ENHANCED ERYTHROPOIESIS WITH CONCOMITANT DIMINISHED GRANULOPOIESIS IN PREIRRADIATED RECIPIENT MICE

Evidence for a Common Stem Cell*

By SAMUEL HELLMAN, M.D., and HELEN E. GRATE

(From the Department of Radiology, Yale University School of Medicine, New Haven, Connecticut 06510)

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Mice recovering from low doses of radiation have an increased capacity to support the erythropoietic proliferation of transplanted bone marrow (1). This, then, is a demonstration of the state of the recipient affecting its capacity to support the proliferation of transplanted bone marrow progenitor cells. The erythropoietic capacity was measured as described by Hodgson (2, 3). We have recently described a method which measures the proliferative capacity of transplanted bone marrow to produce granulocytic progeny (4). This is analogous to the erythropoietic technique. In both methods, recipient mice are irradiated with a dose of X-rays sufficiently large (700 R) to reduce markedly the proliferative capacity of the endogenous bone marrow cells. Subsequently, graded amounts of syngeneic bone marrow cells are injected and, 7 days later, the progeny of the transplanted bone marrow are assayed in the following manner. In the erythrocytic technique, ^{59}Fe is injected, and the incorporation into the peripheral blood is measured 2 days later. This can be shown to be proportional to the number of bone marrow cells injected 1 wk earlier and can be used to assay the injected bone marrow (2). Similarly, in the assay of granulocytic progeny, one injects bacterial endotoxin and measures the white blood cell response to the injected endotoxin. This can be shown to be primarily a granulocyte response and proportional to the number of bone marrow cells injected 7 days earlier (4). Therefore, two comparable techniques are available enabling measurement of the proliferative capacity of bone marrow progenitor cells to form the two major mature peripheral blood elements.

A third technique available for measuring progenitor cells in transplanted marrow is the spleen colony technique of Till and McCulloch (5). In this technique, heavily irradiated recipient animals receive, intravenously, syngeneic bone marrow. After a sufficient period of time, usually 8–10 days, the animals are sacrificed, the spleens removed, and visible nodules on the surface counted. The number of these nodules is found to be proportional to the bone marrow cells injected (5). They appear to be derived from single cells (6) and contain hematopoietic cells of varying maturity (7).

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Since low dose preirradiation of the recipient enhances proliferation of transplanted progenitor cells as measured by the production of erythrocytic progeny, it was of interest to measure such progenitor proliferation by these other methods. The present report describes the influence of a conditioning dose of 150 R to recipient animals on the proliferative capacity of transplanted bone marrow as measured by these three methods.

Materials and Methods

Animals used in all experiments were male C3H/HEJ mice, 8–12 wk old.[1] X-radiation factors were: 250 kv, 15 ma., HVL[2] 1.5 mm of copper, FSD of 50 cm. Dose rate was approximately 120 R per min. Dose measurements were made in a mouse "phantom" using a Baldwin Farmer dosimeter. Before and after irradiation, animals were placed in cages of eight animals each and supplied food and water at lib.

Bone marrow suspensions were prepared by removal of cells from the tibia and femur by flushing the medullary cavity with cold sterile Tyrode's solution. The cell suspensions were kept on ice until used and were injected intravenously into the lateral tail veins not longer than 1.5 hr after removal. A sufficient volume of solution was used so that the injected bolus varied between 0.05 and 0.4 ml. Cell counts were made using a hemocytometer counting at least 200 cells.

