The search for the origin of factor VIII synthesis and its impact on therapeutic strategies for hemophilia A

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Activated factor IX and its cofactor, activated factor VIII, are critical for the proteolytic activation of factor X. Inherited deficiencies of these factors cause the bleeding disorders hemophilia B and hemophilia A, respectively. The development of recombinant expression systems for factor VIII, following the isolation of the *F8* gene,[1] was a major advance that significantly improved the treatment of hemophilia A. Currently, several approaches to alter the hemophilia A phenotype by cell and/or gene therapy are under development.

Although it has been well-recognized from a series of experiments in hemophilia A dogs and human liver transplantation that there are both hepatic and extrahepatic sources of factor VIII,[2,3] the identity of the specific cells responsible for the level of circulating factor VIII has long remained an unresolved issue.[4-6] In this issue of the journal, Zanolini and colleagues provide new insight into both the hepatic and hematopoietic-derived cellular origin of circulating factor VIII.[7] Using multiple sources of hematopoietic cells (bone-marrow derived or cord blood CD34$^+$ cells) from humans, they identified (by both mRNA and immunofluorescence staining of protein) that monocytes and monocyte-derived macrophages are the main cells of the hematopoietic system responsible for factor VIII. Monocytes and monocyte-derived macrophages in peripheral blood also expressed factor VIII mRNA and protein, but at lower levels. Furthermore, upon differentiation of human CD34$^+$ cells to megakaryocytes, mRNA and protein staining also showed factor VIII expression in the differentiated cells. However, the relative contribution of factor VIII from these hematopoietic-derived sources to the total circulating levels remains to be determined.

Similar findings in humans were also obtained in mouse models in the current study as well as in a previously published study by the same group.[8] Notably, in a series of experiments in hemophilia A mice, injection of isolated CD11$^+$ human monocytes resulted in a transient increase in the circulating levels of factor VIII; the protein was biologically active, as demonstrated by the improved survival of animals upon hemostatic challenge to the macrocirculation (tail clip assay). Furthermore, hemophilia A mice that received cord blood-derived CD34$^+$ cells from wild-type donors following total body irradiation experienced a dose-dependent elevation of factor VIII levels and improvement of the bleeding phenotype upon vascular injury.

Recently, three studies addressed the cellular origin of factor VIII synthesis in the liver.[4-6] The overall conclusion was that liver sinusoid endothelial cells (LSEC), not hepatocytes, are the main cellular source of hepatic factor VIII. Here, in a unique set of experiments, the authors demonstrated that transplant of non-parenchymal cells (a mix of Küpffer cells and LSEC), improved circulating factor VIII levels, but infusion of isolated hepatocyte did not. These findings support the emerging understanding that both murine and human hepatocytes do *not* secrete factor VIII protein, despite detectable levels of mRNA.

Several observations on these studies may also be useful to address questions in hemophilia B models. First, it has to be demonstrated that transplant of isolated hepatocytes, but *not* non-parenchymal cells, can increase levels of factor IX. In addition, human factor IX binds to collagen IV on the surface of endothelial cells;[9] whether this is also true for the LSEC is unknown. Secondly, the detection of human factor VIII in dendritic cells raises the question of whether this has any impact on immune responses following protein-based or gene-based therapy. Considering the role of these cells in mediating immune responses, it would also be of interest to determine whether factor IX could be detected in dendritic cells and whether differences in dendritic cell expression of factors VIII and IX may help explain the 5-fold increase in rates of inhibitor formation to factor VIII compared to factor IX therapy.

What are the implications of these findings for cellular and gene therapy approaches for hemophilia A?

This work suggests that gene transfer strategies targeting hematopoietic stem cells, whether lineage-specific (platelets) or non-lineage-specific, for expression of factor VIII should not be considered ectopic.[10-13] It is, therefore, possible that clinical translation of promising preclinical studies utilizing such gene transfer strategies in small and large animal models is, in fact, more tangible.[11] Because the relative contribution of these cells to the total pool of circulating factor VIII is unclear, it may not be feasible to achieve therapeutic levels of factor VIII from unmodified cells. However, the fact that hematopoietic cells make biologically functional factor VIII is encouraging. Indeed, the use of factor VIII variants with enhanced biological activity may circumvent the potential limitation of the relative overexpression of factor VIII in cells with limited capacity for performing critical post-translational modifications.[10,12,14]

How do these findings fit with human hematopoietic stem cells? Data on hemophilia A patients receiving allogeneic bone marrow transplants are limited and may not clearly define the contribution of hematopoietic stem cell-derived factor VIII to circulating levels, especially if very small, due to the comorbidities such as cancer, aplastic anemia and others.[15,16] There is a report of a single case in which a patient with severe hemophilia A, complicated by refractory high titers of inhibitors to factor VIII, was given a hematopoietic stem cell transplant.[17] The transplant was aimed at eradicating the inhibitors; however, the strategy failed to achieve its goal, so the disease phenotype could not be assessed. Thus, the contribution of unmodified hematopoietic stem cell-derived factor VIII to circulating levels in humans has not yet been determined.

To harness the potential of LSEC for therapeutic purposes is

currently challenging because of difficulties in accessing donor tissue, the estimated low yield of residual LSEC, and non-optimized culture systems.[18] However, the growing field of inducible pluripotent cells may provide useful alternatives. In addition, LSEC are also attractive for their ability to induce antigen-specific immune tolerance.

Lastly, the fact that hepatocyte transplantation does not correct the hemophilia A phenotype in mice may have implications for translational studies on liver gene therapy for the disease. To date, the most successful trials for hemophilia B are using hepatocyte-specific promoters for the expression of factor IX. The fact that factor VIII is not normally secreted from human hepatocytes does not prevent the use of gene delivery to the hepatocyte, as promising data from preclinical studies on factor VIII expression in large animals support the concept that targeting hepatocytes has potential for translational studies.[19,20] However, this strategy may be limited by the intrinsic inability of the human hepatocyte to fully synthesize and secrete factor VIII, despite the presence of mRNA.[6] This may explain, at least in part, the higher dose of vectors required for expression of factor VIII compared to factor IX. On the other hand, despite the limiting effect on the efficacy of factor VIII secretion, the use of hepatocyte-specific expression is a favorable strategy in terms of immune tolerance induction to factor VIII, which is the most serious and common complication (~20%) of hemophilia A treatment.[19]

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Dangerous liaisons: cooperation between Pbx3, Meis1 and Hoxa9 in leukemia

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Homeobox (*HOX*) genes have a longstanding association with human acute leukemias. In particular, high expression of the *HOXA9* gene is a highly significant marker of poor prognosis in acute myeloid leukemia,[1] and dysregulation of *HOXA9* appears to play a central role in several distinct leukemias. These include acute myeloid leukemia and acute lymphoblastic leukemias caused by translocations of the *Mixed Lineage Leukemia* (*MLL*) gene,[2-4] fusions of the *HOXA9* gene that produce a novel HOXA9-NUP98 fusion protein in acute myeloid leukemia,[5] and T-cell acute lymphoblastic leukemias that have translocations between the *TCRβ* and *HOXA9/A10* loci.[6] Interestingly however, despite this seemingly central role in a subset of acute leukemias, *Hoxa9* expression alone is only weakly oncogenic in mouse leukemia models and usually requires a second "hit" via overexpression of *Meis1*,[7,8] or in some cases *Pbx3*.[9] Much work has been done trying to understand the molecular function of the HOXA9 protein. MEIS1 and PBX3 are both members of the TALE (three amino acid loop extension) homeodomain-contain-