HEMATOPOIESIS & STEM CELLS

Comment on Abdel-Azim et al, page 4064

Cell expansion and maintenance of stemness

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Manipulation of hematopoietic cells to expand output while maintaining stem-cell potential has been an elusive goal of experimental hematology. The development of a system using a chemically induced dimerizer and modified thrombopoietin receptor has now allowed the expansion of primitive hematopoiesis without sacrificing stem cells.

Hematopoietic-cell expansion represents a much-sought-after therapeutic goal of the biomedical sciences. With the cloning and characterization of a large and growing number of hematopoietic growth factors, a mechanism for hematopoietic expansion seemed to be at hand. However, ex vivo expansion strategies using cocktails of cytokines have failed to expand transplantable hematopoietic stem cells (HSCs). In contrast, most such approaches lead to the differentiation and extinction of the most primitive cells in the cultures. The explanation for these results is the requisite coupling of cell proliferation and differentiation that results when hematopoietic growth factors bind their cognate receptors.

The work of Abdel-Azim and colleagues in this issue of Blood has used a previously described cell-expansion strategy in a new target-cell population to massively expand hematopoietic cells of multiple lineages, including, apparently, the HSC. The approach involves chemically inducing dimerization of the cytoplasmic domain of the thrombopoietin receptor (c-Mpl) in highly purified, primitive human marrow cells. The rationale for this approach began with the discovery that c-Mpl and its ligand, thrombopoietin, provide important and nonredundant support for HSC survival and proliferation.

Hematopoietic growth factors act by binding to their cognate receptors, altering the conformation of the latter, resulting in cross-phosphorylation of 2 tethered Jak signaling kinases. Once phosphorylated, Jak kinases phosphorylate the receptors themselves as well as several secondary survival and proliferation signals, including signal transduction and activator of transcription 3 (STAT3) and STAT5, phosphoinositol-3-kinase (PI3K), and mitogen-activated protein kinases (MAPKs). Ultimately, some of these same signals lead to signal extinction, by inducing receptor internalization and STAT–induced expression of suppressors of cytokine signaling (SOCS) molecules, which block further Jak signaling.

Identification of the FK506 binding protein (FKBP), the target of the commonly used immunosuppressant drug FK506, and the demonstration by Spencer et al that a chemically synthesized dimeric form of FK506, FK1012, could artificially dimerize 2 molecules of FK506,1 led to the first chemical inducer of dimerization (CID) strategy. Following a minor modification in FKBP (F36V) to render it responsive to the nonimmunosuppressive AP20187 compound, the stage was set to use this CID to mimic cytokine-induced cellular signaling. By transducing marrow cells with an FKBP (F36V)–c-Mpl fusion protein, Jin et al first established the ability of the CID approach to influence hematopoietic-cell proliferation.4 These efforts expanded mature blood cell production both in vitro and

### References

1. Sadler JE. Slippery criteria for von Willebrand disease type 1. J Thromb Haemost. 2004;2:1720-1721.

2. Sadler J, Rodeghiero F. Provisional criteria for the diagnosis of VWD type 1; on behalf of the SSC Subcommittee on von Willebrand factor. J Thromb Haemost. 2005;3:775-777.

3. Miller CH, Lenzi R, Breen C. Prevalence of von Willebrand’s disease among U.S. adults. Blood. 1987;78:777.

4. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand’s disease. Blood. 1987;69:454-459.
in vivo; however, HSC expansion was not demonstrable. By using highly purified CD34+/CD38−/CD19−/Lin− human cells, Abdel-Azim and colleagues have moved this technology closer to clinical utility.

Like all studies with clinical implications, the work of Abdel-Azim et al must be repeated by others to verify that the capacity to expand and maintain HSCs can be generalized to transplantation in humans. If so, this work opens a number of new avenues for the manipulation of human HSCs for therapeutic benefit, including the engineering of HSCs with therapeutic proteins, the expansion of HSCs when only small numbers are clinically available, and the ex vivo generation of multiple therapeutic products. But there is an equally important aspect to the mechanism described by Abdel-Azim and colleagues: understanding why it works.

As noted, dimerization of c-Mpl by thrombopoietin results in HSC survival, cellular proliferation and maturation into multiple types of committed hematopoietic progenitor cells, and ultimately, HSC extinction. In contrast, dimerization of a non–cell-membrane-bound form of the cytoplasmic domain of c-Mpl by a CID expands hematopoietic cells and maintains their “stemness.” In their discussion, Abdel-Azim and colleagues suggest that the difference between their study and prior work exists in the constant signaling induced by their engineered construct, one that cannot be down-modulated. Another possible explanation could be the failure of the CID-activated cytoplasmic c-Mpl domain to phosphorylate STAT5, the prerequisite for SOCS activation, an important mechanism for turning off growth signals. Or could it be the cytoplasmic site of origin of the c-Mpl signals?

