Combined Fetal Liver and Thymus for Hematopoietic Reconstitution of Allogeneic Radiation Chimeras[1] (36289)

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(Introduced by Anthony V. Pisciotta)

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In studies aimed at the reduction of mortality in allogeneic radiation chimeras, high 100-day survival rates (87 to 100%) were achieved when lethally irradiated CBA (H-2^k) mice were treated with liver and thymus cells (or a thymic factor) from stain-A (H-2^a) fetal donors (1–3). In contrast, no lethally irradiated CBA mice survived more than 33 days when treated with spleen cells from adult strain-A donors; only 18% survived 100 days when given adult bone marrow; and 69% survived 100 days when treated with fetal liver without thymus cells (3). These results suggested that fetal liver and thymus in combination were highly effective in preventing mortality due to secondary disease, a major cause of death in allogeneic radiation chimeras.

The high survival rates, with absence of obvious graft-versus-host (GVH) disease, prompted us to suggest that fetal liver in combination with fetal thymus might represent a source of "universally" tolerant hematopoietic and lymphoid stem cells for transplantation (2, 3). Because of the observed synergism on immune reactivity of fetal livers plus thymus (4) and the absence of overt infection in chimeras reconstituted with these tissues, we hypothesized that the high survival rates were due, in part, to improved immunological reconstitution of the chimeras (1, 2).

An improved rate and level of hematopoietic reconstitution may have been an additional factor which favorably influenced survival in the fetal liver–thymus chimeras. Goodman and Shinpock have reported that bone marrow plus neonatal or adult thymocytes were synergistic in mice with regard to erythropoiesis (5) and hematopoiesis (6). Also, Hrask *et al.* (7) recently described improved survival and CFU repopulation in murine radiation chimeras if either host or donor strain thymus glands from 4 to 8-day-old donors were transplanted under the renal capsule 2 weeks after allogeneic bone marrow transplantation. The experiments reported here tested the hypothesis that allogeneic chimeras produced with combined fetal liver and thymus cells had more rapid and complete hematopoietic restoration than did chimeras produced with fetal liver alone or with bone marrow from adult donors.

Materials and Methods. The experimental model is shown in Fig. 1. After exposure to 950 R whole-body X-irradiation (TBR), CBA mice were given iv inoculations of adult bone marrow, fetal liver, or fetal liver and thymus from strain-A donors. Groups of chimeras were sacrificed at 1–2-week intervals through day 98. Cell suspensions prepared from the femoral bone marrow of the chimeras were counted and given iv to heavily irradiated strain-A secondary recipients. The secondary recipients were sacrificed 8 days later and their spleens were examined for spleen colony formation (8). Parameters measured to evaluate hematopoietic restora-

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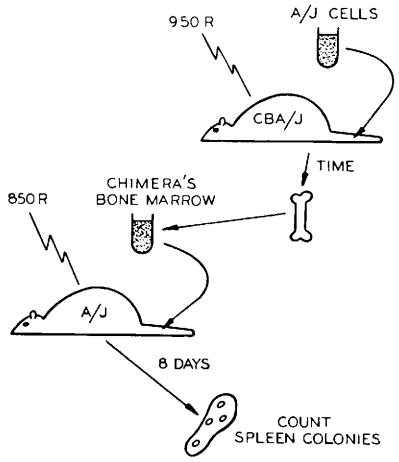

FIG. 1. Lethally irradiated CBA host mice received I-V injections of adult bone marrow, fetal liver, or fetal liver plus fetal thymus cells from strain-A donors. Hematopoietic reconstitution was evaluated by means of periodic determinations of femoral bone marrow cellularity, CFU concentration, and CFU content.

tion included cellularity, colony-forming unit (CFU) concentration, and CFU content in femoral bone marrow obtained from the various chimeras. The CFU concentration was expressed as the CFU ratio or the number of CFU per 10^5 cells inoculated. The formula (CFU ratio $\times$ femoral cell yield)/100,000 was used to compute the CFU content.

Female 10–12-week-old CBA/J and A/J mice and timed-pregnant A/J mice were obtained from the Jackson Laboratory. CBA primary recipients received 950 R TBR ($LD_{100/15}$) and strain-A secondary recipients received 850 R TBR (endogenous CFU $<$ 0.1). To produce chimeras, 2×10^7 viable, nucleated bone marrow cells from strain-A adult donors, 2×10^7 liver cells or 10^7 liver cells plus 10^7 thymus cells from 18-day-old strain-A embryos were given iv within 4 hr following TBR to the CBA hosts. Radiation controls were included with each radiation group. Radiation conditions and the collection and processing of cell suspensions have been described (1, 9).

