Abrogation of Resistance to and Enhancement of DLA-Nonidentical Unrelated Marrow Grafts in Lethally Irradiated Dogs by Thoracic Duct Lymphocytes

By H. Joachim Deeg, Rainer Storb, Paul L. Weiden, Howard M. Shulman, Theodore C. Graham, Beverly J. Torok-Storb, and E. Donnall Thomas

We have previously shown that grafts with low doses of bone marrow (≤ 4 × 10⁸ cells/kg) after 1200 R total-body irradiation (TBI) are successful in DLA-identical, but not DLA-nonidentical littermate or unrelated dogs, suggesting a locus of resistance associated with the major canine histocompatibility complex (DLA). Addition of viable donor blood leukocytes to the marrow inoculum has consistently overcome resistance, either by administration of an increased number of stem cells from the peripheral blood or through an effect mediated by lymphocytes. In-vitro-irradiated leukocytes have been ineffective. In the current study 8 dogs were given 1200 R TBI followed by 2.5 ± 1.4 × 10⁸ marrow cells/kg and 7.7 ± 3.6 × 10⁸ thoracic duct lymphocytes/kg from unrelated DLA-nonidentical donors. All 8 dogs showed prompt and sustained hemopoietic engraftment, and none rejected. Since canine thoracic duct lymphocytes have previously been shown not to contain hemopoietic stem cells, abrogation of resistance to canine marrow grafts appears to be related either to active suppression of residual host immunity or to enhancement of hemopoiesis by cell–cell interaction of thoracic duct lymphocytes with marrow cells. The latter mechanism is suggested by in vitro studies showing that dog thoracic duct lymphocytes cocultured with autologous marrow significantly increase the number of erythroid colonies.

Marrow transplants between DLA-identical littermates have generally resulted in successful engraftment. However, resistance to marrow grafts as indicated by failure of sustained engraftment has been the rule in DLA-nonidentical (littermate and unrelated) donor–recipient combinations. These findings support the concept that resistance to marrow engraftment is associated with incompatibility for DLA. This resistance has been successfully abrogated by addition of viable donor peripheral blood leukocytes to the marrow inoculum, whereas in-vitro irradiated leukocytes have failed to be effective. Possible mechanisms in the production of prompt and sustained marrow grafts when viable blood leukocytes are added to the marrow include (1) the administra-
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RATION OF INCREASED NUMBERS OF HEMOPOIETIC STEM CELLS KNOWN TO BE PRESENT IN CANINE PERIPHERAL BLOOD AND (2) THE ENHANCEMENT OF ENGRAFTMENT BY IMMUNOCOMPETENT CELLS PRESENT IN LARGE NUMBERS IN THE BLOOD. CANINE THORACIC DUCT LYMPH, IN CONTRAST TO PERIPHERAL BLOOD, HAS PREVIOUSLY BEEN SHOWN NOT TO CONTAIN HEMOPOIETIC STEM CELLS CAPABLE OF RESCUING DOGS FROM THE EFFECTS OF OTHERWISE LETHAL TOTAL-BODY IRRADIATION (TBI). TO TEST WHICH OF THESE TWO MECHANISMS IS OPERATIVE, THE PRESENT EXPERIMENT WAS DESIGNED TO EXAMINE WHETHER THE ADDITION OF THORACIC DUCT LYMPHOCYTES WAS AS EFFECTIVE AS PERIPHERAL BLOOD LEUKOCYTES IN OVERCOMING RESISTANCE TO DLA-NONIDENTICAL UNRELATED Marrow GRAFTS.

MATERIALS AND METHODS

DOGS

The dogs used in this study were beagles, hounds, and mongrels obtained from kennels in Washington, Oregon, and West Virginia. They were 7–24 mo old and weighed 6.7–45.0 kg. They had been observed for disease for 2 mo and were dewormed and vaccinated against measles, distemper, hepatitis, and leptospirosis.

The donor–recipient pairs were of opposite sex and were mismatched for the serologically detectable histocompatibility antigens (DLA) determined in a microlymphocytotoxicity assay.

THORACIC DUCT CANNULATION AND MARROW ASPIRATION

Under pentobarbital anesthesia and endotracheal intubation the thoracic duct of the marrow donor was exposed from a left neck incision. The duct was cannulated either directly by insertion of a silastic tube with an external diameter of 2.5 mm or indirectly by insertion of a Teflon-silastic shunt into the left external jugular vein. In the latter case the brachiocephalic and all other branching veins were tied off, leaving the duct as the only afferent vessel. The wound was closed and the cannula was brought through a separate skin incision. Lymph was collected for 48 hr in 300-ml blood administration bags containing sufficient preservative-free heparin for a final concentration of 10 units per 1 ml of lymph. Bone marrow was aspirated from both femora and humeri and processed for infusion as described previously.

