● ● ● **TRANSPLANTATION**

Immune reconstitution after unrelated cord blood transplants in adults

Eliane Gluckman, MD HOSPITAL SAINT LOUIS, PARIS

The article by Komanduri et al in this issue of Blood describes immune reconstitution after human leukocyte antigen (HLA)-mismatched cord-blood transplantation. As expected, a profound reconstitution deficiency was observed; a study of some subpopulations could be predictive of the outcome.

Komanduri et al describe a study of immune reconstitution after unrelated HLA-mismatched cord blood (CB) transplants were given to adults with various malignancies. The use of cord blood as an alternative source of stem cells is increasing markedly, since it was shown that HLA incompatibilities were better tolerated after cord blood transplantation (CBT) than after adult hematopoietic stem cell transplantation because of the reduced frequency of graft-versus-host disease (GVHD). In addition to a lower number of cells infused, cord blood lymphocytes differ from adult cells because they are naïve and immature with different subtype composition. They also have different cytokine requirements and express fewer HLA molecules. The mechanism explaining the reduction of GVHD after CBT is not clearly understood. Because of the T cell's functional immune immaturity, there is a concern about a delay of immune reconstitution and increased risk of malignant relapse due to the loss of the graft-versus-leukemia effect.

Here, the authors studied T-cell reconstitution in 32 adults receiving transplants for various malignancies. They found that in the early posttransplantation period there was a profound lymphopenia associated with a compensatory B and NK expansion, an impaired functional response to CMV and superantigens with some exceptions, and thymopoietic failure with a relative paucity of CCR7^{+} naive and central memory cells after CBT, with an apparent association between the preservation of CD4^{+}CCR7^{+} T cells and an improved posttransplantation outcome. All these data could explain the high rate of infection after unrelated CBT. They differ from some other published results showing that there was a profound immune deficiency in the early period after transplantation but that in the long term (1 year after transplantation) surviving patients had a better immune reconstitution than did patients who had received an HLA-identical sibling bone marrow transplant.[1,2] One interesting finding is the observation that before transplantation they found an impaired baseline thymopoiesis and a relatively diminished naive T-cell repertoire. This is in line with our previously published study showing that pretransplantation host thymic function was a prognostic factor after HLA-identical sibling bone marrow transplantations.[3] In addition to factors related to the source of stem cells, host factors including age, previous treatment, diagnosis, and stage of disease play a major role in outcome after transplantation. Further studies on larger numbers of patients with homogeneous risk factors could determine the individual risks of having delayed immune reconstitution.

Another factor that might modify the results is a study of the influence of number of cells infused and number and type of HLA mismatches. Previous studies have shown that a high number of nucleated cells and CD34^{+} cells in the graft improve the rate of engraftment, decrease transplantation-related mortality, and improve survival. There was also a correlation between the number of HLA mismatches and engraftment, severe GVHD, and survival.[4] The study of the impact of these differences on immune reconstitution should be important for predicting the outcome and developing new methods for improving immune reconstitution.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● **HEMOSTASIS**

microRNAs outwit immune limitations in gene therapy

Mark A. Kay STANFORD UNIVERSITY

In this issue of Blood, Brown and colleagues deliver lentiviral vectors that express human factor IX from the livers of mice.

While this has been accomplished in earlier studies [1,2] the new twist is that they cleverly incorporate the cellular microRNA machinery to inhibit promiscuous lentiviral-mediated transgene expression in hematopoietically derived immune-participatory cells that become inadvertently transduced. As a result, these vectors dampen the cell-mediated immune response directed against human factor IX in vector-transduced cells, prolonging human factor IX expression in immunocompetent animals.

Various flavors of the immune response can limit the efficacy of any gene therapy approach, many of which have been experimentally demonstrated in animals and/or humans. The adaptive immune response can be directed against the vector, vector remnants in

transduced cells, and the transgene product, resulting in a humoral or cell-mediated response, the loss of the transgene product, and a limited period of efficacy. While lentiviral and other vectors show promise in preclinical gene transfer studies, there is no known way to completely restrict the dissemination of a vector when delivered by systemic (eg, intravascular) routes. In addition, even tissue-specific promoters designed to confine transgene expression to a specific cell type can be leaky when placed into some vectors. Taken together, even small amounts of inadvertent transduction can result in transgene expression in nontargeted professional antigen-presenting cells, resulting in an unwanted immune response.

