Effect of Foreign Fetal and Newborn Blood-Forming Tissues on Survival of Lethally Irradiated Mice. (24640)

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The "foreign bone marrow reaction" is a disease syndrome that occurs in lethally irradiated mice given homologous or heterologous (rat) bone marrow(1-5). The disease frequently, but not invariably, results in death from "metabolic starvation." Mice usually died 3 to 8 weeks after irradiation and bone marrow injection. A somewhat similar syndrome occurred in irradiated mice given infant homologous spleen cells instead of bone marrow(6). In certain genetic situations, delayed death pattern for lethally irradiated F_1 mice given bone marrow from one of the parent strains resembles that for mice dying from foreign bone marrow reaction(7-9). Delayed deaths, however, do not occur in all parent-F_1 combinations(10). Prevention or amelioration of foreign bone marrow reaction was first attempted by Cole *et al.*(2), who gave cortisone to lethally irradiated mice injected with rat bone marrow. These authors suggested that immunologic difficulties reduced effectiveness of rat bone marrow for treating irradiated mice. Cortisone did not substantially alter long-term survival. Evidence favoring immunologic basis for foreign bone marrow reaction was reviewed by Makinodan(11). Preliminary experiments by Congdon and Urso (3) indicated that homologous fetal blood-forming tissues were superior to homologous adult bone marrow for producing long-term survival of lethally irradiated mice. This finding has been confirmed in studies from this (12-14) and other laboratories. Lengerová (15) and Barnes *et al.*(16) also reported increase in number of long-term survivors among homologous radiation chimeras given fetal and newborn blood-forming tissues. Uphoff(17) found that long-term survival was more prevalent in irradiated F_1 mice given fetal, parental blood-forming tissues but few mice had long-term survival when given adult parental bone marrow. She also mentions a beneficial effect with homologous (C3H → AKR) fetal tissue not found with adult bone marrow (strain of mouse to left of arrow is the donor animal and to the right, the irradiated recipient). This mss. contains further evidence for superiority of homologous fetal and newborn bood-forming tissues as donor material in treatment of lethal whole-body radiation injury in mice of certain genetic types. Criteria for determining superiority include relatively long-term survival, histologic recovery of lymphatic tissues, and evidence that erythropoietic cells transplant and persist. Heterologous (rat) fetal blood-forming cells and adult rat bone marrow were about equally effective in producing long-term survival in lethally irradiated mice.

Materials and methods. Methods of collecting fetal blood-forming tissues from pregnant mice (12 to 19 days), and rats (12 to 21 days) and blood-forming tissues from newborn donors were similar. Suspensions of pooled liver or spleen tissue in Tyrode's solution were prepared by gentle aspiration through a syringe. After filtration through wire mesh screens, the suspension was passed through a 27-gauge needle. A sample of pooled tissue suspension was removed with red blood cell pipette and diluted with Wright's stain in 0.1 N HCl. Nucleated cells were counted, no differentiation being made between liver cell nuclei and blood-forming cells. A fetal mouse liver yielded 30 to 60 x 10^6 nucleated cells. The suspension was injected intravenously in the tail vein of recipient 1 to 6 hours after irradiation. To allow transfusion of large cell doses, 2 to 4 injections were given within a few minutes. Mice were observed several months, and number of deaths were recorded. Separate homologous and isologous transplantation experiments were

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performed for serial gross autopsy and histologic study of lymphatic tissue changes in lethally irradiated mice treated with adult bone marrow or fetal liver. Bone marrow, spleen, thymus, and lymph nodes were fixed in Zenker-formol, sectioned, and stained with hematoxylin and eosin. Recipient X-irradiated mice were 12-week-old males and females. Factors were 250 kv, 15 ma, 1 mm of Al added filtration, and hvl of 0.5 mm of Cu. Mice were given total-body irradiation of 900 or 950 r, 160 r/min at 60 cm, in revolving lucite cage. Homologous erythrocytes were identified by agglutination technic and a variation of Hildemann's hemolytic system(18).

Results. Mortality after lethal irradiation of (C57L x A)F_1 mice given (101 x C3H)F_1 fetal liver is shown in Fig. 1. Control groups were given (101 x C3H)F_1 adult bone marrow or (C57L x A)F_2 fetal liver. It was not possible to obtain (C57L x A)F_1 fetal liver as true isologous control. Homologous fetal liver was more effective than adult homologous bone marrow in promoting 30-day and longer survival.

