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.1 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.2,3 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.4,5 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.6 Pecora et al7 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.8 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).9,10 We failed to show graft enhancement of unmodified DLA-identical marrow after sublethal irradiation with 9.2 Gy total body irradiation (TBI), transplantation of Leu-Leu-OMe treated marrow from dog leukocyte antigen (DLA)-identical littermates resulted in failure in all mice.9,10 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).9,10 We failed to show graft enhancement of unmodified DLA-identical marrow after sublethal irradiation with 9.2 Gy total body irradiation (TBI), transplantation of Leu-Leu-OMe treated marrow from dog leukocyte antigen (DLA)-identical littermates resulted in failure in all mice.9,10

Selection of DLA-identical littermate pairs for transplantation, preparation, and in vitro treatment of marrow with Leu-Leu-OMe have been described.9,10 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 medium (Waymouth's MB751/1, Fred Hutchinson Cancer Research Center media shared facility) and centrifuged to obtain buffy coat. 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 x 10^6 cells/mL for 15 minutes at room temperature. After recipients were given 9.2 Gv TBI, Leu-Leu-OMe treated donor marrow was infused. Rec-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 re-SCF infusions. All seven dogs given unmodified marrow engrafted compared to three of eight given Leu-Leu-OMe treated marrow and no re-SCF (P = .01).14 Five of 10 dogs given Leu-Leu-OMe treated marrow and postgrafting re-SCF engrafted, a result that was not significantly different from that in dogs not given re-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 re-SCF failed to significantly reduce the high risk of graft failure.

ACKNOWLEDGEMENT

Supported by grants DK42716, CA31787, CA15704, and CA18221 awarded by the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Cancer Institute, National Institutes of Health, Department of Health and Human Services (Bethesda, MD). Laboratory support was also available through a grant from the Josef Steiner Krebsstiftung, Bern, Switzerland, awarded to one of the investigators (R.S.). Rec-SCF was kindly provided by Dr Ian McNiece, Amgen, Inc, (Thousand Oaks, CA).

Hans-Peter Kiem
Fred Hutchinson Cancer Research Center
University of Washington School of Medicine
Wendy Leisening
Robert Raff
Fred Hutchinson Cancer Research Center
H. Joachim Deeg
Friedrich G. Schuening
Frederick R. Appelbaum
Rainer Storb
Fred Hutchinson Cancer Research Center
University of Washington School of Medicine
Seattle, WA

REFERENCES

5. Blazar BR, Thiele DL, VallerA DA: Pretreatment of murine donor grafts with L-leucyl-L-leucine methyl ester treatment of canine marrow and peripheral blood cells: Inhibi-

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 (x10^9/kg)</th>
<th>Rec-SCF</th>
<th>Studied</th>
<th>Acute GVHD</th>
<th>Transient</th>
<th>Fatal</th>
<th>Surviving</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>1.7</td>
<td>No</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>.01</td>
</tr>
<tr>
<td>Yes</td>
<td>1.9</td>
<td>No</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>.64</td>
</tr>
<tr>
<td>Yes</td>
<td>1.7</td>
<td>Yes</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>.64</td>
</tr>
</tbody>
</table>

* 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
Failure of recombinant stem cell factor to enhance engraftment of L-leucyl-L-leucine methyl ester treated canine marrow after irradiation [letter]

HP Kiem, W Leisenring, R Raff, HJ Deeg, FG Schuening, FR Appelbaum and R Storb