Successful Transplantation of Friend Virus-Induced Preleukemia Into Stem Cell-Deficient Fetal Mice

By Roger A. Fleischman

The leukemias induced by the Friend polycythemia virus and other leukemogenic retroviruses have previously not been transplantable until weeks or months after virus inoculation. Because tumor-specific immune mechanisms persist in both irradiated and nude mice, it has not been possible to determine if this result is due to rejection of cells already immortalized by retrovirus infection, or reflects an inherent limitation in the proliferative capacity and malignancy of these "preleukemic" cells. To clarify these issues, we have transplanted virus-infected bone marrow into mouse fetuses that are immunologically immature and thus incapable of graft rejection. We report here that within days of virus inoculation, transplantable cells capable of disease progression in certain fetal hosts can be detected with this technique. These results demonstrate that cells with the capacity for extensive leukemic proliferation arise very early in Friend virus-induced disease. However, successful transplantation was seen only in genetically anemic recipients (W'/W'), which are deficient in hematopoietic stem cells, and not in their normal littermates. Thus, in accord with recent in vitro observations, this in vivo data suggests that normal hematopoietic cells, independent of immune mechanisms, can suppress the malignant progression of transformed cells.

METHODS

Recipient mice. To investigate the requirements for transplantation of FV-infected cells, recipient mice have been studied at two distinct stages of development: in fetal life before the development of the immune system, and in adult life after complete immunity has been achieved (Table 1). These normal recipients, however, do not accept grafts of bone marrow cells without prior radiation or drug conditioning. Mutant W'/W' mice, on the other hand, readily accept transplants of compatible bone marrow due to a deficiency of endogenous hematopoietic stem cells. Moreover, W'/W' fetuses accept engraftment of either syngeneic or allogeneic bone marrow.

All recipient mice were F1 hybrids obtained from matings of inbred W'/+×C57BL/6 females and W'/+×C3H/He male mice, purchased from the Jackson Laboratory, Bar Harbor, ME. Coat color differences due to the W alleles enable the genotypes to be recognized postnatally. The recipient mice (Fv-1++) were resistant to leukemogenesis by the N-tropic FV. The adult W'/W' homozygotes and W'/+ heterozygotes were characterized by additional, nearly complete resistance to FV. The W'/W' genotype is similar, in severity of anemia and postnatal viability, to the more commonly studied W'/W' mice.

Donor mice. Adult mice used as bone marrow donors were 6- to 10-week-old C3H/He and AKR females, purchased from the Jackson Laboratory and housed in the animal facilities of the Health Science Center. Bone marrow cells of the donor C3H/He strain are
not subject to immune or hybrid resistance by adult recipient mice of the W'/W' F1 strain. Because fetal hosts accept allogeneic cells, AKR mice, which carry an electrophoretic variant of glucosephosphate isomerase (GPI), were used for the fetal injections. Quantitative analysis of experimental tumors for the strain-specific enzyme variants, which are present in virtually all cell lineages, provided unequivocal evidence for the donor- or host-origin of the Friend disease.

Virus. N-tropic polycythemia FV, provided by Dr. J. Silver (National Institutes of Health), was maintained by passage in DBA/2J mice and titrated by spleen focus assay. Microinjection of cells from adult bone marrow. Donor AKR/J, normal or leukemic, newborn (stage I or II) mice were inoculated with 1,000 (low-dose) to 5,000 (high-dose) spleen focus-forming units of the virus. Since more than 95% of the hematopoietic organs of the wild-type +/+ fetuses, which have a slight anemia, and nonanemic W'/+ fetuses have been grouped with the strain-specific variants, which are present in virtually all cell lineages, provided unequivocal evidence for the donor- or host-origin of the Friend disease.

RESULTS

Adult recipients. Because earlier studies used normal or lethally irradiated adult recipients to demonstrate the inability to transplant FV-infected cells in the first weeks after virus inoculation, initial experiments were performed to confirm these results in adult mice of the mutant W'/W' genotype. The results of these experiments are presented in Fig 1.