TBI, MARROW AND LYMPH INFUSION, AND POSTGRAFTING CARE

Recipients were prepared for grafting by administration of 1200 R (midline air exposure) of TBI from two opposing 60Co sources delivered at a rate of 9.3 R/min. The day of irradiation was designated day 0. Marrow, 2.5 ± 1.4 (SD) x 10^8 cells/kg, was infused intravenously within 4 hr of irradiation of the recipient. Lymph, containing 7.7 ± 3.6 (SD) x 10^8 mononuclear cells/kg, was infused at frequent intervals on days 0, 1, and 2.

No postgrafting immunosuppression was administered. Details of the postgrafting fluid, electrolyte, and antibiotic therapy, the assessment of allogeneic hemopoietic engraftment by peripheral blood cell counts, marrow examinations, and cytogenetic studies, the clinical and histologic criteria for graft-versus-host disease (GVHD), and the autopsy and histologic examinations of postmortem tissues have been described. Marrow for cytogenetic studies was obtained on day 7.

RESULTS

The results in the recipients in the present study were compared with the results in 17 recipients given 1200 R, an infusion of 3.8 ± 0.6 x 10^8 unrelated DLA-nondetical marrow cells, and no additional viable thoracic duct lymphocytes nor blood leukocytes and the results in 20 recipients given a comparable amount of marrow (3.6 ± 1.6 x 10^8 cells/kg) and additional viable blood leukocytes (5.1 ± 2.6 x 10^8 cells/kg), including 1.0 ± 0.6 x 10^8 lymphocytes/kg. Most of these 37 transplants have been reported previously.

Table 1 summarizes the data. The WBC counts in all 8 recipients declined to very low levels by day 5 after TBI and then started to rise in all except one, thus
Table 1. Data in Dogs Given 1200 R TBI Followed by Marrow and Thoracic Duct Lymphocyte Infusions From DLA-Nonidentical Unrelated Donors

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sex</th>
<th>Marrow (× 10^9/kg)</th>
<th>Thoracic Duct Lymphocytes</th>
<th>Rise in WBC</th>
<th>Cytogenetic Studies† (day 7)</th>
<th>Gross and Histological Evidence of GVHD</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B484</td>
<td>F</td>
<td>4.0</td>
<td>7.0</td>
<td>Yes</td>
<td>11/xy</td>
<td>Skin, gut, liver</td>
<td>7</td>
</tr>
<tr>
<td>B508</td>
<td>F</td>
<td>2.7</td>
<td>8.6</td>
<td>No</td>
<td>20/xy</td>
<td>Gut, liver</td>
<td>7</td>
</tr>
<tr>
<td>B509</td>
<td>F</td>
<td>2.9</td>
<td>8.1</td>
<td>Yes</td>
<td>20/xy</td>
<td>Skin, gut, liver</td>
<td>8</td>
</tr>
<tr>
<td>B170</td>
<td>F</td>
<td>0.3</td>
<td>4.4</td>
<td>Yes</td>
<td>18/xy</td>
<td>Skin(?), gut, liver</td>
<td>9</td>
</tr>
<tr>
<td>B489</td>
<td>M</td>
<td>3.5</td>
<td>13.3</td>
<td>Yes</td>
<td>20/xx</td>
<td>Skin(?), gut, liver</td>
<td>9</td>
</tr>
<tr>
<td>B458</td>
<td>F</td>
<td>1.0</td>
<td>11.7</td>
<td>Yes</td>
<td>N.D.</td>
<td>Skin, gut, liver</td>
<td>10</td>
</tr>
<tr>
<td>B392</td>
<td>F</td>
<td>1.4</td>
<td>2.8</td>
<td>Yes</td>
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<tr>
<td>B460</td>
<td>F</td>
<td>4.0</td>
<td>5.3</td>
<td>Yes</td>
<td>10/xy</td>
<td>Skin, gut, liver</td>
<td>13</td>
</tr>
</tbody>
</table>

*The differential cell count (mean ± 1 SD) showed 93.4 ± 5.7% small lymphocytes, 6.6 ± 5.2% large lymphoid cells, 0.6 ± 0.9% monocytes, and 0.2 ± 0.4% granulocytes.
†Only cells with 78 chromosomes were evaluated. Numbers represent numbers of metaphase spreads studied; all showed the donor karyotype. N.D. = not done.

indicating beginning hemopoietic engraftment. Peak WBC counts before death ranged from 550 to 3,750/cu mm. None of the dogs rejected the graft. Cytogenetic studies of the marrow without mitogen stimulation on day 7 were done in 7 recipients and showed all 101 cells analyzed to be of donor karyotype. At days 6–8, GVHD, as manifested by skin erythema, conjunctivitis, and nasal mucositis, was diagnosed clinically in all dogs. They died between days 7 and 13, with infection in 6 dogs and hemorrhage in 2 dogs. The bone marrow in all 8 dogs showed evidence of hemopoietic engraftment, as demonstrated by the presence of myeloid and erythroid cells at all stages of maturation. Megakaryocytes were present in 3 dogs. All 8 dogs had gross and histologic signs of GVHD.

