Proliferation of Donor Spleen and Bone-Marrow Cells in the Spleens and Bone Marrows of Unirradiated and Irradiated Adult Mice[1] (35231)

AKIKAZU TAKADA, YUMIKO TAKADA, AND JULIAN L. AMBRUS
Springville Laboratories, Roswell Park Memorial Institute, Springville, New York 14141

Hematopoietic stem cells migrate from the bone marrow to the thymus, spleen, and lymph nodes (1), and small lymphocytes in the thoracic duct circulate among lymphoid organs (2, 3). Cytological techniques have greatly facilitated the study of cell migration. Experiments with radiation chimeras (4, 5) indicated that bone marrows, spleens, and thymuses of the irradiated hosts are all recolonized almost exclusively by descendents of injected bone-marrow cells. Studies with parabionts showed that there is an extensive exchange of cells between the two members of each pair (6, 7). Experiments with thymus grafts demonstrated that cells migrate both into and out of thymus grafts (8–12).

In the present paper we report the abilities of donor bone-marrow and spleen cells to proliferate in the bone marrows and spleens of irradiated and unirradiated hosts.

Materials and Methods. Male CBA/H and CBA/HT_6T_6 mice, 8 to 9 weeks old, were obtained from the inbred colonies at the Springville Laboratories. Chromosome analysis was performed according to the methods previously described (13). Two hr after injection of 0.3 ml of 0.04% Colcemid, the animals were sacrificed, and their spleens were removed. Bone marrows were flushed out with 1% sodium citrate solution. The spleens were teased in 1% sodium citrate solution warmed at 37°, and suspensions were fixed with chilled acetic methanol for 1 hr. Several drops of each cell suspension were placed on a separate slide, which was then dried over a flame. Slides were subjected to Giemsa staining.

Results. Unirradiated and irradiated CBA/H mice were each given an injection of 1×10^7 CBA/HT_6T_6 spleen cells, and the numbers of cells with the chromosome marker in the spleen were scored. Table I indicates the results. Few donor cells were found in host spleens if the hosts were unirradiated, but more than 90% of the dividing cells in the host spleens were of donor origin at day 10 if the hosts had received 400 to 800 R of whole-body irradiation. On day 15, the percentage of dividing cells with the T_6T_6 chromosome marker in host spleens averaged 29.3% after 400 R; 42.9% after 600 R; and 91.7% after 800 R. Some of the mice that had received 800 R died by day 20.

Table II shows the results of injecting 1×10^7 CBA/HT_6T_6 spleen cells into irradiated CBA/H mice and scoring the percentages of donor-type cells in the bone marrows of the hosts. Percentages of donor-type cells on days 10 and 15 were less than 10% in the bone marrows of hosts that had received 400 or 600 R of whole-body irradiation. At day 15, the percentage of donor-type cells in the bone marrow was about 10%, still far lower than that in the spleen (Table I). Increasing the radiation dose to 800 R increased the percentage of donor-type cells in the bone marrow, but some of the mice died in less than 20 days.

Table III shows the effects of giving host CBA/H mice 400 to 800 R of X radiation and 1×10^7 CBA/HT_6T_6 bone-marrow cells. Unlike the situation represented in Table II, more than 90% of the dividing cells

[1] This study was supported by General Research Support Grant FR-05648-03 from the National Institutes of Health, U.S. Public Health Service.

TABLE I. Cells of Host and Donor Types in Samples Taken from Host Spleens After Injection of 1×10^7 CBA/HT_6T_6 Spleen Cells into Unirradiated and Irradiated CBA/H Mice.

Radiation dose (R)[a]	Series	Types of cells in host spleens on day of sacrifice[b]								
		Day 8			Day 10			Day 15		
		Host	Donor	(%)	Host	Donor	(%)	Host	Donor	(%)
0	1	100	0	(0)	103	2	(1.9)	70	2	(2.8)
	2	102	0	(0)	100	0	(0)	90	2	(2.2)
	3	100	1	(1)	100	0	(0)	85	1	(1.2)
400	1	56	24	(30.0)	9	80	(89.9)	88	26	(22.8)
	2	11	28	(71.8)	6	76	(92.7)	57	33	(36.7)
	3	50	40	(44.4)	7	72	(91.1)	68	27	(28.4)
	4				13	81	(86.2)			
600	1				3	40	(93.0)	51	29	(36.2)
	2				0	40	(100)	43	32	(42.7)
	3				0	34	(100)	26	26	(50.0)
800	1				0	50	(100)	6	47	(88.7)
	2				0	50	(100)	3	52	(94.6)

[a] Whole-body irradiation.

[b] The number listed for each type of cell was the number of that type in the sample. Day 0 was the day of irradiation.

in the bone marrows of the hosts were of donor origin at day 10 after 400 R of whole-body irradiation. More than 70% of the dividing cells in the bone marrow were of donor origin 15 days after 400 R. Increasing the radiation dose to 600 R or more resulted in exclusive proliferation of donor-type cells in the bone marrows of the hosts on days 10 and 15. (The majority of the dividing cells in the bone marrow continued to be of the donor type for more than 1 month.) Mice that which received 800 R of whole-body irradiation and 1×10^7 bone-marrow cells seldom died early, but survived more than a year.

