Effect of Blood Transfusion in an Experimental Sarcoma Model

Ho-Sheng Lin, MD; Ravi N. Samy, MD; Joanne Lum, BS; Mary Jo Dorie, PhD; David J. Terris, MD

Objective: To study the effect of allogeneic, syngeneic, and autologous blood transfusion on the growth rate of the KHT tumor in a C3H murine model.

Design: Prospective, randomized, and controlled animal study.

Subjects: Sixty-one C3H female mice.

Interventions: The C3H female mice were implanted with $2 \times 10^5$ cells of KHT, a murine sarcoma. Ten days later, 0.3 mL of blood was removed from a retro-orbital site to simulate surgical blood loss. This blood loss was replaced by blood transfusion through a tail vein with the use of allogeneic (major histocompatibility complex incompatible), syngeneic (major histocompatibility complex compatible), or autologous blood. Tumor growth was measured daily for 14 days. The tumor growth curve for each of the animals was constructed and the mean slope of growth calculated for each group.

Results: There were statistically significant differences in tumor growth rate ($P = .001$) when the allogeneic group (mean slope=0.232, n=14), the syngeneic group (mean slope=0.190, n=17), and the autologous group (mean slope=0.202, n=14) were compared. A t test confirmed that there was no significant difference in the tumor growth rate between the groups transfused with syngeneic and autologous blood ($P = .26$). However, the rate of tumor growth in the allogeneic group was found to be significantly higher when independently compared with the syngeneic group ($P < .001$) and the autologous group ($P = .02$).

Conclusions: In this experimental model of a solid murine sarcoma, allogeneic blood transfusion was associated with an increased rate of tumor growth compared with syngeneic and autologous blood transfusion, likely reflecting immunomodulatory effects incurred by the introduction of major histocompatibility complex–incompatible antigens.


TRANSFUSION OF BLOOD from allogeneic (heterologous) donors has a long and interesting history in the practice of medicine. Before the ABO blood group system was discovered by Landsteiner a century ago,1 transfusions were considered ineffective and dangerous. The recognition of the ABO and Rh blood groups and development of preservation techniques for storing allogeneic blood led to the increased application of transfusion therapy with dramatic reductions in morbidity and mortality from hemorrhage during surgery. The impact of blood transfusions on the recipient’s cellular immune system was appreciated in the 1970s, when the beneficial effect of pretransplant allogeneic transfusion on renal transplant survival was described.2 Since the 1980s, the association between transfusion and increased cancer recurrence and decreased survival has been reported in a number of epidemiologic and experimental studies.3,8 Although the preponderance of data suggests an adverse association between allogeneic blood transfusion and cancer outcome, this association remains inconclusive. Nevertheless, this potential adverse effect combined with the small risk of infectious transmission has heightened interest in the use of autologous blood transfusion. The advantages of autologous blood transfusion with respect to cancer outcomes have been suggested in clinical reports.3,8

There is evidence to suggest that the deleterious effect of allogeneic blood transfusion is due to immunosuppression caused by the infusion of incompatible major histocompatibility complex (MHC) antigens.3,8,11 The more dissimilar the MHC antigens between donor and recipient, the greater the immunosuppression and, therefore, the greater the likelihood of adverse effects. This may account for some of the
MATERIALS AND METHODS

EXPERIMENTAL PROTOCOL

Sixty-one C3H female mice were used in this study. The skin over the posterior spine region of these mice was inoculated subcutaneously with $2 \times 10^5$ KHT murine tumor cells. Ten days after tumor implantation, 0.3 mL of blood was removed with the mouse under anesthesia from the plexus of veins in the medial aspect of the orbit with a capillary tube containing preservative-free heparin (1 U/mL) to simulate surgical blood loss. After 1 hour, this blood loss was replaced by blood transfusion through a tail vein. These mice were randomized to receive 1 of 3 different types of transfusions, with the investigator blinded to the identity of the transfusion. The first group ($n=18$) received 0.3 mL of blood removed from C57Bl, a different strain of mice (allogeneic blood transfusion). The second group ($n=23$) received 0.3 mL of blood removed from other C3H mice (syngeneic transfusion). The third group of mice ($n=20$) received the same 0.3 mL of blood that had been removed from them 1 hour earlier (autologous transfusion). Tumor growth was then measured daily. All animals were humanely killed 14 days after transfusion and a final measurement was obtained.

