Studies of the Irradiation Protection Effect of Fetal Liver in Mice. I. Influence of the Gestational Age of the Donor Tissue

By Paul N. Tschetter, John H. Githens and M. Giovannella Moscovici

The prevention of delayed death following transplantation of homologous hematopoietic tissue in irradiated animals remains an unsolved problem. These late deaths usually occur between the 30th and 90th day and many investigators believe that immunologic rejection of the host by the grafted reticuloendothelial cells may be the cause.1-3

It has been suggested that fetal hematopoietic donor tissue might be less likely to cause this immunologic reaction since tolerance can be induced in fetal animals.2 Thus, reticuloendothelial cells from an immature fetus might develop tolerance to the antigens of the host into which they were grafted. Several previous studies support this concept. In 1957, Congdon and Urso1 suggested that the use of fetal instead of adult donor hematopoietic tissue might reduce the incidence of late deaths. Urso,3 in 1958, reported a 70 per cent survival for 90 days in mice receiving homologous fetal liver from 17- to 21-day fetuses as compared with 50-60 per cent survival in recipients of tissue from newborn to 3-day old donors, and a 20-25 per cent survival in animals receiving adult marrow. Barnes et al.6 also reported a lower late mortality in mice receiving fetal tissue as compared to those given adult tissue.

Since there is a time in fetal or neonatal life beyond which tolerance can no longer be induced, the gestational age of the donor fetus may play a role in the incidence of late immunologic deaths. Uphoff7 was able to achieve 90-100 per cent protection for 90 days with tissue from 14- to 16-day old fetuses as compared to 70 per cent protection from fetal cells of 19 to 20 days gestation using (C57BL x DBA/2)F1 hybrid mice as the recipients and fetal hematopoietic tissue of either parental strain as the donor. In 1959, Porter8 reported a reduction in late deaths in irradiated rabbits following injection of a suspension of hematopoietic liver cells of 20- and 27-day old fetal rabbits as compared with adult marrow. Twenty-seven day fetal liver was not as effective as that from 20-day-old fetuses, and newborn liver showed little advantage over adult tissue in preventing the late mortality. On the other hand, Urso, Congdon and Owen in 19599 reported that the long-term survival of mice treated with homologous liver cells was not significantly affected by the gestational age of the donor.

The purpose of the present study was to determine in more detail the effect of the gestational age of homologous fetal hematopoietic tissue on the incidence of both early and late deaths in irradiated mice. Only fetal and neonatal donors

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IRRADIATION OF FETAL LIVER IN MICE

were used, their ages varying from 12 days of gestation through the 5th day of neonatal life.

METHODS AND MATERIALS

The irradiated recipients and the donors were Webster strain mice. The grafts were considered to be homologous rather than isologous since the animals were not bred to be genetically identical and since all attempts at cross skin grafting within the colony had been unsuccessful.

The recipients were given total body irradiation in a dose of 775r measured in air at 50 cm. A 220KV, 15MA Maximar therapy unit was used. The filter was 0.5 mm. copper and 1.0 mm. aluminum with a half-value layer equivalent to 1.35 mm. copper.

The donor tissue was mouse liver obtained from fetuses of 12 to 19 days gestation and newborns up to 5 days of age. The gestational age of the fetus was carefully determined from the external features of the mouse embryo by the method of Gruneberg. The livers of male and female donors of the same age were pooled. Each recipient was given tissue from donors of only one gestational age. The fetal liver was divided into single cells by passage through stainless steel screens and then suspended in Hanks' solution with 10 per cent calf serum. The nucleated cells were counted and diluted to a final concentration of 20,000,000 cells per cc. No attempt was made to distinguish hematopoietic from liver cells. One cc. (containing 20,000,000 cells) was administered intravenously into the tail vein of the irradiated recipients. All of the irradiated animals and the controls were 12-week old female mice.

Following therapy the animals were quartered in individual sterilized cages in an air-conditioned room. With each experiment at least 10 control animals were given 775r total body irradiation but not injected with hematopoietic cells. Another group of non-injected, non-irradiated mice of the same strain and age were maintained in separate cages during the entire course of the experiment to determine whether either early or late deaths might occur from causes unrelated to the conditions of the experiment. At no time were there any deaths in the latter group of animals.

RESULTS

The mortality is reported for irradiated mice injected with tissue from second trimester fetal donors (12 to 14 days gestation); from third trimester donors (15 to 19 days gestation); and from neonatal donors (newborn through 5 days of age).

The mortality is reported separately for early and late deaths. The early deaths include those which occurred within the first 30 days following injection while the late deaths include only those occurring between day 30 and day 90.

Table 1 summarizes the early deaths. Liver tissue from the second trimester donors was used in 50 animals. There were 17 deaths for a 30-day mortality of 34 per cent. In recipients of third trimester tissue, there were 62 deaths in 179 animals for an early mortality of 37 per cent. Seventy mice received neonatal tissue; thirty-two died for a mortality of 46 per cent. One hundred and fifteen of the 129 irradiated but un.injected controls died, an early mortality of 92 per cent. Although the 30-day death rate increased slightly with the increasing age of the donor tissue, the difference between the various injected groups was not statistically significant.

