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The Pattern of Recovery of Erythropoiesis in Heavily Irradiated Mice Receiving Transplants of Fetal Liver* (33988)

BERNHARD KUBANEK,[1] NICHOLAS RENCRICCA,[1] ADOLFO PORCELLINI,[2] DONALD HOWARD, AND FREDERICK STOHLMAN, JR.

St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, Massachusetts 02135

In the past we noted differences between adult and neonatal erythropoiesis in the rodent (1–3). In the newborn rat the red cells are hypochromic and macrocytic (2) whereas, in the adult, hypochromia when it occurs, is preceeded by microcytosis. Moreover, bilateral nephrectomy (2) and starvation (1) which produced red cell aplasia in the adult animal resulted in only a modest decrease in erythropoiesis in the newborn animal. In part, these differences may relate to extrarenal erythropoietin production in the neonatal rat (4) or perhaps in the sensitivity of red cell precursors to erythropoietin. The possibility of a fundamental difference in the regulation of red cell production however, must be considered. Efforts to pursue this problem in the intact newborn animal are hampered by the inability to adequately suppress erythropoiesis prior to the tenth to fifteenth day of life as well as the rapid growth of the animal which is associated with a continuing change in metabolic requirements and hence in the tissue O_2 supply–demand relationships. In an effort to circumvent these problems we initiated studies on red cell production in heavily irradiated adult mice which were transplanted with fetal hematopoietic tissue. In such a system the problems associated with rapid growth of the animal can be circumvented as can some of the technical problems associated with those maneuvers used for suppression of erythropoiesis. In the present study the splenic and femoral iron incorporation and peripheral reticulocyte counts were used as indices of red cell production after transplantation of either fetal liver or adult bone marrow cells. In parallel experiments the numbers of colonies produced by fetal liver cells or bone marrow cells were estimated by the technique of Till and McCulloch (5).

Materials and Methods. Virgin female CF_1

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[1] Research Fellow, St. Elizabeth's Hospital.

[2] NATO Fellow, St. Elizabeth's Hospital.

mice, 10–12 weeks of age, received 800 R whole-body radiation (dose rate 48 R/min; 250 kV, 15 mA with 1.0 mm Cu and 1.0 mm Al filtration). Immediately after irradiation they were injected with 12.0×10^6 nucleated fetal liver cells or 1.8×10^6 nucleated bone marrow cells from mice of the same strain and sacrificed at intervals thereafter. Plasma bound ^{59}Fe was injected intravenously 3 hr prior to sacrifice and the iron incorporation into bone marrow and spleen were measured. Absolute reticulocyte counts were derived as previously described (6). In separate experiments 4.0×10^5 nucleated fetal liver cells or 6.0×10^4 bone marrow cells were given to CF_1 mice immediately after they had received 800 R. The animals were sacrificed on the eighth day and the number of colonies in the spleen were counted. Liver suspensions were prepared, under sterile conditions, from 16.5 day-old fetuses using at least 4 pregnant mothers of the CF_1 strain and 6–10 fetuses/mother. The livers were excised, placed in ice-cold TC199 tissue culture medium and repeatedly drawn into a syringe using needles of decreasing diameter, from 19 to 25 gauge. Adult bone marrow suspensions were prepared from the tibiae of 6–8 mice as previously described (6). The number of nucleated cells were counted in a hemocytometer, the suspensions were appropriately diluted with TC199 and kept at ice-cold temperatures until injection.

Results. The mean number of colony-forming units (cfu) in 12.0×10^6 nucleated liver cells was 517 with a range of 364–779; in 1.8×10^6 bone marrow cells there were 504 cfu with a range of 409–641. These values were derived from 3 separate studies. Splenic iron incorporation values at intervals after transplantation are shown in Fig. 1. Data in this graph are pooled from five separate experiments. The absolute peripheral reticulocyte counts shown in Fig. 2 represent values from three experiments. The femoral iron incorporation values in irradiated recipients of fetal liver and adult marrow are given in Fig. 3. The hematocrits in the recipients of both

