Prevention of Transfusion-Induced Graft-Versus-Host Disease in Dogs by Ultraviolet Irradiation

By H. Joachim Deeg, Theodore C. Graham, Lisa Gerhard-Miller, Frederick R. Appelbaum, Friedrich Schuening, and Rainer Storb

TRANFUSION-RELATED graft-versus-host disease (GVHD) was first described by Hathaway et al in 1965.1 Transfusion-induced GVHD has subsequently been observed with increasing frequency in patients receiving immunosuppressive and myelosuppressive therapy necessitating transfusion support.2,3 GVHD can be prevented if the transfusion product is gamma-irradiated, usually at doses of at least 1,500 to 2,000 cGy before use.4 However, gamma irradiation does not abrogate the ability of transfused cells to induce sensitization and thus can result in transfusion refractoriness.

Earlier studies have shown that ultraviolet (UV) irradiation of blood products eliminates their ability to sensitize the transfusion recipient.5 If UV irradiation also interfered with the ability of immunocompetent cells to trigger a graft-versus-host reaction, this approach could be used to prevent both GVHD and transfusion sensitization. We investigated this question in a canine model of autologous marrow transplantation and transfusion of dog leukocyte antigen (DLA)-incompatible leukocytes post-transplantation. Whereas transfusions of unmanipulated DLA-incompatible leukocytes uniformly induced GVHD, UV irradiation of leukocytes, at appropriate doses, prevented the development of GVHD.

MATERIALS AND METHODS

Dogs. Ten beagles and harriers raised at the Fred Hutchinson Cancer Research Center or bought from commercial kennels were given standard anthelminthic treatment and vaccinations, and observed for disease for at least 2 months. The dogs weighed 9 to 29.9 kg (median, 14.6 kg) and were 7 to 33 months old (median, 8.5 months) at the time of transplantation. Research was conducted according to the principles of the "Guide for Laboratory Animal Facilities and Care" prepared by the National Academy of Sciences-National Research Council. The research protocol was approved by the Internal Animal Care Review Committee of the Fred Hutchinson Cancer Research Center.

Transplantation regimen. Autologous marrow was harvested from femora and humeri, and stored at 4°C for 4 to 8 hours until infusion as described.4 Following the marrow harvest, dogs were given 9.2 cGy of total body irradiation (TBI) from two opposing 60Co sources at an exposure rate of 7 cGy/min. Details of the dosimetry have been described before.7 Following completion of TBI, autologous marrow was infused. The number of marrow cells was 1.2 to 3.7 x 10^8 cells/kg. The day of marrow infusion was designated "day 0."

Leukocyte transfusion. Starting with the day of marrow infusion, the dogs also were given peripheral blood leukocytes obtained via leukapheresis from unrelated DLA-incompatible donors.6 The donors were chosen on the basis of nonidentity for serologically detectable canine histocompatibility antigens DLA-A and DLA-B and mutual reactivity of lymphocytes in mixed leukocyte culture (MLC). An arteriovenous shunt was placed in the neck of the donor dog for manual apheresis.7 The protocol asked for ten aphereses on ten consecutive days (except weekends); in two dogs the shunt became nonoperative prematurely, and only six and eight aphereses, respectively, were carried out. The yield of leukocytes obtained by apheresis was 20.7 to 38.5 (median, 28.2) x 10^7 cells/kg, which corresponded to 11.5 to 36.2 (median, 18.8) x 10^7/kg of recipient weight; 15% to 26% of the cells were lymphocytes.

Post-grafting care. Post-grafting care has been described.8 The dogs received oral nonabsorbable antibiotics (neomycin sulfate and polymyxin B sulfate) three times daily beginning on day 5 until the day the granulocyte count reached 0.5 x 10^9/L postgrafting. No postgrafting immunosuppression was administered.

UV irradiation of leukocytes. Leukocytes obtained by leukapheresis were suspended in phosphate-buffered saline in open petri dishes (Falcon #3003), placed on a rotating platform in a laminar air flow hood and exposed to UV light (200 to 300 nm) from a germicidal lamp (General Electric) at an exposure rate of 750 μW/sec, for a total of 20 or 1,000 mJ/cm². Exposure was determined with a Black Ray UV meter (UV Products, Westbury, NY).8 Following exposure, cells were quantitatively collected into syringes for injection. Concurrent in vitro studies examined the extent to which leukocytes exposed to UV light had lost their ability to serve as responding or stimulating cells in MLC or as responder cells in mitogen-driven cultures.9

Marrow engraftment. Hematopoietic reconstitution was defined as a sustained rise in granulocyte and platelet counts following the postirradiation decline. The development of GVHD was assessed...
by clinical examination as well as ex vivo skin or lip biopsies and postmortem examination.4

RESULTS

Results are summarized in Table 1 and Fig 1. All four dogs in group I given nonirradiated leukocytes developed clinical and pathological evidence of GVHD after six to ten leukocyte transfusions, corresponding to 11.2 to 24.1 x 10^9 cells/kg. GVHD was lethal in one dog; three dogs recovered and survived until completion of the experiment.

