CONCISE REPORT

Ultraviolet Irradiation of Blood Prevents Transfusion-Induced Sensitization and Marrow Graft Rejection in Dogs

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In a canine model using DLA-identical littermate pairs, we have shown that a regimen of three transfusions of donor blood given 24, 17, and 10 days before transplant uniformly leads to marrow graft rejection, presumably due to sensitization to minor (non-DLA) histocompatibility antigens. Untransfused dogs uniformly achieve sustained engraftment. In the present study, we investigated whether the exposure of blood to ultraviolet (UV) light (220–300 nm) prior to transfusion prevented sensitization of the recipient and allowed for successful marrow engraftment. Ten dogs were each given three pretransplant transfusions from the marrow donor. Each transfusion consisted of 50 mL of whole blood exposed in vitro to UV light for a total of 1.35 J/cm². All ten dogs achieved engraftment. In contrast, all four dogs that had received sham-exposed transfusions rejected their grafts. In vitro studies revealed that although cell viability was not affected, leukocytes contained in UV-exposed blood were unable to function as stimulator cells in mixed leukocyte cultures or as accessory cells in mitogen-stimulated cultures. These data are consistent with the hypothesis that accessory cells are involved in transfusion-induced sensitization. We conclude that in vitro exposure of blood to UV light before transfusion prevents sensitization and allows for subsequent marrow engraftment.

Patients receiving a marrow transplant for the treatment of severe aplastic anemia only rarely reject the graft if they are not transfused at the time of transplantation. However, patients who have received transfusions before transplantation are at a high risk for graft rejection, even if the marrow donor is an HLA-identical sibling (see review). The adverse effect of pretransplant transfusions on the outcome of marrow transplantation had been predicted from animal studies (see reviews). Untransfused dogs given 9.2 Gy of total body irradiation (TBI) and marrow grafts from siblings identical at the major histocompatibility complex (DLA) generally achieve sustained engraftment, whereas dogs transfused with whole blood from the marrow donor before grafting uniformly reject the graft as a result of transfusion-induced sensitization of the recipient against minor (non-DLA) antigens of the donor. If, however, leukocyte-depleted red blood cells (RBC) or platelets are transfused, graft rejection is significantly reduced, suggesting that leukocytes are responsible for sensitization. We hypothesized that the effect of transfused leukocytes on the recipient in vivo was comparable to the effect of stimulator leukocytes on responding cells in mixed leukocyte culture (MLC) in vitro. Since it has been shown that exposure of leukocytes to ultraviolet (UV) light abrogates their ability to function as stimulator cells in MLC, we investigated whether in vitro exposure of blood to UV light abrogated its ability to sensitize in vivo.

MATERIALS AND METHODS

Dogs. Purebred and mongrel dogs, 6 to 12 months old, were purchased from commercial kennels and cared for as described. Donor-recipient pairs were littermates, DLA-identical as determined by serologic typing for DLA-A, B, and C and mutual nonreactivity in MLC. Conditioning and marrow transplantation. Recipient dogs were prepared for transplantation by 9.2 Gy of TBI, and donor marrow cells, 2.8 ± 2.1 x 10⁶/kg, were infused within 4 hours of TBI as described. On days 1 and 2, viable leukocytes (18.2 ± 7 x 10⁶/kg), obtained from the donor via arteriovenous shunt, were infused. No postgrafting immunosuppression was given. The postgrafting care has been described. Engraftment was documented by rising granulocyte counts following the postirradiation nadir and by the presence of donor sex karyotype in metaphase spreads from bone marrow and peripheral blood or the conversion to the donor-type RBC antigen pattern. Complete autopsies were carried out in all dogs that died.

Transfusion regimen. Transfusions of 50 mL of blood from the intended marrow donor were given 24, 17, and 10 days before transplantation. Blood was drawn via venipunctune into plastic syringes containing preservative-free heparin, 10 U/mL. UV irradiation of blood. Whole blood was diluted 1:1.5 with Waymouth's minimal medium. Aliquots of 7.5 mL were placed in plastic dishes (Falcon #3003), for a layer of 1.5 mm thickness. Uncovered dishes, on a rotating platform, were exposed for 30 minutes to UV light (220–300 nm) from a germicidal lamp (General Electric) at 750 μW/cm² for 1.35 J/cm² as determined with a Black Ray shortwave UV meter (U.V. Products). Blood was then recovered from the dishes and injected into the recipient dog. Sham-irradiated blood was exposed to visible light instead of UV light.

