The experiments performed by Morales-Tirado et al are puzzling in view of the elevated IgE levels in WAS patients, considering that IgE production is dependent on IL-4. If Th2 cells are deficient in IL-4 secretion, where does IL-4 come from? Morales-Tirado et al show that basophils and the minor T-cell subpopulation γδ T cells can produce IL-4 in a manner independent of WASp. They propose that these cells are responsible for IL-4 production in WAS patients.

The paper by Morales-Tirado et al puts forward new aspects of WASp regulation in T cells. Their findings are interesting but do not always agree with previous data. More studies are needed to fully understand why there is reduced cytokine production in WASp-deficient T helper cells. Whereas Morales-Tirado et al measured steady-state levels of cytokine protein or mRNA, it would be interesting to determine rates of synthesis or degradation. Furthermore, it will be very important to confirm their findings using T cells from WAS patients. The paper by Morales-Tirado et al gives further insights into the immunodeficiency in WAS patients.

REFERENCES


Comment on Klingebiel et al, page 3437

Haploidentical transplantation in children

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In this issue of Blood, Klingebiel and colleagues present a summary of the outcomes of children and adolescents treated with a T cell–depleted haploidentical HCT for ALL. This paper presents one of the largest experiences to date that describes the outcome of 127 children receiving a haploidentical HCT’s using the EBMT registry from 36 pediatric centers.

A constant challenge for pediatric hematopoietic cell transplantation (HCT) centers is finding an acceptable donor for a child with very high-risk acute lymphoblastic leukemia (ALL) in first complete remission (CR1) or relapsed ALL. When no acceptable sibling donor is found, most centers attempt to find an HLA–identical unrelated adult marrow donor or umbilical cord blood (UCB). Unfortunately, initial searches frequently fail to identify acceptable HLA–matched unrelated donors, leading the center to 1 of 2 options. These are either a T cell–depleted haploidentical parent donor or a 4-of-6 HLA–matched unrelated UCB. The chance of finding a parent donor, as long as the parents are in good health, is 100%, and that of a 4-of-6 HLA–matched unrelated umbilical cord blood is as high as 98%. Thus, the availability of an acceptable donor from the 2 sources is essentially identical for a pediatric HCT center.

In this study in this issue of Blood, Klingebiel and colleagues found that smaller pediatric centers performing a T cell–depleted haploidentical HCT had a poorer outcome than did larger centers. Significant differences included a higher rate of cytomegalovirus positivity in donor and recipient, greater use of the Isolox T-cell depletion, less total body irradiation, and more antithymocyte globulin or antilymphocyte globulin as part of the conditioning regimen at the smaller centers. Adjustment for these variables still supported the conclusion that the leukemia-free survival was higher and the relapse incidence was lower at larger pediatric transplantation centers.

Although between 20% to 25% of all allogeneic HCT performed worldwide are in the pediatric population, pediatric HCT centers are predominantly smaller centers. The outcomes of a 4-of-6 HLA–matched UCB transplantation have been evaluated in relatively large series by both the CIBMTR and Eurocord. In the CIBMTR study, Eapen et al evaluated 267 children with ALL who received a 4-of-6 HLA–matched UCB transplant. The 5-year event–free survival (EFS) for these patients was 33% with a transplantation–related mortality of 46% and relapse rate of 19%. Similarly in Eurocord, Roche et al evaluated 290 children receiving an unrelated UCB transplant for ALL with a 2-year EFS of 65% for CR1, 43% for CR2, and 22% for CR3 patients. They also compared outcomes with 118 children receiving a haploidentical transplant and found that, although the children receiving a UCB transplant had a higher graft failure rate and acute GVHD rate, they had a lower relapse rate. The transplantation–related mortality and disease survival rates were similar. The results from these studies evaluating unrelated UCB transplants is almost identical to the outcomes quoted in a recent review of T cell–depleted haploidentical transplants of 3-year disease–free survival of 22% to 48%.

The findings in the Klingebiel paper suggest that medium-sized and small pediatric HCT centers should focus on the use of unrelated UCB transplants rather than attempting the more technologically dependent approach of T cell–depleted haploidentical transplants. One potential option that has not been rigorously evaluated in the pediatric setting is the use of a lower technology–dependent haploidentical donor transplant approach such as post-HCT cyclophosphamide without any ex vivo T-cell depletion. The additional advantage of this approach is that it could potentially be applied to countries outside of Europe and North America where access to UCB is limited and the parents are immediately available.