For comparison, Table 2 shows that as a rule the dogs given marrow only rejected their grafts, whereas dogs given additional viable blood leukocytes showed sustained engraftment. When leukocytes were irradiated in vitro before infusion, failure of marrow engraftment was seen in all cases.

DISCUSSION

The present study shows that the addition of thoracic duct lymphocytes to the marrow inoculum successfully abrogates resistance to marrow grafts from unrelated DLA-nonidentical dogs. We have previously reported abrogation of resistance and enhancement of engraftment by the addition of viable peripheral blood leukocytes. Failure of enhancement of engraftment by in-vitro irradiated leukocytes indicates that the leukocytes must be capable of replication in order to be effective. One possible explanation for the enhancement of engraftment by viable leukocytes is their ability to provide a source of cytokines which act in a paracrine fashion to enhance the growth of bone marrow cells.

Table 2. Data in Unrelated DLA-Nonidentical Dogs Given 1200 R TBI Followed Within Hours by Infusion of ≤4 × 10^8 Marrow Cells/kg Body Weight

<table>
<thead>
<tr>
<th>Additional Therapy</th>
<th>Studied</th>
<th>Marrow Rejection</th>
<th>Sustained Engraftment</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Viable donor leukocytes</td>
<td>20</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Irradiated (2000 rads in vitro) leukocytes</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>
leukocytes is that peripheral blood contains hemopoietic stem cells. However, we have been unable to achieve consistent engraftment even with very large numbers of marrow cells alone, and this argues against that possibility. Also, hemopoietic grafts using peripheral blood as the sole source of stem cells were successful in all instances among unrelated donor-recipient combinations. Since the concentration of stem cells in the peripheral blood is known to be lower than that in the marrow, the consistent abrogation of resistance observed in those previous experiments must be related to the presence of replicating cells other than hemopoietic stem cells among the infused leukocytes. A likely explanation is that immunocompetent cells present in large numbers among the infused leukocytes either suppressed residual host reactivity or in some way interacted with the marrow cells to enhance their growth. The current study fully supports this explanation, since it rules out a contribution by pluripotent hemopoietic stem cells, which are not present in canine thoracic duct lymph in any appreciable number. Canine thoracic duct lymph also lacks CFU-Cs (unpublished observations), which are readily grown from marrow and peripheral blood.

The nature and mechanism of action of the thoracic duct (and peripheral blood) cells that abrogate resistance to and enhance the growth of unrelated DLA-nonidentical canine marrow grafts are currently unknown. It is possible that these lymphocytes are identical with those peripheral blood and thoracic duct cells found to enhance canine erythroid colony growth in vitro and that they enhance engraftment by interacting with the marrow graft. In man, apparently similar cells involved in enhancement of burst-forming erythroid colony growth in vitro have been shown to be T lymphocytes. Alternatively, the cells that abrogate resistance may interact in some way with residual host immune cells and eliminate the host-versus-graft reactivity responsible for resistance to mismatched allogeneic marrow.

Studies similar to ours have been carried out on the phenomenon of hybrid resistance in mice. The addition of thymocytes syngeneic to the marrow (but not of lymph node lymphocytes) overcame resistance and enhanced marrow growth in murine parent-to-F1 hybrid transplants. Data on thoracic duct lymphocytes have not been reported in mice. GVHD appeared not to be a prerequisite for the abrogation of hybrid resistance, since thymocytes from donor mice “tolerant” to the host were reported to be effective as well. The “amplifier” cell among the thymocytes that enhances hemopoiesis has not yet been identified.

Hybrid resistance in the mouse is presumably mediated by surface determinants coded for by recessive genes and expressed only on homozygous hemopoietic cells. The resistance to canine marrow grafts described here and previously can be explained on the basis of products of dominant genes that are associated with the major canine histocompatibility complex. Thus hybrid resistance in the mouse is not strictly comparable to the resistance observed in the dog. Hybrid resistance can be nonspecifically overcome by various manipulations. Only some of these have been examined in the dog. We were unable to abrogate resistance with the macrophage inhibitor silica or with rabbit antidog a thymocyte serum in a model identical with that described in the present study. However, other investigators have reported successful abrogation of resistance in a number of dogs treated with silica. The reasons for this difference are unclear, but they may be related to the degree of genetic disparity among dogs in the various laboratories.
The finding in the present study of consistent enhancement of marrow engraftment by thoracic duct cells suggests that identification and isolation of the cell mediating this effect might make possible consistently successful marrow grafts across major canine histocompatibility barriers without an increased risk of severe GVHD due to other lymphocyte subpopulations.

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