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EDITORIAL

Engineering haploidentical transplants

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Optimizing the conduct of haploidentical hematopoietic stem cell transplants has the potential to expand access to allogeneic transplants for patients in need. The use of a haploidentical transplant is being increasingly utilized for transplant candidates who lack an MHC-matched donor as it has the advantage of a rapidly available donor who is usually highly committed to supplying additional cell therapy products. Moreover, although early studies of haploidentical transplants were associated with high rates of graft failure and GvHD, more recent studies have shown improved outcome with the application of strategies to manipulate or engineer the infused cell products either *ex vivo* or *in vivo* to eliminate the cells that are thought to mediate alloreactivity.[1–3]

T-replete haploidentical transplants have successfully been performed using post-transplant cyclophosphamide (Cy) to eliminate alloreactive T cells *in vivo*[4] or using very high doses of immune suppression and anti thymocyte globulin[5,6] as means to reduce GVHD. These procedures are easy to perform and have been successfully exported to many centers but the rates of relapse with the use of post-transplant Cy[7] and the rates of GvHD with high dose post-transplant immune suppression remain relatively high.[5,6] Another strategy is to use extensive T-cell depletion either by CD34+ cell selection of G-CSF-mobilized, large-volume apheresis or via αβ T-cell deletion, which allows transfer of natural killer (NK) cells and other CD3-negative cells present in the infused product.[8]

As infections are a cause of significant morbidity and mortality after T-cell-depleted grafts, and relapse also remains a major cause of treatment failure after these regimens, there is much interest in approaches to augment antitumor and antiviral immunity after haploidentical transplant. Consequently, efforts have been made to selectively deplete alloreactive T cells[9,10] or to administer regulatory T cells with conventional T cells to suppress alloreactivity while preserving the function of T cells directed to pathogens or malignant cells.[11] Antigen-specific T cells have also been infused to treat viral infections without inducing alloreactivity after haploidentical transplant and several groups are exploring strategies to expand thymic precursors.[12] There is also evidence to suggest that 'alloreactive' KIR-mismatched NK cells can mediate GvL activity in patients with relapsed leukemia[13] and can help eradicate the leukemia cells that might remain after the conditioning regimen.[14] In addition, as NK cells attack primarily hematopoietic cells, they are considered to have a low potential for directly causing GvHD themselves.[15] Adoptive transfer of NK cells after transplant has been evaluated in a few trials,[16–19] and although demonstrated to be safe with some clinical activity observed the benefit of this procedure remains to be proven.[20]

With improved understanding of T-cell development and biology, it is now known that specific subsets of T cells may contribute to the development of GvHD, whereas other T-cell subsets might have a greater role in modulating a GvL effect.[21,22] For example, murine studies suggest that the majority of cells capable of recognizing allo-Ags and therefore responsible for causing GvHD reside in the naive CD45RA+ subset of T cells, whereas CD45 RO+ memory T cells do not induce GVHD.[21,23] These observations led to the hypothesis that the use of CD45RA-depleted grafts might be associated with low rates of GVHD while preserving memory T cells with specificity for viral and leukemia Ags and procedures to engineer such a graft were devised.[24]

In this issue, Triplett *et al.* report on the initial clinical outcomes in pediatric patients with advanced hematological malignancies who received grafts from haploidentical donors depleted of naive CD45RA+ T cells. The regimen used was complex with G-CSF mobilized PBSCs collected from donors on 2 consecutive days to produce a CD34+-selected product that was infused on day 0 followed by a CD45RA-depleted graft on day +1. There was a median of >3log depletion of CD45RA+ cells in the graft and patients received a high total CD34+ cell dose from the combination of both products (median 17.8×10^6 cells per kg). By depletion of CD45RA+ cells, there was also a significant depletion of B cells thereby minimizing the risk of post-transplant EBV lymphoproliferative disease. To further maximize the GVL potential of the graft, Triplett *et al* also infused donor NK cells on day +7 collected from an additional pheresis from the donor without GCSF mobilization. Patients received 13.1 (range 1.65 to 56.1) $\times10^6$ NK cells per kg that were tolerated without complications.

The short-term outcomes described in this small cohort of 17 pediatric patients are impressive. Of note, all patients had very high-risk hematological malignancies with poor prognosis and 10 patients had active disease at time of transplant. All patients engrafted and remarkably despite receiving a median CD3+ cell dose of 121.8×10^6 per kg, no acute GvHD was reported. Six patients developed limited chronic GvHD, which was controlled without systemic therapy in all but one. Although there were a few viral reactivations, there were no deaths related to infectious complications. Thirteen of the 17 patients are alive in remission with 2 patients dying of non-infectious transplant-related mortality and 2 patients experiencing a relapse.

One of the greatest challenges with T-cell-depleted haploidentical transplants is the prolonged period of lymphopenia in the first 100 days post transplant that places patients at increased risk of infections and relapse.[25] Long-term T-cell recovery is largely dependent on *de novo* T-cell production mediated by thymopoeisis and more closely resembles that of age-matched controls rather than donor graft.[26] In this report, patients had robust and rapid immune reconstitution with nearly normal number of CD8+ and CD4+ central and effector memory T cells that were able to mount highly effective proliferative responses to PHA, tetanus, CMV and HSV as early as day +30 providing critical protection against infections, while the presence of large number of Tregs along with negligible numbers of naive CD45RA+ T cells may have contributed to the very low observed rate of GvHD. In the first 100 days post transplant, the phenotype of the reconstituting cells recapitulated the CD45RA-depleted graft content. Interestingly, although the frequency of TRECS was low, there was a wide Vβ repertoire suggesting that a broad TCR repertoire could be achieved by the adoptively transferred memory cells before native thymic output was initiated. This pattern of immune reconstitution may well provide the most optimal immune recovery in the context of T-depleted transplants, bridging the critical period before *de novo* T-cell production is initiated providing immunity against infections with minimal risk of GVHD.

Although these results are encouraging, the number of patients is small and longer-term follow-up with larger patient numbers is

required to confirm that this benefit is consistent and sustainable, and to validate the low acute and chronic GvHD incidence. In addition, the cost and complexity of the procedure in its current form will preclude this approach being evaluated in many other centers. Modifications to simplify the process with perhaps one infusion of a CD45RA-depleted product, as described by Bleakley *et al.*[27] instead of two infusions will make this procedure more accessible. In addition, the NK cell infusion may not be required, given the large numbers of T cells infused. With more advanced graft engineering techniques and the development of 'designer grafts' that can enhance immune reconstitution and separate the GVL effect from GVHD,[28] there is potential for much broader use of haploidentical donor transplantion.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Commentary on rapid memory T-cell reconstitution recapitulating CD45RA-depleted haploidentical transplant graft content in patients with hematologic malignancies.

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