Spleen Weight as a Measure of Marrow Cell Growth in Irradiated Mice.* (30546)

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When bone marrow cells are infused into irradiated mice, they proliferate extensively in the spleen as well as in the marrow cavities. The spleen is the prime organ for assessing rate of growth of transplanted hematopoietic cells because it is well defined and can be easily removed for measurements of weight (1), splenic nodule formation(2), or incorporation of radioactively labeled compounds, such as Fe^{59} or $I^{131}UdR$(3-5). Spleen colony and isotope uptake measurements in the spleen are most sensitive for doses of 10^4 to 10^6 normal marrow cells, although a dose-response is observed in erythrocytes for larger cell inocula if Fe^{59} is injected 5 days after irradiation and marrow treatment(3). Observations on recovery of spleen weight following X-irradiation and isologous marrow treatment (1) suggested that spleen weight might be used to assess growth of 10^5 to 10^7 transplanted marrow cells in mice. A more extensive study was undertaken to determine whether or not a spleen weight assay could be used to study growth of marrow cells in irradiated mice. In this paper, spleen weights were compared both with the number of splenic nodules at 10 days and with the percentages of Fe^{59} uptake at 4 through 10 days after bone marrow treatments. The reason for the comparative study was to determine whether data obtained by each assay system correlated with each other. Studies were done in parent-hybrid combinations in which marrow cells from one parent grow better than cells from the other parent(6-8).

Materials and methods. Male and female 5- to 7-month-old mice of the following inbred strains, C57BL, C3H, C57BL/6, and DBA/2 and F_1 hybrids (C57BL $\times$ C3H)F_1 and (C57BL/6 $\times$ DBA/2)F_1 were used. All mice were obtained from Cumberland View Farms, Clinton, Tenn.

Recipients were irradiated in a partitioned, rotating plastic drum positioned beneath the X-ray target. Physical conditions, except for mice used in the Fe^{59}-uptake experiment, were 300 kvp, 20 ma, hvl 0.5 mm of Cu, 80 r/minute, and tsd 93.7 cm. Physical conditions for mice used in the Fe^{59}-uptake experiment were 250 kvp, 15 ma, hvl 0.5 mm Cu, 150 r/minute, and tsd 60 cm.

Experimental mice received an intravenous injection of a measured number of bone marrow cells, which were flushed from the femurs of normal mice. A hemocytometer was used to make cell counts for each preparation of marrow cells suspended in sterile Tyrode's solution. Cell suspensions were diluted with Tyrode's to the proper cell concentration, and each mouse received an aliquot of 0.5 cc of a marrow cell suspension.

Mice allotted for comparative spleen weight and spleen colony assays received marrow cells within 4 hours after irradiation. Following killing by cervical dislocation, the spleens of the mice were removed, weighed, and placed in Bouin's fixative. Visible colonies were counted(2) for experiments in which the average number of colonies per spleen was less than 20.

Mice used for comparative spleen weight and Fe^{59}-uptake assays received marrow cells 18-22 hours after irradiation. Each of these mice received an intraperitoneal injection of $\sim$0.5 μc of Fe^{59} 24 hours before being killed. Spleens were weighed and placed in test tubes, and the amount of Fe^{59} in each spleen was measured in a NaI crystal scintillator. Results were expressed as percentage uptake of total radioactivity injected into each mouse (4).

Results. Hybrid mice were exposed to different doses of X-rays to determine the spontaneous incidence of spleen colony formation and residual spleen weight. Exposures of 950 and 900 r of X-rays to $BC3F_1$ and $B6D2F_1$ mice, respectively, prevented spontaneous hematopoietic recovery with visible spleen

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TABLE I. Spontaneous Colony-Formation and Spleen Weight Recovery 10 Days After Irradiation.