Femoral bone marrow from the chimeras was administered iv to strain-A secondary recipients which were subsequently studied for spleen colony formation (8). The effect of aging on stem cell populations was accounted for by assaying CFU in the femoral bone marrow of age-control normal strain-A mice. Endogenous CFU controls were included with each radiation group. Strain-A secondary recipients (rather than CBA secondary recipients) were selected as assay animals to test CFU present in the chimeric

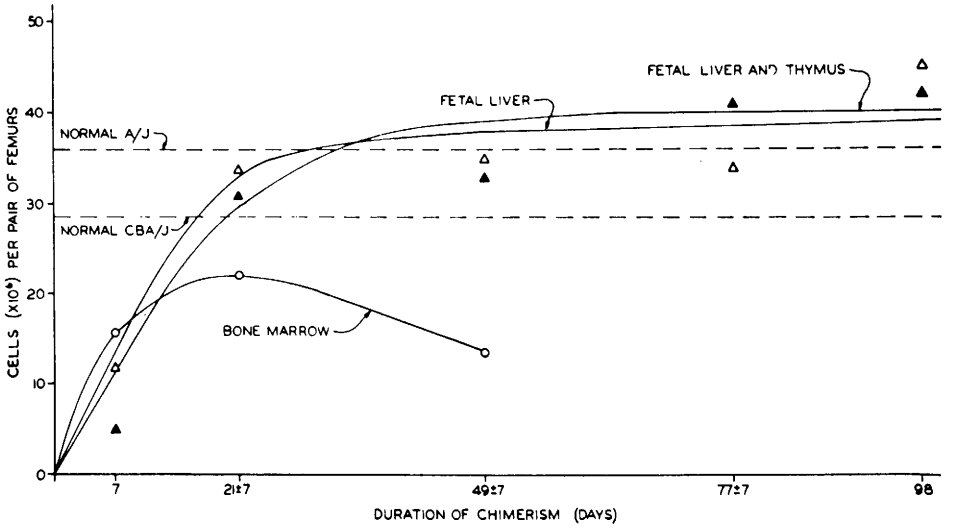

FIG. 2. Femoral cell yields of lethally irradiated CBA mice that received iv injections of cells from strain-A donors: (○) 2×10^7 adult bone marrow cells; (△) 2×10^7 fetal liver cells; (▲) 10^7 fetal liver cells + 10^7 fetal thymus cells.

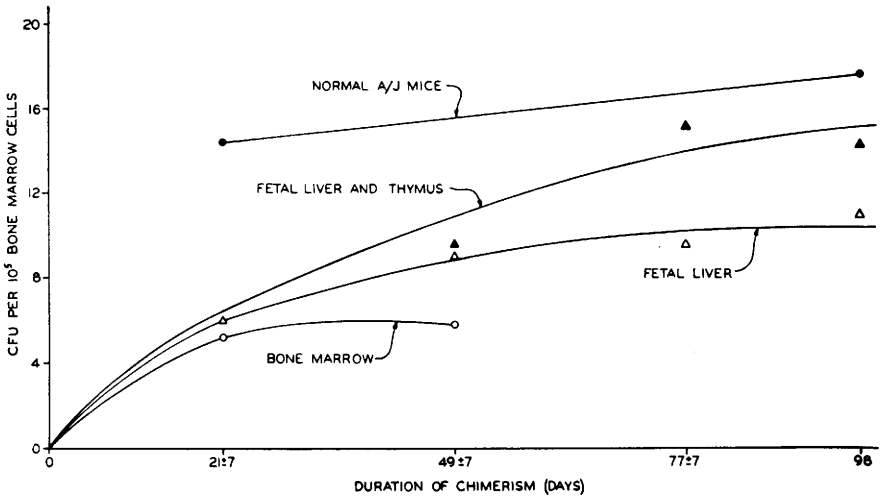

FIG. 3. CFU per 10^5 femoral bone marrow cells in lethally irradiated CBA mice that received iv injections of cells from strain-A donors: (○) 2×10^7 adult bone marrow cells; (△) 2×10^7 fetal liver cells; (▲) 10^7 fetal liver cells + 10^7 fetal thymus cells.

bone marrow in an effort to avoid allogeneic repression of exogenous CFU (10). Spleen colonies were counted as unknowns under the 10-power objective of a dissecting microscope.

