not clear from their findings that these patients necessarily have worse outcomes. If so, prospective studies may be necessary to establish definitions for bone marrow GVHD, possibly based on the degree of T-cell marrow infiltration and expression of κ-deleting recombination excision circles, and evaluate potential interventions. However, it is interesting to note that systemic GVHD correlated with increased marrow T-cell infiltration and that GVHD is often associated with reduced malignant relapse. One could thus be tempted to speculate that bone marrow GVHD may be closely related to graft-versus-leukemia (GVL) responses and the curative potential of the transplant.

Future work should explore the potential relationship between T-cell infiltration of the marrow and prevention of leukemia relapse. Furthermore, for experimental transplanters trying to separate GVHD from GVL in mice, where it is challenging to model transplants in leukemia-bearing hosts, it may be more efficient to study pathophysiology distinguishing epithelial organ GVHD from that in the marrow. Nonetheless, it should be noted how closely the findings of Mensen et al have mirrored the findings in mouse models of GVHD for T-cell infiltration of the marrow, damage to the osteoblast niche, and impairment in B-cell development.[5,6] The authors have made a significant step forward in our understanding of B-cell development after transplant and its impairment in clinical GVHD.

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● ● ● **TRANSPLANTATION**

Comment on Derderian et al, page 973

# Chemotherapy-free conditioning: one step closer

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In this issue of *Blood*, Derderian et al demonstrate that in utero administration of an anti-c-Kit receptor antibody to mice augments engraftment of congenic donor hematopoietic stem cells (HSCs) infused in the neonatal period.[1]

The role of chemotherapy-based conditioning to achieve full immunoreconstitution in patients with severe combined immunodeficiency (SCID) is contentious. Effective, safe alternatives are urgently required. HSC transplantation, and, in some conditions, gene therapy, effectively cure patients with genetic HSC-derived disease including many primary immunodeficiencies, hemoglobinopathies, or other inborn errors.[2,3] In the most serious immunodeficiencies (SCID), transplantation or gene therapy should be performed as soon as possible in the first year of life. For inborn errors, such as Hurler disease, earlier treatment leads to a better outcome, with preservation of neurologic function.[4] The debate over the need for preparative conditioning in patients with SCID continues, with strongly held opinions on each side.[5] In some genetic subtypes with a T and natural killer (NK) lymphocyte–negative, B lymphocyte–positive immunophenotype, the bone marrow space is full of recipient HSCs and mature B lymphocytes, but the thymus is empty of T-lymphocyte precursors. An infusion of donor stem cells permits engraftment of donor T-lymphocyte progenitors in the thymic niche and leads to long-term thymopoiesis with a diverse T-lymphocyte repertoire (panel A). Donor B-lymphocyte engraftment occurs more rarely;

usually, chemotherapy is required to empty the bone marrow niche to enable HSC engraftment, subsequent B-lymphocyte progenitor development, and establishment of B-lymphocyte function and freedom from treatment with immunoglobulin replacement. For other genetic subtypes with T and B lymphocyte–negative, NK lymphocyte–positive immunophenotype, the bone marrow space is full of recipient HSCs and B-lymphocyte precursors, and the thymus is full of T-lymphocyte precursors. An infusion of donor marrow permits engraftment of peripheral T lymphocytes, but no HSC or thymic T-lymphocyte progenitor engraftment with thymopoiesis or B-lymphocyte development, because the bone marrow and thymic niches are occupied (panel B).[6] Peripheral T lymphocytes confer medium-term but finite immune protection, with a limited T-lymphocyte receptor repertoire. Chemotherapy is required to empty the bone marrow and thymic niches to facilitate HSC engraftment and subsequent B- and T-lymphocyte progenitor development.[7] However, chemotherapy is associated with short- and long-term morbidities and serious adverse effects, including infertility, impaired endocrine function, and poor growth.[8]

