Allogeneic Blood Transfusion-Induced Enhancement of Tumor Growth: Two Animal Models Showing Amelioration by Leukodepletion and Passive Transfer Using Spleen Cells

By Morris A. Blajchman, Leslie Bardossy, Raleigh Carmen, Alison Sastry, and Dharam P. Singal

Allogeneic blood transfusions have been reported to induce immunomodulation in recipients of blood products. While the mechanism(s) of this immunomodulatory effect is unknown, it has been suggested that this effect of allogeneic blood transfusions could adversely affect patients with a malignant disorder. These concerns have been supported by a number of nonrandomized, mainly retrospective, clinical studies which indicate that allogeneic blood transfusions can adversely affect prognosis following the surgical treatment of oncology patients. Recently, we have shown that allogeneic blood transfusions enhance primary tumor growth and increase metastatic pulmonary nodule formation in inbred mice. The tumor growth-promoting activity of allogeneic blood transfusions was studied also using outbred rabbits. In this present study, we demonstrate that the tumor growth-promoting effect of allogeneic blood transfusions is mediated by donor leukocytes and that this effect can be abolished by their removal before transfusion. We show also that the allogeneic blood transfusion tumor growth-promoting effect can be passively transferred to naive animals (both mice and rabbits) using spleen cells from allogeneically transfused animals. In these experiments, numbers of metastatic pulmonary nodules were significantly increased in both mice and rabbits that had received spleen cells from allogeneically transfused animals compared with those that had received spleen cells from syngeneically transfused animals, or from animals that had been transfused with leukodepleted allogeneic blood.

To ascertain the mechanism of the tumor growth-promoting effect, we determined the effect of leukodepleting the allogeneic blood before transfusion. In addition, we examined whether this effect could be passively transferred to naive animals using spleen cells from allogeneically transfused animals. Evidence is provided, in both animal models, that the tumor growth-promoting effect of allogeneic blood transfusions can be abolished by leukodepleting the blood before transfusion; and that this effect can be passively transferred to naive animals, using spleen cells from allogeneically transfused animals.

MATERIALS AND METHODS

Effect of blood transfusions on numbers of metastatic pulmonary nodules in mice. Adult male mice of the C57Bl/6J (H-2b) and the BALB/c (H-2d) strains were purchased from the Jackson Laboratory (Bar Harbor, ME). Only male animals were used in these studies to avoid the potential confounding problem of alloimmunization due to a pregnancy. Blood was collected from both strains of mice, into preservative free heparin (1 U/mL), and transfused intravenously directly into the tail vein of recipient C57Bl/6J animals, within an hour of collection. Recipient C57Bl/6J mice were transfused with 0.2 mL of fresh whole syngeneic (C57Bl/6J), allogeneic (BALB/c), or leukodepleted allogeneic (C57Bl/6J) blood, on two occasions (days 0 and 3). Leukodepletion of the allogeneic blood was accomplished using second generation leukocyte filters (Leukotrap Red Cell Storage System; Cutter Biological, Berkeley, CA), as described previously for rabbits. Each filter consists of cellulose acetate fiber in a polycarbonate housing. Appropriate filters with 1 to 5 mL capacity were constructed specifically for these studies by Miles, Inc (Berkeley, CA). On day 10, an intravenous infusion of 2.5 × 10⁶ methylcholanthrene-induced fibrosarcoma (FSL-10) cells, which are syngeneic (H-2b) to C57BL/6J mice, were administered to recipient mice. These animals were killed 21 days later, and the number of metastatic pulmonary nodules counted, as described previously. In all experiments, the enumeration of the pulmonary metastatic nodules performed by an individual who had no knowledge of the source of the blood product received by that animal.

Effect of blood transfusions on numbers of metastatic pulmonary nodules in rabbits. Two different outbred strains of rabbits were used for these studies. California Black (CB) rabbits were used as the allogeneic blood donors and New Zealand White (NZW) rabbits were supported in part by a grant from the Miles/CRCS Research and Development Fund.
used as recipients. The animals were purchased by the Animal Care Facility at McMaster University Medical Centre (Hamilton, Ontario). Again, only male animals were used in all experiments, to avoid the potential confounding problem of alloimmunization due to a pregnancy. Blood was collected, prepared, and administered as described previously. In these experiments, subject NZW rabbits received two intravenous infusions of either syngeneic (NZW) blood, allogeneic (CB) blood, or leukodepleted allogeneic (NZW) blood before receiving an intravenous infusion of VX-2 tumor cells, obtained and propagated as reported by Stetson et al. In these experiments, 15 mL of blood was administered intravenously on each of days 0 and 3. On day 10, VX-2 tumor cells were intravenously administered via a marginal ear vein to recipient NZW rabbits. Twenty-eight days later the animals were killed and the number of metastatic pulmonary nodules counted, as described for the murine experiments. Again, the enumeration of the pulmonary metastatic nodules was performed by an observer without knowledge of the treatment received by that animal.

Effect of blood transfusions on numbers of pulmonary metastases in rabbits. The leukodepletion of the rabbit fresh whole blood was 99.8% effective (mean leukocyte count reduction: 4.595 to 0.009 x 10^9/L). In the rabbit model, allogeneic whole blood also caused a significant (P = .01) enhancement in the number of metastatic pulmonary nodules observed, when compared with rabbits that had received syngeneic whole blood (see Table 2). The leukodepletion of the allogeneic whole blood, before its transfusion, also resulted in a significant (P = .0001) amelioration of the effect of the allogeneic blood transfusion.

The effect of the transfer of spleen cells on the observed numbers of pulmonary metastatic nodules. Table 3 shows the results of three spleen cell transfer experiments from transfused mice to naive animals. In these experiments, the numbers of metastatic nodules were significantly increased in those mice that had received spleen cells from allogeneically transfused animals, compared with those that had received spleen cells from syngeneically transfused animals (P = .0001), or from animals that had been transfused with leukodepleted allogeneic blood (P = .0006).