Strain	No. of mice	X-ray exposure (r)	No. of colonies/spleen*	Spleen wt (mg)*
$BC3F_1$	19	0	—	78.5 ± 2.4
"	18	950	.00	34.5 ± 1.5
"	16	850	.31 ± .14	36.9 ± 1.3
"	16	750	.56 ± .22	33.2 ± .9
"	15	650	1.20 ± .34	29.4 ± 2.1
"	24	550	8.12 ± .81	41.1 ± 2.0
$B6D2F_1$	22	0	—	67.3 ± 2.2
"	20	900	.00	29.7 ± 1.0
"	18	800	.17 ± .09	31.3 ± 1.5
"	19	700	1.79 ± .41	30.9 ± 1.1
"	18	550	6.89 ± .72	46.8 ± 1.6

* Values are mean ± S.E.M.

colonies; the residual weight of spleens of X-irradiated controls was ~30 mg (Table I). Spontaneous hematopoietic recovery with visible splenic nodules occurred within 10 days when these hybrids were exposed to less than 900 r of X-rays. Furthermore, spleen weight did not increase significantly within 10 days in mice exposed to 650 to 800 r of X-rays, although a few colonies developed spontaneously.

Several doses of bone marrow ranging from 10^4 to 10^7 nucleated cells were used to study the relation between spleen colony formation and spleen weight in lethally irradiated mice. A linear relation between cell dose and spleen colony formation was observed (Fig. 1). A significant increase in weight was not observed in spleens with fewer than 5 colonies. However, in spleens with more than 5 colonies, spleen weight increased in relation to the size of the marrow cell inoculum and the number of visible spleen colonies (Fig. 1). Although large cell inocula resulted in confluence of colonies, spleen weight continued to increase in relation to size of the cell inoculum. Linear portions of the plots of spleen weight *versus* number of spleen colonies (Fig. 1) showed that spleen weight increased ~1.5 mg for each macroscopic colony which was observed 10 days after treatment. This relationship held for an increase of approximately 1 log unit in the number of injected cells beyond the point at which colonies became confluent and could not be counted. With increased doses of cells spleen hypertrophy was not so great, and spleen weight was correspondingly less.

Spleen weight and Fe^{59} uptake were compared in a second series of experiments. Fe^{59} uptake in radiation controls was less than 1% of injected dose. Spleen weight and Fe^{59} uptake showed a linear relationship for individuals with spleen weights up to 100 mg; however, as spleen weight increased from 100 to 200 mg, the uptake of Fe^{59} gradually became less than proportional to the increase in spleen weight (Fig. 2). In spleens weighing between 30 and 100 mg, a 10 mg increase in spleen weight corresponded to an increase of ~1.4% in the amount of Fe^{59} incorporated into the spleen.

Recipients of 10^6 or 10^7 parental marrow cells were killed serially to study gain in spleen weight at various intervals after treatment. After both doses of marrow, spleen weight was indistinguishable from that of radiation controls through day 4. A significant increase in spleen weight occurred by day

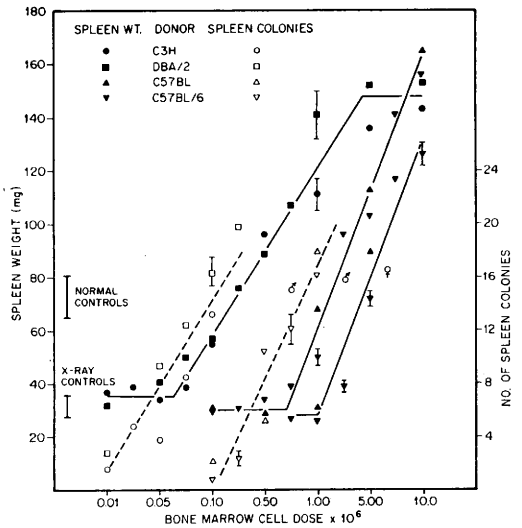

FIG. 1. Spleen-weight and spleen-colony response in lethally irradiated $BC3F_1$ and $B6D2F_1$ recipients of 10^4 to 10^7 parental marrow cells. All mice were killed 10 days after treatment. Each point represents an average from 10 or more mice; standard errors of the mean are shown for a few of the points. Male and female recipients of DBA/2 and C3H parental marrow showed similar spleen weight and spleen-colony response, but the values for male recipients of C57BL or C57BL/6 parental marrow were greater than for females. The solid symbols and solid lines represent spleen weight, and the open symbols and broken lines represent spleen colony.

