Comment on Park et al, page 2071

Sickle marrow: double, double toil and trouble

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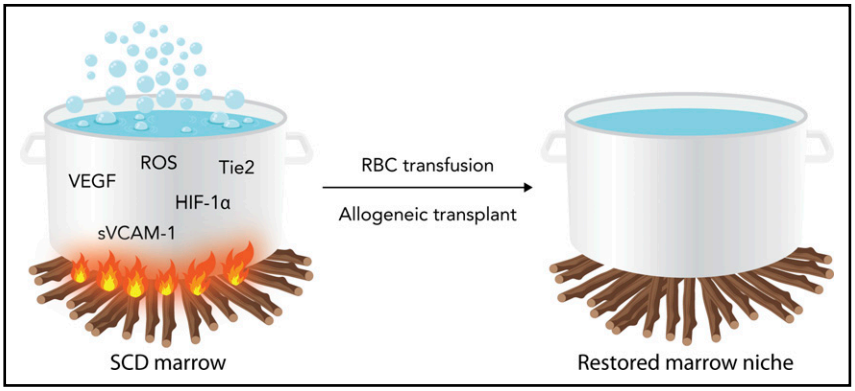
In the report by Park et al,[1] we learn that a cauldron of hypoxic marrow with vascular tortuosity and sinusoidal stasis is the pot from which hematopoietic output in sickle cell disease (SCD) is made. The marrow environment is a unique concoction of oxidative, vasoactive, and neoangiogenesis substances that are associated with collapsed, slow-flowing sinusoids clogged with erythroid and granulocytic aggregates (see figure). It is in this milieu that the hematopoietic niche with its cell-intrinsic (CXCL12-mesenchymal stem cells) and cell-extrinsic (HIF-1α, VEGF, sVCAM1, etc) features is altered.[2] The perturbation prompts hematopoietic progenitor cells to proliferate, egress, and degrade to a certain extent the homeostasis that is critical in maintaining long-term repopulating cell potential. Ineffective erythropoiesis also is induced under oxidative stress and inflammation that characterize the sickle marrow. Remarkably, as shown in this report, these aberrant conditions are transferable upon transplantation of humanized sickle mouse bone marrow into healthy mouse recipients.

The cause and consequences of the sickle mutation are well known. The mutation exchanges the amino acid valine for glutamine at position 6 of the β-globin gene and creates a hydrophobic pocket in the hemoglobin molecule, which under low-oxygen tension promotes the formation of long sickle hemoglobin polymers.[3] The propensity for this intracellular event causes its downstream pathogenic sequelae, including hemolysis, anemia, alterations in RBCs, and vascular endothelium and vascular tone. Together these changes promote vasoocclusion and tissue injury that affect virtually every organ in the body.[4] It is perhaps not surprising that the marrow hematopoietic organ is also adversely affected by these same phenomena. It also stands to reason that therapeutic interventions that mitigate the sickle phenotype might also repair the disordered bone marrow.

This prediction was illustrated by results in Park et al. They observed that the tortuous, slow-flow, collapsed marrow sinusoids of the humanized sickle mice normalized after a 6-week blood transfusion period during which sickle hemoglobin levels declined precipitously.



Schematic representation of the disordered bone marrow milieu in sickle cell disease. The inflammatory stimulus represented by fire under the pot is dampened by regular RBC transfusions or allogeneic bone marrow transplantation, which can correct the disordered milieu. HIF-1α, hypoxia inducing factor-1α; RBC, red blood cell; ROS, reactive oxygen species; sVCAM, soluble (circulating) vascular cell adhesion molecule; Tie2, tyrosine kinase with immunoglobulin-like and EGF-like domains 2; VEGF, vascular endothelial growth factor. Professional illustration by Somersault18:24.

In addition, the elevated levels of soluble neoangiogenic and vasculopathic soluble factors were reversed and oxidative stress in erythroid progenitors also declined. A remarkably similar response was elicited by the transplantation of healthy murine donor marrow in the humanized sickle recipient mice.

These observations have compelling implications for clinical allogeneic hematopoietic cell and autologous gene-modified cell transplantation SCD. With regard to allogeneic transplantation, a critical barrier to a successful outcome is graft rejection and recurrent SCD, which occurs much more frequently in SCD than in hematological malignancies. The reasons for this are not entirely certain, but sensitization to histocompatibility antigens during pretransplant transfusion exposures and immunological rejection of the donor cells has been hypothesized.[5] This report, however, suggests that the marrow niche itself might not be conducive to donor engraftment, perhaps exacerbated by an aggressive chemotherapy conditioning regimen. In autologous transplantation of gene-modified hematopoietic stem and progenitor cells, mobilization and collection of long-term repopulating cells are critical to a successful outcome. The possibility that mobilized hematopoietic cell products might be overrepresented by less-primitive progenitor populations that will not contribute to long-term hematopoiesis also comes to mind.

