CONCISE REPORT

Cyclosporin-A Abrogates Transfusion-Induced Sensitization and Prevents Marrow Graft Rejection in DLA-Identical Canine Littermates

By Rainer Storb, H. Joachim Deeg, Kerry Atkinson, Paul L. Weiden, George Sale, Raymond Colby, and E. Donnall Thomas

Previous studies have shown that marrow graft rejection generally did not occur in untransfused dogs given 900-rad total body irradiation and hemopoietic grafts from DLA-identical littersmates (only 1 of 59 rejected), but was seen in all instances after three preceding transfusions of whole blood from the marrow donor on days –24, –17, and –10 before transplantation (19 of 19 rejected). The present study was undertaken to investigate whether immunization by 3 preceding transfusions of whole blood from the DLA-identical littermate marrow donor could be abrogated by administration of the immunosuppressive agent cyclosporin-A, 20 mg/kg/day intramuscularly on days –5 to 0. Seven of 10 dogs showed sustained marrow engraftment. 2 failed to engraft, and 1 dog died too early to be evaluated. It was concluded that immunization to non-DLA antigens by preceding whole blood transfusions could be abrogated in most cases by a short course of cyclosporin-A before total body irradiation and marrow transplantation, resulting in successful and sustained marrow engraftment.

Untransfused dogs given 900-rad total body irradiation (TBI) and hemopoietic grafts from DLA-identical littersmates have generally shown sustained engraftment (only 1 of 59 rejected) but rejection was the rule after preceding blood transfusions from the marrow donor (all 19 dogs rejected). Presumably, rejection was due to sensitization of recipients to polymorphic “minor” histocompatibility antigens that are outside of DLA, undetected by current histocompatibility typing techniques, and expressed on mononuclear cells in the transfused blood. The present study was undertaken to investigate whether immunization by three preceding transfusions could be abrogated by administration of the immunosuppressive agent cyclosporin-A before transplantation.

MATERIALS AND METHODS

Litters of beagles, labradors, and basenji/labrador crossbreeds were obtained from kennels in the states of Washington, Virginia, and Oregon. The dogs, 7.2-21 kg in weight and 6-13 mo of age, were observed for disease for 2 mo before grafting. They were immunized against distemper, leptospirosis, hepatitis, and parvovirus.

Ten pairs were chosen on the basis of identity for the serologically detectable canine histocompatibility antigens DLA-A and B and mutual nonreactivity in mixed leucocyte culture.

Recipients were conditioned for transplantation by 900-rad midline tissue exposure of TBI given at a rate of 7.1 rad/min from 2 opposing cobalt-60 sources. They were then given marrow, 0.9-7.4 x 10^8 cells/kg body weight intravenously within 4 hr of irradiation. In addition, they were given peripheral blood leukocytes from the marrow donor, 8.2 x 10^7 (median 15.7) x 10^8 cells/kg intravenously on days 1 and 2 after irradiation. No postgrafting immunosuppression was administered. The postgrafting care has been described. The day of TBI and marrow grafting is designated “day 0.” Days before grafting are indicated by a minus sign.

Recipients were given 3 intravenous transfusions of 50 ml heparinized whole blood from the intended marrow donor on days –24, –17, and –10 according to a previously described schedule. From day –5 through day 0 they were given a daily intramuscular injection of cyclosporin-A, 20 mg/kg body weight. Cyclosporin-A was kindly provided by Dr. J. F. Borel, Sandoz Ltd. (Basel, Switzerland).

Marrow engraftment was assessed by promptly rising granulocyte and platelet counts following the postirradiation decline, histologic features of the marrow at autopsy, and the development of graft-versus-host disease (GVHD). Clinical and histologic findings of GVHD have been described. Acute marrow graft rejection was defined as either failure of recovery of granulocyte counts following the postirradiation nadir or, following initial evidence of engraftment, disappearance of granulocytes and platelets from the blood. Autopsy in dogs with rejection showed extreme marrow hypocellularity. Absence of GVHD lesions at autopsy was also evidence for marrow graft failure.

