating trophoblast cell proliferation during development. The impaired trophoblast cell proliferation of the knock-out embryos was linked to altered activation of protease-activated receptors (PARs) at the cell surface, the engagement of PAR-1 by EPCR-bound activated protein C being no longer efficient.<sup>4</sup>

In this issue, Sood and colleagues elegantly show that murine trophoblast cells activate, during differentiation, a gene expression program conferring thromboresistance (see figure). The group of thrombo-

regulatory genes represents a set of candidate genes, transmitted by both parents, which may modulate the risk of adverse pregnancy outcomes experienced by mothers with thrombophilia. The authors also demonstrate that trophoblast cells can sense the presence of activated coagulation factors, which can engage PARs and induce alterations in trophoblast gene expression.

If human and murine physiologies are really similar, the data from Sood et al may open new doors that could prove fascinating and clinically promising. First, the father's genome, as previously suspected,<sup>4</sup> may play a part in poor pregnancy outcomes: the NOHA first study,<sup>1</sup> which systematically included DNA from the father, is currently working in that direction. Second, it may indicate, among the many women carrying frequent thrombogenic polymorphisms, *which* really are at risk, thus leading to a more precise definition of women in need of prophylactic treatment,<sup>5</sup> to a precise development of these treatments, and maybe to primary prophylaxis. Third, it should clarify the link between thrombophilia and a given type of pregnancy complication. A new area is probably in the offing. ■



Cluster display of expression data from TS and DT cells. See the complete figure in the article beginning on page 3173.