Jump-starting immune reconstitution after stem cell transplantation

Recipients of hematopoietic stem cell transplants (HSCT) are at high risk for opportunistic infection. Bacterial and certain fungal infections predominate during the immediate posttransplantation period when patients are neutropenic and have abnormal mucosal barriers. But HSCT recipients continue to be at risk for infections even after their neutropenia and mucositis have resolved. Particularly problematic are infections with cytomegalovirus (CMV) and Epstein-Barr virus, which is associated with the development of posttransplantation lymphoproliferative disease. The problem of immune deficiency is exacerbated by both graft-versus-host disease (GVHD) and procedures to remove mature T lymphocytes to prevent GVHD. Adoptive transfer of mature T lymphocytes as either T-cell clones or polyclonal mixtures of donor cells has been used to confer immunity to either specific pathogens or malignant cells. Adoptive transfer strategies are narrowed by the limited survival and narrow repertoire of antigenic recognition or effector functions of cloned antigen-specific T cells, while polyclonal donor lymphocyte infusions risk triggering GVHD.

The underlying lymphoid defects in HSCT recipients include defective lymphopoiesis, especially thymopoiesis, as well as immune dysregulation. Some of the factors that may restrict the ability of HSCs to reconstitute the immune system are the limited proliferation of HSCs or limitations on commitment to lymphoid lineages. In this issue, Arber and colleagues (page 421) describe a novel approach to immune reconstitution, using common lymphoid progenitors (CLPs). CLPs are a subset of committed progenitors with restricted ability to differentiate into T, NK, and B lymphocytes but not other hematopoietic lineages. Like HSCs, CLPs are prethymic, do not express T-cell receptors, and therefore have not been selected in the thymus for antigenic specificities that could cause GVHD. CLPs are highly proliferative but lack self-renewal capacity. Using fluorescence-based cell sorting to isolate different populations of donor progenitors, Arber et al transplanted either purified HSCs or a combination of HSCs and CLPs into irradiated mice. The mice receiving transplants did not develop GVHD, even when the donor cells were major histocompatibility complex (MHC)-mismatched with the recipients. The mice were challenged with experimental infection with murine cytomegalovirus (MCMV) soon after HSC transplantation. The mortality of HSCT recipients was reduced from nearly 90% to approximately 40% in the recipients of both HSCs and CLPs. The potency of CLPs was demonstrated in experiments showing that transplantation of 3000 CLPs was as protective as adoptive transfer of 10 000 000 lymph node cells. CLP transplantation altered the histopathology of the MCMV infection, resulting in greater inflammatory responses and less viral burden.

While the paper by Arber et al represents a landmark in efforts to improve immune reconstitution after HSC transplantation, a number of important and interesting questions remain. Since the CLPs can contribute to each lymphoid lineage, the exact effects of cotransplantation are likely to be complex. For example, CLPs also protected thymectomized mice from MCMV, suggesting that reconstitution of non-T cells is an important effect of CLP transplantation. The determinants of proliferation and differentiation of CLPs after transplantation need to be elucidated. Translation of the murine experiments to clinical HSC transplantation will be a challenge. Like murine CLPs, CLPs are a rare subset of human marrow. Are they more common in other progenitor sources, for example, mobilized peripheral blood? In experimental transplantations, sufficient numbers of rare donor progenitors can be obtained simply by using multiple donors for each recipient. Since this is not practical in clinical transplantation, strategies to expand CLPs in vitro or in vivo may be necessary to achieve a clinically relevant dose of CLPs to enhance immune reconstitution. The work by Arber et al is also likely to lead to other studies of how committed progenitors can be used to rapidly reconstitute specific hematopoietic lineages.

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DDAVP and interleukin-11: a boosting combination

Desmopressin (DDAVP) has found widespread clinical application in the management of hemophilia A and von Willebrand disease since its description by Mannucci et al in 1977 (Lancet. 1977;1:869-872). DDAVP is attractive because of the absence of risk for the transmission of infections, its lack of serious side effects, and its simple administration by nasal spray, which allows convenient home use. But patients become progressively less responsive upon repeated administration over several days, due to the depletion of storage pools. Furthermore, treatment is limited to patients with only moderately decreased levels of factor VIII (FVIII) or von Willebrand factor (VWF). Thus, new agents improving the application of DDAVP would be of great benefit. One potential candidate is recombinant human interleukin-11 (Neumega), a thrombopoietic growth factor that has been approved for treatment of thrombocytopenia following high-dose chemotherapy. Denis et al have previously shown that administration of interleukin-11 in mice produces a sustained increase of plasma levels of FVIII and VWF (Blood. 2001;97:465-472).