Results. Femoral cell yields. Femoral cell yields of chimeric mice showed marked differences between those mice treated with fetal cell transplants and those that received bone marrow from adult donors (Fig. 2). Each point on the graph represents the median cell yield per pair of chimeric femora at the times indicated. An average of 12 chimeras were used to compute the value at each point. In this allogeneic system, it is apparent that femoral bone marrow cellularity was restored to normal levels when either fetal liver or fetal liver plus fetal thymus were the tissues transplanted. These levels were reached in about 3 weeks and were thereafter sustained. At 49 ± 7 days, the surviving chimeras that had received bone marrow transplants were found to have significantly lower femoral marrow cellularity ($p < .05$). It was not possible to test marrow cell yields or CFU in chimeras produced by bone marrow transplants after day 54 because too few chimeras survived.

CFU concentration. CFU ratios following transplantation of chimeric and normal bone marrow are graphed in Fig. 3. Normal strain-A mice had median CFU ratios of 14.5 and 17.7. The femoral bone marrow of chimeras produced with fetal tissues had significantly higher CFU ratios than those produced with adult bone marrow when tested at 49 ± 7 days ($p < .01$). At 77 ± 7 and 98 days the femoral bone marrow of chimeras produced with fetal liver plus fetal thymus had CFU ratios which were within the normal range. At 98 days, these CFU ratios were significantly higher than those obtained from chimeras that had been produced with fetal liver alone ($p < .05$).

CFU content. The CFU content in the femoral marrow of chimeras treated with bone marrow from adult donors was markedly below normal and below that found in chimeras treated with fetal hematopoietic cell transplants at 21 ± 7 and at 49 ± 7 days (Fig. 4).

Shown in Fig. 5 are the femoral cell yields and CFU ratios of radiation chimeras reconstituted with fetal cells. The data are plotted as a percentage of the values obtained from normal strain-A donors. Each point was computed using combined data from chimeras that received fetal liver plus fetal thymus cells and those which received fetal liver alone. The graph permits a direct comparison between the growth curves for total cells of the femoral bone marrow, and of the CFU