Of the three dogs receiving leukocytes exposed to 20 mJ/cm^2 (group II), one given 11.5 x 10^9 leukocytes/kg showed no evidence of GVHD and survived. The remaining two dogs developed GVHD after six or eight leukocyte transfusions corresponding to 17.5 and 24.1 x 10^9 cells/kg respectively. One dog died of an intervening pneumonitis, and one dog survived. Three dogs given leukocytes exposed to 1,000 mJ/cm^2 of UV light all had sustained engraftment, and none developed GVHD.

In concurrent in vitro studies (data not shown), nonirradiated leukocytes had normal functional abilities in MLC and mitogen cultures. As shown previously, there was only mild impairment after 20 mJ/cm^2 of UV light, whereas those functions were reduced by more than 95% as compared with controls after exposure of leukocytes to 1,000 mJ/cm^2.3

DISCUSSION

With increasing awareness of the occurrence of transfusion-induced GVHD in immunosuppressed patients, gamma irradiation of blood products was introduced into clinical practice.1,4 Despite ongoing debate, most investigators agree that GVHD can be prevented by this approach which, however, does not prevent allosensitization. Allosensitization can occur even in severely immunosuppressed patients.

Previously we have shown in a canine model that UV irradiation of blood products prevents transfusion-induced sensitization.1,6 The current study shows that UV irradiation also prevents transfusion-induced GVHD. In contrast, unirradiated blood products triggered the development of potentially lethal GVHD, a finding similar to observations in human autologous marrow transplant recipients.11 Because 15% to 20% of transfused leukocytes were lymphocytes and approximately 80% of canine peripheral blood lymphocytes represent T lymphocytes,12 dogs received approximately 1.3 to 5 x 10^9 T lymphocytes/kg (contained in four to eight transfusions) before developing GVHD. No attempt was made to establish a dose-response curve to investigate the kinetics of GVHD development. However, even if a single transfusion had been sufficient to induce GVHD, this would amount to a T cell dose 2 to 3 logs higher than that associated with the development of GVHD after allogeneic marrow transplantation in humans (10^7 T cells/kg).3,13,14 This difference may be species-related or may be due to the experimental design. Alternatively these data suggest that the T cell dose required to initiate transfusion-related GVHD may be substantially higher than the minimal dose of marrow T cells necessary to trigger GVHD after allogeneic transplantation.2

The UV doses necessary to abrogate alloreactivity are compatible with normal or close to normal in vivo survival of RBCs or platelets.10 For clinical application it would be desirable to develop a closed system consisting of material that allows for sufficient transmission of UV light.16 On the other hand, one might argue that manipulations such as T

