CONCISE REPORT

Functional Dendritic Cells Are Required for Transfusion-Induced Sensitization in Canine Marrow Graft Recipients

By H. Joachim Deeg, Joseph Aprile, Rainer Storb, Theodor C. Graham, Robert Hackman, Frederick R. Appelbaum, and Friedrich Schuening

Untransfused dogs given 9.2 Gy total-body irradiation and hematopoietic grafts from DLA-identical littermate donors uniformly achieve sustained engraftment, whereas dogs given three transfusions (Tx) of whole blood from the intended marrow donor 24, 17, and 10 days pretransplant uniformly reject their graft. Sensitization appears to be mediated by peripheral blood mononuclear cells and can be prevented by prior irradiation of the Tx product with UV light, known to inactivate leukocytes, in particular, cells with accessory function. In the present study we investigated which leukocyte population was responsible for Tx-induced sensitization and subsequent marrow graft rejection. Surprisingly, neither monocytes nor macrophages or dendritic cells induced sensitization, and all dogs so treated achieved engraftment: however, all four evaluable dogs transfused with UV-exposed blood to which small numbers of normal dendritic cells (12.5 × 10^3/kg) were added rejected their marrow graft. Among five dogs given UV-exposed blood and normal monocytes (12.5 × 10^3/kg) only one rejected its graft, and four achieved sustained engraftment. We conclude that donor dendritic cells are necessary, albeit not sufficient for in vivo sensitization. Sensitization is prevented by elimination or inactivation of dendritic cell.

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PATIENTS requiring prolonged transfusion support frequently become allo sensitized and refractory to platelet transfusions (Tx), which renders further support difficult or impossible. In patients with severe aplastic anemia who subsequently undergo marrow transplantation, prior allo sensitization results in an increased risk of graft rejection even if the donor is an HLA-identical sibling. Patients have postulated that in most instances leukocytes contained in the Tx product are responsible for sensitization. Although it is not known which leukocyte subpopulation is involved, we have previously shown in a canine model that in vitro UV exposure rendered Tx nonimmunogenic, presumably by inactivating leukocytes. Here we present data that indicate that UV exposure mediates its effect by the inactivation of accessory cells and that the immunogenic effect of UV-exposed Tx products can be reconstituted by the addition of normal accessory cells, specifically dendritic cells (DC).

MATERIALS AND METHODS

Dogs. Purebred mongrel dogs, 6 to 12 months old, were purchased from commercial kennels or raised at the Fred Hutchinson Cancer Research Center and cared for as described. Donor-recipient pairs were DLA-identical littermates as determined by serological typing for DLA-A, -B, and -C and mutual nonreactivity of lymphocytes in mixed lymphocyte culture (MLC).3

Conditioning and marrow transplantation. Recipient dogs were prepared for marrow transplantation by 9.2 Gy total-body irradiation (TBI) as described.23 Donor marrow, 2.0 ± 1.7 × 10^6 cells/kg was given intravenously (IV) within four hours of TBI, and viable donor leukocytes, 9.2 ± 6.3 × 10^6/kg were transfused on days 1 and 2. No postgrafting immunosuppression was given. The postgrafting care has been described.1 Engraftment was documented by previously described methods.23 Complete autopsies were performed on all dogs that died.

Tx regimen. On days 24, 17, and 10 before TBI and marrow transplantation recipient dogs were given 50 mL whole blood, purified leukocytes, or both from the intended marrow donor.3 Blood was obtained by venipuncture into plastic syringes containing preservative-free heparin, 10 U/mL. Blood and leukocytes were prepared as described in the following sections.

Purification of canine leukocytes. Peripheral blood mononuclear cells (PBMC) were obtained from the interphase of Ficoll-Hypaque discontinuous density gradients (1.074) as described.4 Percoll (Pharmacia, Uppsala, Sweden) was prepared as described, and concentrations (fraction [F]) of 64% (F1), 56% (F2), 54.3% (F3), 52.7% (F4), 51.1% (F5), and 49.6% (F6) Percoll relative to the stock solution with a final layer of phosphate-buffered saline (PBS, F7) were used for gradients.