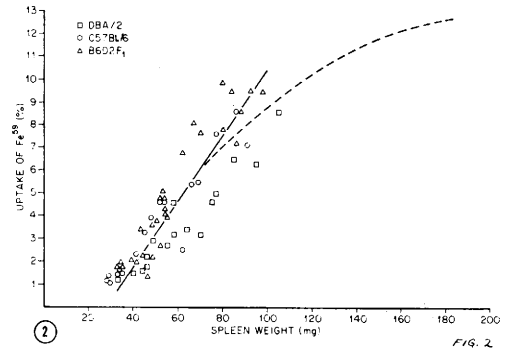

FIG. 2.

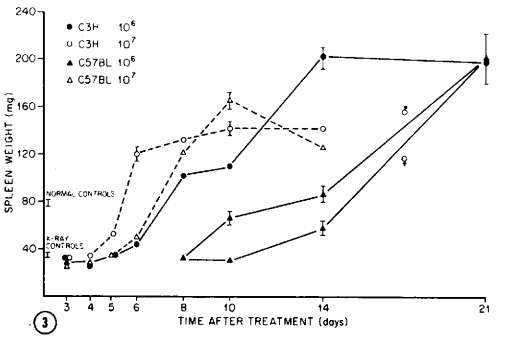

FIG. 2. Spleen weight and Fe^{59} uptake in lethally irradiated $B6D2F_1$ recipients of isologous or parental marrow cells. Irradiated mice received an inoculum of 3.2×10^4 to 3.2×10^7 marrow cells on day 0. Approximately 0.5 μc of Fe^{59} was administered intraperitoneally on day 5, and the mice were killed on day 6. Each point represents an individual mouse killed on day 6. The broken line represents an average of the 24-hour Fe^{59}-uptake values obtained from individuals sacrificed on days 8 and 10 after treatment (individual points are not shown for reasons of clarity).

FIG. 3. Spleen-weight response in lethally irradiated $BC3F_1$ recipients at intervals after receiving 10^6 or 10^7 parental marrow cells. Each point represents an average weight of 6 or more spleens; standard errors of the mean are shown for a few of the points. Solid symbols and solid lines represent spleen weight in recipients of 10^6 parental marrow cells, and open symbols and broken lines represent spleen weight in recipients of 10^7 parental marrow cells.

6 in recipients of 10^6 C3H marrow cells, but not until day 14 in recipients of 10^6 C57BL marrow cells (Fig. 3). Recipients of 10^6 C57BL marrow cells, which were killed on day 21, had donor-type erythrocytes in their peripheral blood. Increased spleen weight began earlier, increased more rapidly, and reached maximum earlier in recipients of the larger dose of parental marrow cells (Fig. 3). Nevertheless, a significant difference between the growth of 10^7 C3H and C57BL parental cells was observed between days 5 and 8 after marrow infusion.

Discussion. Donor-recipient combinations in which the 2 parental marrows have different abilities to proliferate in F_1 hybrids (6-8) were chosen to investigate the possibility of using spleen weight to assess the growth of transplanted marrow cells. If spleen weight is a good index of proliferation of transplanted hematopoietic cells in irradiated mice, spleen weight should indicate that C57BL and C57BL/6 cells do not grow as well as C3H and DBA/2 cells in $BC3F_1$ and $B6D2F_1$ mice, respectively, as has been shown by the spleen colony technique(2), I^{131}UdR uptake(5), and Fe^{59} uptake(9). Comparative studies reported in this paper on spleen weight, spleen colony formation, and Fe^{59} uptake (Fig. 1 and 2) in spleens of lethally irradiated recipients of parental bone marrow cells indicate that spleen weight is a good index of growth of transplanted hematopoietic cells. Till and McCulloch(2) have shown that the number of spleen colonies that develop in the spleen is cell-dose dependent. Hodgson(3) and Smith(4) have reported that the percentage uptake of Fe^{59} in erythrocytes and spleen is a function of the number of marrow cells injected and the time after marrow injection. Furthermore, Cudkowicz (5) has shown that I^{131}UdR uptake and spleen colony formation are positively correlated with size of the marrow inoculum. Vogel(10) has suggested that spleen weight could be used to assess spontaneous radiation recovery in mice. We must conclude, therefore, that spleen colony formation, Fe^{59} uptake, I^{131}UdR uptake, and spleen weight are all suitable assays for measurement of proliferation of transplanted hematopoietic cells, and that results of each assay system can be compared with results of any of the others. This is what would be expected because the number of spleen colonies, Fe^{59} uptake, and I^{131}UdR uptake are cell-dose dependent, and the weight of the spleen is a reflection of the number of cells in the spleen which arise from colony-forming cells in the marrow inoculum to produce clones of cells that incorporate Fe^{59} or I^{131}UdR. Fe^{59} assay is restricted to erythroid cells synthesizing hemoglobin,