### Table 1. Dogs Given Autologous Marrow Grafts and Leukocyte Transfusions From DLA-Incompatible Donors

<table>
<thead>
<tr>
<th>Group (mJ/cm^2)</th>
<th>Dog No.</th>
<th>No. of Days</th>
<th>Total No. of Cells (x 10^9)</th>
<th>Per kg (x 10^9)</th>
<th>Neutrophils ≥0.5 x 10^9/L (d)</th>
<th>Platelets ≥20 x 10^9/L (d)</th>
<th>BM % Cellularity (No. of Tx)</th>
<th>Organ</th>
<th>Day of Onset (No. of Tx)</th>
<th>Survival (d)</th>
<th>Cause of Death</th>
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<tr>
<td>I (0)</td>
<td>C495</td>
<td>6</td>
<td>32.6</td>
<td>36.2</td>
<td>NR</td>
<td>NR</td>
<td>Focal</td>
<td>S, E, I</td>
<td>6 (4)</td>
<td>8</td>
<td>Septicemia</td>
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<tr>
<td></td>
<td>C500</td>
<td>10</td>
<td>38.5</td>
<td>30.7</td>
<td>8</td>
<td>36</td>
<td>100</td>
<td>S, E, I</td>
<td>6 (5)</td>
<td>&gt;89</td>
<td>K</td>
</tr>
<tr>
<td></td>
<td>C664</td>
<td>10</td>
<td>32.6</td>
<td>15.5</td>
<td>9</td>
<td>22</td>
<td>100</td>
<td>S, E, I</td>
<td>10 (8)</td>
<td>&gt;79</td>
<td>K</td>
</tr>
<tr>
<td></td>
<td>C666</td>
<td>8</td>
<td>20.2</td>
<td>14.7</td>
<td>8</td>
<td>20</td>
<td>100</td>
<td>S, E, I</td>
<td>8 (6)</td>
<td>&gt;63</td>
<td>K</td>
</tr>
<tr>
<td>II (20)</td>
<td>C553</td>
<td>10</td>
<td>35.8</td>
<td>17.5</td>
<td>10</td>
<td>21</td>
<td>100</td>
<td>S, E, I</td>
<td>12 (6)</td>
<td>&gt;49</td>
<td>K</td>
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<tr>
<td></td>
<td>C561</td>
<td>10</td>
<td>28.7</td>
<td>24.1</td>
<td>15</td>
<td>NR</td>
<td>80</td>
<td>S, E, I</td>
<td>8 (6)</td>
<td>21</td>
<td>Pneumonia</td>
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<td>C549</td>
<td>10</td>
<td>25.3</td>
<td>11.5</td>
<td>11</td>
<td>18</td>
<td>100</td>
<td>—</td>
<td>NA</td>
<td>&gt;41</td>
<td>K</td>
</tr>
<tr>
<td>III (1,000)</td>
<td>C658</td>
<td>10</td>
<td>24.6</td>
<td>16.8</td>
<td>10</td>
<td>20</td>
<td>100</td>
<td>—</td>
<td>NA</td>
<td>&gt;99</td>
<td>K</td>
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<td></td>
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<td>NA*</td>
<td>100</td>
<td>—</td>
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<td>&gt;42</td>
<td>K</td>
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<tr>
<td></td>
<td>C718</td>
<td>10</td>
<td>30.1</td>
<td>20.1</td>
<td>9</td>
<td>11</td>
<td>100</td>
<td>—</td>
<td>NA</td>
<td>&gt;51</td>
<td>K</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; NR, not reached; NA, not applicable; S, skin; E, eyes; I, intestinal tract; K, killed; Tx, transfusion; d, day.

*The platelet count never fell below 20 x 10^9/L.
cell depletion of bone marrow are also being carried out in open systems without undue side effects.

The mechanism by which UV light mediates its effects is not entirely clear. UV light is known to induce DNA strand breaks, and thereby can cause cell death. However, more recent data indicate that UV light also induces alterations of cell surface structures and calcium mobilization which may lead to changes in the immune-function of UV-treated viable cells. We had interpreted our data on the prevention of sensitization by UV irradiation of transfused cells as an indication that UV-modified cells escaped immune recognition by the recipient. The data presented here suggest that UV-treated leukocytes also lack alloaggressive ability, rendering them incapable of triggering the GVHD. It is also conceivable that UV treated cells are tolerogenic and induce an active mechanism of tolerance in the recipient. We have not formally investigated the possibility that untreated allo-geneic transfusions sensitize the autologous graft leading to an autologous GVHD-like reaction. Because UV treatment prevents sensitization it also might prevent such an autolo-gous GVHD reaction.

In addition to the data presented here, these observations suggest the possibility that UV manipulation of donor mar-row can prevent GVHD after transplantation. Theoretically, UV treatment would allow engraftment even of HLA-incompatible marrow (because histocompatibility differences could not be recognized), and omission of postgrafting immunosuppression in vivo might accelerate immune recon-stitution. We have recently shown in a human in vivo system that it is possible to expose bone marrow cells to doses of UVB light which completely eliminate T lymphocyte func-
tion while preserving hematopoietic colony formation. Preliminary data in a murine model show that spleen cells exposed to doses that completely eliminate lymphocyte function in vitro are capable of forming normal colony forming units—spleen (CFU-S) in lethally irradiated recipients (Deeg HJ, unpublished results). Moreover, Pepino et al could show that rats transplanted with UVB treated bone marrow became healthy, stable, chimeras.

These and other ongoing studies have raised questions about the safety of UV irradiation. In one study UV irradiation was shown to activate latent viruses, even in the absence of the appropriate promoter; however, UV doses used in that study were substantially higher than those used here. Other workers have been concerned about UV-induced mutations and carcinogenesis, and mutagenesis has, indeed, been shown in various models. It is of note, however, that in a murine model transplanted UV-induced skin tumors were rejected even by syngeneic recipients, suggesting that the recipient may be able to recognize and eliminate abnor-
mal (mutated) cells. Additional studies are necessary to confirm the safety of UV-treated blood products in clinical use.

In conclusion, UV irradiation prevents in vivo sensitization and GVHD in animal models. Clinical studies aimed at exploiting this phenomenon appear warranted.

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REFERENCES


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