To the Editor:

Graft failure is rare (<1% incidence) in leukemic recipients of unmodified HLA-identical sibling marrow. True failure of engraftment and graft rejection have been seen in two settings. The first occurs with marrow from donors who are HLA-nonidentical. The second setting is observed with T-cell–depleted marrow grafts. Presumably, T cells are needed for consistent marrow engraftment to occur.

Understanding the mechanisms of graft failure and rejection is important for designing ways to overcome the problem. Animal studies have shown that the mechanisms depend upon the particular donor/recipient combination. Studies in the canine model have implicated radiation-insensitive, large granular lymphocytes with natural killer function in playing a role in resistance to dog leukocyte antigen (DLA)-nonidentical grafts while host T cells may be involved in rejection of DLA-identical grafts. A number of treatment modalities have been effective in raising the incidence of engraftment of DLA-nonidentical marrow following pretreatment of the animals with antibodies to framework structure antigens of Ia or to CD44.

The most effective way of enhancing engraftment across a major histocompatibility complex (MHC) barrier was the addition of viable lymphocytes from the marrow donor to the marrow inoculum. Engraftment rose to close to 95%. In vitro irradiation of the donor lymphocytes abrogated the beneficial effect. Although effective in enhancing engraftment, the addition of viable lymphocytes led to uniform hyperacute graft-versus-host disease (GVHD) and mortality. The exact mechanism by which lymphocytes work to enhance engraftment is unclear. It could involve removal of host cells causing graft resistance via a GVH effect or a growth enhancing influence of donor lymphocytes on pluripotent stem cells, for instance, through the release of hematopoietic growth factors. An observation supporting the latter suggestion has been made in mice where it was shown that lymphocytes tolerant to host antigens were also effective in enhancing engraftment.

In the current study, we tested whether engraftment of marrow from DLA-nonidentical unrelated donors could be enhanced after raising the dose of TBI from 920 to 1,800 cGy. The most effective way of enhancing engraftment was raising the dose of TBI from 920 to 1,800 cGy. The most effective way of enhancing engraftment across a major histocompatibility complex (MHC) barrier was the addition of viable lymphocytes from the marrow donor to the marrow inoculum. Engraftment rose to close to 95%. In vitro irradiation of the donor lymphocytes abrogated the beneficial effect. Although effective in enhancing engraftment, the addition of viable lymphocytes led to uniform hyperacute graft-versus-host disease (GVHD) and mortality. The exact mechanism by which lymphocytes work to enhance engraftment is unclear. It could involve removal of host cells causing graft resistance via a GVH effect or a growth enhancing influence of donor lymphocytes on pluripotent stem cells, for instance, through the release of hematopoietic growth factors. An observation supporting the latter suggestion has been made in mice where it was shown that lymphocytes tolerant to host antigens were also effective in enhancing engraftment.

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significantly increased numbers of circulating leukocytes on days 7 of the study, mice administered G-CSF for 14 days after transplant had poor engraftment of histoincompatible marrow (BALBlc into C57BL/6) after TBI (7.5 Gy at 0.45 Gy/min). In contrast to the results in the current murine data, though, neither increases in leukocyte numbers nor improvement of survival were observed in this canine study. This difference in results supports the need for testing promising agents in large random bred animal species before applying them to human patients.

**ACKNOWLEDGMENT**

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Research was conducted according to the principles enunciated in “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources of the National Academy of Sciences/National Research Council.

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**Table 1. Dogs Administered 9.2 Gy TBI, DLA-Nonidentical Unrelated Marrow Transplants and Subsequent Treatment With Either Recombinant Human G-CSF or Recombinant Canine G-CSF**

