CONF-790524--5

BNL-26107

MASTER

HEMOPOIETIC STEM CELL NICHES, RECOVERY FROM RADIATION AND BONE MARROW TRANSFUSIONS

E.P. CRONKITE, A.L. CARSTEN

Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

G. BRECHER

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California

L. FEINENDEGEN

Kernforschunganlage, Jülich, West Germany

There are many models for stem cell behavior in the normal steady state. In Figure 1 is shown a concept for which we claim no originality. It is a useful model

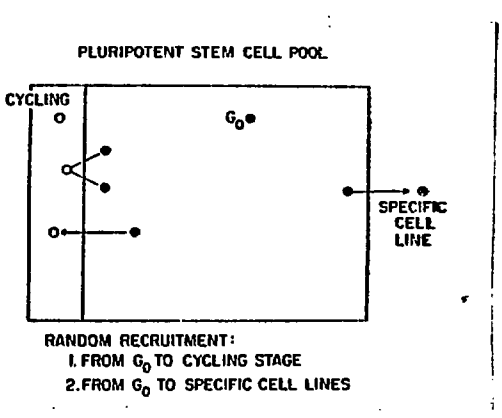

Figure 1. Schematic presentation of the pluripotent stem cell pool in normal steady state blood cell production. On the left is the cycling compartment and on the right is the resting G_0 compartment. Most investigators' data suggest that the cycling compartment is about 10% of the total. When a G_0 cell is induced down a specific cell line, another G_0 cell is triggered into cycle. The daughters of each mother cell move into G_0 stage and mix randomly.

from which to plan studies and discuss experimental results. The model shows the pluripotent stem cell pool (PSCP) as consisting of two compartments. On the left side of the compartment it is in cycle, producing two stem cells for each mitosis. We

believe that the two newly-produced stem cells enter the noncycling resting pool, G_o state, and mix randomly with the cells in this compartment. To maintain population size of both compartments and the total pool, there is a random recruitment from the G_o compartment of one cell into the cycling compartment for each mitosis and the recruitment of one G_o cell into a defined hemopoietic cell (erythropoietic, granulopoietic, thrombopoietic and perhaps, also lymphocytopoietic). The birth rate (K_B) of the PSC is

$$K_B = f\frac{N}{t_s}\,2 = \frac{N}{t_G} \times 2$$

where t_s = DNA synthesis time; f = fraction in DNA time; t_G = generation time (time for $G_1 + S + G_2 + M$); N = total number in PSC pool.

Since one cell is recruited into the cycling compartment from the G_o compartment, the flux from the cycling to the G_o and the flux out of G_o into all hemopoietic cell lines is N_s/t_s. It can be seen that K_B can be maintained constant as the total PSCP diminishes in size by compensatory increase in the fraction of pluripotent stem cells (PSC) in cycle). If in the normal steady state, 10% of PSC are in cycle and a hypothetical stem cell pool contains 1000 cells with t_s - 1 for simplicity

$$K_B = \frac{0.1 \times 1000}{1} = 100 \quad .$$

If the total PSC pool is reduced to 11% of its size or 110 cells, and if it is possible for the fraction in DNA synthesis to increase to a maximum of 0.9, the K_B would still maintain the steady state needs

$$K_B = \frac{0.9 \times 110}{1} = 100 \quad .$$

The above simplistic example assumes that t_s remains constant and that the fraction in s cannot exceed 0.9. In fact, the highest fraction in s observed to data for bone marrow (BM) cells is 0.9 (1) and the fraction of CFU-S killed by tritiated thymidine or hydroxyurea is about 0.8. Boggs *et al.* (2) have observed that the CFU-S do not allow cells to be directed down identifiable cell lines until the CFU-S pool exceeds 10% of its steady state size. Lajtha (3) believes that during regeneration about 40% of the daughter cells are lost from the stem cell regrowth, being diverted for continuing differentiation down the diverse hemopoietic cell lines (3,4). All of the preceding appears to be consistent with Mackey's (5) sophisticated mathematical modeling of the stem cell behavior in aplastic states.