In this issue, Olsen and colleagues (page 436) show that interleukin-11 treatment of heterozygous VWF-deficient and normal dogs results in increased VWF mRNA levels. This may explain why interleukin-11...
administration is associated with elevated VWF plasma levels, and it indicates that interleukin-11 and DDAVP modulate VWF plasma levels in a mechanistically distinct manner. Interleukin-11 pretreated dogs indeed still exhibit the characteristic response to DDAVP. Moreover, this response is now much stronger and perhaps not as easily exhausted. Apparently, interleukin-11 treatment not only results in elevated VWF plasma levels but probably also increases the amount of VWF available from DDAVP-responsive storage pools. This raises the question of how this extra VWF is stored: are there more Weibel-Palade bodies per cell, or do more cells contain them?

The promising data justify the initiation of clinical studies of the use of interleukin-11, alone or in combination with DDAVP, and point to new avenues for the exploration of fundamental aspects of VWF and FVIII biosynthesis. Several issues regarding efficacy and safety obviously need to be addressed, and special attention should be given to fluid retention in patients, since both DDAVP and interleukin-11 possess antidiuretic properties.

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Another advance for globin gene therapy

In this issue, Persons and colleagues (page 506) report another advance in the now rapidly moving field of gene therapy for the β-chain hemoglobinopathies, β-thalassemia (β-thal), and sickle cell disease (SCD). The hemoglobinopathies are the most common human inherited diseases, and while the milder forms are increasingly amenable to drug therapies, the only real cure for the severe forms of these diseases has been bone marrow transplantation (BMT). But the scarcity of human leukocyte antigen (HLA)-matched donors and the high morbidly associated with complete myeloablation have limited the use of BMT for the treatment of SCD and β-thal. Recent breakthroughs in vector design allowing stable transfer of globin genes that can be expressed at therapeutic levels in red cells (May et al, Nature. 2000;406:82-86; Pawlick et al, Science, 2001;294:2368-2371) have made the hemoglobinopathies candidates for gene replacement therapy. In mouse models, the introduction of a globin gene (β-globin for β-thal and γ-globin to inhibit sickling in SCD) into a portion of autologous hematopoietic stem cells (HSCs) has led to permanent cures of β-thal or SCD mice receiving transplants (May et al, Blood. 2002;99:1902-1908; Rivella et al, Blood. 2003;101:2932-2939; Persons et al, Blood. 2003;101:2175-2183). Combined with the observations that stable mixed chimerism was associated with the successful cure of severe β-thal or SCD in a subset of human recipients of transplants, it appears that 25% to 50% of corrected cells are sufficient for a full cure. However, the low frequency of gene transfer into human hematopoietic stem cells (about 1%) and the morbidity of full myeloablation in patients with hemoglobinopathies have prevented the application of the recent advances in globin gene therapy to humans.

Persons et al have addressed these problems using 2 different mouse models of stem cell gene transfer. In the first model, they gave β-thal intermedia mice a non-myeloablative conditioning regimen and transplanted into them a small number of normal bone marrow cells that were transduced with a retrovirus vector containing the MGMT gene, which confers resistance to O6-benzylguanine (BG). The resulting bone marrow chimeras resemble low-level engrafment of HLA-matched normal cells after partial myeloablation and a low frequency of gene transfer. Prior to treatment the animals receiving transplants were indistinguishable from β-thal mice. Following treatment with BG, the level of transduced normal cells rose from less than 10% to 56% in 6 of 10 animals with a concurrent normalization of all red cell indices.

In the second model, Persons et al introduced a lentivirus vector containing both a human γ-globin producing red blood cells increased from a pretreatment level of less than 1% to more than 60% in 5 of 7 animals.

The 2 studies demonstrate a conservative approach that dramatically lowers the risk of transplantation-related complications to the patient and does not require high rates of HSC transduction. The most pressing issue facing Persons et al and other investigators in this field is to develop vectors that express higher levels of globin per vector copy, so that the maximum amount of globin protein can be produced from the minimum number of insertion events. In addition, while the MGMT selection is quite powerful, the frequency of gene transfer to human cells must still be improved to allow the most efficient treatment. I predict that these problems will be solved in the near future and that the first clinical trials for β-thal and SCD will resemble those described in this issue by Persons et al.

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Collagen-mediated platelet activation and PI3K

Phosphatidylinositol 3-kinase (PI3K) is a phospholipid kinase that is involved in diverse cellular events, including the prevention of apoptosis, regulation of glucose metabolism, chemotaxis, and cell proliferation. It is far from obvious what role this enzyme would play in a platelet—a terminally differentiated anucleate cell. Studies using less-than-specific pharmacologic inhibitors have suggested that PI3K might participate in both collagen-induced platelet activation and the irreversible phase of platelet aggregation (Kovacsovics et al, J Biol Chem. 1995;270:11358-11366; Pasquet et al, Biochem J. 1999;342(pt 1):171-177).

Most cells have multiple isoforms of PI3K that are composed of a regulatory subunit and a catalytic subunit. Using cells obtained from genetically modified mice,