<table>
<thead>
<tr>
<th>Origin and Dose (μg/kg) of G-CSF*</th>
<th>Recovery of Granulocyte Count &gt; 500/μL</th>
<th>GVHD</th>
<th>Survival (dogs)</th>
<th>Cause of Death</th>
<th>Marrow Cellularity at Autopsy (% of normal)</th>
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<tbody>
<tr>
<td>Human, 100</td>
<td>No</td>
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<td>13</td>
<td>Sepsis</td>
<td>0</td>
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<tr>
<td>Human, 100</td>
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<td>Yes</td>
<td>13</td>
<td>Pneumonia</td>
<td>0</td>
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<tr>
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<td>Yes</td>
<td>13</td>
<td>Pneumonia, GVHD*</td>
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<td>No</td>
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<tr>
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<td>No</td>
<td>12</td>
<td>Pneumonia†</td>
<td>0</td>
</tr>
<tr>
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<td>No</td>
<td>No</td>
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<td>Pneumonia†</td>
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<td>Canine, 10</td>
<td>Yes</td>
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<td>Pneumonia</td>
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</table>

* Scheduled to be given subcutaneously twice daily from day 0-20.
† Skin, gut, liver.
‡ Killed due to poor condition.

Hutchinson Cancer Research Center (FHCRC; Seattle, WA). All dogs were immunized against canine distemper, hepatitis, leptospirosis, and parvovirus, and were disease-free before entry into the study. They were housed in an American Association for Accreditation of Laboratory Animal Care approved facility in standard indoor runs and provided commercial dog chow and chlorinated tap water ad libitum. The protocol of this study was approved by the Institutional Animal Care and Use Committee of the FHCRC. Peripheral blood mononuclear cells obtained from the unrelated donor/recipient pairs were typed for serologically defined dog leukocyte antigens (DLA-A and B) and were tested for mutual stimulatory reactivity in mixed leukocyte culture as described. Pedigrees of donors and recipients showed no common ancestry for at least five generations.

Recipient dogs were conditioned for transplantation with 920 cGy TBI administered from two opposing Cobalt sources at a dose rate of 7 cGy/min with supportive care given as described. Between 1.9 and 4.1 (median 3.2) × 10⁹ marrow cells/kg body weight were obtained from femora and humeri under general anesthesia and infused within 4 hours of TBI. Dogs were not given postgrafting immunosuppression. Complete blood counts were obtained before TBI and then daily to the end of the study. Complete autopsies were performed on all dogs to assess marrow cellularity and histologic evidence of GVHD. Engraftment was defined as increase of peripheral blood neutrophils above 500/μL, clinical and/or histologic signs of GVHD, and a marrow cellularity at autopsy of greater than 5% of normal cellularity. Recipients were given G-CSF subcutaneously twice a day from the day of TBI and marrow transplantation (day 0) until day 20 or until death as indicated in Table 1. Two of the 10 dogs showed sustained increases in granulocyte counts above 500/μL. These two and another dog developed clinical and/or pathological signs of GVHD involving skin, liver, or gut. The two dogs with granulocyte recovery died of GVHD associated complications on days 16 and 28, respectively. Marrow cellularity at autopsy was 30% and 60%, respectively. The remaining eight dogs had no evidence of engraftment. This result was not different from that in 57 historical and concurrent control dogs among which 10 engrafted (P = .42) (and unpublished data, May 1996).

Studies in mice have examined the effect of posttransplant infusion of human recombinant G-CSF on engraftment of T-cell-depleted histoincompatible marrow (BALBlc into C57BL/6) after TBI (7.5 Gy at 0.45 Gy/min). In contrast to the results in the current study, mice administered G-CSF for 14 days after transplant had significantly increased numbers of circulating leukocytes on days 7 and 14 after transplant as well as significantly improved survival compared with controls given phosphate-buffered saline infusions. Donor cell engraftment, though, was not enhanced, similar to current results. We conclude that, similar to results with murine marrow allografts across an H-2 barrier, canine grafts across a major histocompatibility barrier cannot be enhanced by G-CSF treatment. In contrast to the murine data, though, neither increases in leukocyte counts nor improvement of survival were observed in this canine study. This difference in results supports the need for testing promising agents in large random bred animal species before applying them to human patients.
REFERENCES


DLA-nonidentical unrelated marrow grafts after high-dose total body irradiation: granulocyte colony-stimulating factor treatment after transplant does not enhance engraftment [letter]

FG Schuening, TC Graham, R Hackman, HJ Deeg and R Storb