Similar data were obtained in an experiment in which spleen cells from allogeneically transfused rabbits (n = 12 per group) were passively transferred to naive rabbits (data not shown). In this experiment, the number of metastatic nodules in rabbits that had received spleen cells from allogeneically transfused animals were significantly greater (P = .0015) than those that had received spleen cells from syngeneically transfused animals.

### Table 1. Effect of Syngeneic, Allogeneic, and Leukodepleted Allogeneic Blood Transfusions on Numbers of Pulmonary Metastases in Transfused C57B1/6J Mice

<table>
<thead>
<tr>
<th>Type of Blood Transfusion</th>
<th>No. of Mice</th>
<th>No. With Metastatic Tumors</th>
<th>No. of Pulmonary Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic (a)</td>
<td>22</td>
<td>18</td>
<td>1.0 0–14</td>
</tr>
<tr>
<td>Allogeneic (b)</td>
<td>34</td>
<td>30</td>
<td>5.0 0–41</td>
</tr>
<tr>
<td>Leukodepleted allogeneic (c)</td>
<td>35</td>
<td>25</td>
<td>1.0 0–11</td>
</tr>
</tbody>
</table>

* Statistical significance (Mann-Whitney test) (a) v (b), P = .01; (b) v (c), P = .0002; (a) v (c), not significant.

### Table 2. Effect of Syngeneic, Allogeneic, and Leukodepleted Allogeneic Blood Transfusions on Numbers of Pulmonary Metastases in Transfused New Zealand White Rabbits

<table>
<thead>
<tr>
<th>Type of Blood Transfusion</th>
<th>No. of Rabbits</th>
<th>No. With Metastatic Tumors</th>
<th>No. of Pulmonary Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic (a)</td>
<td>36</td>
<td>36</td>
<td>9.0 3–87</td>
</tr>
<tr>
<td>Allogeneic (b)</td>
<td>36</td>
<td>36</td>
<td>15.0 3–143</td>
</tr>
<tr>
<td>Leukodepleted allogeneic (c)</td>
<td>23</td>
<td>23</td>
<td>6.0 1–18</td>
</tr>
</tbody>
</table>

* Statistical significance (Mann-Whitney Test) (a) v (b), P = .01; (b) v (c), P = .0001; (a) v (c), not significant.

### Table 3. Effect on Naive C57B1/6J Mice of the Passive Transfer of Spleen Cells from Syngeneically, Allogeneically, or Leukodepleted Allogeneically Transfused C57B1/6J Mice

<table>
<thead>
<tr>
<th>Source of Spleen Cells</th>
<th>No. of Mice</th>
<th>No. With Metastatic Tumors</th>
<th>No. of Pulmonary Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneically transfused mice (a)</td>
<td>16</td>
<td>16</td>
<td>8.5 1–31</td>
</tr>
<tr>
<td>Allogeneically transfused mice (b)</td>
<td>18</td>
<td>18</td>
<td>63 14–174</td>
</tr>
<tr>
<td>Leukodepleted allogeneically transfused mice (c)</td>
<td>10</td>
<td>10</td>
<td>17 4–37</td>
</tr>
</tbody>
</table>

* Statistical significance (Mann-Whitney Test) (a) v (b), P = .0001; (b) v (c), P = .0006; (a) v (c), not significant.
DISCUSSION

The present data, from both inbred and outbred animal models, demonstrate that the tumor growth-promoting effect of allogeneic blood transfusion is mediated by donor leukocytes. Thus, the tumor growth-promoting effect of allogeneic blood transfusion is ameliorated by the removal of the donor allogeneic leukocytes before transfusion. In addition, the results show that this effect can be passively transferred to naive animals using spleen cells from allogeneically transfused animals. These data suggest that the tumor growth-promoting effect of allogeneic blood transfusions is mediated immunologically.

Immunological mechanisms have been proposed previously to explain the enhanced renal allograft survival seen in patients undergoing allogeneic renal transplantation.5,9,21,22 This postulated immunomodulatory effect of allogeneic leukocytes has also been used advantageously in clinical transplantation and the treatment of women with spontaneous recurrent abortions.4,6,21 We extend these observations to clearly demonstrate that this immunomodulatory effect is also operable in patients with a malignancy.

This is the first report that demonstrates clearly that the tumor growth-promoting effect of allogeneic blood transfusion is related to the presence of donor leukocytes in the allogeneic blood. These data therefore provide new insights into the mechanism of the tumor growth-promoting effect of allogeneic blood transfusions. More importantly, it provides information as to how to potentially ameliorate this deleterious effect.

A number of reports have provided evidence for the deleterious effects of allogeneic blood transfusions on the recurrence of a malignancy in patients undergoing curative surgery.1,3,8,12 The results of the present study indicate that this deleterious effect on tumor recurrence might be avoided by the leukodepletion of the allogeneic blood before transfusion. Although the amelioration, by leukodepletion, of tumor growth has not yet been reported in humans, a recent prospective randomized study demonstrated a statistically significant abrogation of the incidence of postoperative infection in colorectal cancer patients who received leukodepleted blood, compared with those that received nonleukodepleted blood.23 Thus, while data from experimental animals cannot necessarily be extrapolated to the clinical situation, these present studies suggest that patients with a malignant disorder are likely to benefit from the transfusion of leukodepleted blood products, to avoid the potential deleterious tumor growth-promoting effect of allogeneic blood transfusions.

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REFERENCES

Allogeneic blood transfusion-induced enhancement of tumor growth: two animal models showing amelioration by leukodepletion and passive transfer using spleen cells [see comments]

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