ANIMALS

The C3H female mice and C57Bl mice (The Jackson Laboratory, Bar Harbor, Me) ranged in age from 10 to 11 weeks. The mean weight of the mice was 24.2±1.8 g, which correlates with a total blood volume of 1.7±0.1 mL. The surgical blood loss of 0.3 mL in these mice is therefore equivalent to a blood loss of 80±66 mL in a 70-kg human. The human MHC is equivalent to the murine histocompatibility-2 (H-2) complex. The C3H mice have the major H-2 complex antigens H-2k/H-2k, whereas the C57Bl mice have the major H-2 complex antigens H-2b/H-2b. They were housed in a climate-controlled, virus-free environment with regulated light-dark cycles and permitted free access to standard pellet chow and water.

The allogeneic blood transfusion was achieved by transfusion of major H-2-incompatible blood (H-2b/H-2b) from C57Bl mice to C3H mice (H-2k/H-2k) (Figure 1A). Syngeneic blood transfusion was achieved by transfusion of major H-2-compatible blood from one C3H mouse (H-2k/H-2k) to another C3H mouse (Figure 1B). These are inbred mice that have the same major H-2 complex antigens but may differ in minor histocompatibility complex antigens. Finally, autologous blood transfusion (transfusion of the mouse’s own blood; Figure 1C) represented preservation of the same major and minor histocompatibility complex antigens. This study was approved by the Stanford University Administrative Panel on Laboratory Animal Care, Stanford, Calif, and strict guidelines for the care and use of laboratory animals were followed.

TUMOR

The tumor cells were originally derived from the mouse KHT sarcoma line. They were maintained in culture in α-minimal essential medium supplemented with 10% fetal calf serum, 1% l-glutamine, and penicillin-streptomycin in 80-cm² plastic tissue culture flasks and cultured in a humidified incubator with 5% carbon dioxide at 37°C. The cells were harvested by trypsinization for 3 to 5 minutes. The cell suspension was washed in Hanks solution and centrifuged. The cell pellet was then resuspended in Hanks solution and counted with a hemocytometer. The cell solution was then diluted to approximately 4×10⁶ cells per milliliter. The cell viability was checked by means of eosin exclusion. The cell suspension was then maintained in ice until injection. A total of $2 \times 10^5$ KHT murine tumor cells (0.05 mL) were injected intradermally.

ASSESSMENT OF TUMOR GROWTH

After transfusion, the size of the tumor was measured daily for 14 days. Since the tumor shape approximated a sphere (Figure 2), the volume was calculated by means of the method of Attia and Weiss: $V = (0.4/3) \times (ab^2)$, where $V$ is tumor volume in cubic millimeters, $a$ represents the length of the major axis, and $b$ represents the length of the minor axis.

STATISTICS

The Microsoft Excel statistical program (Microsoft Corp, Redmond, Wash) was used to generate the growth curve and to calculate the slope of tumor growth in each animal. The slopes were then averaged to determine the mean slope of growth for each of the 3 groups, and SDs were calculated. Any individual slope more than 2 SDs away from the mean value of the slope was eliminated from the final statistical calculation to minimize the incorporation of aberrant values stemming from unrecognized cannibalization of tumors. An analysis of variance was used to detect significant differences in tumor growth rates among the 3 groups. Differences between pairs of groups were then evaluated with a paired, 2-tailed t test.

RESULTS

Data from 45 of the 61 mice were able to be evaluated. Twelve mice (3 from the allogeneic group, 5 from the syngeneic group, and 4 from the autologous group) died of various causes before the end point of the experiment was reached (14 days after transfusion). The size of the tumor could not be determined in 2 mice because of tumor cannibalism (1 from the allogeneic group and 1 from the syngeneic group). Finally, the data from another 2 mice (1 from the syngeneic group and 1 from the autologous group) were eliminated from evaluation because they were more than 2 SDs outside the mean of the growth slope.

The mean slope of the growth curve was calculated to be $0.232±0.035$ for the allogeneic group ($n=14$),...
0.190±0.025 for the syngeneic group (n=17), and 0.202±0.032 for the autologous group (n=14) (Table and Figure 3). The analysis of variance showed the difference in the value of the mean slope between the 3 groups to be statistically significant (P=.001). Further analysis with the t test showed no significant difference in mean slope when the syngeneic and autologous transfusion groups were compared (P=.26). Both of these groups were transfused with blood containing the same major H-2 complex antigens. The allogeneic group, which was transfused with blood containing incompatible major H-2 complex antigens, was associated with a faster rate of tumor growth, with a mean growth slope of 0.232. This value was significantly higher than the mean slope of 0.190 for the syngeneic group (P<.001) and the mean slope of 0.202 for the autologous group (P=.02).