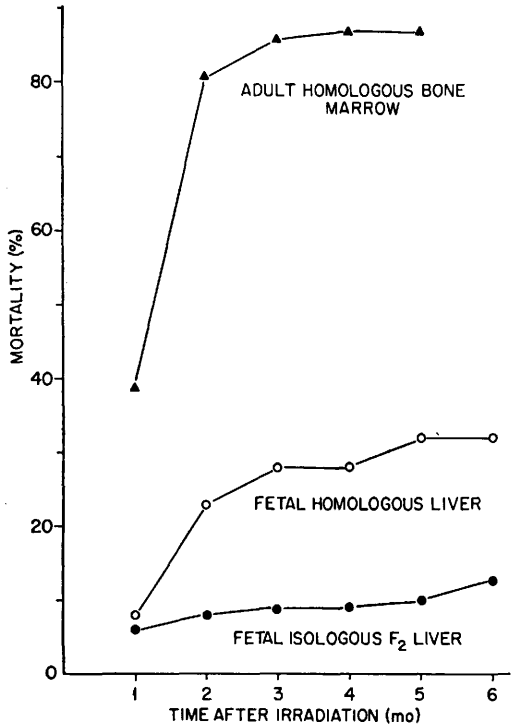

FIG. 1. Mortality in (C57L × A)F_1 mice given 900 r plus adult bone marrow or fetal liver. ▲, control group, 88 mice: dose, 30 to 50 × 10^6 (101 × C3H)F_1 adult bone marrow cells. ○, 47 mice: dose, 50 to 65 × 10^6 (101 × C3H)F_1 fetal liver cells. ●, 78 mice: dose, 38 to 60 × 10^6 (C57L × A)F_2 fetal liver cells.

In general appearance, behavior, rate of graying, mice given homologous fetal liver had a closer resemblance to those of isologous control group than to mice treated with adult homologous bone marrow.

Donor-type cells completely replaced red blood cells in 33 mice surviving homologous fetal liver treatment; about half the mice were tested 68 to 97 days after exposure, and the remainder at 123 to 130 days. Similar tests on 12 mice living 118 to 134 days after adult homologous bone marrow treatment, showed complete replacement by donor type cells.

Gross autopsy and histologic examination were performed on 20 (C57L x A) F_1 females given homologous (101 x C3H)F_1 fetal liver (80 x 10^6 cells) after 900 r. One mouse/day was killed and examined for first 7 days after treatment. This procedure was repeated at frequent intervals thereafter through day 63. One control group of 15 (C57L x A)F_1 females was given homologous (101 x C3H)F_1 adult bone marrow (30 x 10^6 cells) after 900 r. The same procedure was followed as in the experimental group, until day 32. The 17 (C57L x A)F_1 females in the other control group were given an equal amount of isologous adult bone marrow. Sacrifice and examination followed approximately the same plan as before from day 1 through day 57 after irradiation.

Mice given homologous fetal liver and those given adult homologous bone marrow, showed secondary loss in weight about 3 weeks after irradiation. At 4 weeks, body weight of all fetal tissue-treated animals had increased to that of mice given isologous bone marrow. 9 to 10 days after treatment, spleen weight in mice given each type of homologous cell treatment, paralleled that in mice treated with isologous bone marrow. Return to normal weight from extreme hyperplasia at day 10, did not occur after homologous cell treatment. With isologous bone marrow therapy, spleen weight returned to near normal levels within 3 weeks after exposure.

Histologic examination of mice given adult homologous bone marrow showed same changes in bone marrow, thymus, spleen, and lymph nodes previously reported for foreign bone marrow reaction(3). Granulomatous lymph node reactions leading to extreme atrophy were present within 7 days. Fibrinoid necrosis in white pulp of spleen was first seen in mice killed on day 10. Regional loss of bone marrow in the femur at day 32 indicated partial loss of graft at this site.

In contrast, mice given homologous fetal liver showed relatively mild granulomatous reactions in some lymph nodes. These reactions regressed or persisted to a very limited extent. Extreme atrophy of nodes was not observed, and normal regeneration was striking. At 32 days, lymph node structure was normal. Splenic white pulp also returned to normal appearance. Fibrinoid necrosis was not seen. After 50 days, lymph nodes and splenic white pulp did not appreciably differ from these tissues in irradiated controls given isologous bone marrow.

Further to check these morphologic findings, we studied 2 other genetic combinations. In each instance, mice treated with homologous fetal liver were compared with those given homologous adult bone marrow. In one experiment, each of 22 irradiated (900 r) (C57L x A)F_1 mice was given 103 x 10^6 fetal liver cells from BDF_1 mice, and 22 were each given 40 x 10^6 bone marrow cells from adult BDF_1 donors. At frequent intervals from 1 to 64 days after exposure, some mice were killed and autopsied. In the other experiment, 16 irradiated (950 r) (101 x C3H)F_1 mice were each given 70 x 10^6 fetal liver cells from BDF_1 mice, and 18 were each given 39 x 10^6 bone marrow cells from adult BDF_1 mice. Mice were killed and autopsied at frequent intervals from 1 to 50 days in the fetal group and 1 to 64 days in the adult.

