Failure of Recombinant Stem Cell Factor to Enhance Engraftment of L-Leucyl-L-Leucine Methyl Ester Treated Canine Marrow After Irradiation

To the Editor:

T-cell depletion from marrow has been used to reduce the risk of graft-versus-host disease (GVHD) in patients undergoing allogeneic marrow transplantation; however, this was accomplished at the price of an increased incidence of graft failure. L-leucyl-L-leucine methyl ester (Leu-Leu-OMe), a lysosomotropic compound, has been shown to selectively deplete cytolytic T cells and their precursors, natural killer cells, and monocytes, but not helper T cells from peripheral blood and marrow. Treatment of donor marrow with Leu-Leu-OMe prevented GVHD in murine transplants across major histocompatibility complex class I and class II disparities without interfering with engraftment. In dogs, Leu-Leu-OMe treatment of marrow resulted in a substantial reduction of progenitor cell colonies even when accessory cells were added back to the culture. Pecora et al showed a reduction of committed progenitors when human marrow was incubated with Leu-Leu-OMe. Although Leu-Leu-OMe treatment did not interfere with engraftment of autologous marrow after conditioning with 9.2 Gy total body irradiation (TBI), transplantation of Leu-Leu-OMe treated marrow from dog leukocyte antigen (DLA)-identical littermates resulted in graft failure in most dogs. Studies in mice have suggested improved engraftment of T-cell-depleted histoincompatible marrow after sublethal TBI by treating donor marrow with recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) and by administration of recombinant human interleukin-1 (IL-1). We failed to show graft enhancement of unmodified DLA-identical marrow after sublethal conditioning with 9.2 Gy total body irradiation (TBI), transplantation of Leu-Leu-OMe treated marrow from dog leukocyte antigen (DLA)-identical littermates resulted in graft failure in most dogs. Studies in mice have suggested improved engraftment of T-cell-depleted histoincompatible marrow after sublethal TBI by treating donor marrow with recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) and by administration of recombinant human interleukin-1 (IL-1). We failed to show graft enhancement of unmodified DLA-identical marrow after sublethal TBI with a combination of IL-1 and GM-CSF, as well as with canine granulocyte colony-stimulating factor, canine stem cell factor (rcSCF); or a combination thereof. Here we investigate whether posttransplant treatment with rc-SCF enhances engraftment of 'T-depleted' Leu-Leu-OMe treated marrow from DLA-identical littermates.

Selection of DLA-identical littermate pairs for transplantation, preparation, and in vitro treatment of marrow with Leu-Leu-OMe have been described. Briefly, aspirated marrow was diluted in medium (Waymouth's MB751/1, Fred Hutchinson Cancer Research Center media shared facility) and centrifuged to obtain buffy coat cells. Cells were washed once with hemolytic buffer, twice with medium, and then incubated with 1,000 μmol/L Leu-Leu-OMe at a concentration of 20 × 10⁶ cells/mL for 15 minutes at room temperature. After recipients were given 9.2 Gy TBI, Leu-Leu-OMe treated donor marrow was infused. Rc-SCF, 100 μg/kg BID subcutaneously was given until day 21 or until engraftment (absolute granulocyte count greater than 1,500/μL). Results are shown in Table 1 and compared to those in dogs given unmodified (n = 7) or Leu-Leu-OMe treated (n = 8) marrow and no rc-SCF infusions. All seven dogs given unmodified marrow engrafted compared to three of eight given Leu-Leu-OMe treated marrow and no rc-SCF (P = .01). Five of 10 dogs given Leu-Leu-OMe treated marrow and postgrafting rc-SCF engrafted, a result that was not significantly different from that in dogs not given rc-SCF (P = .64).

In conclusion, the study confirmed previous observations of an increased risk of graft failure with Leu-Leu-OMe incubation of the grafted marrow. Treatment of recipients with rc-SCF failed to significantly reduce the risk of graft failure.

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REFERENCES


Table 1. Dogs Given 9.2 Gy TBI and Marrow Grafts from DLA-Identical Littermates

<table>
<thead>
<tr>
<th>Leu-Leu-OMe Treatment</th>
<th>Median No. of Donor Cells (Infused x10⁶/kg)</th>
<th>RC-SCF Studied</th>
<th>Graft Failure</th>
<th>Acute GVHD</th>
<th>Transient</th>
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* P value compares proportion of dogs failing to engraft.
tion of proliferative responses with maintenance of the capacity for autologous marrow engraftment. Transplantation 46:655, 1988


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