From the studies of Rytomaa and Kiviniemi (6) and Kivalakso and Rytomaa (7) and Lord *et al.* (8-12), it is apparent that there are inhibitors of erythrocytic and granulocytic identifiable cell lines. These act on the amplifying non-selfrenewing chain of hemopoietic cell proliferation. In contrast, the inhibitors and stimulators of PSC described by Lord *et al.* (13) act specifically on the CFU-S as measured in liquid culture. They have no effect on descendants of the PSC. It is claimed by Lajtha and associates (3) that the stem cell inhibitor will completely shut off proliferation of all hemopoietic cell lines by terminating continuing differentiation into the different lineages. The removal of the stem cell inhibitor does not permit the CFU-S to proliferate. This requires that the specific stem cell proliferation stimulator be present in the liquid culture medium. These studies on CFU-S inhibitor and stimulators are all based on *in vitro* culture system analogous to studies on CSF.

With this background and concepts on the behavior of the PSC pool and factors that may operate *in vivo* in regulating its proliferation, let us consider other aspects of the PSCP.

The PSCP is radiosensitive and decreases in number in direct proportion to the dose of radiation and is measured by the colony-forming unit spleen (CFU-S)(14). The recovery in number of PSC after a single dose of sublethal radiation is generally not

complete (15,16). However, the total marrow cellularity returns to normal as does the granulocyte level in the blood. The return to normal production rates is accomplished by a smaller number of CFU-S having a higher fraction in DNA synthesis, the logic of which was discussed earlier, thus allowing a smaller number of CFU-S to produce the needed number of stem cells for direction down the diverse cell pathways required for maintenance of life. These and other observations (3,17) led to the concept that there is a defined number of niches within the body for stem cells, and when these are filled further stem cells cannot be accepted from transfusion or through regenerative processes after induction of marrow hypoplasia. This notion is shown schematically, Figure 2. On the left side are shown the niches with a PSC comfortably ensconced in

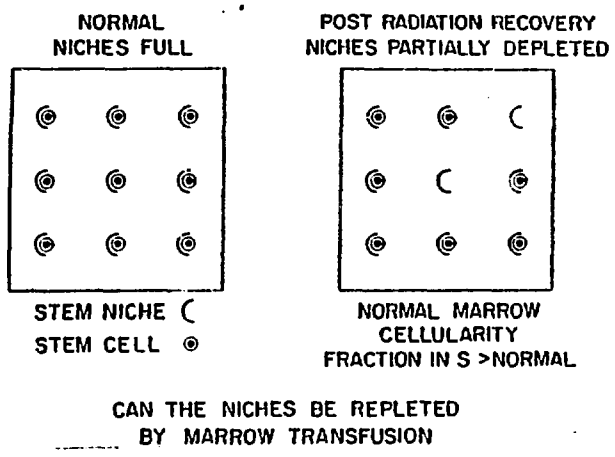

Figure 2. Schematic presentation of stem cell niches, their depletion and failure to replete completely after recovery from sublethal irradiation with normal marrow cellularity and peripheral blood counts.

its hiding place. On the right side after recovery from radiation, when marrow cellularity has returned to normal along with blood counts, empty niches are shown. The questions that arose in our minds were as follows: 1) Can one replete the assumed empty niches by massive syngeneic bone marrow transfusion? 2) Why doesn't the irradiated animal replete all of the assumed niches? Is the anatomic basis (if any) for some of the niches destroyed by irradiation or other forms of marrow injury? 3) If the niches are intact and present, why does the animal maintain normal cell production with a smaller number of CFU-S with greater fraction in s rather than replete niches, reduce fraction in s and allow a longer time in G_0 for "genetic housekeeping" or repair of injury to DNA, a concept of Lajtha (3)?

These problems have been approached two ways. Brecher (18) has been studying the appearance of cells in recipient BM with chromosome markers after BM transfusion to recipients that have had different treatments. Carsten and Cronkite (22) have tried to replete the BM CFU-S at diverse times after recovery from maximal sublethal doses of x-ray or during continuous exposure to tritiated water. Brecher has also searched for the sex chromosome in our animals receiving marrow transfusions from the opposite sex. In addition, Brecher et al. (18) have studied the effect of diverse treatments upon the acceptance of BM transfusions as shown by chromosomal markers. The experimental details, materials and methods are published elsewhere (18,19).

