Recovery of Immunocompetence of Bone Marrow and Spleen Following Sublethal Irradiation[1] (34406)

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Mouse spleen cell suspensions can make antibody when they are transferred to irradiated syngeneic recipients and stimulated with an antigen such as sheep erythrocytes (SRBC) (1). Thymus and marrow cells are by themselves not immunocompetent; however, suspensions of *both* thymus and marrow cells are immunocompetent and can respond to SRBC in such a transfer experiment (1–3). Thus, the cells needed to make antibody to SRBC are present in the spleen and also thymus–marrow cell combinations. In the latter case, it appears that the antibody is actually manufactured by marrow-derived cells while the thymus-derived cells act in some as yet unknown auxiliary fashion (4).

The identity of the bone marrow cell which is the precursor of the antibody-making cell is not known, and cell separation techniques have so far failed to isolate it. However, knowledge of the behavior of the bone marrow immunocompetent cell may be gained by studying its responses in various situations.

Thymus and marrow immunocompetence is radiosensitive (2, 3) as is that of the spleen (5). The experiments reported here measure the kinetics of recovery of immunocompetence of the spleen and marrow following sublethal irradiation. This type of experimental model gives a more physiological picture of cellular interaction involved in the immune response. The recovery of cell numbers and hematopoiesis was also measured.

The immunocompetence of irradiated transferred spleen cells was measured by the number of direct plaque-forming cells (PFC) in the recipient spleen. The immunocompetence of marrow cells was measured similarly by their ability to interact with normal thymus cells and produce PFC in recipient spleens (1, 2, 3).

Methods. Donor LAF_1 male mice, 10–12 weeks old received 500 R of ^{60}Co whole-body irradiation. On each of days 2, 4, 7, and 11 following irradiation, a group of donor mice was sacrificed (together with a similar group of control nonirradiated mice). Spleen and marrow cell suspensions were made, counted, and separately injected with SRBC (0.1 ml of 40% suspension) intravenously into groups of lethally irradiated (900 R of ^{60}Co) recipient LAF_1 mice, 10–12 weeks old. Cell suspensions were adjusted so that each recipient received the mean number of cells from one donor spleen or two femurs, either normal or irradiated. Therefore, the response was looked at in terms of recovery of a whole donor organ (spleen or marrow). Recipients of spleen cells were sacrificed 6 days after transfer. Recipients of marrow cells all received a standard dose of 3.5×10^7 normal thymus cells. These recipients of marrow and thymus and SRBC also received a booster intraperitoneal injection of 0.5 ml of 10% SRBC 4 days after transfer, and were sacrificed 4 days later. Twenty-four hr before sacrifice, each group received 0.5 μCi of ^{59}Fe as $FeCl_3$ intravenously. At sacrifice, recipient spleens were weighed and the spleen index calculated: spleen index = spleen wt (mg)/mouse wt (g) (6). The ^{59}Fe uptake per spleen was measured in a well-type gamma scintillation counter and expressed as counts per minute per recipient spleen. The number of PFC per recipient spleen was determined in duplicate aliquots (7, 8).

