Donor-specific transfusion via the portal venous route induces prolongation of H-2-compatible but not H-2-incompatible cardiac graft survival

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Abstract. In the H-2-compatible donor-recipient combination (BALB/c→DBA/2), pretransplant donor-specific blood transfusion (DST) via the portal venous (PV) route significantly prolonged cardiac graft survival. DST via the intravenous (IV) route (systemic circulation) also showed a marked prolongation of heart tissue transplant survival in this model. In the H-2-incompatible combination (BALB/c→CBA/H), DST via the IV - but not via the PV - route resulted in accelerated graft rejection.

Key words: Transfusion effect, intraportal route - Transfusion effect, experimental, heart - Heart transplantation, experimental - Portal venous route, transfusion effect.

It has been documented both in animal models and in clinical studies that pretransplant donor-specific blood transfusion (DST), with [15, 16, 33, 36] or without [8, 17, 24, 29] concurrent immunosuppression, exerts a beneficial effect on the survival of allografts. However, the immunologic mechanisms implicated in mediating this beneficial effect have not been fully elucidated, although several hypotheses have been presented [4, 18, 23].

A number of studies have suggested that orally presented antigen has been associated with suppression of systemic immune responses [3, 5, 35], a phenomenon referred to as oral tolerance. Observations showing that allogeneic cells injected into the portal circulation induced systemic hyporesponsiveness to the appropriate alloantigens [26, 27], as well as data suggesting increased responses to a variety of antigens after disruption of hepatic architecture [12, 34], have tended to support the notion that the liver plays an important role in the induction of oral tolerance. This phenomenon may be potentially useful in preventing the rejection of transplanted tissue.

In the present study, we examined the effect of pretransplant DST via the portal venous route on allogeneic graft survival in mice.

Materials and methods

Animals

The following inbred mouse strains were used: BALB/c (H-2d), DBA/2 (H-2b), and CBA/H (H-2k). Breeding pairs were originally obtained from Dr. A.Czarnomska (Institute of Oncology, Warsaw, Poland). All mice were kept in standard conditions during the experimental period. Recipients (DBA/2 and CBA/H) were 12-14 weeks old and heart donors (BALB/c) 2-4 days old at the time of grafting. Only males were used as graft recipients.

Blood transfusions

Recipient mice were given blood on the -9th day before grafting. Transfusions of 0.25 ml fresh, sex-matched, heparinized blood were given via the tail vein or via the portal venous route.

Injection via portal venous route

Animals were anesthetized with chloral hydrate given IP. A right side abdominal incision was made and the viscera exposed. Blood was slowly injected through a superior mesenteric vein with a 27-gauge needle. The hemostasis was performed using a cotton wool swab. Mice were occasionally excluded because of bleeding. The abdominal incision was secured with a chromic catgut 4/0 suture (Ethicon, Edinburgh, UK).

Sham operation

The sham operation was performed step-by-step like injection via the portal venous route except that mice were given 0.25 ml heparinized physiological saline.
Table 1. The effect of pretransplant donor-specific blood transfusion (DST) via the portal venous (PV) or the intravenous (IV) route on cardiac allograft survival in H-2 compatible donor-recipient combination (BALB/c→DBA/2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Recipient pretreatment</th>
<th>Graft survival (days)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DST PV</td>
<td>7, 8, 10, 11, 11, 12, 13, 14, 15, 16, 18, 20, 28, &gt;100, &gt;100, &gt;100, &gt;100, &gt;100</td>
<td>1 vs 2: &lt;0.005, 1 vs 3, 4: NS, 1 vs 5: &lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>7, 7, 7, 9, 10, 10, 11, 11, 11, 12, 12, 12, 12, 13, 14, 14, 14, 16</td>
<td>2 vs 1: &lt;0.005, 2 vs 3: &lt;0.01, 2 vs 5: NS</td>
</tr>
<tr>
<td>3</td>
<td>DST IV</td>
<td>7, 8, 9, 9, 9, 9, 9, 11, 11, 11, 12, 12, 13, 15, 15, 16, 20, 24, 26, 30, 30, 36, 75, &gt;100</td>
<td>3 vs 1: NS, 3 vs 2: &lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>DST IV + sham operation</td>
<td>7, 10, 10, 11, 11, 13, 20, 22, 22, 30, 32, 54</td>
<td>4 vs 1: NS</td>
</tr>
<tr>
<td>5</td>
<td>Sham operation only</td>
<td>8, 10, 11, 11, 11, 12, 12, 14, 14</td>
<td>5 vs 1: &lt;0.01, 5 vs 2: NS</td>
</tr>
</tbody>
</table>