Discussion. Table I indicates that increasing the radiation dose given to the hosts increases the percentage of donor-type spleen

TABLE II. Cells of Host and Donor Types in Samples Taken from Host Bone Marrow After Injection of 1×10^7 CBA/HT_6T_6 Spleen Cells into Irradiated CBA/H Mice.

Radiation dose (R)[a]	Series	Types of cells in host bone marrow on day of sacrifice[b]								
		Day 10			Day 15			Day 18		
		Host	Donor	(%)	Host	Donor	(%)	Host	Donor	(%)
400	1	56	2	(3.4)	107	9	(7.7)	100	30	(23.1)
	2	50	4	(7.4)	95	7	(6.8)	100	12	(10.7)
	3	90	8	(8.2)	63	5	(7.3)			
600	1	90	4	(4.2)	32	4	(11.1)	50	1	(1.9)
	2	40	3	(6.9)	33	3	(8.3)	52	15	(22.3)
	3	45	7	(13.5)	34	4	(10.5)			
800	1	0	50	(100)	6	50	(89.2)	12	50	(80.6)
	2	0	50	(100)	11	50	(81.9)	10	50	(83.3)
	3							3	50	(94.3)

[a,b] Footnotes as in Table I.

TABLE III. Cells of Host and Donor Types in Samples Taken from Host Bone Marrow After Injection of 1×10^7 CBA/HT$_6$T$_6$ Bone-Marrow Cells into Irradiated CBA/H Mice.

Radiation dose (R)[a]	Series	Types of cells in host bone marrow on day of sacrifice[b]					
		Day 10			Day 15		
		Host	Donor	(%)	Host	Donor	(%)
400	1	2	60	(96.9)	32	63	(66.3)
	2	6	63	(91.3)	38	64	(62.7)
	3				16	75	(82.4)
600	1	0	50	(100)	0	50	(100)
	2	0	50	(100)	0	50	(100)
	3	0	50	(100)	2	50	(96.1)
	4	0	50	(100)	1	50	(98.0)
800	1	0	50	(100)	0	50	(100)
	2	0	50	(100)	0	50	(100)
	3	0	50	(100)	0	50	(100)
	4	0	50	(100)	1	50	(98.0)

[a,b] Footnotes as in Table I.

cells in the spleens of the hosts. Thus suppression of the division of host spleen cells by irradiation brings about a larger percentage of dividing donor spleen cells in host spleens.

Table II demonstrates that less than 10% of the dividing cells in the host bone marrows were of the donor type after spleen cells were injected into irradiated hosts. This experiment indicates that injected spleen cells do not proliferate well in the bone marrows of hosts exposed to 400 or 600 R of irradiation. Increasing the radiation dose of 800 R almost completely suppressed host mitosis, resulting in a majority of donor dividing cells in the bone marrow.

On the other hand Table III indicates that injection of bone-marrow cells subsequent to irradiation resulted in exclusive proliferation of donor-type cells in the host marrows (and spleens) on days 10 and 15. The difference between bone-marrow cells and spleen cells in this regard may be due to a difference between the dividing capacities of these two kinds of cells.

Bennett and Cudkowicz (14) reported that the size of the pool of stem cells relative to the pool of precursor cells is much greater in the bone marrow than in the spleen in adult mice. The greater dividing capacity of bone-marrow cells may thus be related to the greater size of the pool of stem cells in the bone marrow. In addition, stem cells are produced more rapidly in the bone marrow.

The present results can be explained by the assumption that the division of hematopoietic cells is largely regulated by the other dividing cells that surround them. Accordingly, bone-marrow or spleen cells injected into unirradiated hosts did not proliferate in the bone marrow or spleen in the hosts because of a regulatory barrier imposed by the dividing host cells. Likewise, bone-marrow or spleen cells injected into irradiated hosts divided and proliferated in the host organs, but suppression of the mitosis of host cells by donor spleen cells was not great enough to result in exclusive proliferation of donor spleen cells in the spleens of the hosts, and host spleen cells started to divide by day 15. Furthermore, bone-marrow cells injected into hosts irradiated with 400 R completely suppressed the mitotic capacity of the hosts, which would have returned to normal by day 10 after irradiation if the bone-marrow cells had not been injected (13). Finally, spleen cells injected into mice irradiated with 400 or 600 R did not proliferate well in the bone marrow of the hosts, possibly because irradiated host marrow cells still maintained in the bone marrow a strong regulatory barrier for the division of donor spleen cells, which did not have as great mitotic capacity as did the

injected bone-marrow cells.

Feedback regulation of mitosis imposed by currently dividing cells over the surrounding cells may be very important in keeping the cell population constant. Impairment of this mechanism could result in unlimited cell proliferation.