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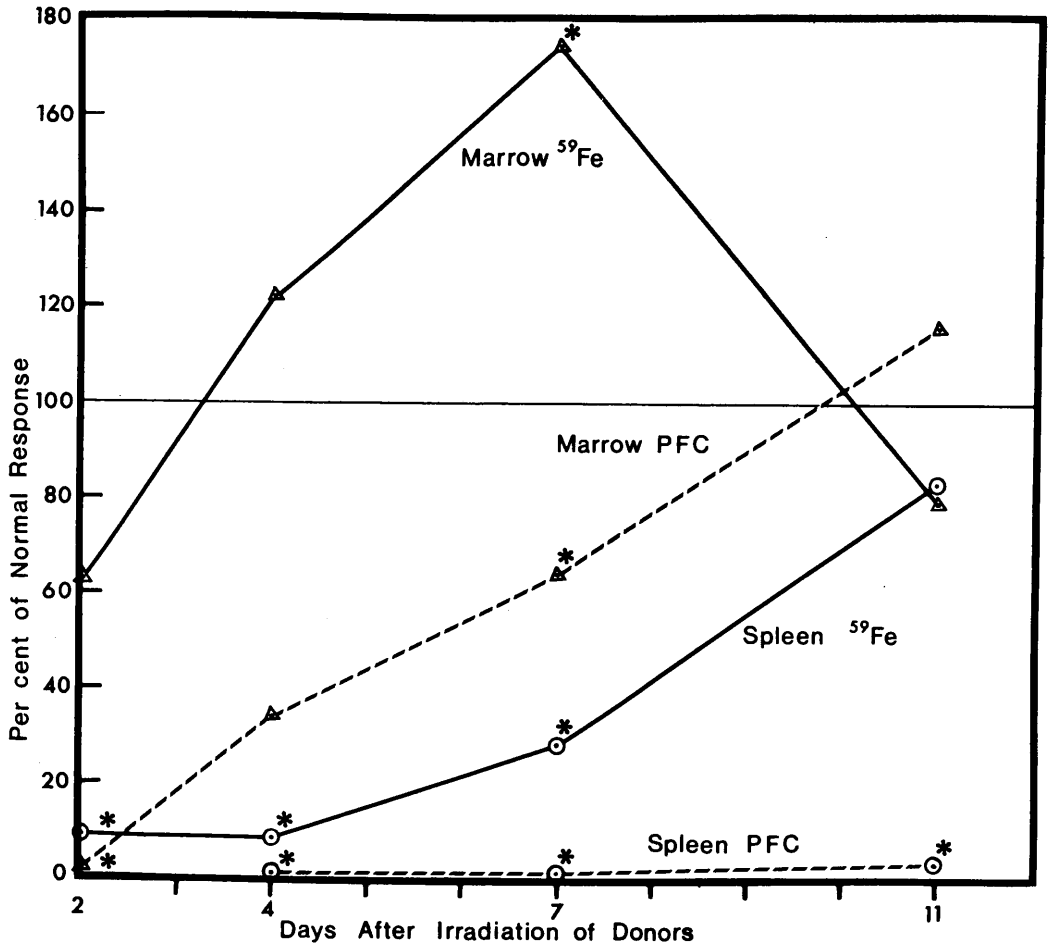

FIG. 1. Comparison of the mean ^{59}Fe uptake and the mean PFC produced in recipient spleens by transfer of normal or irradiated spleen or bone marrow (plus thymus) together with SRBC antigen. The data are expressed as percentage of normal. Each point represents: [(geometric mean of test mice/geometric mean of normal mice) × 100]. ^{59}Fe uptake: after spleen transfer; O—O; after marrow transfer; Δ—Δ. PFC after spleen transfer; O---O; after marrow transfer; Δ---Δ. *Significantly different from normal at the 95% level.

Results. The results were statisticaly evaluated using geometric means and upper and lower 95% confidence limits (9) and are summarized in Table I and Fig. 1. Table I gives the actual data. Since the normal mice sacrificed at each interval had varying levels of immunocompetence and hematopoiesis, Fig. 1 compares the mean values of irradiated and control mice sacrificed that *same day*.

The proliferative ability of donor cells (measured by spleen index) was parallel to the hematopoietic ability of these cells (measured by ^{59}Fe incorporation). The hematopoietic ability of the irradiated spleen recovered slowly and by day 11 was within normal limits. That of irradiated marrow, however, recovered much faster, with a pronounced "overshoot" on day 7 and a return towards normal on day 11.

The discrepancy between spleen and marrow recovery of immunocompetence was even more striking. By day 11, irradiated spleen cells produced only 3.4% of the PFC of normal spleen cells, although the number of irradiated nucleated spleen cells transferred was 33.3% of normal. Irradiated bone mar-

TABLE I. Recovery Rate of Cell Number, Ability to Replicate in Recipient, Hematopoiesis and Immunological Responsiveness of Donor Spleen and Marrow Cells Following 500 R of ^{60}Co.

After 500 R of ^{60}Co (days)	Cell type transferred:	No. of cells transferred/lethally irradiated recipient $\times 10^{-7}$				Spleen index[a]			
		Spleen		Marrow		Spleen		Marrow	
		Treated	Normal	Treated	Normal	Treated	Normal	Treated	Normal
2		.73	4.93	.216	1.85	1.4(5)[b] (1.2–1.6)[c]	4.2(6) (3.4–5.2)	3.6(5) (2.8–4.5)	5.7(6) (4.7–6.9)
4		.67	4.43	.56	1.86	1.1(6) (1.0–1.2)	4.9(6) (4.1–5.9)	4.9(6) (4.3–5.6)	5.0(6) (4.6–5.4)
7		1.11	4.9	1.37	1.55	1.8(4) (1.5–2.2)	4.2(5) (2.9–6.0)	6.4(6) (5.3–7.6)	5.6(4) (4.8–6.4)
11		2.33	7.0	2.2	2.6	2.9(5) (2.3–3.6)	4.2(5) (3.2–5.6)	5.2(6) (4.3–6.2)	4.8(2) (—)
		^{59}Fe uptake/recipient spleen (cpm)				PFC/recipient spleen			
2		181 (114–286)[c]	3821 (2310–6351)	2561 (1570–4160)	4041 (3651–4460)	Not done	Not done	222 (141–348)[c]	11100 (2250–48300)
4		207 (81–535)	4921 (3920–6190)	5012 (3273–7674)	4111 (3882–4365)	63 (29–135)	9861 (5990–16200)	698 (222–2188)	2042 (1178–3532)
7		1114 (625–1982)	3917 (2094–7328)	4178 (3945–4436)	2382 (1950–2917)	133 (67–265)	9462 (3793–23600)	9036 (6209–13120)	14030 (9333–21130)
11		2218 (1400–3524)	2685 (2153–3350)	2239 (1754–2858)	2780 (—)	329 (163–662)	9683 (8185–11460)	2404 (1730–3342)	2070 (—)

