Enhancement of stem cell engraftment on a WHIM

Hal E. Broxmeyer
Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana, USA.

WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome is a genetic autoimmune disorder that results from gain-of-function mutations in the gene encoding chemokine receptor CXCR4. A previous study characterized a patient with WHIM who underwent a chromothriptic event that resulted in spontaneous deletion of the WHIM allele in a single hematopoietic stem cell and subsequent cure of the disease. In this issue of the *JCI*, Gao et al. extend this work and show that Cxcl4-haplosufficient bone marrow has a selective advantage for long-term engraftment in murine WHIM models. Moreover, successful engraftment occurred without prior conditioning of recipients. Together, these results have important implications for improving hematopoietic stem/progenitor cell transplant not only for patients with WHIM but also for all patients who may require the procedure.

Introduction

Chemokine receptor CXCR4 and one of its two ligands, CXCL12 (also known as stromal cell derived factor 1 [SDF-1]), are involved in the migration, homing, mobilization, and survival of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) (1). The genetic disorder WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome manifests as a primary immunodeficiency and involves an autosomal dominant gain-of-function CXCR4 mutation. In this issue, Gao et al. (2) provide a follow-up on previous studies that have advanced the understanding of the pathophysiology and treatment of WHIM and a role for CXCR4 in the disease. Allogeneic BM transplantation has been used to cure several patients with WHIM (3, 4), and an antagonist of CXCR4 (5), originally called AMD3100 and more recently termed plerixafor (Mozobil, Sanofi), is currently being evaluated in a clinical trial for this disorder (6–8). In their previous study, McDermott et al. reported on one patient with WHIM in whom chromothripsis (known as chromosome shattering) resulted in spontaneous deletion of the WHIM allele in a single HSC and subsequent cure of WHIM syndrome in the individual (9). While 164 genes were deleted by this chromothriptic event, Gao et al. have now focused on CXCR4 and found a selective growth advantage of Cxcr4-haplosufficient (Cxcr4+/o) mouse BM HSCs that yielded almost total chimeraism of myeloid cells in a congenic mouse competitive transplant setting (2). Cxcr4+/o BM cells resulted in stable long-term engraftment beyond that of BM from either WHIM model mice (Cxcr4+/w) or WT Cxcr4+/o animals in the setting of competitive transplantation following lethal irradiation of congenic mouse recipients. Moreover, recipient Cxcr4+/w WHIM mice were able to accept the engraftment of Cxcr4+/o HSCs without any conditioning, and a small percentage of donor Cxcr4+/o HSCs were able to support more than 70% long-term chimeraism of myeloid cells in unconditioned Cxcr4+/o mice, resulting in correction of the blood myeloid deficiency in Cxcr4+/o WHIM mice (Figure 1A).

Based on these data, Gao et al. suggested future gene editing possibilities to delete the WHIM allele in donor cells for curative autologous transplantation that would not require conditioning of the patient. In fact, the actual implications of these studies may go well beyond selective correction of the clinical WHIM syndrome, as engraftment involves the homing characteristics of donor cells and the nurturing capacity of the BM microenvironment. Additional translational aspects of the improved engraftment of Cxcr4-haplosufficient HSCs that extend beyond WHIM (Figure 1B) are discussed below.

Role of CXCR4

Cord blood (CB) hematopoietic cell transplantation (HCT) is a viable alternative source of HLA- and partially HLA-matched donor cells for treatment of malignant and nonmalignant genetic diseases, with over 40,000 CB HCTs done to date (10). A continuing disadvantage of CB HCT is the length of time it takes for recovery of donor neutrophils, platelets, and immune cells, as compared with BM or mobilized peripheral blood HCT. Attempts to enhance engraftment of CB for more efficient HCT have included ex vivo expansion of HSCs and HPCs and/or enhancing the homing efficiency of HSCs (11). Although Gao et al. (2) did not demonstrate that loss of one Cxcr4 allele in Cxcr4+/o mice enhances the speed of engraftment in their mouse models, the demonstration that decreasing, without complete loss of, CXCR4 expression on donor cells may enhance homing and engraftment may be a lesson to incorporate for improving CB HCT. It may seem counterintuitive to decrease CXCR4 expression of donor cells for enhanced engraftment, as CXCR4 is implicated in survival and homing of HSCs and HPCs (1). However, CXCR4 is not necessarily of absolute importance itself in these functions, as expression levels of CXCR4 do not always correlate with engraftment efficiency. Collection of mouse BM or human CB under hypoxic conditions results in increased numbers of functionally engrafting HSCs, yet these cells exhibit significant decreases in CXCR4 expres-
Role of DPP4 and CXCL12

