Defect Extrinsic to Stem Cells in Spleens of Steel Anemic Mice (36032)

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Reports in the literature (1–4) suggest that the basis of the hemopoietic defect in steel anemic mice ($S1/S1^d$) is extrinsic to their colony-forming cells, resulting from an improperly functioning hemopoietic-tissue matrix. This concept developed from the following observations: (i) Steel stem cells were shown to be normal in their ability to repopulate irradiated wild-type or W/W^v anemic mice and to cure the anemia of unirradiated W/W^v mice. (ii) As tested by transplantation into lethally irradiated normal mice, $S1/S1^d$ marrow and spleen cell suspensions contained the normal proportion of colony-forming units (CFU). (iii) Injections of normal marrow cells into irradiated (900 R) steel anemic mice did not lead to appearance of splenic colonies. (iv) Steel erythroid cells were normally responsive to erythropoietin when implanted into W/W^v recipients. (v) Although normal peripheral hematological values characterized both partners when $S1/S1^d$ and normal mice were joined in parabiosis, splenic hemopoietic activity was observed only in the normal, never in the $S1/S1^d$ partner, suggesting that the $S1/S1^d$ hemopoietic defect cannot be attributed either to lack of proper humoral stimulation or to unusual humoral depression. The observation that grafted whole normal spleens were capable of alleviating the anemia of steel mice, although injections of suspensions of normal spleen cells were completely ineffective, offered further support for this view.

Another index of effective function of the splenic cellular matrix is its capacity to support growth of hemopoietic colonies, originating from injected marrow cells, in heavily irradiated recipients whose indigenous stem cells have been destroyed. This capacity is known to be impaired in $S1/S1^d$ spleens residing in $S1/S1^d$ bodies, since no splenic colonies appeared following injection of normal marrow cells into heavily irradiated intact $S1/S1^d$ mice (1). In the present study we have analyzed this response further, comparing colony-supporting capacities of $S1/S1^d$ vs $+/+$ or W/W^v spleens which resided in bodies of each of the three genotypes. This was achieved by using mice bearing grafted spleens of a foreign genotype, either in addition to, or in place of, their own spleen. All of the mice used in these studies received the same dose of whole-body irradiation, and all were given intravenous injections of normal marrow cells. In most of the cases, $S1/S1^d$ and $+/+$ or W/W^v spleens resided side by side in the same abdominal cavity, and thus experienced the same humoral influences and the same radiation, and were presented with the same inoculum of injected marrow cells. Our basic experimental difference was between genotypes of splenic matrices, and our experimental observation was the number of visible hemopoietic colonies which were supported by spleens of each genotype.

Materials and Methods. Mice. The mice were bred and treated surgically at The Jackson Laboratory. The principles of laboratory animal care as promulgated by the National Society of Medical Research are observed in this Laboratory. All $S1/S1^d$ and many of the $+/+$ mice were of WCB^6F_1 hybrid genetic background, and all W/W^v and the remainder of the $+/+$ mice were of WBB^6F_1 hybrid genetic background. Although the two genetic backgrounds differ slightly, marrow and spleen grafts between them are readily accepted (6). The breeding schemes used in production of these mice have been presented

elsewhere, as have the surgical procedures (2). Treatment groups, including animals of both sexes unless otherwise stated, consisted of: intact $S1/S1^d$ mice; intact wild-type mice (+/+) from the two genetic backgrounds; $S1/S1^d$ mice implanted with W/W^v spleens; W/W^v mice implanted with $S1/S1^d$ spleens; splenectomized W/W^v male mice bearing $S1/S1^d$ spleen transplants: splenectomized $S1/S1^d$ male mice bearing W/W^v spleen transplants; and $S1/S1^d$ female mice grafted with +/+ spleens. Some of the grafted spleens were attached to the peritoneal lining, and others were anastomosed to the surface of the host spleen. Mode of splenic transplant did not appear to affect colony-supporting capacity. Some, but, not all, of the animals with grafted spleens had been observed in previous experiments, which, in our best judgment, in no way affected their response to the present experimental treatment. It should be pointed out that, although W/W^v mice have defective hemopoietic stem cells, their spleens are fully capable of supporting colony formation (5).

Hematological values obtained just prior to shipment of experimental animals from The Jackson Laboratory to Stony Brook were characteristic for their particular condition. The untreated $S1/S1^d$ mice were severely anemic, but all of the other mice had normal or nearly normal hematocrit percentages, red cell numbers, and mean cell volumes.

X-Irradiation of recipients. A General Electric Maxitron-250 machine was used to deliver 650 rads of whole-body X-radiation. Conditions were: target–subject distance, 60 cm; filtration, 0.5 mm Cu, 1.0 mm Al; calibration, Victoreen 100 meter; dose rate, about 105 rads/min. Animals were radiated in a circular Lucite container, usually about 10 at a time, on a rotating turntable.

Marrow injections. Within 4 hr after irradiation, all mice received marrow cell injections via the tail vein. Marrow cell suspensions were prepared by a slight variation of the method of Stoner and Bond (7) and Carsten and Bond (8). Aliquots of each suspension, containing cells from two like-sexed donors, one WCB^6F_1-+/+ and one WCB^6F_1-$S1/S1^d$, were injected into irradiated recipients of the same sex, to avoid histocompatibility problems associated with the H-y antigen. Half of the radiated mice in each treatment group received 5×10^4, the other half 5×10^5, marrow cells suspended in 0.2 ml of saline G.