Assay of the Erythrocytic Repopulating Ability of the Transplanted Bone Marrow.—Recipient animals were exposed to 700 R whole body irradiation and within 2 hr injected with graded numbers of syngeneic bone marrow cells suspended in Tyrode's solution. 7 days were allowed for the cells to proliferate, and then the animals received intravenously 0.5 μc of ^{59}Fe as ferrous citrate. 2 days later a cardiac puncture was made and 0.5 ml of blood removed and counted in a well scintillation counter. The per cent of ^{59}Fe incorporation was then calculated assuming a blood volume of 0.66 cc per 10 g body weight. In the preirradiation group the technique was identical, except that these randomly selected littermates of the control animals were exposed to 150 R whole body irradiation 7 days before 700 R and bone marrow transplantation. Both the control and preirradiated animals were transfused with the same bone marrow at the same time (Fig. 1A). Six to eight surviving animals were used for each dose of bone marrow in both control and preirradiated groups.

Assay of the Granulocytic Repopulating Ability of the Transplanted Bone Marrow.—Recipient animals were exposed to 700 R whole body irradiation and within 2 hr injected intravenously with graded numbers of syngeneic bone marrow. 7 days were allowed for the bone marrow cells to proliferate, and then the animals were given 10^{-3} gamma of Pyrexal,[3] a bacterial endotoxin isolated from *Salmonella abortus-equi*. The white blood cell count of each animal was measured immediately before and at 2, 4, and 6 hr after the injection using a Coulter electronic counter, model B. The maximum white blood cell response in each animal was then grouped and plotted against the number of cells injected 1 wk earlier. The preirradiated animals were randomly selected littermates of the controls. They were exposed to 150 R 7 days before 700 R and bone marrow transplantation. These animals received the same bone marrow cells at the same time as did the controls (Fig. 1B). Two replicate experiments were performed; the results were similar and thus were pooled. This resulted in 9–10 surviving animals for each dose of bone marrow in both the control and preirradiated groups.

Spleen Colony Method.—Recipient mice were exposed to 850 R whole body radiation and,

[1] Supplied by Jackson Laboratories, Bar Harbor, Maine.

[2] HVL, half-value layer; FSD, focus-to-skin distance.

[3] Originally prepared by the Dorsey Laboratories, Lincoln, Nebr.

within 2 hr, injected with syngeneic bone marrow. 9 days later the surviving animals were sacrificed, the spleens removed and placed in Bouin's solution. 24 hr later, using a dissecting microscope, all colonies 0.5 mm in greatest diameter or larger were scored and measured. The preirradiated animals were treated in a similar fashion and received the same bone marrow as their littermate controls. However, they were pretreated with 150 R whole body irradiation 7 days before the 850 R and bone marrow. (Fig. 1C) Two replicate experiments were performed with 20 mice in each experimental group.