### COMMENT

A large body of clinical and experimental evidence supports an association between allogeneic blood transfusion and poor cancer outcome.3,6-8 However, the effect of autologous blood transfusion on patients undergoing curative resection of cancer is less clear. The often contradictory literature on this issue likely reflects the poor understanding of the complex immunomodulatory processes associated with blood transfusion.

The first evidence pointing to the immunosuppressive effect of blood transfusion was reported in the transplant literature when it was noted that allogeneic transfusion improved renal transplant survival.1,2 Since then, multiple clinical and laboratory studies have demonstrated transfusion-induced up-regulation of suppressor T-cell activity and down-regulation of natural killer cell activity, cytotoxic T-lymphocyte antitumor activity, and T-cell proliferative activity and decreased secretion

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Allogeneic (n = 14)</th>
<th>Syngeneic (n = 17)</th>
<th>Autologous (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.243</td>
<td>0.203</td>
<td>0.186</td>
</tr>
<tr>
<td>2</td>
<td>0.233</td>
<td>0.166</td>
<td>0.187</td>
</tr>
<tr>
<td>3</td>
<td>0.297</td>
<td>0.209</td>
<td>0.152</td>
</tr>
<tr>
<td>4</td>
<td>0.272</td>
<td>0.156</td>
<td>0.248</td>
</tr>
<tr>
<td>5</td>
<td>0.180</td>
<td>0.211</td>
<td>0.149</td>
</tr>
<tr>
<td>6</td>
<td>0.191</td>
<td>0.138</td>
<td>0.201</td>
</tr>
<tr>
<td>7</td>
<td>0.228</td>
<td>0.225</td>
<td>0.184</td>
</tr>
<tr>
<td>8</td>
<td>0.261</td>
<td>0.211</td>
<td>0.215</td>
</tr>
<tr>
<td>9</td>
<td>0.228</td>
<td>0.179</td>
<td>0.237</td>
</tr>
<tr>
<td>10</td>
<td>0.204</td>
<td>0.226</td>
<td>0.218</td>
</tr>
<tr>
<td>11</td>
<td>0.261</td>
<td>0.190</td>
<td>0.191</td>
</tr>
<tr>
<td>12</td>
<td>0.223</td>
<td>0.196</td>
<td>0.195</td>
</tr>
<tr>
<td>13</td>
<td>0.243</td>
<td>0.206</td>
<td>0.213</td>
</tr>
<tr>
<td>14</td>
<td>0.182</td>
<td>0.193</td>
<td>0.254</td>
</tr>
<tr>
<td>15</td>
<td>0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.168</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.193</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean slope</td>
<td>0.232</td>
<td>0.190</td>
<td>0.202</td>
</tr>
<tr>
<td>SD</td>
<td>0.035</td>
<td>0.025</td>
<td>0.033</td>
</tr>
</tbody>
</table>

*The equation used for the calculation is \( y = (c)(e^b x) \), where \( b \) = slope, \( e \approx 3.14, \( c \) = 0 intercept, \( x \) = day number, and \( y \) = tumor volume in cubic centimeters.
of cytokines.\textsuperscript{1,4,5,14,15} Evidence then accumulated that the immunosuppressive effect of allogeneic blood transfusion may be due to the infusion of incompatible MHC antigens present on the leukocytes.\textsuperscript{16} For example, suppression of T-lymphocyte-mediated renal graft rejection occurred only when leukocytes were present in the transfused blood. Since MHC antigens are not present on erythrocytes, leukocyte-depleted blood failed to exert any immunosuppressive effect in protecting grafts.\textsuperscript{16} Furthermore, several studies have shown that the reduction or elimination of leukocytes from allogeneic blood, or the use of autologous blood, prevents the negative impact on cancer recurrence and survival associated with blood transfusion.\textsuperscript{3,6,8}

We set out in this study to investigate the effect of transfusing blood of different histocompatibility complex antigens on the growth rate of tumor in a murine model. Our data demonstrated an association between transfusion of incompatible major H-2 complex antigens and faster rate of tumor growth (allogeneic group). Conversely, transfusion of compatible major H-2 complex antigens was associated with a slower rate of tumor growth (syngeneic and autologous groups). In addition, our data confirmed the absence of a statistically significant difference in tumor growth rate between the syngeneic and autologous groups. Both groups received blood containing the same major H-2 complex antigens, but with some differences in the minor H-2 complex antigens. This finding suggests that the deleterious effect of blood transfusion is primarily due to the infusion of incompatible major H-2 complex antigens. This is supported by a study on the growth of MH 134 hepatoma, in which C3H mice were transfused with either syngeneic blood from C3H mice (H-2k/H-2k) or allogeneic blood from BDF mice (H-2b/H-2d) or AKR mice (H-2k/H-2k).\textsuperscript{11} The authors described a significant increase in the growth rate of hepatoma in the group that was transfused with incompatible major H-2 blood from BDF mice. On the other hand, the groups that received the blood with compatible major H-2 complex antigens from C3H mice or AKR mice had slower tumor growth.