RESULTS

In Figure 3 is shown the effect of a single 250 Kvp x-ray dose of 525 rads (120 rads/min) upon CFU-S content of the leg (femur plus tibia) in Hale-Stoner male mice.

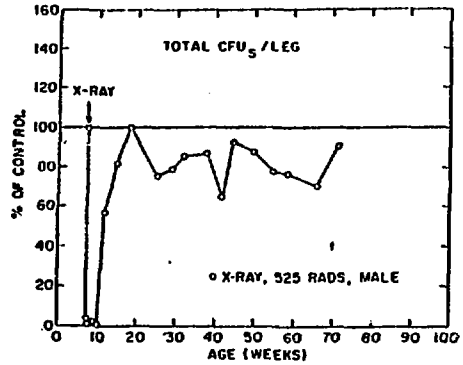

Figure 3. The CFU-S content of a leg of a mouse is expressed as the percent of its age-matched control non-irradiated mouse for a period of 70 weeks from birth and 62 weeks after irradiation with 525 rads.

The results are expressed as percent of the control animals of the same age. Note that the CFU-S content drops to nearly 0 a few days after this maximal sublethal dose of x-ray. Between two and three weeks after irradiation, there is a rapid recovery nearing normal level by about 10 weeks after exposure. Thereafter, the CFU-S content of the irradiated mice drops and remains about 20% below the age-matched controls for 70 weeks.

In Figure 4, the effect of chronic exposure to tritiated water, 3.0 μCi/ml in drinking water, chronic exposure to ^{137}Cs gamma irradiation at ∿0.7 rads/day and a single dose of 525 rads x-ray upon the CFU-S content of BM are shown. The CFU-S content is expressed as percent of the number of CFU-S observed in age-matched controls. With the exception of one aberrant or inexplicable observation in chronic tritiated exposed mice at 60 weeks, all observations show a consistent depression below age-matched controls of between 10 and 46%. The depression is present in the animals that

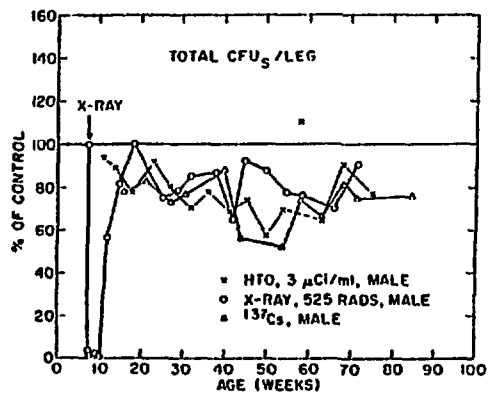

Figure 4. The CFU-S content of mice exposed to ^{3}HOH 3.0 μCi/ml drinking water and chronic ^{137}Cs gamma radiation (∿.7 rads/day) is compared to that of mice given a single maximal sublethal exposure of 525 rads x-ray. Results are expressed as percent of the number of CFU-S in the age-matched controls.

had only a single exposure to 525 rads and in those chronically exposed to ^{137}Cs gamma rays at a dose rate of ∿0.7 rads/day. Between 42 and 60 weeks, the chronically

exposed mice had CFU-S levels below that of the single-maximal sublethally-exposed mice, but from 60 to 80 weeks of age all were about equally depressed, around 80% of the age-matched controls.

In Figure 5, the total marrow cellularity is shown as a function of age, from two weeks of age to 100 weeks and after exposure of mice to 525 rads x-ray at seven weeks of age. By about one week after irradiation, the total number of marrow cells

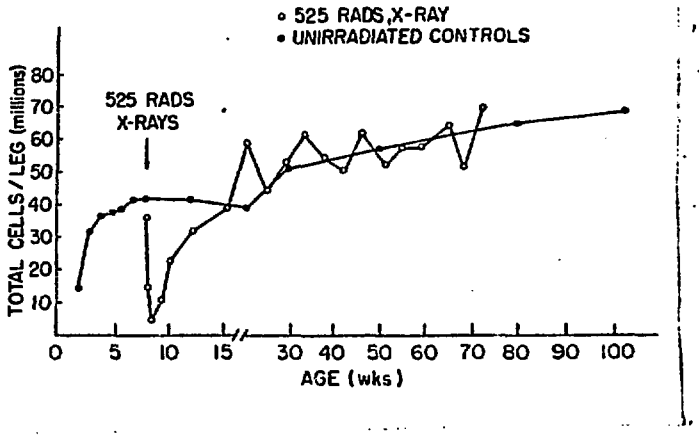

Figure 5. The increase in marrow celluarity is shown as a function of age from two to 100 weeks of age. Also, the effect of 525 rads whole body x-ray upon marrow cellularity is shown.

