A Difference Between Stem Cells from Marrow and Spleen in Initiating Splenic Megakaryocytopoiesis[1] (35723)

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The compartment of differentiated, recognizable megakaryocytes is dependent upon continuous influx of cells from unrecognizable precursor compartments. There also is evidence that megakaryocytes ultimately arise from the same pluripotential stem cell compartment that produces granulocytes and erythroid cells (1), but that there are intermediate "stem cells" interposed between the pluripotential and the differentiated compartments (2). In the present studies, differentiation of megakaryocytes from pluripotential stem cells was observed, and it was found that stem cells of splenic origin differ from those of marrow origin in their tendency to produce megakaryocytes after transplantation.

Materials and Methods. Female mice of the CF_{1s} strain (Carworth Farms) were used as donors and recipients of spleen or bone marrow cells. All mice were 12–14 weeks of age.

Transplantation of bone marrow and spleen cells. The technique of Till and McCulloch (3) was used to assay for colony-forming units (CFU) as an estimate of pluripotential stem cell numbers. Suspensions of femoral bone marrow or spleen cells were made in ice-cold TC 199 (pH 7.2–7.4) using techniques described by Kubanek *et al.* (4). Each suspension was derived from 5 femurs or 5 spleens. Nucleated cell counts were done in quadruplicate by hemocytometer, and dilutions were made immediately before injection. Each recipient mouse was injected intravenously with 10^5 marrow or 10^6 spleen cells in 0.25 ml within 6 hr after irradiation.

Radiation. Recipient mice were given 800 R of total body X-radiation before injection of donor cells (dose rate 48 or 62 R/min with 1.0-mm Cu and 1.0-mm Al filtration, 250 kV, and 15 or 13 mA). The $LD_{50/30}$ in these mice is 650 R.

Colony counting. Ten days after irradiation and transplantation, recipient mice were killed, and their spleens were fixed in Bouin's solution. The numbers of splenic colonies were counted with the aid of a dissecting microscope and 15× magnification.

Megakaryocyte counting. After the colonies were counted, sections of the recipients' spleens were prepared. Sections of 12-μ thickness were cut longitudinally. About 5 consecutive sections were cut and mounted on one slide, then approximately 20 sections were discarded before another set was mounted. This was continued through the entire spleen. The slides were then stained with hematoxylin and eosin, examined microscopically for megakaryocytes, and the total number of megakaryocytes per section was recorded. To avoid duplicate counting of the same cells, megakaryocytes were not counted in adjacent sections. On each slide, every third section was counted, so that the sections in which megakaryocytes were counted were at least 24 μ apart. From these values, the average number of megakaryocytes per section was calculated for each spleen. It can be grossly estimated that about 5–10% of the spleen is examined microscopically by this technique.

Results. There were 70 recipient mice in 8 different experimental groups that survived 10 days after 800 R of irradiation and transplantation with 10^5 normal marrow cells. After transplantation with 10^6 normal spleen cells, 113 mice in 15 experiments survived.

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TABLE I. Splenic Colonies and Megakaryocytes 10 Days After Irradiation and Transplantation.