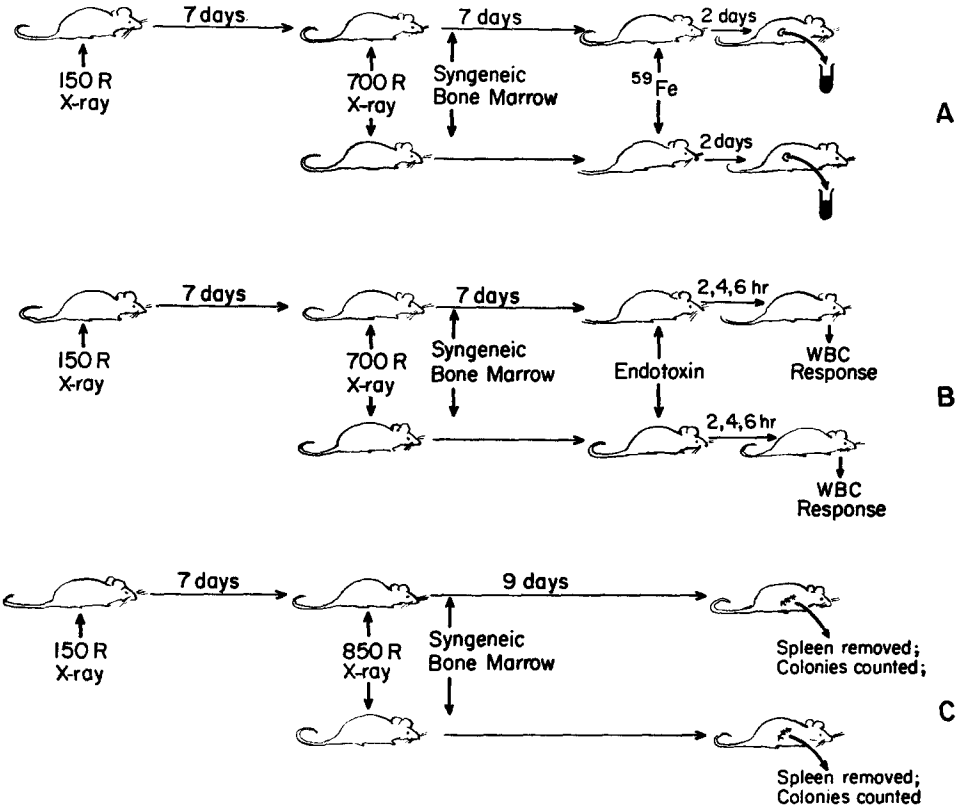

FIG. 1. A. Measurement of erythrocytic progeny of transplanted bone marrow in preirradiated and control recipients. B. Measurement of granulocytic progeny of transplanted bone marrow in preirradiated and control recipients. C. Measurement of spleen colonies produced by injection of syngeneic bone marrow in preirradiated and control recipients.

RESULTS

Erythropoiesis.—The enhancement of erythropoiesis by preirradiation was shown in C57/Bl mice allowing 5 days after bone marrow transplantation before injecting of ^{59}Fe (1). In the present experiment, a different strain of mice is used, and 7 days are allowed for proliferation and differentiation before ^{59}Fe is injected. The results can be seen in Table I. There is consistently higher ^{59}Fe

incorporation in those recipient animals who were preirradiated than in non-preirradiated control animals receiving no bone marrow cells, indicating either that there were more surviving stem cells after 700 R in the preirradiated group or, alternatively, that the recipient animals allow greater proliferation of these survivors. These results are consistent with the previous report (1).

Granulopoiesis.—The effect of preirradiation of recipient animals on the granulopoietic capacity is shown in Fig. 2. In this figure, the maximum white blood cell response to endotoxin is plotted against the number of bone marrow cells injected 7 days earlier. In both the test and the control groups, a linear relationship is obtained. The slope as well as the absolute counts at each dose of bone marrow are reduced in the preirradiated recipients. This indicates that the preirradiated animals are less able to support the granulopoietic ability of

TABLE I

^{59}Fe Incorporation into Newly Formed Erythrocytes 7 Days after 700 R Whole Body Radiation and Transfusion of Graded Numbers of Syngeneic Bone Marrow Cells

No. cells injected 1 wk earlier	Per cent ^{59}Fe uptake	
	Preirradiated	Control
0	4.3 (±0.7)	2.0 (±0.2)
0.48×10^6	18.0 (±2.1)	7.3 (±2.1)
0.96×10^6	32.3 (±2.1)	24.5 (±2.7)
1.92×10^6	41.3 (±5.8)	31.3 (±2.6)

The preirradiated recipients received 150 R 7 days before the 700 R and bone marrow. All figures represent mean of 6 to 8 recipients. Number in parenthesis represents standard error of the mean. The statistical significance of the difference between the control and preirradiated recipients is $P = >0.001$ (15).

the transplanted bone marrow than are the controls. This occurs at all dose levels of marrow shown, even in those animals who received no bone marrow. The preirradiated recipients appear to have inverse proliferative capacities when measured by these two systems. Such recipients have an enhanced ability to support erythropoiesis and an impaired ability to support granulopoiesis.