For DC enrichment light buoyant density cells (F12) were incubated overnight at 37°C in a 5% CO2/air/H2O-saturated atmosphere on plastic dishes (Falcon Labware no. 3003, Oxnard, CA) prepared as described.34 Nonadherent (NA) (F12) cells were removed and used as the “DC-enriched population.” Monocytes were obtained as described before.4 Briefly, F12 cells were suspended in Waymouth’s medium at 10 × 10^6 cells/mL. An equal volume of monoclonal antibody (DLy6, IgM, diluted 1:100) reactive with canine lymphocytes and DC was added and incubated for 30 minutes at room temperature. After the addition of rabbit complement, monocytes suspended in DR and incubated for 30 minutes at room temperature. After the addition of rabbit complement (screened for DR typing; Pel-Freeze, Rogers, AZ), incubation was continued for 60 minutes. The remaining cells (F12, DLy6 –) were washed twice, resuspended in minimal medium, and used as “monocyte-enriched cells.”

Alveolar macrophages were obtained by bronchoalveolar lavage as described.1 Under anesthesia, 200 mL of sterile saline was installed gently and aspirated under low suction. The cells were washed in Hanks’ balanced salt solution, counted, and incubated on plastic dishes as described for DC earlier. After discarding the NA
DENDRITIC CELLS CAUSE SENSITIZATION

cells, the plates were incubated with fresh medium containing 1% lido- 
caine for 15 minutes. Adherent cells were recovered by gentle 
strokes with a rubber policeman and rinsing of the plates. This 
suspension was then washed twice in Waymouth's medium and used 
as "alveolar macrophages."

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 no. 3003) for a layer 1.5 mm thick. Uncovered 
dishes on a rotating platform were exposed for 30 minutes to UV 
light (220 to 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 quantita-
tively recovered from the dishes and transfused into the recipient 
dog.

In vitro studies. Enrichment for and purity of monocytes or 
macrophages was determined by cell morphology, phagocytosis and 
cytochemistry as described.4 DC were identified by both phase-
contrast and electron microscopy as described.4 6 DC were identified 
by both phase-

RESULTS

In vivo results are summarized in Table 1. Sensitization 
appeared to be mediated by small numbers of mononuclear 
cells contained among Ficoll-Hypaque-separated peripheral 
blood leukocytes (group 1). Because we had shown pre-
viously that accessory cell-depleted peripheral blood leuko-
cytes and thoracic duct lymphocytes failed to induce 
sensitization,8 we next investigated the effect of cells with 
presumptive accessory function, ie, monocytes; however, Tx 
of neither enriched monocytes (group 2) nor macrophages (group 3), 
even at a 100-fold increase (group 2) as compared 
with the number of unfractio- 
nated mononuclear cells suffi-
cient for sensitization (group 1 13), 
resulted in marrow graft 
rejection.

 Concurrent in vitro studies showed that monocyte/macro-
phage populations after the enrichment procedure as used for 
these studies no longer functioned as accessory cells in 
mitogen-driven cultures.4 Rather, accessory function was 
mediated by other light bouyant density cells with the 
appearance of DC, which had been eliminated in the process 
of monocyte enrichment.6 However, two dogs transfused with 
purified DC also achieved sustained engraftment (group 4).

We then hypothesized that antigen-presenting cells such as 
monocytes and DC were necessary but not sufficient to 
induce sensitization. Therefore, dogs were given three Tx of 
UV-exposed blood (nonsensitizing)2 to which autologous 
non–UV-exposed accessory cells were added. As shown in 
Table 1, among five dogs given monocytes (group 5) one 
rejected the graft, whereas four had sustained engraftment.

In contrast, among dogs given DC (group 6), four unequivo-
cally rejected their grafts. One dog was killed on day 34 when 
its platelet count was 1 × 10⁹/L, its WBC count was 2.1 × 10⁹/L, 
and the marrow showed 30% cellularity. No markers 
to distinguish donor and host cells were available, and it was 
impossible to determine whether the dog's incomplete hema-

topoietic recovery was autologous or donor derived.