	10^5 Marrow cells				10^6 Spleen cells			
Expt.	No.	Colonies/spleen	Megakaryocytes/ section	*M/C*	No.	Colonies/spleen	Megakaryocytes/ section	*M/C*
271	10	20 ± 2.5[a]	5.2 ± 1.48	0.26 ± 0.084	10	10 ± 1.9	10.1 ± 3.00	1.01 ± 0.327
272	8	13 ± 1.3	3.4 ± 1.74	0.22 ± 0.106	8	8 ± 1.2	6.7 ± 2.95	0.91 ± 0.330
336	9	12 ± 2.8	6.7 ± 2.02	0.42 ± 0.134	7	11 ± 2.1	4.8 ± 1.61	0.40 ± 0.156
511					8	14 ± 1.9	11.2 ± 3.12	0.77 ± 0.164
512					5	13 ± 3.8	14.8 ± 3.44	2.34 ± 1.350
513					5	16 ± 2.8	12.2 ± 3.27	0.68 ± 0.175
531					4	15 ± 1.5	18.4 ± 11.26	1.05 ± 0.599
689	9	18 ± 1.8	7.6 ± 4.15	0.47 ± 0.259	8	15 ± 2.7	7.2 ± 1.60	0.46 ± 0.058
705					11	12 ± 1.5	15.4 ± 3.78	1.28 ± 0.300
718					10	14 ± 1.6	8.5 ± 2.61	0.60 ± 0.174
735					10	25 ± 1.2	23.3 ± 4.93	0.93 ± 0.181
845	10	17 ± 2.2	4.0 ± 1.53	0.24 ± 0.095	8	13 ± 1.6	7.8 ± 3.60	0.78 ± 0.331
477	5	14 ± 3.3	1.3 ± 0.39	0.11 ± 0.050	4	16 ± 2.4	9.6 ± 3.17	0.58 ± 0.213
481	10	20 ± 1.2	7.9 ± 1.36	0.40 ± 0.073	10	12 ± 1.4	9.3 ± 2.04	0.84 ± 0.235
487	9	21 ± 1.5	6.7 ± 1.12	0.32 ± 0.052	5	10 ± 1.6	12.0 ± 5.63	1.43 ± 0.627
Av		17 ± 0.8	5.6 ± 0.7	0.32 ± 0.04		14 ± 0.6	11.4 ± 1.0	0.91 ± 0.11

[a] Average ± SEM.

These numbers represent 82 and 73% survival, respectively. Bone marrow recipients had 0–30 colonies/spleen with an average of 17; recipients of spleen cells had 1–30 colonies/spleen with an average of 14. Data from individual experiments are presented in Table I.

Megakaryocytes were present in the spleens in several geographic locations. They occurred as pure colonies of recognizable megakaryocytes, as mixed colonies with granulocytes, erythroid cells and/or undifferentiated cells, or as isolated cells or small clusters of two or three. Colonies that appeared to be composed entirely of megakaryocytes on one section often were not pure colonies on nearby sections but rather were mixed with other cell types. Consequently, assignment of megakaryocytes to various morphological types of colonies from examination of subserial sections proved to be unreliable, and only the total number of megakaryocytes for each section was determined. The average values for each experiment are shown in Table I. Recipients of 10^5 bone marrow cells generally had about the same number of splenic colonies as recipients of 10^6 spleen cells, but the spleen cell recipients tended to have more splenic megakaryocytes than did the marrow cell recipients.

Figure 1 shows the individual observations with the average number of megakaryocytes per splenic section plotted on the ordinate and the number of surface colonies on the same spleen plotted on the abscissa. Visually, the values appeared to be related, and the regression lines for megakaryocyte number as a function of CFU number are shown. The correlation coefficient (r) for transplanted marrow cells was 0.21 and for spleen cells was 0.42. The probability that there was no correlation between the two values was $<10\%$ but $>5\%$ for marrow cells and $<0.1\%$ for spleen cells. Therefore, the number of megakaryocytes formed was, in large part, determined by the number of stem cells that implanted and grew in the spleen. In 83 uninjected, irradiated control mice the average number of colonies per spleen was 0.9 ($\pm$ 0.23) and the average megakaryocytes per section was 0.39 ($\pm$ 0.12).

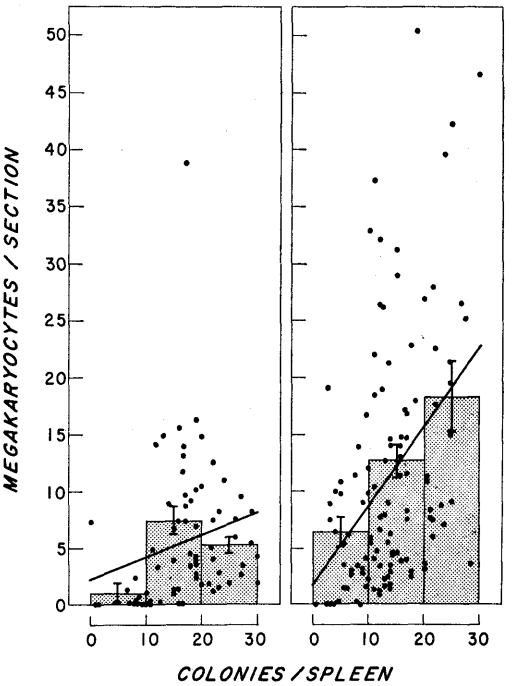

FIG. 1. Average number of megakaryocytes per splenic section plotted as a function of the number of surface colonies for each recipient spleen. The results in recipients of 10^5 marrow cells are shown on the left and recipients of 10^6 spleen cells on the right. Each point represents a single mouse. Regression lines for megakaryocyte number as a function of number of colonies are shown. The bars show the mean number of megakaryocytes per section ($\pm$SE for combined experiments) for groups of spleens with 0–10, 11–20, and 21–30 surface colonies/spleen.