Examination of spleens. The animals were killed 7 to 8 days after irradiation and marrow cell injection. Their spleens were removed carefully and fixed in Bouin's solution for at least 72 hr before colony counts were made under a magnifying glass. Scoring of colonies in grafted spleen tissue was of necessity somewhat uncertain, owing to the inevitable occurrence of capsular adhesions.

Results and Discussion. Our results confirm and extend previous observations on the abilities of irradiated mice of various genotypes to support hemopoietic colony growth in their own spleens (Table I). When heavily irradiated $S1/S1^d$ mice (first column, Expts. I, II, and III) were given small (5×10^4 cells) or large (50×10^4 cells) injections of competent marrow (column 2), no colonies developed in their native $S1/S1^d$ spleens (columns 3 and 4). When +/+ and W/W^v mice were similarly treated (Expts. V, VI), small countable numbers of colonies appeared in the native spleens of mice which had received small marrow inocula. The native spleens of such mice which had received large marrow injections were very much enlarged, containing large masses of confluent hemopoietic colonies. These characteristic responses were observed whether or not the treated +/+ or W/W^v mouse also contained a grafted spleen. Hemopoietic colonies also appeared in +/+ and W/W^v spleens grafted into $S1/S1^d$ mice (columns 5 and 6, Expts. III, IV, V); their number was dependent on the size of the marrow inoculum and independent of presence or absence of a native $S1/S1^d$ spleen. Small numbers of colonies were observed in 3 of 9 $S1/S1^d$ spleens implanted in W/W^v hosts which received small numbers of marrow cells, but no colonies were observed in 10 $S1/S1^d$ spleens implanted in W/W^v hosts which received large injections of marrow cells (columns 5 and 6, Expts. VI and VII). We have no explanation for the very few hemopoietic colonies which were

TABLE I. Defective Colony Formation in Sl/Sl^d Spleens, Compared to +/+ or W/W^v Spleens in Irradiated Sl/Sl^d, +/+, and W/W^v Hosts.

Expt.	Irradiated host mice: Genotype	Irradiated host mice: No.	Normal marrow cells injected (No. × 10^4)	Colony formation in: Native spleen: Genotype range	Native spleen: CFU spleen	Grafted spleen: Genotype range	Grafted spleen: CFU spleen
I	Sl/Sl^d	4	5	Sl/Sl^d	0	None	
	Sl/Sl^d	4	50	Sl/Sl^d	0	None	
II	Sl/Sl^d	4	5	Sl/Sl^d	0	W/W^v	2 to 13
	Sl/Sl^d	4	50	Sl/Sl^d	0	W/W^v	All confluent[a]
III	Sl/Sl^d	6	5	Sl/Sl^d	0	+/+	3 to confluent
	Sl/Sl^d	4	50	Sl/Sl^d	0	+/+	All confluent
IV	Sl/Sl^d	5	5	splx[b]	—	W/W^v	5 to confluent
	Sl/Sl^d	6	50	splx	—	W/W^v	All confluent
V	+/+	3	5	+/+	1, 7, 15	None	—
	+/+	6	50	+/+	All confluent	None	—
VI	W/W^v	3	5	W/W^v	1 or 2	Sl/Sl^d	0 to 1
	W/W^v	4	50	W/W^v	All confluent	Sl/Sl^d	0
VII	W/W^v	6	5	splx	—	Sl/Sl^d	0 to 3
	W/W^v	6	50	splx	—	Sl/Sl^d	0

[a] "Confluent" denotes coalescence of colonies too numerous to count precisely.
[b] "splx" denotes splenectomized host.

seen in $S1/S1^d$ spleens implanted in W/W^v hosts, but their appearance clearly was not positively related to the size of the marrow inoculum nor to the presence or absence of a native W/W^v spleen. The general situation was still inhibition of hemopoietic colony growth in implanted $S1/S1^d$ spleens. This finding is particularly interesting because prior to irradiation all of these W/W^v mice with implanted $S1/S1^d$ spleens had acquired normal, rather than anemic, blood values through the functioning of $S1/S1^d$ hemopoietic cells which had migrated from the implanted spleen into the W/W^v recipient hemopoietic tissue matrix (3).

The results of these experiments strengthen the hypothesis that the site of the anemia-producing defect of steel mice is the hemopoietic tissue matrix. The physiological and biochemical basis of the defect remains to be determined.

Summary. The $S1/S1^d$, +/+ and W/W^v mouse spleens, residing *in situ* or transplanted into recipient mice of identical or alternate genotypes, were compared with respect to their abilities to support hemopoietic colony formation by exogenous bone marrow cells inoculated intravenously after whole-body x-irradiation. In contrast to the normal numbers of colonies formed consistently in +/+ and W/W^v spleens, few or no colonies were formed in $S1/S1^d$ spleens, even when spleens of $S1/S1^d$ and W/W^v types resided side-by-side in the same abdominal cavity. These findings strengthen the hypothesis that the anemia of the steel ($S1/S1^d$) mouse results from a defect in its hemopoietic tissue matrix rather than in its hemopoietic stem cells or humoral regulatory apparatus.

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