inside blood

Stem cells take a shortcut to the bone marrow

One of the major problems limiting stem cell–based therapies, beyond simple transplantation of mononuclear cells or positive selection of $CD34^+$ cells, is the absence of a clear understanding of the composition of the stem cell (HSC) pool in humans. The right cell must be targeted for the right therapy. For gene therapy, HSCs capable of permanent repopulation must be transduced, whereas for cancer therapy an HSC capable of rapidly generating granulocytes, platelets, and erythroid cells is also required. Xenotransplantation of human cells into preimmune sheep or, more commonly, immune-deficient mice provides powerful assay systems to characterize the HSC compartment. Clonal tracking of retrovirally transduced cord blood cells has identified individual HSCs (termed SCID repopulating cells, or SRCs) with short-term (ST-SRCs) and long-term (LT-SRCs) repopulation capacity. Cell purification studies indicate that LT-SRCs are highly enriched in the $Lin^-CD34^+CD38^-$ cell fraction, while the $Lin^-CD34^+CD38^+$ fraction contains ST-SRCs that lack self-renewal potential. Thus a picture of the human HSC compartment is emerging in terms of functional repopulation properties of different classes of HSCs, their frequency, cell surface markers, cell cycle status, and response to in vitro cytokine stimulation. Importantly, the mechanism of migration and homing/adhesion of HSCs within the xenoenvironment is being defined. The chemokine SDF-1, expressed by both human and murine bone marrow (BM) endothelium and stroma, and its cognate receptor, CXCR4, expressed on human progenitors play a key role. The SRCs possess the capacity for migration to SDF-1 and the in vitro motility of human $CD34^+$ cells correlates with their repopulation potential in patients receiving transplants.

All HSC repopulation assays rely on intravenous (IV) injection; a complex process involving circulation through blood, recognition and extravasation through BM vascular endothelium, and migration to a supportive microenvironment. It is possible that cells with intrinsic HSC function might exist but either do not survive in the circulation or do not possess the machinery for homing and retention, rendering them poorly detectable by traditional IV-based HSC assays. Along this line, a rare class of human $CD34^-$-SRCs, found within the $Lin^-CD34^-CD38^-$ fraction, was tentatively identified. These $CD34^-$-SRCs have low CXCR4 expression, poor responsiveness to SDF-1–mediated migration, and very low in vivo homing, leading to very limited repopulation. But upon in vitro culture with cytokines and/or stroma these $Lin^-CD34^-CD38^-$ cells generate high numbers of $Lin^-CD34^+CD38^-$ cells that now possess high levels of SDF-1–mediated migration and repopulation capacity, suggesting that these HSCs possess significant intrinsic HSC potential. Complicating this story is the realization that expression of CD34 on both murine and human HSCs is subject to modulation either developmentally (murine fetal HSC are $CD34^+$; adult HSC, mostly $CD34^-$), after culture, or following transplantation. Thus we currently do not know whether these rare human $CD34^-$-SRC exist as a distinct class of HSC and whether they have clinical significance.

One approach to overcome these inherent limitations of IV-based repopulation assays is to deliver cells directly into the BM. In this issue, 2 manuscripts elegantly describe a more sensitive assay for human HSCs involving intra–bone marrow transplantation (IBMT) into immune-deficient mice. Yahata and colleagues (page 2905) used limiting dilution analysis and retroviral-mediated clonal analysis to show that the IBMT method detected 15-fold more $Lin^-CD34^+CD38^-$-derived LT-SRCs than did IV injection (1 SRC per 44 cells vs 1 SRC per 660 cells, respectively). In addition, treatment with neutralizing anti-CXCR4, anti–VLA-4, or anti–VLA-5 antibodies alone or in combination indicated that SRC retention was still dependent on SDF-1/CXCR4 and VLA-4/VLA-5 interactions with their respective ligands. Wang and colleagues (page 2924) demonstrate that IBMT of Lin^-CD34^- cells resulted in repopulation of all mice receiving transplants, in contrast to no engraftment following IV injection. The $CD34^-$-SRCs were engrafted with somewhat slower kinetics were 10-fold less frequent than $CD34^+$-SRCs, suggesting that these 2 HSC classes are distinct. Interestingly, the IBMT method also detected low levels of $CD34^+$-SRCs within a fraction of Lin^-CD34^+ cells that were unable to migrate in vitro to SDF-1 and consequently were unable to engraft NOD/SCID mice when injected intravenously. These nonmigrating $CD34^+$-SRCs are likely those shown to contain intracellular CXCR4 that oscillates to the cell surface.

Thus, direct delivery by IBMT reveals cells with intrinsic stem cell function that were previously poorly detectable by the traditional IV assay. Collectively, these studies indicate that the human HSC compartment might be more complex than previously thought and that novel classes of HSCs might still await discovery using this new assay. With the flurry of recent excitement concerning putative transdifferentiation of organ-specific stem cells and the induction of embryonic cells along various lineages, one can imagine that the IBMT method may provide a generalized method to detect cells with intrinsic HSC function. IBMT may also potentiate engraftment of other cell types such as mesenchymal progenitors to cure inherited bone diseases. Finally, although human experience with intraosseous transplantations goes back to the 1930s, there is little strong underlying experimental basis. These studies provide such a framework and some specific stem cell–mediated therapeutic strategies might

benefit from the clinical use of IBMT. For example, if HSCs are delivered more efficiently into humans via IBMT as the xenotransplantation studies suggest, it may be possible to extend the use of cord blood transplantations to adults.