In the irradiated animals, space was made available by the destruction of the host marrow, so that donor cells could lodge and divide in the marrow. This being the case, proliferation of donor marrow cells may result in filling most of the space in the marrow with donor marrow cells, so that the host marrow cells cannot proliferate. The difference between the results produced by injection of marrow cells and spleen cells may be due to a difference between the rates of proliferation of these two kinds of cells, and thus a slow proliferation of injected spleen cells allows the host marrow cells to proliferate. Since the spleen enlarges after irradiaiton, space should be available for host spleen cells to divide in the spleen following irradiation with 400 or 600 R, regardless of whether donor marrow cells or spleen cells are dividing. The results of the present study indicate that injected spleen cells initially settled and divided in the host spleen, but were soon replaced with host cells, whereas injected marrow cells continued to comprise the majority of the dividing cells in the spleen for more than a month, and suppressed the division of host spleen cells. Under the circumstances, it seems likely that both feedback mechanisms and availability of space regulate proliferation of injected cells.

A study involving parabiosis (6) revealed that chromosomally marked cells slowly penetrate into the unlabeled marrow. This finding may indicate that stem cells migrate not only from the marrow to the peripheral hematopoietic organs, but also from one region of the marrow to another. Stem cells engaging in the latter type of migration start to divide wherever the regulatory barrier imposed by the local dividing cells is not too great. Since stem cells are not likely to be evenly distributed in the marrow, it seems probable that there are always sites where few actively dividing cells are present, and hence immigrant cells can always divide.

Summary. CBA/HT_6T_6 spleen or bone-marrow cells were injected into unirradiated or irradiated CBA/H mice, and the percentages of donor-type cells in the spleen and the bone marrow were scored at various intervals after irradiation.

When CBA/HT_6T_6 spleen or bone-marrow cells were injected into unirradiated CBA/H hosts, a few donor cells were found in the bone marrows and spleens of the hosts. When CBA/HT_6T_6 bone-marrow cells were injected into CBA/H mice irradiated with 400 R, more than 90% of the dividing cells in the bone marrow were of donor origin at day 10, and more than 70% were still of donor origin at day 15. Increasing the radiation dose to 600 or 800 R resulted in exclusive proliferation of donor cells in the bone marrow at days 10 and 15. When CBA/HT_6T_6 spleen cells were injected into CBA/H mice irradiated with 400 or 600 R, more than 90% of the dividing cells in the host spleens were of donor origin at day 10, but most of the donor cells were replaced with host cells by day 15. Increasing the radiation dose to 800 R resulted in a higher percentage of donor-type cells in the spleens at day 15, but some of the mice died by day 18. When CBA/HT_6T_6 spleen cells were injected into CBA/H irradiated with 400 or 600 R, less than 10% of the dividing cells in the bone marrow were of donor origin at days 10 and 15. Increasing the radiation dose resulted in dividing cells mainly of donor origin in the bone marrow at days 10 and 15, but some mice died by day 18.

These results suggest that stem cells compete with one another in mitosis, and that division of stem cells is largely regulated by that of other dividing cells.

The authors are grateful to R. Church and P. M. Peters for their technical assistance. Dr. M. Bennett's criticisms are appreciated.

1. Miller, J. F. A. P., and Osoba, D., Physiol. Rev. 47, 437 (1967).

2. Gowans, J. L., and Knight, E. J., Proc. Royal Soc. Sect. B 159, 257 (1964).

3. Gowans, J. L., Int. Rev. Exp. Pathol. 5, 1 (1966).

4. Ford, C. E., Hamerton, J. L., Barnes, D. W. H., and Loutit, J. F., Nature (London) 177, 452 (1956).

5. Micklem, H. A., Ford, C. E., Evans, E. P., and Gray, J., Proc. Roy. Soc. Ser. B **165**, 78 (1966).

6. Harris, J. E., Ford, C. E., Barnes, D. W. H., and Evans, E. P., Nature (London) **201**, 886 (1964).

7. Metcalf, D., and Brumby, M., Proc. Soc. Exp. Biol. Med. **124**, 99 (1967).

8. Miller, J. F. A. P., Ann. N.Y. Acad. Sci. **99**, 340 (1962).

9. Miller, J. F. A. P., Lancet **1**, 43 (1963).

10. Harris, J. E., and Ford, C. E., Lancet **1**, 389 (1963).

11. Harris, J. E., and Ford, C. E., Nature (London) **201**, 884 (1964).

12. Ford, C. E., and Micklem, H. S., Lancet **1**, 359 (1963).

13. Takada, A., Takada, Y., Huang, C. C., and Ambrus, J. L., J. Exp. Med. **129**, 445 (1969).

14. Bennett, M., and Cudkowicz, G., Proc. Soc. Exp. Biol. Med. **129**, 99 (1968).

Received June 11, 1970. P.S.E.B.M., 1971, Vol. 136.