DISCUSSION

Dogs given three Tx of whole blood from the marrow 
donor before TBI uniformly reject their grafts,3 whereas dogs 
transfused with UV-exposed blood uniformly achieve sus-
tained engraftment.2 The present study shows that the addi-
tion of functional accessory cells, specifically DC, to UV-
exposed blood reconstitutes the sensitizing ability of the Tx 
product whereas the Tx of accessory cells by themselves did not 
result in graft sensitization. Presumably antigens 
expressed on cells other than DC and not affected by UV 
exposure are also necessary for sensitization to occur. 
These observations provide further evidence that UV exposure 
renders blood nonimmunogenic through inactivation of 
accessory cells, specifically DC.

Table 1. Results in Dogs Given 9.2-Gy TBI and Hematopoietic Grafts From DLA-Identical Littermates 

<table>
<thead>
<tr>
<th>Group</th>
<th>Type of Transfusion (Number of Cells/kg)</th>
<th>Number of Dogs</th>
<th>With Graft Rejection</th>
<th>With Engraftment</th>
<th>Percent Engraftment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBMC (FH)*</td>
<td>12.5 × 10⁸</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Monocytes†</td>
<td>12.5 × 10⁸</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Alveolar macrophages‡</td>
<td>12.5 × 10⁸</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>DC</td>
<td>12.5 × 10⁸</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Whole blood</td>
<td>UV exposed + monocytes‡</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Whole blood</td>
<td>UV exposed + DC§</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Obtained from the interphase of Ficoll-Hypaque (FH) gradients. Dogs in this group were included in a previous report.8
† Cells consisted of 80% ± 12% monocytes and 19% ± 5% lymphocytes and granulocytes; no DC were identified.
‡ Cells consisted of 88% ± 7% macrophages and 11% ± 10% lymphocytes.
§ Cells consisted of 25% ± 13% DC and 2% ± 3% monocytes; the remainder were lymphocytes and granulocytes.
|| One dog was killed on day 34 with minimal hematopoietic reconstitution and could not be evaluated for engraftment (see text).
Lechler and Batchelor observed that rats accepted DC-depleted kidney grafts but rejected grafts when reconstituted with DC. Lau et al reported that rat pancreatic islet cells UV exposed in vitro survived in the recipient for prolonged periods of time whereas untreated islet cells were rejected promptly. These investigators interpreted their results as an indication that functional DC provided a crucial signal resulting in rejection of the graft.

In in vitro studies, canine lymphocytes, after triggering by mitogen or allogeneic cells, form dense clusters with DC before blast transformation and proliferation. UV exposure of DC before use in these assays completely prevents cluster formation and lymphocyte proliferation. Conceivably the loss of DC function was related to a disappearance of class II histocompatibility antigens from the cell surface; however, since class II antigen expression decreases only gradually over time, it is likely that other signals, also affected by UV exposure, play a role during the early steps of coculture and cluster formation. Almost instantaneously UV exposure results in a profound inhibition of intracellular Ca\(^{2+}\) mobilization, an early signal of cell activation, both in T lymphocytes and in accessory cells. Because cluster formation is Ca\(^{2+}\) dependent (unpublished observations), it is conceivable that UV exposure prevents cluster formation through Ca\(^{2+}\) inhibition. In agreement with this notion is the observation that the addition of calcium channel blockers prevents cluster formation and proliferation even in the presence of normal DC. Therefore, the addition of normal DC to UV-exposed blood would reestablish conditions for normal interaction of accessory cells and antigen and lead to sensitization.

In conclusion, functional DC in the Tx product are responsible for Tx-induced sensitization. Sensitization can be prevented by prior UV irradiation, presumably by interference with antigen presentation to the recipient.

**REFERENCES**

Functional dendritic cells are required for transfusion-induced sensitization in canine marrow graft recipients

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