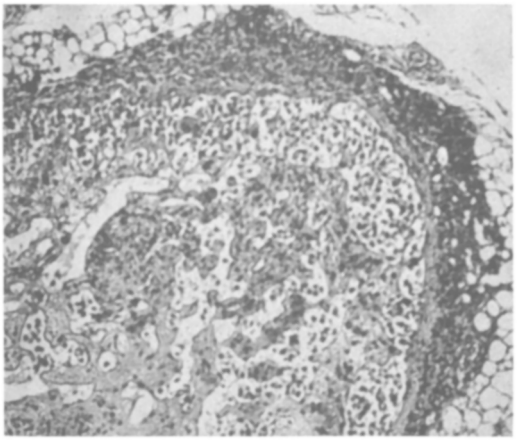

FIG. 2. Atrophic skeleton of a peripheral lymph node from a (C57L × A)F_1 mouse 50 days after 900 r and BDF_1 adult bone marrow treatment. Hematoxylin and eosin × 40.

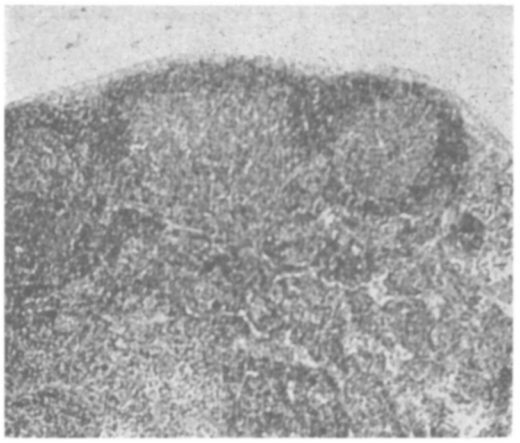

FIG. 3. Normal appearing peripheral lymph node from a (C57L × A)F_1 mouse 50 days after 900 r and BDF_1 fetal liver treatment. Hematoxylin and eosin × 40.

In both genetic combinations, autopsy and histologic studies indicated great superiority of homologous fetal liver treatment over that of adult homologous bone marrow. Figs. 2 and 3 show lymph node recovery in 1 experiment. The so-called 3-day swelling reaction of splenic white pulp was much weaker with homologous fetal liver(5) than with adult homologous bone marrow.

In another experiment of same design, equal amounts (22 x 10^6 cells) of isologous fetal liver or adult bone marrow were given to irradiated (950 r) (101 x C3H)F_1 mice. No significant differences between adult and fetal isologous tissue therapy were observed at autopsy and histologic study.

In other survival studies, pooled (101 x C3H)F_2 fetal liver was greatly superior to pooled (101 x C3H)F_2 adult bone marrow for treatment of lethally irradiated (C57L x A)F_1 mice. Similarly, survival was

higher in irradiated (C57L x A)F_1 mice given BDF_1 fetal liver than in those given BDF_1 adult homologous bone marrow. There were fewer delayed deaths, however, in this strain with BDF_1 adult homologous bone marrow than with (101 x C3H) F_1 or F_2 adult homologous marrow.

Fetal homologous spleen and liver were about equally effective in preventing delayed deaths in 11 (C57L x A)F_1 mice. One-day-old newborn homologous spleen (36 mice) or liver (32 mice) was as effective as fetal tissue in preventing delayed deaths. However, 2- to 4-day-old newborn homologous liver (33 mice) and 2- to 6-day-old newborn homologous spleen (53 mice) caused many delayed deaths, comparable in number to those resulting from treatment with adult homologous bone marrow. All homologous donors in these experiments were (101 x C3H)F_1 or F_2 mice. Isologous fetal and newborn liver and spleen in (101 x C3H)F_1 mice, promoted survival from radiation injury, as found earlier by Jacobson *et al.*(19) and Duplan(20).

In preliminary experiments, studies with fetal homologous liver cells gave no obvious significant differences in survival for 5 months after irradiation. Cell doses ranged from 19 to 117 x 10^6 nucleated cells. Donors at all stages of gestation were included. Donor livers were from (101 x C3H)F_2 mice, and 152 recipients were (C57L x A)F_1 mice. The long-term survival of mice treated with homologous fetal liver cells (30 to 60 x 10^6), was not significantly affected by gestation age of donor; cells taken at 12 to 17 and 16 to 20 days of gestation had about the same effect. The 72 recipient mice were the same types as those used for cell-dose study.