Numerous studies suggest that the subcellular site from which a signal emanates plays a major role in the physiological effect of that signal. Or is it, perhaps, the geometry of the induced receptor dimerization, which differs between thrombopoietin and the CID? Wilson et al, in their work with erythropoietin mimetic peptides and the erythropoietin receptor, revealed that as little as a 20° difference in homodimer conformational rotation turns a peptide agonist into an antagonist.

Thus, like all excellent studies, the work of Abdel-Azim and colleagues answers some questions, raises many others, and prods us to move a new technology forward; hopefully, such work will help us fulfill the goal of expanding hematopoietic cells for therapeutic benefit.

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**REFERENCES**

1. Kaushansky K. Thrombopoietin: accumulating evidence for an important biological effect on the hematopoietic stem cell. Ann N Y Acad Sci. 2003;996:39-43.

2. Kaushansky K, Drachman JG. The molecular and cellular biology of thrombopoietin: the primary regulator of platelet production. Oncogene. 2002;21:3359-3367.

3. Spencer DM, Wandle TJ, Schreiber SL, Crabtree GR. Controlling signal transduction with synthetic ligands. Science. 1993;262:1019-1024.

4. Jin L, Sriuranarak N, Emery DW, et al. Targeted expansion of genetically modified bone marrow cells. Proc Natl Acad Sci U S A. 1998;95:8093-8097.

5. Wilson IA, Joliffe I. The structure, organization, activation and plasticity of the erythropoietin receptor. Curr Opin Structural Biol. 1999;9:696-704.

**CLINICAL OBSERVATIONS**

Comment on Bower et al, page 3986

**AIDS, T cells, chemotherapy: HAART-breaking?**

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Chemotherapy depletes T cells in AIDS patients on HAART, but preexisting lymphopenia limits the scale compared with non-AIDS patients. Thus T-cell recovery time to baseline may be rapid, but the risk of additional AIDS-related complications persists.

Treatment advances for AIDS-related lymphoma (ARL) have largely been predicated on highly active antiretroviral therapy (HAART). Before HAART, the combined immune injury of AIDS and chemotherapy made effective cancer treatment unavailable.

Chemotherapy is now more well-tolerated secondary to immune preservation with HAART. Conventional wisdom stresses that successful administration of anticancer therapy is dependent on concomitant HAART. This inference is challenged by data indicating favorable outcomes in patients who stopped HAART until completion of cancer therapy. A study of 105 ARL patients reported by Bower and colleagues in this issue of *Blood* does not resolve this debate, but the authors do detail virological and immunological changes seen in a subset of 68 patients treated concomitantly with HAART and chemotherapy who survived 3 months or longer.

Importantly, the fact that analysis of lymphoid recovery was restricted to survivors favors selection of subjects with a higher number of CD4+ cells. However, this selection bias is justified because it reduces spurious results that may occur when subjects with low numbers of CD4+ cells die disproportionately early in the study timeline, shifting the mature dataset to represent the remaining subjects—mainly those with higher numbers of CD4+ cells. In such a scenario, the appearance of later CD4+ cell increases could be unrelated to any actual changes in individual patient counts. The analysis by Bower and colleagues likely has avoided this confounding element. Consequently, the data they present inform important concepts regarding AIDS, T cells, and chemotherapy.

To better appreciate the issue at hand, recall that lymphocytotoxic chemotherapy is a more potent T-cell destroyer than is HIV by several orders of magnitude. HIV-seronegative adults typically lose approximately 600 cells/mL within 2 treatment cycles, reaching a nadir at 150 to 600 cells. Recovery from this degree of depletion usually takes well over 12 months. Importantly, most patients with ARL have far fewer than 600 CD4+ cells/mL at the time of lymphoma diagnosis—a consequence of years, not weeks, of ongoing HIV replication. In this study, the survivors’ median baseline CD4+ cell count was 178 cells/mL, and ranged from 8 cells/mL to 636 cells/mL. Thus, when starting with a damaged immune system, even minor additional CD4+ cell loss can be life-threatening. Conversely, small, rapid CD4+ cell increases can substantially reduce morbidity. As with febrile neutropenia, opportunistic illness (OI) risk is, in part, dependent on the depth and duration of T-cell depletion. The
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