A number of clinical studies have concluded that the benefits of autologous transfusion are maintained in the human model.\textsuperscript{3,6,8} We previously published a retrospective analysis of the effect of blood transfusion on recurrence rate in 165 patients with squamous cell carcinoma of the head and neck treated surgically at Stanford University Medical Center.\textsuperscript{3} Patients who received allogeneic blood transfusion had a 59% recurrence rate compared with 33% and 35% recurrence rates for patients who received autologous blood and no transfusion, respectively. Similar findings were reported with a prospective, randomized clinical trial comparing allogeneic and autologous blood transfusions in 120 patients with colorectal cancer.\textsuperscript{17} The relative risk of recurrence was 0.96 in the group transfused with autologous blood, in contrast to the group transfused with allogeneic blood, which had a relative risk of recurrence of 7.01.

The conclusion that autologous blood transfusion does not impact cancer outcomes is not uniformly accepted, however.\textsuperscript{8,20} Busch et al.\textsuperscript{20} for example, reported on a prospective, randomized clinical trial involving 475 patients with colon cancer and concluded that both autologous and allogeneic blood transfusions increased the risk of cancer recurrence. Survival rates were 67% for the allogeneic transfusion group, 62% for the autologous transfusion group, and 88% for the group of patients without transfusion. Unfortunately, this study was confounded by the fact that a third of the patients in the autologous group also received allogeneic blood (since they required more than the 2 U of autologous blood that was donated preoperatively). When these patients with a mixed transfusion profile were excluded from consideration, the autologous recipients had a 15% lower recurrence rate and 23% lower death rate than the allogeneic group.

Further distorting the interpretation of clinical trials is the fact that, although whole blood transfusion has been used in most animal studies, the blood products used in prospective and retrospective clinical trials reported from different institutions have been variable and sometimes undefined. The blood components transfused to patients have changed during the evolution of transfusion therapy and blood storage. Twenty years ago, transfusions were often of whole blood, containing $2 \times 10^9$ to $3 \times 10^9$ white blood cells (WBCs) per unit.\textsuperscript{19} Patients now receive red blood cell transfusions that are partially leukocyte depleted and vary in the amount of leukocyte concentration, depending on the filtration method.\textsuperscript{4,21-23} Low-performance leukodepletion methods such as the buffy-coat method widely used in Europe reduce the residual WBC count to between $10^6$ and $10^8$ per unit (10- to 100-fold reduction of WBCs found in whole blood). High-performance leukodepletion methods can reduce the residual WBC count to between $1 \times 10^6$ and $5 \times 10^6$ per unit (1000-fold reduction). The detrimental effect on cancer recurrence and survival rates associated with MHC-incompatible blood transfusion suggested a possible beneficial effect from reducing leukocyte content in allogeneic blood transfusion.\textsuperscript{8} Recently, mainly because of the fear of Creutzfeldt-Jakob disease, several European nations have implemented universal WBC reduction to less than $1 \times 10^6$ WBCs per unit.\textsuperscript{21-23} Transfusion of this leukocyte-depleted blood may ameliorate the potentially deleterious effect on tumor growth by minimizing the infusion of incompatible MHC antigens.
Our data suggest that the adverse effect of blood transfusion on cancer outcome is likely due to the infusion of incompatible MHC antigens. However, caution must be exercised in extrapolating findings from animal studies to clinical situations. Although animal studies have historically measured the growth rate of implanted cancer cells, clinical trials typically assess recurrence, metastasis, and survival rates after surgical resection. These end points measured by clinical trials are obviously much more complex and can be confounded by multiple variables. Nevertheless, the clinical and experimental data available support the use of either autologous blood or leukocyte-depleted allogeneic blood in place of the conventional allogeneic blood for transfusion in cancer patients.

Accepted for publication August 16, 2001.
This study was presented at the annual meeting of the American Head and Neck Society, Palm Desert, Calif, May 14, 2001.

Corresponding author and reprints: David J. Terris, MD, Division of Otolaryngology/Head & Neck Surgery, Edwards Building, R135, Stanford University Medical Center, Stanford, CA 94305-5328 (e-mail: dterris@stanford.edu).

REFERENCES