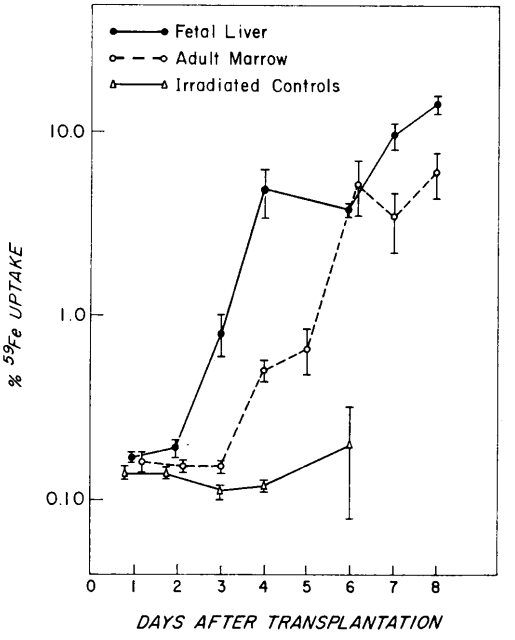

FIG. 1. Splenic ^{59}Fe uptake 3-hour in lethally irradiated mice receiving fetal liver or adult bone marrow: each point represents the mean ± 1 SE of 5–24 animals.

fetal and adult tissues were similar.

Discussion. In these experiments the numbers of colony-forming units transplanted into heavily irradiated recipients were similar for adult and fetal hematopoietic tissue but the splenic and femoral iron incorporation curves as well as the appearance of reticulocytes in the peripheral blood differed. In recipients of hematopoietic cells of fetal origin, there was, when compared with controls, a slight but significant increase in splenic iron uptake on the second day after transplantation and a striking increase in iron incorporation by the third day. These changes in red cell production, as judged from splenic iron incorporation, were reflected in the peripheral reticulocyte count 24 hr later. In those irradiated animals receiving adult bone marrow, the regeneration of erythroid tissue, as judged by splenic ^{59}Fe uptake and peripheral reticulocytes occurred 24 hr later than in the recipients of fetal liver cells. With the exception of the sixth posttransplant day (which will be discussed below) splenic erythropoiesis in the fetal transplants appeared to be

[3] Carworth Farms.

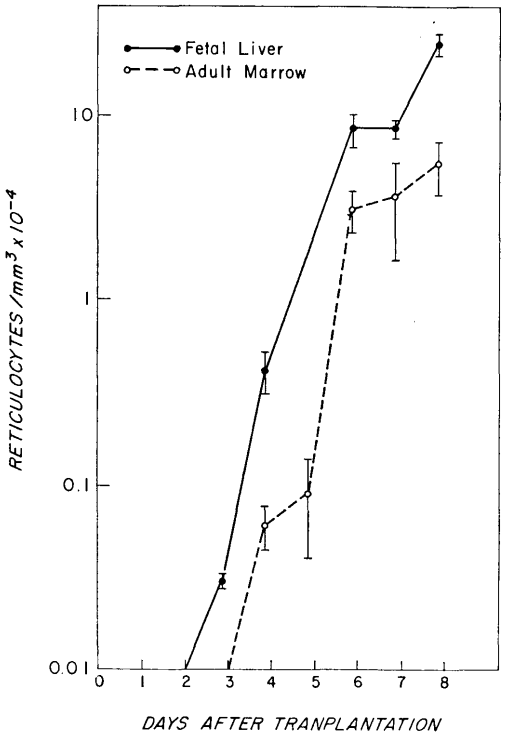

FIG. 2. Peripheral reticulocytes/mm^3 in lethally irradiated mice receiving fetal liver or adult bone marrow: each point represents the mean ± 1 SE of 5–22 animals.

greater throughout the study than that seen in recipients of adult bone marrow.

The pattern of recovery of erythropoiesis in the bone marrow differed from that seen in the spleen. In recipients of both adult and fetal tissues femoral iron uptake decreased through the second posttransplant day, as the differentiated compartment of the irradiated animal matured. On the third day there was a slight increase in femoral iron uptake in both transplanted groups. Thereafter there was a significant increase in erythropoiesis in the bone marrow of recipients of fetal liver through the sixth posttransplant day. At that time red cell production, as judged by iron incorporation, plateaued out at a level of about 50% of that which is seen in normal mice of this strain. In contrast erythropoiesis in the recipients of adult bone marrow was only slightly greater than that of irradiated controls throughout the period of study.