—John E. Dick
University of Toronto
—Tsvee Lapidot
The Weizmann Institute of Science

Lack of *BCL-6* self-control fuels lymphomagenesis

Chromosomal translocations affecting the *BCL-6* proto-oncogene, which are the most common, specific genetic alteration found in diffuse large B-cell lymphomas (DLBCLs), were first identified by Dalla-Favera and coworkers. Targeted deletion of *BCL-6* revealed its critical physiologic role for the formation of the germinal center (GC) and the production of memory T cells. Signals emanating from pathways associated with lymphocytic differentiation, such as IgM engagement or CD40-CD40L interaction, down-regulate *BCL-6* expression. The Bcl-6 protein, a transcriptional repressor that is expressed in the mature GC, suppresses genes involved in apoptosis, cell cycle arrest, and in lymphocytic mitogenesis and differentiation. These observations indicate that *BCL-6* inhibits lymphocytic differentiation and, hence, *BCL-6* deregulated expression in DLBCLs contributes to the maintenance of the undifferentiated lymphoma phenotype.

In addition to the chromosomal translocations activating *BCL-6* in DLBCLs, the *BCL-6* locus is altered by somatic mutations that are intriguingly also found in normal GC B cells. While the genesis of these *BCL-6* mutations, which cluster around the transcription initiation site, are associated with the normal somatic hypermutation mechanism responsible for immunoglobulin variable sequences, the significance of these mutations in lymphomagenesis has remained unclear. In this issue, Pasqualucci and coworkers (page 2914) report mutations of *BCL-6*, which are not found in the normal GC, that disrupt negative autoregulation of *BCL-6* in 40% of DLBCL cases. In particular, the 2 Bcl-6 DNA binding sites located in the noncoding exon 1 are mutated such that Bcl-6 protein is unable to bind the mutant sites. These observations are similar to those recently reported by Wang et al (Proc Natl Acad Sci U S A. 2002;99:15018-15023) supporting the hypothesis that lack of self-control by *BCL-6* contributes to the genesis of DLBCLs.

—Chi Van Dang
Johns Hopkins University
School of Medicine

Toward gene therapy for β-thalassemia: new models, new approaches

Gene therapy for hemoglobinopathies has suffered from problems of vector instability, low viral titers, and variable expression for over a decade. In a pioneering study, Sadelain's group had shown that a lentiviral vector was able to stably transmit the human β-globin gene and its regulatory elements, resulting in therapeutic correction of β-thalassemia in $Hbb^{th3/+}$ mice (May et al, Nature. 2000;406:82-86). But the $Hbb^{th3/+}$ mice carry deletion of the β^{major} and β^{minor} genes only on one allele (Ciavatta et al, Proc Natl Acad Sci U S A. 1995;92:9259-9263; Yang et al, Proc Natl Acad Sci U S A. 1995;92:11608-11612) and resemble the human thalassemia intermedia phenotype. The degree of correction accomplished by the TNS-9 vector (about 3 g/dL increase in hemoglobin level per proviral copy) would be subtherapeutic in humans with Cooley anemia (β-thalassemia major). Homozygous deletion of both the β^{minor} and β^{major} globins is embryonic lethal in mice because, unlike in humans, the switch to adult globin production occurs in utero.

In this issue, Rivella and colleagues (page 2932) have developed a model of mouse thalassemia major by transplanting fetal liver stem cells from thalassemia homozygous fetuses into lethally irradiated healthy adults. They report a recapitulation of thalassemia major phenotype observed in humans, starting as early as 6 weeks following transplantation. Genetic correction of the thalassemia major bone marrow with the TNS-9 vector followed by a transplantation rescues the otherwise lethal anemia. But increases in hemoglobin level are no higher than those previously reported by this group using the same vector (May et al), predictably converting the thalassemia major phenotype to that of severe thalassemia intermedia. Nevertheless, this model of murine β-thalassemia major is representative of human disease and, eventually, may become the ultimate model to test therapeutic strategies.

Using a different approach, Persons and colleagues (Blood. 2003;101:2175-2183) have used the human γ-globin gene in a lentiviral vector, driven by a minimal β-globin promoter and the β-globin locus control region (LCR) elements to correct murine thalassemia intermedia. They show therapeutic increases in fetal hemoglobin production with 2 or more copies of provirus per cell in $Hbb^{th3/+}$ mice. The levels of expression are less robust than were levels of β-globin or a mutant β-globin expression previously observed by the Sadelain (May et al) and Leboulch (Pawliuk et al, Science. 2001;294:2368-2371) laboratories, respectively, probably due to a smaller β-globin gene promoter or LCR fragments. Nevertheless, their vector produces the highest levels of fetal hemoglobin protein reported in primary cells from an integrating viral vector. Additionally, γ-globin vectors have the advantage of a therapeutic potential both in thalassemia and sickle cell disease. Persons et al have taken gene therapy for β-thalassemia a step further: they have used self-inactivating lentiviral vectors, where the viral long-terminal repeat is deleted upon integration into cells, inactivating viral transcription and improving their biosafety.

While both these studies represent important strides toward gene therapy for thalassemia, they also highlight the obstacles yet to be conquered. Both studies show presence of chromatin position effects and underscore the need for better vectors, which would yield higher and predictable