Spleen Colony Technique.—Table II shows the results of the spleen colony technique. There is no difference in the number of spleen colonies produced when the same bone marrow suspension is injected into preirradiated or control recipients. This indicates that the number of progenitor cells able to lodge and grow in the spleen is the same in either recipient group. This was true in both replicate experiments. Measurement of the diameter of the colonies revealed significantly larger colonies in the preconditioned recipients. This suggests that while the number of stem cells lodging and growing in the spleens is the same, the proliferative capacity of such cells is greater in these surface colonies in preirradiated recipients.

DISCUSSION

Preirradiation of recipient animals results in enhanced production of erythrocytic progeny from transplanted bone marrow. Concomitant with this is a decrease in the ability of such recipients to produce granulocytic progeny. This inverse relationship can be explained by hypothesizing a limited number of common hematopoietic stem cells on which competing proliferative demands

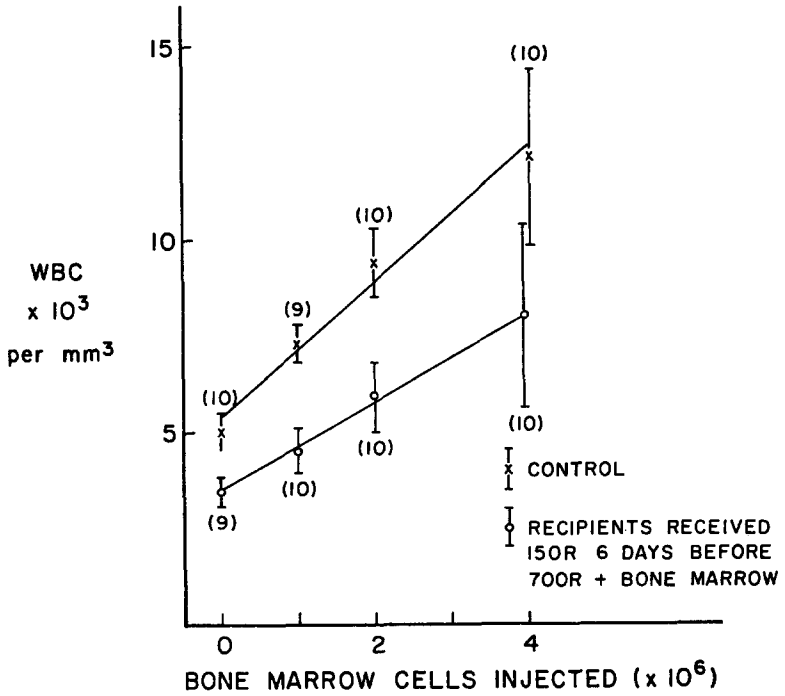

FIG. 2. Maximum white blood cell response to endotoxin in irradiated (700 R) recipient animals plotted against dose of bone marrow injected 7 days earlier.

Number of animals at each point shown in parenthesis. Lines drawn by regression analysis using method of least squares. Statistical significance of the difference between the control and preirradiated recipients is $P = >0.001$ (15). Ratio of the slopes = 1.52.

are made by both erythropoiesis and granulopoiesis. We have obtained other evidence for such a phenomenon in recipient animals in whom the demand for erythropoiesis has been increased (8). In those experiments, bled recipients or recipient animals receiving erythropoietin have a decreased ability to support granulopoiesis compared to control animals. Harris et al. (9) have found a reduced number of granulocytic precursors in guinea pig bone marrow after an acute hemorrhage. Dividing cells, of both granulopoietic and erythropoietic

series present in a single spleen colony, are reported to be the progeny of a single cell as determined by chromosomal analysis (10). Such results are consistent with the notion of a population of cells able to differentiate along either granulocytic or erythrocytic lines.