MicroRNAs are a class of short (20-22 nt long) regulatory RNAs that represent up to 4% of the mammalian genome, encoding more than 400 transcripts that are believed to regulate as many as 30% of all genes. Some of these microRNAs are tissue specific and fine-tune genetic circuits, which are critical for normal development, cellular differentiation, and normal cellular homeostasis. In mammals, most of the currently known microRNA targets are localized to the 3′ untranslated region of mRNAs and contain sequence mismatches with their corresponding microRNA. In the absence of perfect complementarity, the primary mode of gene control is at the level of translational inhibition of the corresponding mRNA, by mechanism(s) that are still not well understood. If the target and microRNA have perfect complementarity, the mRNA is eliminated by a RNA degradation pathway. MicroRNA expression profiles are being harnessed as diagnostic tools and as means to predict possible reactions to different treatment options being considered for complex diseases such as cancer, while individual micro RNAs are being targeted to treat diseases such as hepatitis C.

Naldini and colleagues suggest a new therapeutic endeavor for microRNAs. The group previously inserted a modified microRNA 142–3p target that contained several copies of a perfect complement to the corresponding microRNA into the 3′ untranslated region of a test reporter gene[3]; they have now done so with the human factor IX expression cassette. After intravenous infusion, most of the lentiviral vector is taken up by the intended target, the liver, although some hematopoietic-derived cells become transduced. Since the 142–3p microRNA is only expressed in hematopoietic cells, the reporter gene or factor IX mRNAs are degraded, thwarting antigen production and the potential immune response against the transgene product. Moreover, because the microRNA is not produced in liver cells, transgene expression is unabated, resulting in a sustained therapeutic level of the coagulation factor.

The current approach has its limitations. It will not ameliorate cell-mediated responses directed against vector particles taken up by antigen-presenting cells or transgene product synthesized in the target cells but then taken up by other cells that may participate in evoking an immune response. The former process was believed to limit factor IX gene expression from the liver when the transgene was delivered in a recombinant AAV-2 vector in a clinical trial,[4] although the mechanisms for such responses have not been fully characterized. In addition, the Naldini study used a cross-species transgene, human factor IX in a mouse, a scenario that is not likely to be tried in clinical trials. Nonetheless, this study shows the proof of concept that the strategic inclusion of microRNA targets in a transgene expression cassette can help thwart at least one of the robust processes that can result in an immune-mediated elimination of the therapeutic. The utility of this approach for use in humans will need to be further evaluated.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● ● **HEMOSTASIS**

Comment on Kilic et al, page 4223

Is CEACAM1 a lymphangiogenic switch?

Björn Öbrink   KAROLINSKA INSTITUTET

Newly formed lymphatic capillaries closely associated with malignant tumors have a key role in metastasis, but much remains to be learned about the trigger and mechanisms of lymphangiogenesis. In this issue of *Blood*, Kilic and colleagues report that CEACAM1, recognized as a possible lymphangiogenic switch, is expressed in newly formed tumor-associated lymphatic capillaries, and that it can trigger reprogramming of microvascular endothelial cells to lymphatic endothelial cells.

Knowledge about lymphangiogenesis has been lagging behind that about blood vessel angiogenesis. However, with the identification of lymphatic endothelial cell-specific proteins, such as the homeobox transcription factor Prox1 and the plasma membrane proteins podoplanin (a mucin-type glycoprotein), LYVE-1 (a hyaluronan receptor of the CD44 family), and VEGFR-3 (a receptor for VEGF-C and VEGF-D), it has become possible to study the mechanisms of lymphangiogenesis in vivo.[1] Prox1 is required for lymphatic development, and VEGF-C/VEGFR-3 is essential for sprouting and proliferation of lymphatic endothelial cells in embryonic development. Studies of lymphangiogenesis in health and disease have attracted attention in recent years, not least because formation of new lymphatics early in the development of malignant tumors is of prime importance for metastasis of many types of human cancer. We know that tumors can induce formation of lymphatic vessels, but the trigger of lymphangiogenesis has not yet been identified.

CEACAM1, a member of the immunoglobulin superfamily, is a homophilic, trans