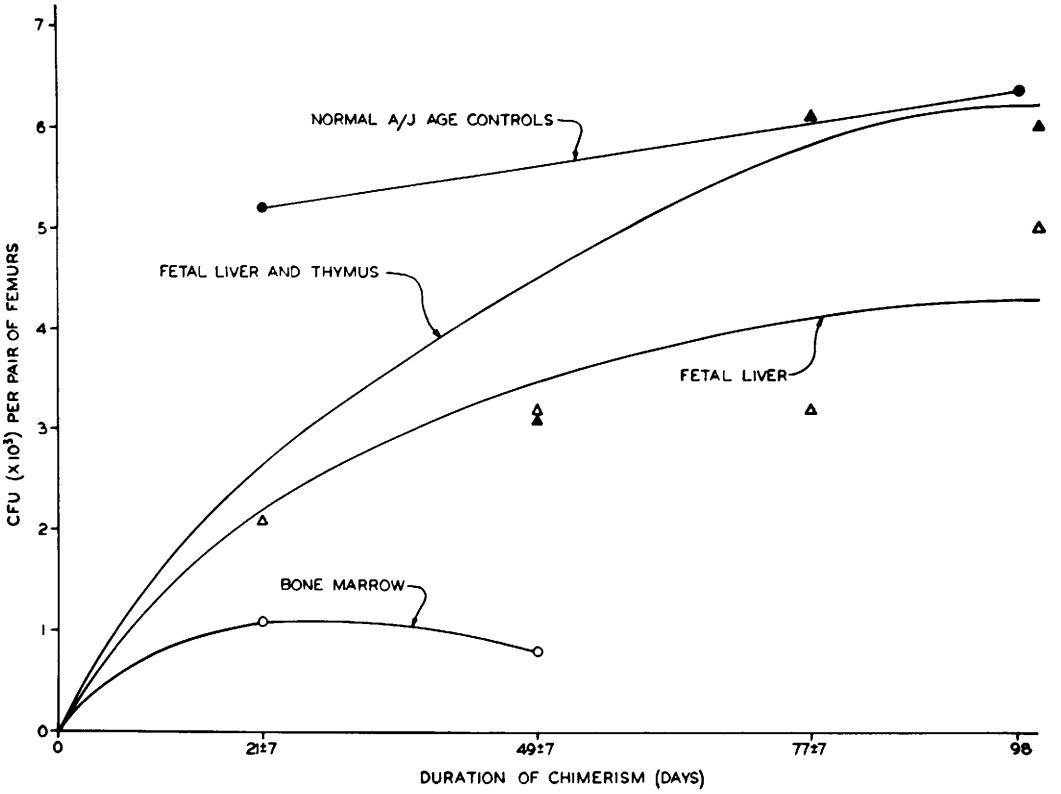

FIG. 4. CFU content in femoral bone marrow of lethally irradiated CBA mice that received iv injections of cells from strain-A donors: (○) 2 × 10^7 adult bone marrow cells; (△) 2 × 10^7 fetal liver cells; (▲) 10^7 fetal liver cells + 10^7 fetal thymus cells.

within that cell population. When allogeneic radiation chimeras were produced with fetal cells, femoral bone marrow cellularity reached normal levels within 3 weeks. However, at this same point in time, CFU in these femurs were only restored to 40% of the normal value for strain-A mice. The CFU ratios in the chimeras rose more gradually than did bone marrow cellularity. Thus, in the hematopoietic restoration of these allogeneic radiation chimeras, a difference in growth rate was found between the differentiated and undifferentiated cell lines.

Discussion. In terms of the indices studied, treatment with the allogeneic fetal tissues provided the chimeras witth hematopoietic reconstitution which was superior to that found when bone marrow from adult donors was the tissue transplanted. This superior hematopoietic reconstitution of mice treated with fetal tissues as opposed to adult bone marrow was of sufficient magnitude to suggest that it probably is an important factor in the higher long-term survival seen when cells from fetal donors have been used for the production of allogeneic radiation chimeras (3, 11).

In the present study it was shown that femoral bone marrow from normal adult strain-A mice had a median CFU ratio of 16.1. Previously we found that the CFU ratio for liver cell suspensions from 18-day-old strain-A embryos was 5.73 and for fetal thymus 0.025 (12). Extrapolation of these data to the present study disclosed that each lethally irradiated CBA host received about 3200 CFU in the transplanted inoculum of 2 × 10^7 adult bone marrow cells; each chimera produced with fetal liver alone received about 1200 CFU in the 2 × 10^7 cells administered; and each chimera treated with 10^7 fetal liver cells plus 10^7 fetal thymus