RESULTS

Table 1 summarizes the data. Two dogs failed to show engraftment. One of these did not show rises in granulocyte and platelet counts after the postirradiation decline and died on day 9 with septicemia and autopsy findings of an extremely hypocellular marrow. The other had a transient rise in granulocytes and died on day 9 with an extremely hypocellular marrow. One dog (B704) died on day 11 with pneumonia. This dog's
Table 1. Data on Ten Recipients Given Three Whole Blood Transfusions From Their Marrow Donors Before 900-rad Total Body Irradiation and Hemopoietic Grafts From DLA-Identical Littermates

<table>
<thead>
<tr>
<th>Recipient Number</th>
<th>No. of Cells Infused per kg x 10^8</th>
<th>Rise in Counts</th>
<th>Marrow Rejection</th>
<th>Survival (Days)</th>
<th>Marrow Histologic Findings at Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marrow</td>
<td>Blood Leukocytes</td>
<td>Granulocytes</td>
<td>Platelets</td>
<td></td>
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<tr>
<td>B738</td>
<td>6.3</td>
<td>25.0</td>
<td>No</td>
<td>No</td>
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<tr>
<td>B734</td>
<td>3.7</td>
<td>13.4</td>
<td>Yes</td>
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<tr>
<td>B704</td>
<td>7.4</td>
<td>16.4</td>
<td>Yes</td>
<td>No</td>
<td>—</td>
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<tr>
<td>B761</td>
<td>1.6</td>
<td>11.2</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>B699</td>
<td>5.8</td>
<td>12.8</td>
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<tr>
<td>B737</td>
<td>4.5</td>
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<tr>
<td>B753</td>
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<tr>
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<td>0.9</td>
<td>18.0</td>
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</tbody>
</table>

Granulocytes had begun to rise. The marrow at autopsy was moderately cellular with all hemopoietic precursors present. The dog died too early to assess the fate of the graft. Seven dogs showed sustained engraftment. Of these, 2 died on days 20 and 24 with clinical and histologic evidence of GVHD and associated infections and autopsy findings of cellular marrows with all hemopoietic precursors present. One died on day 68 with a septicemia; although this dog's platelet count never rose to high levels, the autopsy marrow was moderately cellular with erythropoietic and granulocyte-megakaryocyte precursors and megakaryocytes present. The 4 remaining dogs with sustained engraftment have survived for more than 200 days.

DISCUSSION

Our previous studies have shown prompt and sustained marrow engraftment and prolonged survival in 58 of 59 untransfused recipients given 900-rad TBI and hemopoietic grafts from littermates that were DLA-identical on the basis of serologic histocompatibility testing and mutual nonreactivity in mixed leukocyte culture. In contrast, when recipients were given three preceding fresh blood transfusions from their intended marrow donors before irradiation and grafting, rejection of the marrow and early death of the recipients with marrow hypoplasia was seen in all 19 cases. Even a single preceding transfusion from the marrow donor resulted in 75% graft rejection, and 30% of the dogs rejected after random transfusions. Apparently, 900-rad TBI was not sufficient to destroy the immunity to non-DLA histocompatibility antigens. Similar data were reported for H-2-compatible, x-irradiated mice, and mice treated with cyclophosphamide.

The observations in the dog are in agreement with the findings in human patients with aplastic anemia treated with high-dose cyclophosphamide and marrow grafts from HLA-identical siblings. Rejection rates ranging from 25%-60% have been reported in multiply transfused patients, while untransfused patients have generally shown sustained engraftment. The present study showed that six doses of cyclosporin-A given immediately before TBI were capable of abrogating an established immune response to non-DLA antigens induced by 3 preceding blood transfusions in at least 70% of the dogs studied. Previous studies using a direct and indirect hemolytic plaque-forming system in rats had already suggested that a memory response could be suppressed by cyclosporin-A. Our present results in DLA-identical dogs contrast with those reported by Tutschka et al. in RT-H-incompatible rats. These authors conditioned Lewis rats with busulfan and cyclophosphamide and gave the animals infusions of ACI spleen cells. Recipients were either sensitized or not by preceding infusions of ACI spleen cells. Animals were given cyclosporin-A, 25 mg/kg/day subcutaneously on days 1-18. Rats that were not immunized had sustained engraftment, while rats that had preceding spleen cell infusions all rejected their grafts, regardless of whether they received additional treatment with cyclosporin-A or not. Apparently, cyclosporin-A was not able to abrogate transfusion-induced sensitization and graft rejection across major histocompatibility barriers in rats.

It is not known whether the encouraging results seen in the present study in DLA-identical littermate dogs can be extrapolated to the human marrow transplant situation. Nevertheless, the lack of marrow toxicity of cyclosporin-A and its potential additional usefulness in preventing GVHD after marrow transplantation make it a candidate drug for a prospective clinical trial in marrow transplantation for aplastic anemia.
REFERENCES


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