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

Ten dogs were given 9.2 Gy of total body irradiation and autologous bone marrow infusion followed by ten daily transfusions of leukocytes for a total of 11.5 to 36.2 (median, 18.8) x 10^9/kg obtained via leukapheresis from histoincompatible unrelated donors. Four dogs were given unirradiated leukocytes, and all developed graft-versus-host disease (GVHD). In contrast, only two of three dogs given leukocytes irradiated with 20 mJ/cm^2 of ultraviolet (UV) light (200 to 300 nm), and none of three dogs given leukocytes irradiated with 1,000 mJ/cm^2 developed GVHD. These data indicate that UV irradiation abrogates the alloreactive potential of transfused leukocytes, and suggest that UV irradiation can be used to prevent the development of transfusion-induced GVHD.

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From the Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, DC, and the Fred Hutchinson Cancer Research Center, Seattle, WA.

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Address reprint requests to H. Joachim Deeg, MD, Vancouver General Hospital, LSP, Histocompatibility, 855 W 12th Ave, Vancouver, BC V5Z 1M9, Canada.

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UV IRRADIATION PREVENTS GVHD

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 10^8 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 10^8 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 10^8 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.

DISCUSSION

With increasing awareness of the occurrence of transfusion-induced GVHD in immunosuppressed patients, gamma irradiation of blood products was introduced into clinical practice. 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. 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. Because 15% to 20% of transfused leukocytes were lymphocytes and approximately 80% of canine peripheral blood lymphocytes represent T lymphocytes, dogs received approximately 1.3 to 5 x 10^8 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). 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. The UV doses necessary to abrogate areactivity are compatible with normal or close to normal in vivo survival of RBCs or platelets. For clinical application it would be desirable to develop a closed system consisting of material that allows for sufficient transmission of UV light. On the other hand, one might argue that manipulations such as T

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**Table 1. Dogs Given Autologous Marrow Transplants and Leukocyte Transfusions From DLA-Incompatible Donors**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tx of Leukocytes</th>
<th>No. of Cells</th>
<th>Neutrophils ( \geq 0.5 \times 10^9/L ) (d)</th>
<th>Platelets ( \geq 20 \times 10^9/L ) (d)</th>
<th>BM Cellularity</th>
<th>Organ</th>
<th>Day of Onset (No. of Tx)</th>
<th>Survival (d)</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (0)</td>
<td>C495</td>
<td>32.6</td>
<td>36</td>
<td>8</td>
<td>NR</td>
<td>S, E, I</td>
<td>6 (4)</td>
<td>8</td>
<td>Septicemia</td>
</tr>
<tr>
<td></td>
<td>C500</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 K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C664</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 K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C666</td>
<td>14.7</td>
<td>8</td>
<td>20</td>
<td>100</td>
<td>S, I</td>
<td>8 (6)</td>
<td>&gt;63 K</td>
<td></td>
</tr>
<tr>
<td>II (20)</td>
<td>C553</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 K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C561</td>
<td>24.1</td>
<td>15</td>
<td>NR</td>
<td>80</td>
<td>S, I</td>
<td>8 (6)</td>
<td>21</td>
<td>Pneumonia</td>
</tr>
<tr>
<td></td>
<td>C549</td>
<td>11.3</td>
<td>11</td>
<td>18</td>
<td>100</td>
<td>—</td>
<td>NA</td>
<td>&gt;41 K</td>
<td></td>
</tr>
<tr>
<td>III (1,000)</td>
<td>C658</td>
<td>16.8</td>
<td>10</td>
<td>20</td>
<td>100</td>
<td>—</td>
<td>NA</td>
<td>&gt;99 K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C691</td>
<td>34.1</td>
<td>7</td>
<td>NA*</td>
<td>—</td>
<td>—</td>
<td>NA</td>
<td>&gt;42 K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C718</td>
<td>30.1</td>
<td>9</td>
<td>11</td>
<td>100</td>
<td>—</td>
<td>NA</td>
<td>&gt;51 K</td>
<td></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 \( 20 \times 10^9/L \).*
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- genic transfusions sensitize the autologous graft leading to an autologous GVHD-like reaction. Because UV treatment prevents sensitization it also might prevent such an autologous GVHD reaction.

In addition to the data presented here, these observations suggest the possibility that UV manipulation of donor marrow 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 reconstitution. We have recently shown in a human in vitro system that it is possible to expose bone marrow cells to doses of UVB light which completely eliminate T lymphocyte function 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-irradiated skin tumors were rejected even by syngeneic recipients, suggesting that the recipient may be able to recognize and eliminate abnormal (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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