Thus in these experiments the recovery of erythropoiesis in recipients of fetal liver differed in two respects from that observed in animals transplanted with adult marrow. There was earlier recovery of splenic erythropoiesis and perhaps more importantly there was significantly greater erythroid colonization of the bone marrow in the recipients of fetal transplant. These observations suggest that there is in fact a difference in regulation if not in the nature of the stem cell for erythroid tissue in fetal life. In this respect it is to be noted that Feldman (7) reported that plethora does not completely suppress erythroid colony formation on the sixth and ninth posttransplant day in recipients of fetal liver, but does after transplantation with adult bone marrow. The earlier appearance of red cell production, as judged by splenic iron incorporation and peripheral reticulocytes, in our studies would be compatible with these observations. A clear-cut transition from fetal to adult erythropoiesis, however, could not be detected from the slopes of the splenic or myeloid iron incorporation curves

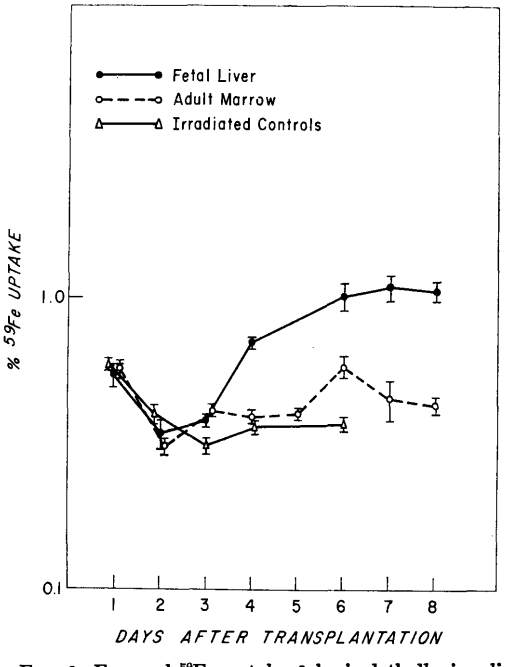

FIG. 3. Femoral ^{59}Fe uptake 3-hr in lethally irradiated mice receiving fetal liver or adult bone marrow: each point represents the mean ± 1 SE of 5–25 animals.

or from the peripheral reticulocytes. The observed differences between the time of onset of splenic red cell production in fetal and adult transplants could be attributed to a difference in generation time but the more extensive colonization of the marrow cannot be explained on the basis of generation time alone and appears to reflect a more fundamental difference in control.

It is of interest that Lucarelli[4] derived a generation time of ~7 hr for neonatal erythroid elements from evaluation of labeled mitoses in newborn animals. In adults of the same strain they obtained a generation time of 10-12 hr. Definitive information is not available on the comparative generation times of adult and fetal colony-forming cells, although preliminary data in our laboratory suggests that the generation time for fetal cfu may be somewhat shorter. An alternative explanation, namely that the differences in the recovery pattern of erythropoiesis were due to the fact that the fetal liver is in an actively "regenerating" state whereas the adult bone marrow is in a steady state, must await studies on the patterns of recovery after transplantation of regenerating adult hematopoietic tissue.

It is perhaps noteworthy that between days 4 and 6 posttransplantation in recipients of fetal liver and between days 6 and 7 in mice given adult marrow there was a change in the slope of the splenic ^{59}Fe uptake curve. This was also reflected in the peripheral reticulocyte curve. At this time the femoral ^{59}Fe incorporation curve appeared to plateau. One can only speculate on the cause of the phenomenon, which was observed in all experiments. The most likely explanation to us appears to be that there is a temporary diversion of stem cells from erythroid to myeloid differentiation. Thus during the initial phase of recovery stem cell differentiation was predominantly along erythroid lines and an exponential increase in red cell production resulted. As myelopoiesis increased there was a temporary diversion of a portion of stem cells previously directed toward erythropoiesis into myelopoiesis resulting in a change in the slope of red cell production. Changes in erythropoietin production might also have affected erythropoiesis. Although erythropoietin was not directly measured, the differences in the degree of anemia between days 4 and 7 as judged by peripheral hematocrits did not seem to be sufficiently great to lend support to this as the cause of the change in the slope of the splenic iron incorporation curve.

Summary. The recovery of erythropoiesis in heavily irradiated mice receiving fetal liver was compared with that of mice receiving adult bone marrow. The number of cells was adjusted so that each group received a comparable number of cfu. Red cell production as judged by both iron incorporation and peripheral reticulocyte values began earlier in the fetal liver transplant than after adult bone marrow. Further, the extent of erythroid colonization of the bone marrow appeared greater in recipients of fetal tissue than adult. These differences in the recovery pattern suggest that stem cell regulation of fetal erythropoiesis differs from that of the adult.

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