For comparative purposes, it was necessary to express the number of megakaryocytes per section as a function of the number of stem cells which, in turn, was estimated by the number of surface colonies. Therefore, the M/C value was calculated for each spleen. In this calculation, the average number of megakaryocytes per splenic section was divided by the number of surface colonies on the same spleen. Average values for each experiment are shown in Table I. In this determination, values for one marrow recipient (Expt. 689) were discarded, because there were no surface colonies, but 7.2 megakaryocytes/section. The overall average M/C values for all other

recipients were 0.32 when marrow cells were injected and 0.91 for spleen cells, indicating that a given number of stem cells of splenic origin will form about three times as many splenic megakaryocytes as the same number of marrow stem cells.

Platelet counts were done in 6 experiments at the time of sacrifice (10 days after irradiation and transplantation). In 46 marrow recipients, the average platelet count was 3300/mm^3 (range, 0–12,500), and in 37 spleen recipients it was 2500/mm^3 (0–12, 000). Thus, the thrombocytopoietic stimulus should have been of comparable intensity in both groups.

Discussion. Pluripotent hematopoietic stem cells are considered to be the source of megakaryocytes as well as erythroid and granulocytic cells (1). Similar cells also appear to be the transplantable colony-forming units (CFU) in mouse tissues that form visible colonies of hematopoietic cells in the spleens of irradiated recipient mice (3, 5, 6). However, it has become apparent that cells which differ in other respects may share the capability for colony formation. CFU from spleen, marrow, and peripheral blood have been reported to form colonies of similar morphology (7–9), while those from fetal liver differ (10). Transplanted stem cells from adult bone marrow reproduce themselves in the spleens of the recipients at a slower rate than do stem cells from fetal liver (11, 12). Splenic stem cells have been variously reported to have more (12) or less (13) tendency to replicate themselves after transplantation than marrow CFU. Within the bone marrow, CFU of large size have more of a capacity for self-replication than those of small size (14). Stem cells of different origins also differ in their ability to prevent radiation mortality, those of marrow origin being more efficient than those from the spleen (9).

Radiation mortality is, in part, related to thrombocytopenia. Since bone marrow transplants are more effective in reducing mortality as compared to spleen, it could be suggested that they would also produce more platelets, thus permitting long-term survival after irradiation and transplantation. In contrast to this notion, our data indicated that splenic CFU formed more megakaryocytes in recipients' spleens than did marrow CFU. However, we did not attempt to quantify total body thrombocytopoiesis, and survival has been correlated more with hematopoietic recovery in the marrow than in the spleen (7). Differentiation of transplanted cells is influenced by the microenvironment (15), and it is possible that the bone marrow may offer a more favorable environment for differentiation of marrow CFU into megakaryocytes than does the spleen.

Humoral factors in the host animals did not appear to be a reason for the difference in megakaryocytopoiesis, as all animals were treated the same and blood platelet counts were comparable. The only host factor that has been reported to influence differentiation of transplanted hematopoietic stem cells into megakaryocytes is age (16). In our experiments, age of the mice was not varied.

The only conclusion that seems reasonable from these data is that CFU in the spleens of normal mice are intrinsically different from those in the marrow. This difference is expressed in the number of splenic megakaryocytes that form after transplantation into irradiated recipients. This finding adds support to the findings of others, which were reviewed above, that all cells capable of forming hematopoietic colonies in the spleens of irradiated mice are not identical in all other respects.

Summary. Bone marrow and spleen cells were transplanted from normal mice to lethally irradiated recipient mice. Comparable numbers of splenic surface colonies were formed from 10^5 marrow or 10^6 spleen cells. However, more splenic megakaryocytes were produced by stem cells from spleen than marrow. This finding indicates that there is an intrinsic difference between stem cells in the marrow and those in the spleen of normal mice.

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