decreased to about 10% of the normal level. By 15 weeks of age, or eight weeks after irradiation, the total nucleated marrow cellularity had returned to normal at which time CFU-S content was still 30% below normal (see Figure 3). To accomplish this certainly requires that the CFU-S production rate per CFU-S present be substantially greater than normal in order to expand the CFU-S pool from nearly zero and also supply cells for the regeneration of three major hemopoietic amplifying cell lines. Hence, these cells exposed to a mutagenic agent-radiation are dividing rapidly and have little time for "genetic housekeeping" as defined by Lajtha (3) and thus may be susceptible to retaining defects that may lead to later development of leukemia.

In Table I is shown the tritiated thymidine cytocide for CFU-S at different times after recovery from the sublethal irradiation for mice exposed to a single whole body x-ray dose of 525 rads at 8 weeks of age. The tritiated thymidine cytocide is consistently higher at 10, 18 and 36 weeks after irradiation.

TABLE I

Percent ^{3}Htdr Cytocide

Time after x-ray (weeks)	525 rads WB x-rays	Unirradiated controls
10	24.30	16.20
18	24.30	16.13
36	20.53	15.40

from Carsten and Cronkite (16)

TABLE II

The effect of transfusion of 2 x 10^8 bone marrow (BM) cells upon the depressed CFU-S content of mice having received 525 rads 12 weeks earlier or ^{3}HOH (3.0 µCi/ml) in drinking water for 10 weeks

	Col/60,000	S.E.	^{3}Htdr cytocide	Total cells per leg	Total CFU-S per leg
525 rads to male mice 12 weeks before injection of 2 x 10^8 female BM cells					
day 1 BM cells	15.5	0.55	12.3%	53.4	13,795
media-injected controls	16.3	0.71	14.1%	59.3	16,096
day 7 BM cells	16.6	0.70	9.6%	61.1	16,891
media-injected controls	18.7	0.63	12.8%	56.9	17,718
Female mice on 3.0 µCi HTO for 10 weeks before injection with 2 x 10^8 BM cells or medium					
day 1 BM cell-injected	15.6	0.55	18.6%	57.5	14,937
media-injected controls	16.8	0.53	18.5%	55.4	15,498
day 7 BM cell-injected	13.6	0.55	16.2%	46.8	10,608
media-injected controls	13.2	0.55	10.6%	54.2	11,913

In Table II is shown the effect of transfusion of 2 x 10^8 BM cells upon the CFU-S content of mice that have received 525 rads 12 weeks earlier or that had been on tritiated water, (3.0 µCi/ml of drinking water) for 10 weeks. The CFU-S content of 525 rads mice 12 weeks after exposure would be about 80% of normal. The CFU-S content of mice on tritiated water (3.0 µCi/ml drinking water) would be about 80% of normal. The CFU-S content of the BM in each case was assayed one and seven days after the BM transfusion and compared to mice that received only the suspending tissue culture medium. In each instance, the CFU-S ratio to total marrow cells and the total CFU-S content per leg was greater in the mice that received only tissue culture medium. However, with the observed standard errors, the difference is not significant. In this study, there is no indication of CFU-S entering any assumed empty nighes even though about 2 x 10^5 CFU-S were contained in the injected BM.

Tritiated thymidine cytocide was also performed. There were no consistent changes amenable to interpretation.

Several animals were given 2 x 10^8 female BM cells into male donors and male donors were examined by Brecher et al. (18). Not a single karyotype of the opposite sex was observed.