Infants with SCID commonly present with life-threatening, persistent viral, or opportunistic infection. The outcome of
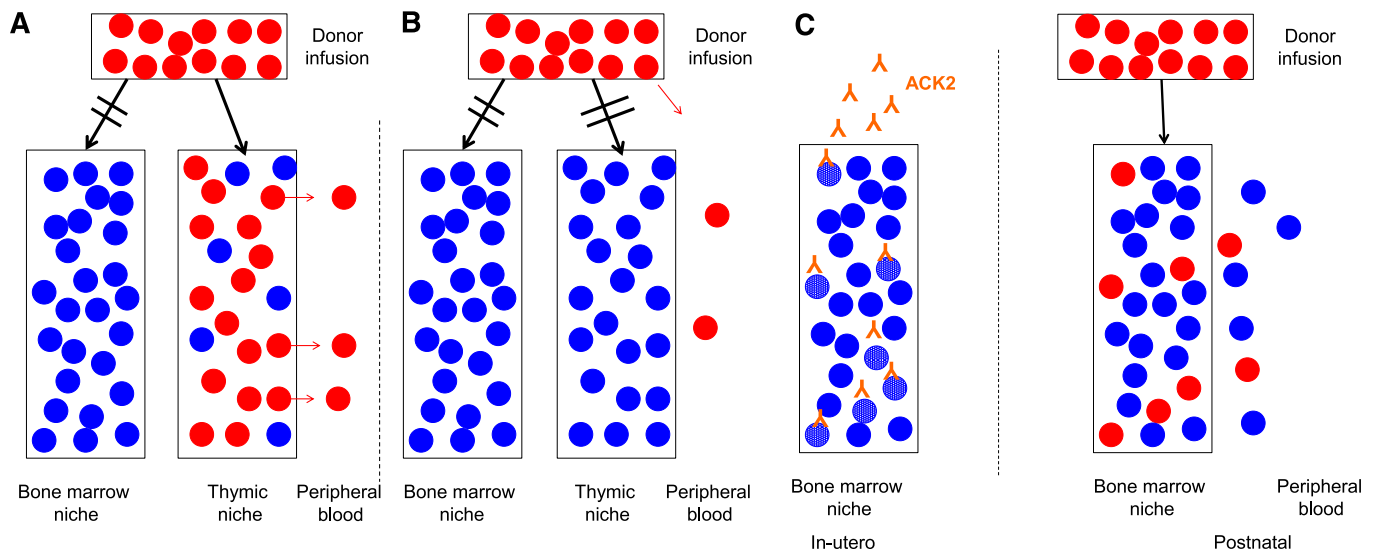
(A) Infusion of donor HSCs in patients with T and NK lymphocyte–negative and B lymphocyte–positive SCID leads to long-term thymopoiesis with a diverse T-lymphocyte receptor repertoire secondary to donor T-lymphocyte progenitor engraftment in the thymus. Donor HSCs do not engraft due to lack of space in the stem cell niche. (B) Donor HSCs infused into patients with T and B lymphocyte–negative and NK lymphocyte-positive SCID fail to engraft due to lack of space in the stem cell niche or in the thymus. Neither T nor B lymphopoiesis occurs, and patients are left with a nonrenewable limited T-lymphocyte repertoire and poor lymphoid immuno-reconstitution. (C) Fetal administration of anti-c-Kit receptor (ACK2) antibody to mice clears space within the HSC niche, facilitating engraftment of congenic donor HSCs in the neonatal period, permitting long-term immuno-reconstitution.

transplanting such patients is impaired by the presence of end-organ damage.[9] Recently, many states within the United States have implemented newborn screening, using the neonatal blood spot to detect T-lymphocyte receptor excision circles. A number of infants have been diagnosed at only a few days of age using this technique, before severe or recurrent infection develops.[10] The optimum treatment of such infants remains hotly debated—with concerns about the toxicity of chemotherapy administered to such young infants vs the long-term outcome of incomplete immune reconstitution after infusion of donor HSCs in infants treated without conditioning. Ideally, engraftment of donor (or autologous gene-corrected) HSCs would be achieved without requirement of toxic chemotherapeutic agents.

Derderian et al[1] administered a murine anti-c-Kit receptor antibody (ACK2), which interrupts a key signaling pathway in the homing, adhesion, maintenance, and survival of HSCs in the hematopoietic niche, to fetal mice, with the aim of depleting recipient HSCs and making space for donor HSC engraftment. B6.CD45.2 fetal mice received predetermined concentrations of ACK2, injected directly into the fetal liver, in utero. Pretreated murine pups and controls were subsequently transplanted with congenic CD45.1/CD45.2 fetal liver mononuclear cells on the first day of life. A further dose of

ACK2 was administered to the pretreated group at 2 weeks neonatal age by retro-orbital injection, and both treatment and control groups were retransplanted 7 days later. Peripheral blood chimerism was determined from each transplant, measured from 5 weeks of age. Although ACK2 was toxic in utero at high concentrations, at lower concentrations, survival of treated mice to birth was the same as controls. The higher concentrations of ACK2 were also more toxic after neonatal transplantation, leading to worse survival. At lower concentrations, toxic effects were confined to coat discoloration secondary to the effect of ACK2 on melanocytes. Importantly, given the expression of c-Kit receptor on germ cells, both male and female mice in the treatment arm appeared fertile. Anemia and leukopenia appeared transiently in utero in treated mice, but there was no effect on maternal hematopoiesis.

Treatment with ACK2 led to selective depletion of HSCs with no effect on differentiated progenitor or mature cell lineages (panel C). Control and treated mice engrafted following transplantation, but in mice treated with ACK2, donor chimerism increased over time with equal multilineage engraftment, including HSCs, stable over time, and higher than in controls, particularly when ACK2 was administered at lower doses. Unfortunately, administration of AKC2 postnatally had no significant influence on the

degree of donor chimerism, with no differences between treated mice and controls following the second transplant. Furthermore, pretreatment with ACK2 treatment did not lead to detectable engraftment after neonatal transplantation with allogeneic HSCs.

So, how does this study contribute to the debate regarding chemotherapy vs infusion for nonmalignant genetic disease? Well, mice are not men, and results need to be interpreted with caution and possibly replicated in nonhuman primate models. However, for those diseases where gene therapy is available, and autologous gene-corrected HSCs are reinfused, sustained and significant gene-corrected autologous HSC chimerism may be possible, without the use of chemotherapy, particularly in the SCID setting, where gene-corrected cells have a selective advantage. For patients with metabolic disease, where early correction of the defect leads to better clinical outcome, in utero transplantation may eventually be possible for families where the disease has already been identified, thus enabling early enzyme replacement, even in utero. For diseases where gene therapy is not yet possible, more work is required to achieve engraftment of allogeneic HSC, and augment engraftment in the postnatal period. How that leads to reconstitution of thymic and B-lymphocyte niches within the different genetic diseases remains to be seen. The debate over which treatment strategy to use for the

treatment of neonatal SCID and for inborn errors looks set to continue, but the goal of effective, nontoxic antibody-based treatments to achieve engraftment of HSCs has moved a little closer.

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