

EDITORIAL

Determinants of Engraftment After Allogeneic Marrow Transplantation

By Paul J. Martin

DESPITE RECENT improvements in posttransplant immunosuppressive regimens, acute and chronic graft-versus-host disease (GVHD) remain significant obstacles to successful marrow transplantation, particularly in patients not having an HLA-identical related donor. These findings have led to continued interest in preventing GVHD by depleting T cells from the donor marrow. Initial animal experiments in the 1970s demonstrated the feasibility of this alternative approach but gave little indication that T-cell depletion could lead to complications. Data from extensive clinical trials during the 1980s led to widespread recognition of increased graft failure among other problems associated with T-cell depletion.¹ The problems encountered in clinical marrow transplantation have helped to refocus efforts in the laboratory, leading to a much more sophisticated appreciation of the complex cellular interactions involved in allogeneic marrow engraftment.

Both host and donor factors can influence allogeneic marrow engraftment. The conditioning regimen used to prepare the host represents a major determinant of transplant outcome, depending on the number and types of cells that survive.^{2,3} The conditioning regimen not only creates "space" or microenvironmental niches for development of donor hematopoietic stem cells, but also reduces the number of cells that could cause rejection. Regimens that ablate both hematopoietic stem cells and the cells that cause rejection enable full allogeneic engraftment, and regimens that fail to ablate host immunity while sparing host hematopoietic stem cells result in rejection with autologous hematopoietic reconstitution. Regimens that spare hematopoietic stem cells while eradicating or inactivating cells that cause rejection might result in mixed chimerism by enabling competitive repopulation with both donor and host hematopoietic elements. Regimens that spare cells capable of causing rejection while eradicating hematopoietic stem cells would be expected to cause death with aplasia.

Pretransplant immunization of the host against alloantigens of the donor can increase the risk of rejection⁴ both by increasing the number of donor-reactive T cells that survive the conditioning regimen and also by causing the host to generate alloantibodies against donor cells.⁵ In both rodents and humans, host CD8 cells can mediate allogeneic marrow graft rejection, presumably through recognition of major histocompatibility complex (MHC) class I disparity or through recognition of minor histocompatibility antigens presented by MHC-class I molecules. In mice, rejection can be prevented by administration of a CD8-specific antibody.⁶ In humans, host CD8 cells with specific antidonor cytotoxic or suppressive activity have been identified in the blood of patients with graft failure after T-cell-depleted marrow transplantation.^{7,8} Evidence implicating CD4 cells in causing rejection has come from studies showing that treatment of recipients with both CD4 and CD8-specific antibodies was necessary to enable engraftment of fully allogeneic

T-cell-depleted marrow in sublethally irradiated mice.⁹ In rodents, marrow graft rejection can also be mediated by host T-lineage cells that express a natural killer (NK) phenotype.¹⁰ These cells recognize "hematopoietic histocompatibility" (Hh) antigens expressed on donor hematopoietic cells and also on donor lymphoid cells.¹¹ Hh genes appear to show dominant negative transregulation such that Hh antigen expression occurs in certain homozygous strains but not in their heterozygous F1 hybrids. Thus, certain F1 strains of mice and rats can recognize Hh antigens expressed on parental hematopoietic or lymphoid cells. This cannot occur with conventional transplantation antigens that show codominant expression. Unlike conventional T cells, the NK-like effectors that mediate rejection through recognition of Hh antigens are radioresistant and do not require prior sensitization or priming.

Several donor factors can influence allogeneic marrow engraftment, but their relative importance remains highly controversial. These factors can be broadly classified as stem cell mass, antigen mass, and lymphoid components. The number of stem cells can have a critical effect, depending both on the type of disparity between the donor and host and also on the conditioning regimen. In most cases, rejection mediated by Hh recognition in mice can be overcome by transplanting more than 5×10^6 marrow cells, although in certain strain combinations even 1×10^7 cells are not sufficient to enable engraftment.¹² Rejection mediated by recognition of minor histocompatibility antigens in mice prepared with 11 Gy total body irradiation (TBI) can be overcome by transplanting more than 1×10^5 T-cell-depleted marrow cells, whereas at least 100-fold more marrow cells may be required to enable engraftment in MHC-disparate recipients.¹³ Under ordinary circumstances, the "antigen mass" of a marrow graft is proportional to the number of hematopoietic stem cells. Increasing the antigen mass by adding irradiated spleen and marrow cells to the graft can increase the risk of rejection.⁶ From this finding it follows that decreasing the antigen mass by removing antigen-presenting cells or by stem cell enrichment procedures might decrease the risk of rejection, but this hypothesis has yet to be tested.