Of interest, the routine use of preventive exchange RBC transfusions administered before commencing treatment has been instituted in clinical transplantation protocols for SCD.[6] The principal intention of the RBC transfusion is to reduce the risk of experiencing a clinical vasoocclusive event during or after the transplantation procedure. In addition, hematopoietic cell harvesting for autologous gene modifications therapies in SCD has shifted broadly from marrow harvesting to plerixafor-mobilization and peripheral blood cell harvesting.[7-9] This procedure also is preceded by exchange RBC transfusion to reduce the risk of a clinical vasoocclusive event after the plerixafor-mediated leukocytosis that accompanies mobilization. It is serendipitous that another potential benefit of RBC transfusions before mobilization and transplantation as reported by Park et al is that transfusions might also favorably alter the sickle marrow milieu and improve hematopoietic progenitor

cell harvesting and promote engraftment of allogeneic donor and autologous gene-modified hematopoietic cells.

This interesting report appears to directly address the difficult problem of graft rejection that has challenged allogeneic hematopoietic cell transplantation for SCD. It also suggests that in gene therapy for SCD, in which there is transfer of both modified and unmodified hematopoietic cells, the gene-modified cell product must contain a sufficient fraction of corrected hematopoietic stem cells to re-model the marrow niche and ensure production of erythroid cells without oxidative stress. In addition, the short-term institution of RBC transfusions to reduce the fraction of sickle RBCs several months in advance of stem cell harvesting and transplantation might be prudent.

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TRANSPLANTATION

Comment on Burroughs et al, page 2094

Children with WAS: prefer early transplant!

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In this issue of *Blood*, Burroughs et al show impressive results in 129 pediatric patients who have undergone allogeneic hematopoietic stem cell transplantation (HCT) in North America between 2005 and 2015.[1]

Wiskott-Aldrich syndrome (WAS) is an X-linked disorder caused by hemizygous mutations in the *WAS* gene leading to reduced/absent expression of the WAS protein, a major regulator of the actin cytoskeleton in hematopoietic cells. Affected boys experience severe microthrombocytopenia, eczema, and progressively deteriorating immune functions leading to autoimmunity, infections, and cancer. WAS is a life-threatening disease that can only be cured by allogeneic HCT[1-4] or gene therapy.[5]

Infants and children aged <5 years benefited most from HCT, with a 5-year overall survival (OS) of 94%, whereas patients aged ≥5 years achieved poorer outcomes (66%). Myeloid donor chimerism (DC) >50% was associated with superior platelet production. Preexisting autoimmune disease, mainly autoimmune cytopenias, responded favorably to HCT. The preparative regimen consisted of mainly busulfan-based reduced intensity/toxicity conditioning or myeloablative conditioning regimens, including serotherapy. Excellent results were obtained with HLA-matched sibling donors (MSDs), 9/10-10/10 HLA–matched unrelated donors (MUDs), and unrelated cord blood transplants (UCBTs).

I remember in 2008 when one of my patients, at the age of 3 months, was diagnosed with WAS. Because of rare HLA alleles with no available MUD, at 2 years of age, the patient received a 7/10 (5/6) HLA–mismatched UCBT. He was negative for cytomegalovirus (CMV)/Epstein-Barr virus, and no virus-specific T cells were needed in his graft. The conditioning was myeloablative with busulfan/fludarabine. Therapeutic drug monitoring of busulfan was performed and resulted in a 50% dose reduction. Serotherapy with rabbit antithymocyte globulin (thymoglobulin; 4 × 2.5 mg/kg) was given to reduce the risks of graft-versus-host disease (GVHD). The patient achieved engraftment, and the HCT was uneventful. At latest follow-up in 2019, he exhibited 100% DC, normalized humoral immunity, and thymic function. Growth and length (both 75th-90th percentile), platelets (227 × 10^9/L), and results of lung function tests were all normal, and there was no chronic GVHD. This was a satisfactory clinical outcome.

Burroughs et al show on a much larger scale that UCBT (30% of their reported transplant cases) has become a good alternative to MSD/MUD transplants in WAS and achieved a 5-year OS of 90%. Very young children and infants who are less likely infected with viruses such as CMV and with less disease burden can benefit from timely UCBT. Previous reports of UCBT in 90 patients reported inferior results, reaching OS rates of 75% mainly due to infectious deaths.[4] The other finding of Burroughs et al that patients aged ≥5 years had poorer outcomes is not new. It is noteworthy, however, that the group of patients aged ≥5 years consisted of only 12 patients (9%), whereas 117 patients (91%) were <5 years of age at HCT. Statisticians do not favor age thresholds in medicine, but clinicians love them because they are helpful to facilitate therapeutic decisions.

It is far more interesting to understand why very young patients with WAS do better after HCT. WAS is undisputedly a progressive disease of the immune system, and risk scores as well as disease burden clearly increase with age.[2,3] But what are the reasons to wait with HCT until the patient becomes ≥5 years of age? A milder clinical course or