The 90-day or late mortality was calculated by determining the number of deaths in the second and third month among the animals which had survived the first 30 days. Table 2 summarizes the late mortality. Thirty-three recipients
Table 1.—Early (30-day) Mortality in Lethally X-Irradiated Female Webster Mice Receiving Homologous Liver Tissue

<table>
<thead>
<tr>
<th>Donor Tissue</th>
<th>Total No. Mice</th>
<th>30-day Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent</td>
</tr>
<tr>
<td>2nd Trimester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12–14 days gestation)</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(15–19 days gestation)</td>
<td>179</td>
<td>62</td>
</tr>
<tr>
<td>Neonatal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(newborn to 5 days)</td>
<td>70</td>
<td>32</td>
</tr>
<tr>
<td>Controls (non-injected)</td>
<td>127</td>
<td>115</td>
</tr>
</tbody>
</table>

The difference between the injected groups are not statistically significant.

Table 2.—Late (90-day) Mortality in Lethally X-Irradiated Female Webster Mice Receiving Homologous Liver Tissue

<table>
<thead>
<tr>
<th>Donor Tissue</th>
<th>Total No. Mice</th>
<th>90-Day Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent</td>
</tr>
<tr>
<td>2nd Trimester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12–14 days gestation)</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(15–20 days gestation)</td>
<td>117</td>
<td>46</td>
</tr>
<tr>
<td>Neonatal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(newborn to 5 days)</td>
<td>38</td>
<td>15</td>
</tr>
<tr>
<td>Controls (non-injected)</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>

The difference in the mortality of the 2nd trimester group is significant at the 0.1 level.

of second trimester tissue survived 30 days. Of these, 8 died by day 90 for a late mortality of 24.2 per cent. There were 46 late deaths in the 117 30-day survivors that had been injected with third trimester tissue for a 39.3 per cent late mortality. In animals receiving neonatal tissue, 15 of the 38 early survivors died by day 90 for a late mortality of 39.4 per cent. There were 3 late deaths among the 12 surviving uninjected controls.

**DISCUSSION**

The mortality is reported separately for early and late deaths because they probably have different causes. The early deaths are due to either a failure in transplantation of hematopoietic tissue or other acute effects of the irradiation. The late deaths, however, may be due to the immunologic rejection of the host by the graft.

The age of the donor tissue did not influence the incidence of early mortality. This suggests that there was no difference in the antigenic competence of fetal and neonatal liver. It also suggests that the liver of older donors contained a sufficient number of hematopoietic cells since the degree of protection was the same as that obtained with liver tissue from younger donors.

The data suggest that the gestational age of the donor tissue did influence the late mortality. Although the death rate was almost identical (39.3 per cent and 39.4 per cent) for the recipients of tissue from third trimester fetuses and newborn animals respectively, it was 16 per cent lower in animals receiving hematopoietic tissue from second trimester donors. Statistical analysis of this difference, however, gives a P value of only 0.1.
The variation in the results of the few reported studies on the influence of the gestational age of the donor tissue on late mortality may be due to several factors. First, there has been variation in the degree of immaturity of the fetal donor tissue with very little evaluation of tissue from donors in the second trimester. Second, the sex of the donor and the recipient animals may be an important factor since Eichwald, et al.\textsuperscript{11} have demonstrated evidence for a sex-linked antigen located on the Y-chromosome. In our experiments this histoincompatibility would be expected since the recipients were all female while the donor tissue was partially from male animals. Third, the animal species and strain has been different in each report. Fourth, it has been demonstrated that late deaths may occur in irradiated animals that have been given isologous transplants.\textsuperscript{11} This suggests that x-ray alone may be responsible for some of the late mortality.

The effect of x-ray as a cause of late deaths is substantiated in our study by the fact that 3 of the 12 non-injected but irradiated 30-day survivors died by the 90th day. We have also observed a 17 per cent late mortality in a group of 59 C57BL mice that received isologous grafts following 775 r total body irradiation. This suggests that a considerable number of the late deaths in our homologously injected animals may also have been due to the late effects of irradiation rather than on an immunologic basis.

**Summary**

1. The gestational age of donor hematopoietic tissue appeared to have no influence on the 30-day mortality following homologous transplants in irradiated mice.
2. Recipients of second trimester homologous donor tissue had a late (90-day) mortality that was 16 per cent lower than that observed in animals receiving tissue from third trimester and neonatal donors, but statistical analysis showed a low level of significance.
3. Irradiation alone appeared to cause a late mortality in non-injected irradiated animals and in isologously transplanted irradiated animals.

**Riassunto in Interlingua**

1. Le etate gestational del tissu hematopoietic donatori pareva haber nulle influentia super le mortalitate de 30 dies post transplantationes homologe in irradiate muses.
2. Recipientes de tissu homologe prendite durante le secunde tertio del gestation habeva un mortalitate tardive (post 90 dies) que esseva 16 pro cento plus basse que illo observate in animales recipiente tissu prendite durante le tertie tertio del gestation e ab donatores neonate, sed le analyse statistic monstrava un basse nivello de signification.
3. In animales sin transplantationes e in animales con transplantationes isologe, le irradiation per se pareva causar un mortalitate tardive.

**References**


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