As expected, proliferation of both uninfected and virus-infected cells was undetectable after transplantation into wild-type (+/+ ) recipients. None of these mice developed Friend disease during a subsequent 6-month period of observation. On the other hand, all of the stem cell-deficient W'/W' recipients showed evidence of engraftment by the second week after transplantation. Complete replacement occurred by 6 to 8 weeks, although the FV-infected cells exhibited a slight but significant delay in the rate of repopulation.

However, despite complete reconstitution with donor erythroid cells, none of the W'/W' animals developed polycythemia, erythroblastosis, or enlarged spleens. When autopsied at 16 weeks after transplantation, serum and spleen lysates from mice did not produce spleen foci in DBA/2J mice, suggesting that they were not viremic at the time of death. In addition, the bone marrow from these animals repopulated secondary W'/W' recipients without induction of leukemia and with kinetics similar to those observed in the primary recipients (data not shown). These preliminary studies demonstrated that preleukemic hematopoietic cells do not give rise to progressive leukemia after transplantation into immune-competent adult recipients, even when the recipients are mutants that lack normal hematopoietic stem cells.

Fetal recipients. To further elucidate the role of the immune system and endogenous stem cells, similar experiments were performed via placental injection of fetal recipients. As shown in Table 2, survival of the fetal recipients from injection to birth was approximately 80% for both uninfected control and infected experimental groups. Under these conditions, then, the presence of virus-infected cells did not increase prenatal mortality, probably because the titer of free virus present at this stage of the infection was low.

Similarly, the rate of initial engraftment for preleukemic bone marrow, 0% in the nonanemic mice and 35% in

![Fig 1](https://www.bloodjournal.org)
the W'/W' mice, was not significantly different than that obtained with control bone marrow (0% and 45%, respectively). These results are consistent with our prior reports that demonstrate a 30% to 50% rate of successful engraftment with the placental injection technique in severely anemic mouse fetuses.

Friend disease in fetal recipients. Four of the initially engrafted W'/W' recipients of FV-infected bone marrow, however, rapidly developed typical FV-induced disease that proved to be donor in origin (Table 3). In three cases (A, B, D) smears of the peripheral blood revealed markedly increased numbers of immature "smudge" cells and erythroblasts. Analysis of the spleens and buffy coats, which contain the circulating nucleated cells, demonstrated that the leukemic cells were predominantly, if not completely, donor-derived. Mosaicism in the blood, liver, and spleen of case C was more limited; however, gross examination revealed a single, white 1 mm nodule within otherwise normal-appearing spleen. Imprints of the nodule showed immature blasts consistent with FV-induced disease, and electrophoretic analysis showed that the cells were more than 80% donor in origin. In contrast, the splenic foci induced in adult mice by FV-infected cells are exclusively host in origin.

The failure to observe, after more than 1 year of observation, any similar cases of donor-derived disease in the nonanemic genotypes (+/+, W'/+, W'/+) is statistically significant ($\chi^2 = 9.1, P < .01$). Although virus-infected donor cells were unable to proliferate in the nonanemic fetuses, Friend disease of host origin was observed subsequently in 20 (12%) of the high-dose recipients at 2 to 4 weeks of age and in two of the low-virus group at 3 to 4 months of age, confirming the successful injection of these hosts with virus-infected cells. Apparently, in the fetal hosts, Fv-1 resistance provided incomplete protection from viral leukemogenesis.