The other concern raised by the Klingebiel study is the very poor outcome for ALL in...
CR1 after receiving a T cell–depleted haploidentical transplantation. Whether there is greater dependence on an early T-cell response or another mechanism, the data suggest that T cell–depleted haploidentical transplantation should be used only for children with ALL at CR2 or higher, no matter the center size.

In summary, this study suggests that for the majority of pediatric HCT centers, UCB transplantation should be the preferred option. T cell–depleted haploidentical transplantation should be used only for children with ALL at CR2 or higher at larger centers that have a high level of experience with T cell–depleted haploidentical transplantation. Less technology–dependent approaches to haploidentical transplantation, such as pharmacologic approaches with cyclophosphamide after transplantation, potentially should be considered as a future strategy for many pediatric HCT centers, especially those that do not have an easy access to unrelated UCB donors.

**HEMATOPOIESIS & STEM CELLS**

Comment on Tulpule et al, page 3453

Fanconi anemia strikes early in utero

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In this issue of *Blood*, Tulpule and colleagues describe the use of lentiviral vectors to knock down FANCA and FANCDC2 in hESC lines, which results in early hematopoietic defects reminiscent of the human disease.

Fanconi anemia (FA) is an autosomal recessive disorder characterized by bone marrow failure, birth defects, and a predisposition to malignancy. A plethora of new scientific information has clearly established that the 13 known FA gene products are involved in the regulation of DNA repair (reviewed in de Winter and Joenje). It is currently believed that 8 of the FA proteins (FANCA, B, C, E, F, G, I, and M) form a nuclear complex that functions “upstream” in the pathway to enzymatically monoubiquitinate a heterodimer pairing between FANCI and FANCDC2 (referred to as the ID heterodimer). Following this biochemical activation, the ID heterodimer is targeted to nuclear foci that contain BRCA1, RAD51, and BRCA2/FANCDC1, which ultimately participate in homology-directed DNA repair. FANCDC2, FANCI, and BRCA2/FANCDC1 thus act “downstream” in the pathway from the core complex, and may have additional functions in maintaining genomic integrity as well. This view of the FA pathway certainly explains the hypersensitivity of patients’ cells to DNA cross-linking agents, which still forms the basis for diagnosing FA.

Knowledge gained of the molecular function of the FA gene products has not yet translated, however, into an understanding of the pathophysiology or ontogeny of the bone marrow failure that is the clinical hallmark of the disease. A clear picture has been elusive thus far, largely because mouse knockout models do not exhibit spontaneous marrow failure. Furthermore, because nearly all of the insights obtained have relied on experiments on postnatal hematopoietic cells from the peripheral blood or bone marrow, not much is known about the ontogeny of hematopoiesis in utero.

To summarize many years of research on FA mouse models, individual knockouts of Fancb, Fancd, Fanca, and Fancd2 genes have been generated, but none spontaneously develops bone marrow failure, aplastic anemia, leukemia, or skeletal abnormalities (reviewed in Parmar et al). Although the mice exhibit subtle hematopoietic cell abnormalities including decreased long-term repopulating ability and hypersensitivity to oxidative stress and inhibitory cytokines such as tumor necrosis factor-alpha, progressive pancytopenia is not seen but can be induced after DNA damage with mitomycin C treatment.

A popular theory accounts for many aspects of the knockout mouse data is summarized in the figure (top panel). In this scenario, FA hematopoietic stem cells are initially normal in number at birth but suffer a steady decline as a result of a high rate of apoptosis, which leads to bone marrow failure. Due to strong selective pressure for stem cells resistant to apoptosis, secondary mutations in apoptosis-regulating genes can lead to clonal escape and expansion of preleukemic cells.

The work from Tulpule et al contradicts several key tenets in this theory and leads to a different picture (bottom panel of the figure). First, Tulpule et al demonstrated that knockdown of FANCDC2 in hESCs led to a reduction in hematopoietic fates and progenitor numbers that could be rescued by expression of the wild-type FA gene product. In these assays, human embryonic stem cells (hESCs) were induced to differentiate to embryoid bodies (EBs) and then to hematopoietic lineages. Hematopoiesis was impaired from the earliest stages of blood cell formation, which contrasts with the view of progressive stem cell loss during early childhood. A second unexpected finding was an apparent absence of significantly increased apoptosis rates in FA hematopoietic progenitors, suggesting that the observed hematopoietic defect might not be a result of direct cell loss at all.

Other unexpected findings included possible intrinsic developmental differences between “upstream” and “downstream” FA mutants. Knockdown of FANCDC2 resulted in a much greater impairment of hematopoietic development from hESCs than knockdown of FANCA, suggesting greater deficiencies in embryonic hematopoiesis and accelerated progression to marrow failure in FA-D2 patients.

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**REFERENCES**


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