[a] Spleen index = [spleen wt (mg)/body wt (g)].

[b] Number of mice tested per group.

[c] 95% confidence limits.

row cells, on the contrary, recovered immunocompetence steadily and achieved a normal level by day 11.

Discussion. The present results show that the recovery rate of bone marrow following sublethal irradiation is much more rapid than that of the spleen. Others, studying recovery of erythropoiesis, have reported similar findings (10–12) and suggested that during the first 24 hr following sublethal irradiation any marrow repair may represent recovery of sublethally damaged cells. Any subsequent repair probably is due to cell proliferation. Because our study was concerned not only with recovery of erythropoiesis, but also with recovery of immunocompetence, selective reduction by irradiation of a given cell population must be considered. It seems likely that if there were a selective reduction then there should be a remarkable reduction of one given biological function and no or only a slight change in others. Our data show that proliferation properties (spleen index), erythropoiesis, and immunocompetence are significantly reduced in both irradiated spleen and bone marrow. Although the recovery rates of these parameters were different, the data indicate that the amount of irradiation given significantly reduces all cell types studied. The fact that the spleen hematopoietic activity was slow in returning is at least partly a reflection of the rate of migration of bone marrow stem cells to the spleen (5).

The intact spleen has all of the cellular elements necessary for evoking an immune response (1). However, if this mechanism is disrupted, by irradiation for example, the spleen seems not to be able to reestablish itself immunologically within 11 days. Perhaps then, the spleen is dependent on cells migrating from other sources. The fact that hemapoietic activity (^{59}Fe uptake) returns to normal levels by day 11 suggests that bone marrow cells migrate to the spleen (5). These cells may be preferentially directed into erythropoiesis, as suggested by Brecher and Smith (13). Alternatively, the lag in recovery of immunocompetence could be caused by absence of "thymic-derived cells." It is known that bone marrow-derived cells are capable of repopulating the thymus following thymic injury (14). Also, the rate of recovery of the thymus following irradiation has been shown, by marrow transfer studies, to be directly proportional to the number of bone marrow cells injected (15, 16). Thus, the slow recovery rate of spleen immunocompetence may then depend on two functions: (i) bone marrow recovery and "stem cell" migration to the spleen, and (ii) bone marrow contributing to the reestablishment of a population of thymus-derived cells. Once reestablished, these cells would be free to migrate to other lymphoid tissues such as the spleen. It is suggested then, that the recovery of immunocompetence of the spleen depends not only on bone marrow repopulation, but also on thymus reestablishment and the migration of both of these cells types to the spleen. Once in the spleen, and in the presence of antigen, they can participate in cell–cell interaction which is required for a cell line activity producing specific antibody.

Which thymic cell is the antigen recognition cell and which bone marrow cell is the "effector cell" is not known. Also, what the antigenic information is and how it is transferred is not understood. Answers to some of these questions may come from more detailed migration studies which are presently in progress.

Summary. The rates of recovery of mouse spleen and marrow cells after sublethal irradiation were studied. Cells from donors given 500 R were transferred to lethally-irradiated recipients at various times after donor irradiation. Donor spleen cells were given with sheep erythrocyte antigen (SRBC), while donor marrow cells were given with normal thymus cells and SRBC. The recovery rates of cellular proliferative ability, hematopoiesis (by ^{59}Fe incorporation) and immunocompetence (by antibody formation to SRBC) were measured. The cellular proliferative ability and hematopoietic recovery were parallel; marrow cells had recovered by day 11 after irradiation (following an "overshoot") while spleen cells had also recovered by day 11 but without overshoot. The marrow and spleen recovery rates of immunocompetence were markedly different, however. By day 11, marrow immunocompetence had reached

normal levels while spleen immunocompetence was only 3.4% of normal.

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