The results with the spleen colony technique in the present experiments can be interpreted as being consistent with the other two assay procedures. The fact that the colony number is the same in control and preirradiated animals indicates that the "seeding" efficiency in preirradiated recipients is unchanged. The larger colony size indicates more progeny per transplanted cell. The spleen

TABLE II

Spleen Colony Size and Number in Control and Preirradiated Recipients

Exp.	Cells injected	Preirradiation of recipients	No. of spleens counted	Colonies per spleen	Mean colony diameter
		R			*mm*
I	0	0	19	0.3 (±0.2)	
	0	150	10	0.6 (±0.3)	
	9.7×10^4	0	19	9.7 (±1.1)	1.24 (±0.03)
	9.7×10^4	150	11	9.5 (±1.2)	1.37 (±0.05)
II	0	0	8	0.1 (±0.1)	
	0	150	7	0.4 (±0.3)	
	1.05×10^5	0	20	11.2 (±1.0)	0.78 (±0.01)
	1.05×10^5	150	17	10.0 (±1.3)	1.07 (±0.02)

All animals were exposed to 850 R whole body radiation and then injected with syngeneic bone marrow cells. The spleens were removed 9 days later. The preirradiation dose of X-ray was given 7 days before the 850 R and bone marrow. The number in parenthesis represents the standard error of the mean.

In both experiments there was no significant difference in the colony number; however, the difference in colony size was statistically significant: Exp. I, $P > 0.01$; Exp. II, $P > 0.001$.

in the mouse recovering from radiation is primarily concerned with erythropoiesis (11, 12). Discrete surface colonies are most often erythocytic (13, 14). Therefore, most of the colonies being measured are primarily erythropoietic, and their increase in size in the preirradiated recipients is consistent with the increased production of erythropoietic progeny demonstrated by the ^{59}Fe repopulation technique. Because of this predilection for erythropoiesis, the spleen colony technique is not useful in measuring the whole animal's ability to support myelopoietic proliferation.

Of interest is why, 1 wk after a low dose of whole body radiation, the animal favors erythropoiesis at the expense of granulopoiesis. This is being actively investigated at present. Preliminary results suggest that the initial response to

radiation by the stem cell pool is a preferential differentiation toward the granulocyte. Perhaps the animal recovering from irradiation 1 wk later is compensating for this initial sparing of granulopoiesis. Blackett and coworkers[4] are studying the time course of this enhancement of erythropoiesis by preirradiation of the recipient. Preliminary data indicate an observable effect in animals preirradiated 3 days before radiation and bone marrow transplantation. This reaches a maximum at 7 to 10 days and then gradually diminishes.

The present study demonstrates that changes in the recipient can affect the ability of progenitor cells to give rise to differentiated progeny. This relationship of enhancement of erythropoiesis with concomitant decrease in granulopoiesis is similar to the decrease in granulopoiesis observed in recipient animals having an increased demand for erythropoiesis (8). Such data argue for a common stem cell whose path of differentiation can be directed by the milieu in which it is proliferating.

SUMMARY

Three different methods of measuring the proliferative capacity of transplanted mouse bone marrow were used to study the effects of preirradiation of the recipient. Recipient mice were exposed to 700 R and given graded numbers of syngeneic bone marrow. 7 days were allowed for proliferation of these cells, and then the granulocytic or erythrocytic progeny was measured. The former was determined by the response to endotoxin, and the latter by the incorporation of radioactive iron into newly formed red blood cells. Erythropoiesis, therefore, could be measured independently from granulopoiesis by these techniques. The third method used was the spleen colony method of Till and McCulloch (5).

Recipient animals exposed to 150 R preirradiation, 7 days before 700 R and bone marrow transplantation, demonstrated an increase in erythropoiesis with a concomitant decrease in granulopoiesis compared to similar recipients not preirradiated. The spleen colony technique showed that while the number of colonies were the same in both groups, the colonies themselves were significantly larger in the preirradiated animals. Since such colonies are primarily erythropoietic, this finding is consistent with the other methods. The results can be explained by assuming the presence of a hematopoietic stem cell which, in these preirradiated recipients, is directed towards erythropoiesis at the expense of granulopoiesis.

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