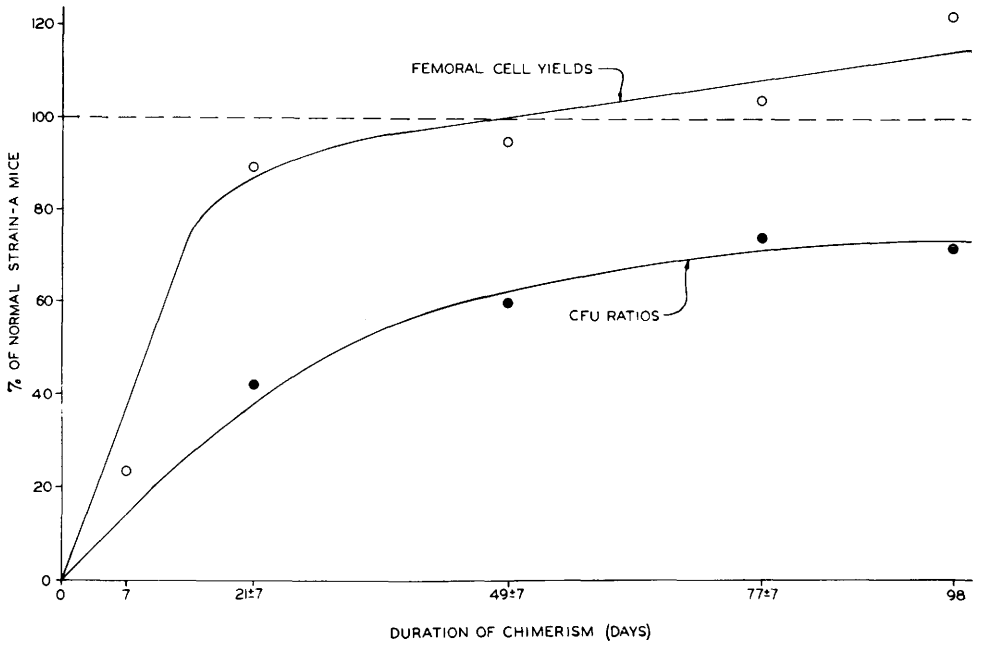

FIG. 5. Allogeneic radiation chimeras exhibited more rapid growth of bone marrow cells than of CFU within that population: Shown is a comparison of femoral cell yields and CFU ratios in chimeras produced with fetal cells.

cells received a total of about 600 CFU. Although the chimeras produced with adult bone marrow received the largest inoculum of hematopoietic stem cells they had the poorest long-term survival and the poorest hematopoietic reconstitution, as measured in these experiments. In contrast, chimeras produced with fetal liver had intermediate long-term survival and femoral CFU repopulation. Highest survival rates and femoral CFU restoration occurred in allogeneic chimeras produced with combined fetal liver and thymus cells, despite the fact that these mice had received the fewest stem cells. Thus, an inverse relationship appeared to exist between the degree of hematopoietic reconstitution (and long-term survival) of the chimeras and the number of hematopoietic stem cells transplanted.

At least two factors might explain the poor hematopoietic restoration of chimeras produced with adult bone marrow: (i) loss of donor hematopoietic stem cells as a result of the "innocent bystander" effect (13); and (ii) prevention of growth of residual host hematopoietic stem cells because of the GVH reaction.

It is possible that large numbers of donor hematopoietic stem cells (the innocent bystanders) might have been killed, in the presence of a GVH reaction, because of the release and accumulation of toxic breakdown products from host target cell injury and death (13). Significant donor stem cell loss was presumably avoided when cells from immunologically immature fetal donors were transplanted because of the absent or minimal GVH reaction.

Although all A → CBA chimeras tested previously were truly chimeric and tolerant of strain-A skin grafts (1), discriminant Simonsen spleen weight-gain assays (14) invariably disclosed a roughly equal mixture of donor and host lymphoid cells in bone marrow, fetal liver, and fetal liver thymus A → CBA chimeras (3). Tests for erythroid chimerism were not performed, but the finding of mixed lymphoid chimerism makes it reasonable to assume that host stem cells also participated in the hematopoietic restoration of the chimeras. Because host hematopoietic tissue is a target of the GVH reaction

(14), participation of host cells in hematopoietic restoration would be expected to be more depressed in chimeras produced with adult bone marrow rather than the fetal tissues.

The differences in growth rates of femoral bone marrow cells and of femoral bone marrow CFU (Fig. 5) suggest that homeostatic control mechanisms regulated their proliferation. It seems plausible to explain these differences on the basis of a feedback mechanism (of unknown type) which directed the stem cells toward the early preferential production of urgently needed differentiated cells, rather than toward self-replication which was not as urgent a necessity.

The role of thymus cells (or a thymic humoral factor) to potentiate hematopoiesis as a therapeutic tool in clinical hematologic disorders is not yet known. Experiments, utilizing a mouse model, are in progress to estimate the potential of fetal liver plus thymus as a source of "type-O" stem cells for clinical transplantation.

Summary. Femoral bone marrow cellularity, CFU concentration, and CFU content were markedly subnormal in allogeneic radiation chimeras that had been produced with bone marrow cells from adult donors.

2. Marrow cellularity was restored to normal within 3 weeks when liver or liver plus thymus from fetal donors was the tissue transplanted.

3. In chimeras produced with fetal cell transplants, marrow CFU increased at a slower rate than did differentiated marrow cells. However, at 77 ± 7 days, chimeras produced with fetal liver plus fetal thymus cells had normal numbers of femoral CFU and this level was significantly higher than in chimeras produced with fetal liver without thymus cells.

4. Hematopoietic repopulation of allogeneic radiation chimeras using combined fetal liver and thymus cell transplants was equal or superior to that achieved with fetal liver alone or adult bone marrow despite the fact that with the latter tissues 2 to 5 times as many hematopoietic stem cells had been transplanted.

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