<sup>a</sup> 0.25 ml blood was transfused on the −9th day before transplantation
<sup>b</sup> Moses test for reciprocal values of survival times

Table 2. The effect of pretransplant donor-specific blood transfusion (DST) via the portal venous (PV) or intravenous (IV) route on cardiac allograft survival in H-2 incompatible donor-recipient combination (BALB/c→CBA/H)

<table>
<thead>
<tr>
<th>Group</th>
<th>Recipient pretreatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Graft survival (days)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DST PV</td>
<td>7, 7, 9, 9, 11, 11, 11, 11</td>
<td>1 vs 2: &lt;0.05, 1 vs 3: NS</td>
</tr>
<tr>
<td>2</td>
<td>DST IV</td>
<td>5, 6, 7, 7, 8, 8, 9</td>
<td>2 vs 1: &lt;0.05, 2 vs 3: &lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>7, 8, 8, 8, 8, 8, 9, 9, 10, 10, 11, 12, 13, 17</td>
<td>3 vs 1: NS, 3 vs 2: &lt;0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> 0.25 ml blood was transfused on the −9th day before transplantation
<sup>b</sup> As determined by Wilcoxon rank sum test

**Technique of heart transplantation**

The technique of heterotopic cardiac transplantation described by Fulmer et al. [9] was adopted. Briefly, hearts were removed from neonatal (2–4 day-old) mice and cut into two equal parts longitudinally. Each part of the heart tissue was placed into cold minimal essential medium (MEM). Meanwhile, recipient mice were anesthetized with chloral hydrate. A pocket on the dorsal side of the left ear lobe was made by an incision in the skin at the base of the ear lobe. The tips of small curved forceps were forced into the skin, bluntly dissecting the skin and the cartilage toward the distal edge of the ear. The heart fragment was placed into this pocket within 1 min of removal of the heart from the donor. Graft function was evaluated starting on the 5th or 7th day after grafting. For this, an electrocardiograph enhancer, recording electric heart impulses and transforming them into visual and sonic signals, was used. Animals were anesthetized with chloral hydrate. Electrodes made from thin pins were inserted into the ear on both sides of the graft. The grafts were recorded daily during the first 2 weeks after grafting and thereafter on every second day; beginning on the 36th day, this occurred once a week. Grafts were regarded as rejected if no impulses were recorded on three consecutive examinations.

**Statistical analysis**

The reciprocal values of survival times for the H-2-compatible donor-recipient combination (BALB/c→DBA/2) were calculated and are presented in Table 1. For the graft surviving longer than 100 days, the reciprocal value of survival time was assumed to be zero [2]. Such transformed data were compared by means of the nonparametric Moses test of extreme reactions. In Table 2 these values for the H-2-incompatible donor-recipient combination (BALB/c→CBA/H) are shown. Here, the Wilcoxon rank sum test was used.

**Results**

The effects of DST via the PV or IV route on the survival of BALB/c heart tissue transplanted into H-2-compatible DBA/2 recipients are summarized in Table 1. In this model, DST via the PV route induced significant prolongation of graft survival resulting, in several cases, in permanent cardiac tissue survival (group 1 vs 2, P<0.005; group 1 vs 3, P<0.01). DST via the IV route also showed a marked prolongation of heart tissue transplant survival (group 3 vs 2, P<0.01). There was no statistically significant difference in the donor-specific transfusion effect induced via the PV or IV route (group 1 vs 3 and group 1 vs 4). In this model, surgical stress (sham operation) did not exert any influence on graft survival (group 2 vs 3 and group 4 vs 3).