In vitro studies. Aliquots of UV- or sham-exposed blood were fractionated on Ficoll-Hypaque gradients, and interface cells (PBMC) were processed and used in MLC and mitogen cultures as described. For further analysis, 100–300 x 10⁶ PBMC were fractionated on discontinuous Percoll gradients. The two lowest density fractions (49.6% and 51.1% Percoll) were pooled, washed twice, suspended in Waymouth's medium containing 20% normal canine serum. Purified lymphocytes were obtained from Percoll fraction 5 (F5).

RESULTS

Results are summarized in Table 1. All ten dogs given UV-exposed blood before transplantation had marrow...
engraftment. All four dogs transfused with sham-exposed cells, in a quasi-autologous MLC, functioned as potent cytotes. However, DLA-identical cells enriched for dendritic exposed leukocytes were not. As expected, no stimulation their grafts.

whereas dogs transfused with normal blood uniformly reject showing that untransfused dogs achieve engraftment, blood rejected their grafts and died with marrow aplasia. Included for comparison are results from previous studies,4 engraftment was documented by rising ‘anLocyte counts following the postirradiation nadir and by the presence of erythroid and myeloid precursor cells (total cellularity 10–25% of normal) on marrow samples obtained at autopsy. Seven dogs are surviving > 100 days; in these, sustained engraftment was documented by the presence of the donor sex karyotype in all metaphase spreads from bone marrow and peripheral blood (4 dogs) or the conversion to the donor erythrocyte antigen pattern (3 dogs).

†These dogs died on days 10, 11, 11, and 13, respectively, with septicemia. Marrow cellularity at autopsy was less than 5% in all dogs. Cells were composed of plasma cells and reticulum cells; hematopoietic precursor cells were absent. In vitro results are summarized in Fig 1. While 131I-irradiated leukocytes were potent stimulators in MLC, UV-exposed leukocytes were not. As expected, no stimulation was seen with 131I-Cs- or UV-exposed DLA-identical leukocytes. However, DLA-identical cells enriched for dendritic cells, in a quasi-autologous MLC, functioned as potent stimulators after 131I-Cs but not after UV exposure. In addition, mitogen responsiveness of accessory cell–depleted lymphocytes (F5) was restored by the addition of normal or sham-exposed PBMC but not by UV-exposed PBMC.

**DISCUSSION**

We have shown in this study that dogs transfused with UV-exposed blood from the intended marrow donor uniformly achieved sustained engraftment of a subsequent marrow graft and thus behaved like untransfused dogs. In contrast, dogs transfused with sham-exposed blood, as with unmanipulated blood, uniformly rejected their grafts. Abrogation of the sensitizing ability of blood was not due to a destruction of cells but, presumably, to a modification of their immunogenicity. In vivo RBC survival (data not shown) was comparable to that of unmanipulated RBC, and in vitro studies revealed viable (viability >90%), structurally intact leukocytes.

We have shown previously that thoracic duct lymphocytes, devoid of accessory cells, and cotton-wool nonadherent cells depleted of Ia-positive accessory cells fail to induce sensitization. We have also shown that purified monocytes are poor accessory cells, whereas dendritic cells provide potent stimulator and accessory function, which, similar to findings in other species, is abrogated by UV exposure. Lau et al9 exploited this finding in a rat model of pancreatic islet cell transplantation and showed that pretransplant exposure of islets to UV light resulted in significant prolongation of graft survival. The implication was that UV exposure inactivated dendritic cells, thus eliminating a critical dendritic cell–dependent signal (from the graft) that normally triggers graft rejection by the recipient.

The mechanism by which UV exposure modifies immunogenicity is not clear. Results in murine models suggest that the injection of UV-exposed cells leads to the activation of suppressor cells.10 In the present study, however, we found no evidence for a role of suppressor cells (data not shown). Others have shown that UV exposure interferes with interleukin production,11 but it is not clear why interleukins should not be provided in vivo by the transfusion recipient. Also, we have no evidence for a decrease of the expression of Ia-like antigen after UV exposure, as suggested by others,11 although it is possible that Ia function was altered.12 This, in turn, could result in defective presentation of minor (non-DLA) histocompatibility antigens to the recipient and a lack of sensitization. Alternatively, non-DLA antigens on donor cells could be modified by UV exposure, thus escaping recognition by the recipient. Therefore, we cannot implicate a single cell population in sensitization.

Regardless of the mechanism involved, the present results suggest that UV-exposed blood can be transfused without jeopardizing the success of a subsequent DLA-identical marrow graft. These observations may be relevant for trans-
fusion support in general. Preliminary results in a canine platelet-transfusion model indicate that UV exposure of platelets can prevent refractoriness and facilitate platelet transfusion support.\textsuperscript{14}

REFERENCES

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