Failure of Interleukin-1 and Granulocyte-Macrophage Colony-Stimulating Factor to Enhance Allogeneic Marrow Engraftment and Survival in Irradiated Dogs

To the Editor:

A publication by Blazar et al in Blood described enhancement of engraftment of H-2 incompatible murine marrow by interleukin-1α (IL-1α) after 600 to 750 cGy of total body irradiation (TBI) delivered at 41 cGy/min. Equally impressively, mice receiving a suboptimal dose of IL-1α along with granulocyte-macrophage colony-stimulating factor (GM-CSF) had significantly higher levels of donor alloengraftment (92%) than mice receiving either IL-1α (57%) alone, GM-CSF (18%) alone, or no growth factor (8%). Their findings are of potential interest for clinical application of these growth factors in individuals exposed to radiation accidents. In that setting, marrow allografts would be more successful if they could be used along with those hematopoietic growth factors that have the potential to stimulate engraftment.

We have developed a canine model for radiation accidents using a barely supralethal dose of 450 cGy of TBI. At that dose, all dogs not receiving marrow rescue die. With a DLA-identical marrow infusion, virtually all dogs show initial allogeneic engraftment. Subsequently, grafts are rejected in 65% of cases, and most of these dogs die during the ensuing second period of pancytopenia. Overall, 60% of the dogs survive and, of those, a quarter show autologous recovery, whereas three-quarters have persisting allografts. We investigated the usefulness of canine recombinant granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) in this model and found that overall survival of dogs was improved to 88%, including all dogs receiving G-CSF and SCF combined. The survival advantage of G-CSF- and SCF-treated dogs was not due to an increase in allogeneic engraftment but rather to a decrease in infections resulting from higher granulocyte counts during the entire posttransplant course.

In the current study, we used DLA-identical grafts after 450 cGy of TBI delivered at 7 cGy/min from two opposing 60Co sources to study the combination of recombinant human IL-1α and recombinant canine GM-CSF. We asked whether the survival of dogs so treated could be improved and allogeneic engraftment enhanced by the growth factor combination. IL-1α was administered as a continuous subcutaneous infusion at 2.5 μg/kg/day and GM-CSF at 30 μg/kg/day subcutaneously in divided doses, both from day 9 to 11. The littermate donor-recipient pairs were selected on the basis of identity for the serologically detectable canine histocompatibility antigens DLA-A and B and by identity for restriction fragment length polymorphism patterns for canine major histocompatibility complex class II genes. Recipients received 2.1 to 4.0 (median, 2.4) × 10⁶ nucleated marrow cells per kilogram of body weight by intravenous infusion within hours of TBI. Dogs did not receive postgrafting immunosuppression. Postgrafting care has been described.

Table 1 compares the results in the four current dogs with those in historical (n = 5) and concurrent (n = 12) control dogs not receiving growth factor after transplant. All four dogs showed initial evidence of allogeneic engraftment as demonstrated by promptly increasing peripheral blood granulocyte counts after the postirradiation nadir. After early recovery, granulocyte counts declined again, and marrow aplasia developed, findings that were consistent with acute graft rejection. Three of the four dogs were euthanized between days 17 and 22 because of uncontrollable intercurrent infections. They had no evidence of graft-versus-host disease. Their marrows were profoundly hypocellular. One of the four dogs showed ultimate hematopoietic recovery. This dog was euthanized on day 145, at the end of the study. Examination of the marrow and peripheral blood cells in this dog by (CA), dinucleotide markers showed a mixture of donor and host cells on day 20. A repeat examination on day 60 showed only cells of host type. Results in the current dogs were not statistically significantly different from those in controls. It could be argued that the number of dogs receiving transplants is too small to draw firm conclusions. However, given the uniform graft failure observed, even if 10 dogs received transplants under the current protocol, and the additional six all showed sustained allografts, there would still not have been statistically significant evidence for graft enhancement by IL-1α/GM-CSF.

<table>
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<tr>
<th>IL-1α and GM-CSF</th>
<th>No. of Dogs</th>
<th>Early Death With Aplasia</th>
<th>Complete Allograft</th>
<th>Mixed Chimerism*</th>
<th>Autologous Recovery</th>
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<td>3</td>
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<td>17</td>
<td>11</td>
<td>7</td>
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</table>

* Mixed chimerism was assumed if either cytogenetics, dinucleotide (CA), repeat markers, or both showed mixtures of host and donor hematopoietic cells.
We conclude that, in contrast to data with murine marrow allografts across an H-2 barrier, canine grafts across a minor histocompatibility barrier cannot be enhanced by IL-1α and GM-CSF. Whether the difference in results is due to differences in the histocompatibility settings studied remains conjectural. The only other difference is the use of recombinant human GM-CSF in mice versus a recombinant canine product in dogs. The difference in study outcome points toward the necessity of testing promising drug combinations in large randombred animal species before applying them to human patients.

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REFERENCES


5. Yu C, Ostrander E, Bryant E, Burnett R, Storb R: Use of (CA)n polymorphisms to determine the origin of blood cells after allogeneic canine marrow grafting. Transplantation 58:701, 1994
Failure of interleukin-1 and granulocyte-macrophage colony-stimulating factor to enhance allogeneic marrow engraftment and survival in irradiated dogs [letter; comment]

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