In Table III are shown the results of karyotyping BM of mice that had been restored by 4 x 10^6 BM cells of the same or opposite sex. In addition, one to four months after the first BM transfusion where the animals had normal blood counts, a second transfusion of 4 x 10^7 BM of either sex was given to determine if one could get

injury and functional recovery of the BM in these groups. The reduction in the number of BM stem cells might be attributed to stromal injury in the marrow such that it cannot support as many stem cells as previous to the radiation exposure. This concept of an insufficient number of "niches" or supportive environment could explain the results. The first notion on possible anatomical areas for stem cells is suggested by Micklem and Loutit(20) who used the term "proliferative sites". Subsequently, Schofield(17) and Lajtha(3) have elaborated on the notion and Patt and Maloney(21) have presented evidence defining the BM volume or domain for the stem cells.

As an alternate to the "niche" hypothesis, injury to the stem cell pool such that self-replication was not sufficient to restore normal cell concentrations is a possibility. Since, by necessity, the stem cell must have self-replicative potential, at least for the larger single doses the concept of insufficient niches makes the most reasonable explanation. The question of whether there are niches waiting to accept stem cells or whether the niches themselves have been destroyed is the concept which was investigated in this study. It appears from our observations that if empty niches do exist they are not filled through transfusion of BM cells as done here. This inability to restore the normal PSC content does not, of course, mean that transfusions of PSC's are ineffective in restoring hematopoiesis to the severely irradiated animal. This is well established by the protective effect of marrow transfusions in lethally irradiated animals where evidence of restoration of depleted marrow is apparent through the establishment of hematopoietically active foci in both the BM and the spleen, as well as by survival of the animal.

The time sequence of transfusion of the marrow may be important to the ultimate effect. Attempts to fill empty niches at 10 and 12 weeks following a single and severe radiation injury may be impossible due to stromal changes which in effect have elminated the niches. This may not be the case when stem cells are transplanted shortly after irradiation and the animal has not yet compensated for its loss in marrow cellularity. Although the reduction in stem cells is essentially equivalent for the chronic and acute exposure, the mechanisms causing this reduction may not be identical and the inherent injury in the animal may also differ in nature when, in one case, a severe depletion followed by rapid regeneration is apparent as compared to a continuing insult which is compensated for by a continuing mechanism.

The studies presented in Table III show that the BM of animals rescued by transfusion of 4×10^6 BM cells will accept from 0 to 25% of the second transfusion of BM cells given one to four months after the first transfusion and examined two to three weeks after the second transfusion of 4×10^7 cells. Whether this is due to the second transfusion filling up empty niches or whether the CFU-S's in the second transfusion have exchanged with the blood remains to be seen.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Arthur Gremillion, Mr. Larry Cook, and Mr. James Bullis for their most competent laboratory assistance. Research supported by the U.S. Department of Energy.

REFERENCES

1 CRONKITE, E.P., BOND, V.P., FLIEDNER, T.M., KILLMANN, S.A., CIBA Foundation Symposium on Hemopoiesis, Churchill, London (1960)
2 BOGGS, S.S., CHERVENICK, P.A., BOGGS, D.R., Blood 40:375 (1972)
3 LAJTHA, L.G., GILBERT, C.W., GUZMAN, E., Brit. J. Haematol. 20:343 (1971)
4 VOGEL, H., NIEWISCH, H ., MATIOLI, G., J. Cell Physiol. 72:221 (1968)
5 MACKEY, M.C., Blood 51:941 (1978)

TABLE III

Transplantability of isogeneic marrow into previously irradiated, hematologically normal mice

	Sex of Recipients	No. of Animals	Sex of Initial Donor	Sex of Second Donor	Percent Bone Marrow Cells (Mean and Range) Male	Female
1	female	11	female	male	7.6 (1-25)	92.4
2	female	3	male	female	88.3	11.7 (1-23)
3	female	3	male	--	98.0	2.0 (0-6)
4	male	11	male	female	98.8	1.2 (0-4)
5	male	3	female	male	17.3 (15-20)	82.7
6	male	2	female	--	1.0 (0-2)	99.0

penetration of sex marked CFU-S into the recipient. Lines 3 and 6 express the control results from the first rescue transfusion after an otherwise fatal dose of 900 rads whole body x-ray. Note that 98% of the BM cells are male donor in origin and only 2% are female from endogenous regeneration in line 3. Line 6 shows that 99% of the mitoses are in the sex karyotype of the rescue BM.