Several types of lymphoid cells can facilitate allogeneic marrow engraftment. Murphy et al¹⁴ showed that an absence of T cells in murine marrow grafts leads to an increased susceptibility to rejection by NK cells and T cells. Rejection by either mechanism could be overcome by adding 20×10^6 alloreactive thymocytes to grafts containing

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1.0 to 1.5×10^6 marrow cells. Similarly, Lapidot et al¹⁵ showed that engraftment of MHC-disparate marrow could be facilitated by as few as 8×10^4 immunocompetent thymocytes. Engraftment could also be facilitated by nonalloreactive thymocytes from F1 donors tolerant of both donor and host histocompatibility antigens. With grafts containing 8×10^6 marrow cells, approximately 1×10^6 F1 thymocytes were required to have an effect comparable to that seen with 8×10^4 thymocytes from alloreactive donors. With grafts containing 2 to 4×10^6 marrow cells, 4 to 8×10^6 F1 thymocytes were required to facilitate engraftment. Thus, thymocytes from tolerant donors appeared to be substantially less effective than those from nontolerant donors.

Other investigators have also found that allogeneic marrow engraftment can be facilitated by donor cells that do not recognize host alloantigens. Sykes et al^{16,17} reported that certain cells reactive with a rabbit antiserum against mouse brain were able to facilitate engraftment of F1 marrow in lethally irradiated parental recipients. Results were similar whether F1 donor cells could recognize Hh antigens of the parental recipient or not. Experiments with antibodies against CD4, CD8, Thy-1, and NK1.1 suggested that neither T cells nor NK cells could account for the graft enhancement. Thus, the cells responsible for the effect in this model remain unidentified. Pierce et al¹⁸⁻²⁰ showed that marrow grafts containing 30×10^6 cells from allogeneic C57BL/6 and semiallogeneic BALB/c \times C57BL/6 F₁ donors had equivalent ability to establish mixed lymphoid chimerism and skin graft tolerance in sublethally irradiated BALB/c recipients. Lymphoid engraftment and tolerance induction required the presence of Thy-1⁺ cells in the marrow, and CD8 cells appeared to be more important than CD4 cells. Depletion of NK cells, cytotoxic T cells, and precursor cytotoxic T cells by treatment of the marrow with L-leucyl-L-leucine methyl ester (LLOME) had no effect on lymphoid engraftment or tolerance induction. In this strain combination, cells of the F1 donor can recognize Hh antigens of the BALB/c host. Finally, Nakamura and Gress²¹ showed that T-cell-depleted C57BL/6 \times DBA/2 F1 marrow that had been incubated in interleukin-2 (IL-2) could promote engraftment of fresh F1 marrow in C57BL/6 parental recipients. In this strain combination, cells of the F1 donor can recognize Hh antigens of the C57BL/6 host, but this is an unlikely explanation for the graft enhancement because IL-2-activated T-cell-depleted C57BL/6 \times C3H F1 marrow cells had no effect despite a similar ability to recognize Hh antigens of the C57BL/6 host. In this model, marrow graft facilitation required administration of IL-2 after transplantation.

In a recent issue of *Blood*, Uharek et al²² have extensively explored relationships between the conditioning regimen, marrow cell dose, and alloreactive or tolerant donor T lymphocytes as determinants of allogeneic engraftment in a rat transplant model. As might have been expected, they found that reducing either the pretransplant immunosuppressive conditioning or the number of marrow cells caused a stepwise increase in rejection rates. Reductions in the

number of marrow cells could be compensated by increased immunosuppression, and reductions in the pretransplant conditioning could be compensated by an increased number of marrow cells. Unexpectedly, they found that results were similar with marrow either from fully allogeneic donors or from semiallogeneic F1 donors, despite the fact that the former caused GVHD, while the latter could not. From these findings they concluded that marrow cell dose represents a critical determinant of engraftment and that the presence or absence of alloreactive T cells is relatively unimportant.

How can this provocative conclusion be reconciled with the findings previously published by Murphy et al¹⁴ and Lapidot et al¹⁵ which clearly demonstrated the importance of alloreactive donor T cells as a determinant of allogeneic marrow engraftment? First, it should be recognized that the approach taken by Uharek et al did not isolate a single variable for close examination. Both the marrow cell dose and the number of T cells were increased exactly in parallel. In contrast, both Murphy et al and Lapidot et al added alloreactive or tolerant T lymphocytes to a fixed marrow inoculum scrupulously designed not to contain T lymphocytes. With very large numbers of marrow cells, it may not be possible to discern any effect of donor T cells because of high background engraftment,¹⁵ and with small numbers of marrow cells the numbers of endogenous T cells may not be sufficient to have an effect. Second, the conclusions advanced by Uharek et al rest on the premise that lymphocytes in the marrow from F1 donors could not recognize the parental host cells responsible for rejection. While this is certainly true of T lymphocytes, it may not be the case for cells that recognize Hh antigens. Notably, Murphy et al and Lapidot et al were careful to select strain combinations in which donors cannot recognize Hh antigens of the host. Unfortunately, it has not been determined whether such recognition can occur in the rat strain combination used by Uharek et al, and this issue remains an open question. Thus, the marrow graft facilitation by F1 lymphocytes in their model could reflect antihost reactivity mediated by NK cells. Alternatively, the F1 effect could reflect the activity of another cell type, similar to the findings of other investigators.¹⁶⁻²¹