Fetal and newborn blood-forming tissues from Sprague-Dawley rats were given to male and female (101 x C3H)F_1 mice after 950 r for comparison with the effect of adult rat bone marrow treatment. Cell doses of fetal (15 to 22 days of gestation) and 1-day-old newborn rat liver (equal to dose of adult rat bone marrow), did not increase the number of 30-day (and longer) survivors (Fig. 4). Rather low 30-day survival was obtained with somewhat smaller doses of fetal and newborn (1- to 6-day-old) rat spleen. Rat red blood

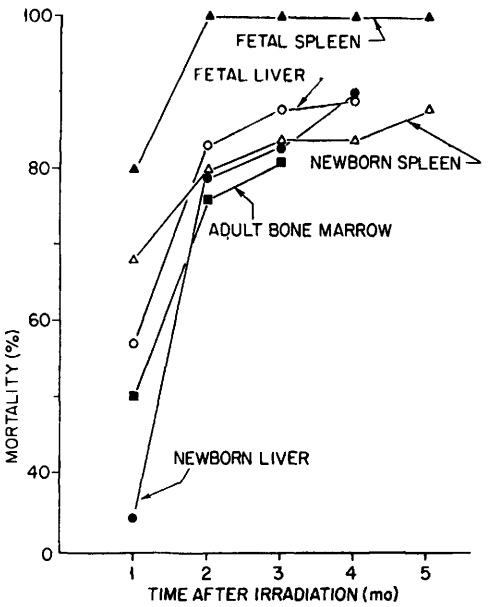

FIG. 4. Mortality in (101 × C3H)F_1 mice given 950 r plus rat fetal or newborn liver or spleen after irradiation. ■, controls, 101 mice: dose, 50 to 180 × 10^6 adult bone marrow cells. ○, 161 mice: dose, 80 to 180 × 10^6 fetal liver cells. ●, 29 mice: dose, 60 to 120 × 10^6 newborn 1-day-old liver cells. ▲, 10 mice: dose, 8 to 50 × 10^6 fetal spleen cells. △, 25 mice: dose, 10 to 60 × 10^6 newborn (1- to 6-day-old) spleen cells.

cell chimerism was observed in 7 mice tested 240 days after rat fetal liver treatment by hemagglutination technic described by Makinodan(21).

Discussion. Our observations show that therapy with fetal and 1-day-old newborn homologous liver and spleen, alters foreign bone marrow reaction in mice of these genetic combinations. Reports by Uphoff(17), Lengerová(15), and Barnes *et al.*(16) indicate that fetal liver and spleen in other genetic combinations are also capable of altering delayed mortality in foreign bone marrow experiments.

Owen and Urso did not find good long-term survival in C57BL/10D2 → (C3H x 101)F_1 mice treated with homologous fetal liver. (D. Uphoff and J. Trentin have also commented on genetic homologous combinations in which fetal blood-forming tissues were not superior to adult bone marrow in promoting long-term survival after irradiation.) Fetal rat liver was not superior to adult rat bone marrow cells when used in irradiated mice. More exten-

sive investigation into cell-dose requirement, age of gestation, and specific histocompatibility relations are probably needed for understanding of these unfavorable results.

Porter and Moseley(22) used 1- to 3-day-old newborn rabbit liver in irradiated homologous rabbits with 50% 14-day and 25% 56-day survival. This therapy seems inferior to previous results reported by Porter and Murray(23) with homologous rabbit bone marrow. Lacassagne *et al.*(24) had few survivors among irradiated rats, given a small dose of rat fetal liver.

Primary mechanism of action in favorable experiments with fetal and 1-day-old newborn tissues, is of considerable importance. Reports by Uphoff(17), Lengerová(15) and Barnes *et al.*(16) suggest a reverse "actively acquired tolerance" in which grafted fetal cells are presumed to have no antibody-forming ability. When antibody-forming cells do develop from the graft, they become "tolerant" of host antigens. The mechanism of actively acquired tolerance is itself not understood, and hence this becomes a kind of experimental analogy. Antibody-forming potentiality of blood-forming cells in fetal liver is not known.

Another idea for a primary mechanism would be the possibility of antigenic immaturity of fetal cells. These would be less able to evoke an immune reaction in the irradiated host.

In these experiments, fetal liver cells had no obvious effect on liver or other tissues of irradiated host.

Summary. Lethally irradiated mice that received blood-forming cells from homologous fetal liver showed better long-term survival and lymphatic tissue recovery, than those treated with blood-forming cells from adult homologous bone marrow. In both cases there was transplantation and persistence of erythropoietic cells.

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