A component not critically addressed by Gao et al. is the CXCR4 ligand CXCL12, which is one of a number of proteins that have a truncation site for the enzyme dipeptidylpeptidase 4 (DPP4, also known as CD26) (13, 14). DPP4 is present on the surface of HSCs, HPCs, and other cells, and is found within cells and in serum (14, 15). DPP4 removes two amino acids from the N-terminus of proteins, usually when the second amino acid is a proline or alanine (13, 14). Truncation of CXCL12 by DPP4 results in decreased chemotaxis, homing, and engraftment of HSCs and HPCs (15, 16); yet, little has been done to determine what the ratio and functional implications of full-length versus DPP4-truncated CXCL12, or other proteins, are in mice and humans. A role for DPP4-truncated molecules has been inferred in studies using DPP4-induced truncation of selective cytokines and chemokines and/or DPP4 inhibitors (e.g., diprotin A, a tri-peptide; ILE-PRO-ILE; or the orally active small-molecule sitagliptin [Januvia], which is used to treat type 2 diabetes) or Cd26<sup>Δ−/−</sup> cells and mice (15, 16). Unfortunately, there are no antibodies available to distinguish between DPP4-truncated and full-length proteins. The production and use of such antibodies may be telling as biomarkers and useful for modulating the functional activity of CXCL12-CXCR4 interactions and other protein-ligand interactions in malignant and nonmalignant disorders, as some DPP4-truncated proteins can block the activities of their full-length counterparts at the receptor level (14, 15).

Role of hypoxia collection

A recent study that evaluated collection and processing of BM and CB cells in an hypoxic environment of about 3% oxygen has potential relevance for the engraftment of genetically modified WHIM and other patient donor HSCs and/or HPCs (12). Hypoxic collection and processing of cells yields increased numbers of phenotypically defined and engrafing HSCs compared with those collected in ambient air conditions (12). In ambient air, the phenomenon of extra physiologic oxygen shock/stress (EPHOSS) causes rapid differentiation of HSCs through a mitochondrial permeability transition pore/p53/cyclophilin D axis and subsequent release of ROS (12). Collection and processing of cells in hypoxia is cumbersome and not easily adapted for routine clinical use; however, EPHOSS can be counteracted in ambient air with cyclosporine A (12) or by combinations of antioxidants and/or epigenetic enzyme inhibitors (17). None of these measures to counteract EPHOSS during ambient air collection of cells have yet to be adapted for clinical utility, though such efforts would not only increase the collection of HSCs but also potentially elucidate more physiological information regarding the genetic and intracellular characteristics of these cells. It is not yet clear how such efforts in the context of gene therapy might turn out. Hypoxia-collected cells might be in a deeper non-cycling state and harder to transduce with gene therapy vectors. But cells collected under hypoxic conditions do engraft well, even though surface expression of CXCR4 on these cells is decreased (12). It will be interesting to determine if decreased CXCR4 expression on these cells may be related to the observations of Gao et al. (2), regarding enhanced engraftment of Cxcr4<sup>+/−</sup> mouse BM cells, and what role CXCR4 plays, either alone or with other cell surface-homing molecules.

Modifying BM microenvironment for enhanced engraftment

An interesting aspect of the Gao et al. paper (2) was the demonstration that engraftment of Cxcr4<sup>+/−</sup> mice with congenic BM cells occurred without conditioning, a result with clinical implications for HCT to treat patients with WHIM. However, it is not absolutely clear if this nonconditioning effect is solely due to the gain-of-function of CXCR4 in the Cxcr4<sup>+/−</sup> mice or to other aspects of a primary immunodeficiency. Patients with primary immunodeficiencies may be more accept-
ing of autologous or allogeneic donor cells because of decreased immune cell activities. Nevertheless, it is of interest to see if modulating CXCR4 in the BM of recipients might allow recipients to be more accepting of donor cells. Several groups have tested effects of AMD3100/plerixafor treatment of animal and human recipients prior to or shortly after HCT for enhancement of donor cell engraftment (18, 19). These results, not yet in routine clinical practice, were intriguing, and mainly based on the HSC/HPC mobilizing effects of AMD3100 that could then potentially open up space in the BM for better acceptance of donor cells. In addition to the CXCR4 antagonistic activity of AMD3100, there is a report of it also having agonistic activity (20). Thus, an intriguing question is whether or not antagonism or agonism of CXCR4 in the BM of recipients with AMD3100 or similar small molecules might be a viable way to enhance engraftment of donor cells, and if AMD3100-enhanced engraftment is through the opening up of space in the BM or through other mechanisms. The use of AMD3100 or other such molecules might be more effective if CXCL12 activity could also be increased, as CXCL12 is produced by BM stromal cells and is a guiding molecule for CXCR4-expressing donor HSCs/HPCs (I). One way to enhance CXCL12 activity is to prevent its truncation by DPP4 with DPP4 inhibitors (15, 16). Could use of AMD3100 and DPP4 inhibitors together, in an appropriate sequence, to treat recipients, result in enhanced engrafting capability of donor HSCs/HPCs? This question may be worthy of further investigation. Together, the results of Gao et al. (2) have opened up a plethora of future possibilities, and it will be of great interest to see where and how far this information takes us.

**Acknowledgments**

The papers cited in the reference section from the author’s own laboratory or collaborations were supported by the following Public Health Service grants from the NIH to the author: R35 HL139599, R01 DK109188, R01 HL056416, R01 HL112669, and U54 DK106846.

Address correspondence to: Hal E. Broxmeyer, Indiana University School of Medicine, Department of Microbiology and Immunology, 950 West Walnut Street, R2-302, Indianapolis, Indiana 46202-5181, USA. Phone: 317.274.7510; Email: hbroxmey@iupui.edu.


**Blood. 2014;123(15):2308-2316.**