Lines 1, 2, 4 and 5 show the penetration of BM cells of the opposite sex into the restored BM when 4×10^7 BM cells are transfused and the BM is examined two to three weeks later. In each instance, karyotypes corresponding to the sex of the second donor were observed varying from an average of 1.2 to 17.3%. The incidence of second donor karyotypes was significantly less when the second donor was female and the recipient was male.

DISCUSSION

The long term hematologic effects of a single whole body sublethal x-ray exposure (525 rads) and low level chronic exposure from ^{137}Cs gamma rays and ingested HTO have been investigated in mice. The single x-ray exposure had an early severe effect on the BM both in terms of total cellularity and number of PSC's. The animal possesses sufficient reserves in the PSCP to maintain normal cellularity in the chronically exposed animals or after an immediate sharp depression, restore normal cellularity in the single x-ray exposed animal. This is done in the presence of a reduced total number of PSC's. These observations present at least two questions. 1) How does the animal maintain normal cellularity in the absence of a normal number of PSC's? 2) Is the failure to restore normal levels of PSC's due to damage to the PSC pool or to the supporting environment in the BM such that it cannot support a normal number of PSC's? The first of these questions can be answered by at least three different mechanisms: 1) additional divisions in the cytologically identifiable mitotable pool of the BM; 2) a shortening of cycle time allowing more divisions in the same time with greater amplification of a smaller number of CFU-S's, and 3) recruitment of G_o stem cells into proliferation. Of these three possibilities, the third was tested by the tritiated thymidine cytocide technique. Results of these measurements on the 525 rad x-rayed animals indicate that the mouse does compensate, at least in part, by an increase in number of cells in active DNA synthesis. The results in the case of the chronically exposed animals are not as definitive. While there may be a similar compensation in the chronically exposed animals, the results are not sufficiently well defined to attribute the observed effects to this explanation. These observations lead to the second major question of whether or not different mechanisms may be involved in the

6 RYOTOMAA, T., KIVINIEMI, K., Cell Tissue Kinet. 1:329 (1968)
7 KIVALAASKO, E., RYOTOMAA, T., Cell Tissue Kinet. 4:1 (1971)
8 LORD, B.I., CERCEK, L., CERCEK, B., SHAH, G.P., DEXTER, T.M., LAJTHA, L.G., Brit. J. Cancer 29:168 (1974)
9 LORD, B.I., CERCEK, L., CERCEK, B., SHAH, G.P., LAJTHA, L.G., Brit. J. Cancer 29:407 (1974)
10 LORD, B.I., MORI, K.H., WRIGHT, E.G., LAJTHA, L.G., Blood Cells 3:451 (1977)
11 LORD, B.I., SHAH, G.P., LAJTHA, L.G. Cell Tissue Kinet. 10:215 (1977)
12 LORD, B.I., MORI, K.J. WRIGHT, E.G., LAJTHA, L.G., Brit. J. Haematol.34:441 (1976)
13 LORD, B.I., MORI, K.J., WRIGHT, E.G., Biomedicine 27:223 (1977)
14 TILL, J.E., MCCULLOCH, E.A., Radiat. Res. 14:213 (1961)
15 CARSTEN, A.L., BOND, V.P., Normal and Malignant Cell Growth, Fry, R.J.M., Griem, M.L. and Kirsten, W.H., eds., p. 21, Springer-Verlag, NY (1969)
16 CARSTEN, A.L., CRONKITE, E.P., IAEA - International Symposium on Biological Implications of Radionuclides Released from Nuclear Industries, Vienna (1979)
17 SCHOFIELD, R., Blood Cells 4:7 (1978)
18 BRECHER, G., TJIO, J.H., HALEY, J.E., NARLA, J., BEAL, S.L., Blood Cells (in press)
19 CARSTEN, A.L., CRONKITE, E.P., Proc. Soc. Exp. Biol. Med. 137:948 (1971)
20 MICKLEM, H.S., LOUTIT, J.F., Tissue Grafting and Radiation, Academic Press, NY (1977)
21 PATT, H.M., MALONEY, M.A., Blood Cells 4:27 (1978)
22 CRONKITE, E.P., CARSTEN, A.L., Unpublished Data (1979)