and $I^{131}UdR$ incorporation gives information on the number of cells synthesizing DNA. Spleen colony formation is an index of the number of stem cells in the inoculum, and spleen weight provides information on proliferation of colony-forming cells over a period of time. Analyses of spleen weights of mice which are killed at intervals during the growth phase should give data comparable to that of isotope-incorporation studies. Since comparable results are obtained with each method, the choice of assay depends in part upon techniques available to the investigator; however, the simplest and most economical assay for most studies would be spleen weight. A combination of assays which count the number of spleen colonies at lower cell doses (10^4 to 10^5 marrow cells) and measure spleen weight at higher cell doses (10^5 to 10^7 marrow cells) permits assessment of growth of marrow cells over increments of 3 log units in the donor-recipient combinations used.

One observation deserves further comment. Lethally irradiated $BC3F_1$ recipients of 10^6 C57BL marrow cells showed a delayed increase in spleen weight (Fig. 3). The 21-day survivors had donor-type erythrocytes in their peripheral blood, which suggests that 10^6 C57BL marrow cells did proliferate, but the growth phase occurred later than for 10^6 C3H cells. A similar, but less pronounced, delay was also observed after infusion of 10^7 cells (Fig. 3). The time required to attain complete replacement of host erythrocytes in (C57BL $\times$ 101)F_1 recipients of 101 (50 days) or C57BL (63 days) marrow cells probably results in part from differences in the lag phase periods of these parental cells in lethally irradiated F_1 mice(11). Moreover, ability of 101 to outgrow C57BL marrow cells, when injected as mixtures in $B1F_1$ mice, can be explained on the basis of the difference in time required for these parental cells to begin logarithmic growth in F_1 hybrids(7). McCulloch and Till(6) have reported that $C3BF_1$ recipients of 10^5 C57BL marrow cells survived much better than radiation controls although few colonies developed. Reasons for the prolonged lag phase and the beneficial effect of C57BL marrow, even when it does not show overt signs of proliferation in the spleen, are not clear.

Summary. C57BL, C3H, C57BL/6, and DBA/2 parental marrow cells were injected at varying doses into lethally irradiated $BC3F_1$ and $B6D2F_1$ mice. Spleen weight in recipients was compared to spleen colony formation and Fe^{59} uptake. Results showed that increased spleen weight was proportional to formation of increased numbers of splenic nodules and percentage of Fe^{59} uptake. Data suggest that spleen weight, spleen colony formation, and Fe^{59} uptake are equally suitable assays, within appropriate limits, for studies on proliferation of marrow cell transplants in irradiated mice. Spleen weight is a useful index of growth for doses of transplanted marrow cells which promote recovery of hematopoietic injury in lethally irradiated mice.

1. Urso, P., Congdon, C. C, Blood, 1957, v12, 251.
2. Till, J. E., McCulloch, E. A., Radiat. Res., 1961, v14, 213.
3. Hodgson, G. S., Blood, 1962, v19, 460.
4. Smith, L. H., Am. J. Physiol., 1964, v206, 1244.
5. Cudkowicz, G., Upton, A. C., Smith, L. H., Gosslee, D. G., Hughes, W. L., Ann. N. Y. Acad. Sci., 1964, v114, 571.
6. McCulloch, E. A., Till, J. E., J. Cell. Comp. Physiol., 1963, v61, 301.
7. Popp, R. A., Cosgrove, G. E., Popp, D. M., Ann. N. Y. Acad. Sci., 1964, v114, 538.
8. Cudkowicz, G., Stimfling, J. H., Immunology, 1964, v7, 291.
9. Goodman, J. W., Mathews, M. C., Am. J. Physiol., in press.
10. Vogel, H. H., Jr., Exp. Hematol., 1964, v7, 81.
11. Popp, R. A., Cosgrove, G. E., Proc. Soc. Exp. Biol. and Med., 1959, v101, 754.

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