### Table 2. Frequency of Engraftment and Donor-Derived Friend Disease in Recipients of Normal and Preleukemic Bone Marrow

<table>
<thead>
<tr>
<th>Donor Cells</th>
<th>No. Injected</th>
<th>No. Born (%)</th>
<th>Engraftment*</th>
<th>Donor Disease†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no FV)</td>
<td>133</td>
<td>105 (79)</td>
<td>0/74</td>
<td>14/31</td>
</tr>
<tr>
<td>Virus (low FV)</td>
<td>140</td>
<td>102 (73)</td>
<td>0/81</td>
<td>6/21</td>
</tr>
<tr>
<td>Virus (high FV)</td>
<td>248</td>
<td>221 (89)</td>
<td>0/166</td>
<td>20/55</td>
</tr>
</tbody>
</table>

The results are expressed as the number of animals initially engrafted or developing donor leukemia over the numbers of animals injected.

*All animals with detectable donor cells (> 1%), as determined by analysis of blood from one-day-old newborn mice for strain-specific variants of GPI, were considered to be engrafted.

†Friend disease was considered donor in origin if the GPI enzyme activity from the spleen, buffy coat cells, or tumor nodules was more than 80% donor-derived.

### Table 3. Blood Analysis and Percent Tissue Mosaicism in W'/W' Mice With Friend Disease of Donor Origin

<table>
<thead>
<tr>
<th>Age at death</th>
<th>WBC</th>
<th>Blood smear</th>
<th>Spleen</th>
<th>Percent donor GPI</th>
<th>Bone marrow</th>
<th>Spleen nodule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>23 d</td>
<td>Leukemia</td>
<td>8 x</td>
<td>50%</td>
<td>Bone marrow</td>
<td>Spleen nodule</td>
</tr>
<tr>
<td>High</td>
<td>3 d</td>
<td>Leukemia</td>
<td>10 x</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1 d</td>
<td>Normal</td>
<td>1 x</td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>4 d</td>
<td>Leukemia</td>
<td>5 x</td>
<td>80%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The experiments described in this report demonstrate that within days of virus infection transplantable cells capable of disease progression can be detected by injection into immunologically immature mouse fetuses. However, this result was seen only in the W'/W' mutant fetuses that are deficient in hematopoietic stem cells, and not in their normal fetal littermates. The deficiency of host stem cells, although necessary, is not sufficient for successful transplantation. Donor-derived leukemias were not observed in the immune-competent W'/W' adult recipients, despite injection with cell inoculums that were ten to 20 times larger than the inoculums for fetal recipients.

The most cogent explanation for the experimental data is that, early after virus infection, the fate of the transformed cells is determined not only by immune mechanisms but also by the hematopoietic environment of the host. Within the hypoplastic environment of stem cell-deficient fetuses, certain virus-infected cells are capable of extensive proliferation and progression to leukemia. The very early presentation of these donor-derived leukemias, as compared with host-derived leukemias, strongly suggests that viral transformation occurred before, rather than after, transplantation. Moreover, the results are not attributable to differences in the incidence or numbers of bone marrow cells and stem cells
of a normal embryonic environment to suppress growth by self-renewal may limit the self-renewal of virus-infected cells. Alternatively, self-renewal may be regulated by interactions with the rapidly proliferating normal hematopoietic cells of the fetal host. Previous in vivo studies have demonstrated the ability of a normal embryonic environment to suppress growth by teratocarcinoma cells or tumor induction by Rous sarcoma virus. More recent experiments have shown that normal cells can also inhibit the growth and tumorigenicity of cells transformed by oncogenes in vitro.

In conclusion, transplantation of transformed cells into mouse fetuses represents a useful model system for the analysis of viral leukemogenesis in vivo. In addition, unlike irradiation of adult recipients, the present approach readily discriminates the role of host stem cells from that of host immunity. The experimental results suggest that growth of preleukemic hematopoietic cells is subject not only to rejection by host immune mechanisms but also to suppression by normal endogenous hematopoietic cells. In the absence of both constraints, donor-derived cells capable of transplantation and leukemic evolution can be detected within days of virus infection.

REFERENCES

Successful transplantation of Friend virus-induced preleukemia into stem cell-deficient fetal mice

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