The ability to enhance allogeneic marrow engraftment by genetically nonalloreactive T-cell populations provides important impetus for attempts to isolate such cells from nontolerant donors. Thus, it has become critical to identify these cells and to define the functional mechanisms by which they facilitate engraftment. It is possible that tolerant T cells could facilitate engraftment through production of lymphokines or cytokines that promote the growth and differentiation of hematopoietic stem cells. However, at present there are no published data that directly support this hypothesis. As an alternative explanation, tolerant T cells could facilitate engraftment through "veto activity."²³ Host T cells usually develop an immune response when they recognize alloantigens expressed on donor cells. Veto activity can specifically prevent an immune response when host T cells recognize donor cells. The veto activity of donor

cells depends on passive recognition by host cells and can be mediated by certain T lymphocytes and lymphokine-activated killer (LAK) cells. Thus, donor cells with veto activity cannot prevent immune responses against cell surface antigens not expressed by the veto cell itself. The findings reported by Nakamura and Gress²¹ are consistent with veto activity because graft enhancement was mediated by LAK cells syngeneic with the marrow donor and not by LAK cells from a third party donor that did not express the antigens involved in rejection.

Published data have not adequately addressed whether donor NK cells can facilitate allogeneic marrow engraftment. The importance of this question stems from the fact that NK cells do not initiate GVHD, although they may participate during the effector phase.²⁴ Blazar et al²⁵ found that removal of NK cells from the marrow had no effect on engraftment. These negative results could be explained by the fact that the NK-depleted marrow contained T cells that were shown to facilitate engraftment. In addition, the donor strain used for these experiments could not recognize Hh antigens of the host. On the other hand, Uharek et al²⁶ have developed a mouse marrow transplant model for experiments similar to those described in their rat transplant model. In these experiments, they compared different numbers of marrow cells transplanted from C57BL/6 donors into lethally irradiated BALB/c recipients. The incidence of rejection (measured as death with granulocyte counts $< 500/\text{mm}^3$) was the same whether the marrow was unmanipulated or depleted of Thy-1⁺ cells, CD4 and CD8 cells, or treated with LLOME to deplete cytotoxic T cells, precursor cytotoxic T cells, and NK cells. Although they concluded that marrow inoculum size represents the most important determinant of engraftment, other interpretations are possible. In this model, the marrow graft enhancement could have been mediated by NK-like effectors of the C57BL/6 donors that are known to recognize BALB/c Hh antigens. One might expect that LLOME treatment should have ablated this mechanism, but it remains possible that the number of alloreactive T cells remaining after LLOME

treatment was sufficient to facilitate engraftment. Alternatively, NK cells might have been regenerated from the marrow soon after LLOME treatment.

What conclusions should be drawn from the studies of Uharek et al? It remains undeniable that the number of marrow cells can influence graft outcome, but it remains uncertain whether this represents the most important determinant of engraftment. Even if this were the case in rodents, extrapolation of this conclusion to other species might not be valid because of possible quantitative differences in the behavior of hematopoietic stem cells and lymphoid cells among different species. Thus, these data should not be used by themselves to justify heroic attempts to aspirate large numbers of marrow cells from donors. Such efforts might be expected to yield disproportionately more T cells than hematopoietic stem cells due to coaspirated blood. In addition, the donor could be jeopardized by excessive trauma, hypovolemia, or by otherwise unnecessary transfusions. Certainly it seems sensible and appropriate to avoid nonspecific loss of stem cells during marrow manipulations to deplete T cells, but a recent analysis of International Bone Marrow Transplant Registry data showed no discernable relationship between marrow cell dose and risk of graft failure.²⁷

Whether NK cells can mediate a graft-enhancing effect deserves further scrutiny. Ciccone et al^{28,29} have reported that specific recognition by a human NK clone required that allogeneic T-cell targets express a gene termed EC1 which maps to the HLA class I region and appears to show autosomal recessive expression. These findings are highly reminiscent of Hh recognition. Such NK-mediated recognition would not have significance for HLA-genotypically identical marrow transplantation because both the donor and recipient would have identical EC1 alleles. On the other hand, it might be possible to exploit this type of recognition in securing a graft-facilitating effect without GVHD in certain patients transplanted with T-cell-depleted marrow from an HLA-mismatched relative or from an unrelated donor.

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