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Bone Marrow Transfusions in Previously Irradiated, Hematologically Normal Syngeneic Mice (41079)[1]

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Abstract. Transfusion of syngeneic marrow into normal, nonirradiated recipients results only in minimal proliferation of donor cells. However, irradiated recipients, restored to hematologic normalcy by an initial marrow transfusion, subsequently sustain proliferation which replaces approximately 10% of endogenous marrow after a single transfusion of 4×10^7 marrow cells of the same strain as the host. Cells from histoincompatible donors proliferate only rarely or minimally in the marrows of these irradiated, but hematologically normal recipients without reirradiation. Syngeneic male donor cells proliferate in irradiated and restored female mice, while female donor cells fail to proliferate in the marrow of syngeneic male recipients. A possible explanation is that transfused female cells respond immunologically to the abundant H-Y antigen in the male environment and are eliminated as a result.

Bone marrow transfusions from syngeneic donors into normal, nonirradiated mice result in only minimal seeding and proliferation of donor cells. The observations were originally made in CBA mice transfused with 2×10^7 marrow cells from syngeneic T6T6 donors which can be identified by their marker chromosomes. An average of 2.5% of mitoses in the recipients' marrow were found to be of donor origin 1–12 weeks after transfusion. The percentage was stable over that time (1). In a second, similar study 2% or less of mitoses were of donor origin (2, 3). We confirmed these results, using first another marker chromosome and more recently transfusion of 4×10^7 marrow cells from male donors into female recipients of the C3H, C57B1, and CBA strains (4). Of 50 normal animals so transfused, the recipients' marrow contained 2% or less of mitoses of donor origin in 48. In a single animal 12% of donor cells were found and in another 3%. An immunologic rejection is highly unlikely, particularly in the CBA strain which tolerates prolonged parabiosis of males and females without evidence of incompatibility (J. M. Dorie: personal communication).

The accepted explanation for the nonproliferation of syngeneic donor cells in the normal, nonirradiated recipient is the lack of empty proliferative sites (1), niches (5), or domains (6) in which the transfused cells could establish themselves.

We report now that irradiated mice, restored to hematologic normalcy by postradiation marrow transfusion, subsequently accept and maintain proliferation of transfused donor cells, provided the donor is of the same strain and sex as the recipient; except that male donor cells will proliferate in female recipients of the same strain. This acceptance is thus distinct from the immediate postradiation marrow transfusion which extends to allogeneic and often xenogeneic donors. The present data do not appear explainable by the niche theory.

Materials and Methods. Adult CBA or C57B1 mice, at least 8 weeks old, of either sex were lethally irradiated and restored with isogeneic bone marrow. Irradiation was at 1.22 m from a ^{60}Co source with a total dose of 900 R and a dose rate of 22 R/min measured with a Victoreen con-

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denser R-meter. Bone marrow for restoration was obtained from femurs and tibias of donor mice by flushing with cold Medium-199 (Gibco). Cells were dispersed and counted in a Coulter counter, and 4×10^6 cells were injected intravenously into the irradiated mice within two hours.

Beginning at 4 weeks, blood counts were done weekly, or biweekly on restored mice to assure hematologic normalcy. This included hematocrits, white cell counts, platelet counts, and differentials. When these counts were in the range of unirradiated controls, the restored mice received another transfusion without reirradiation of either isogeneic or CBA/HT6J marrow, prepared as outlined above, except that 4×10^7 cells were given. Fourteen to sixteen days after the second bone marrow transfusion, mice were killed and cell suspensions of bone marrow and spleen were obtained for chromosome analysis. Colcemid (Gibco) 0.05 μg/ml was added to *in vitro* suspensions of marrow or spleen in RPMI 1640 medium with 15% fetal calf serum. Suspensions were incubated 2–4 hr at 37°. Slides were prepared and stained with a Giemsa C-banding method, and the Y chromosome was differentiated from the autosomes by the lack of a dark heterochromatic area at the centromere. The T6 chromosome markers were evident by their size. One hundred metaphases were scored in each preparation.

CBA/J and C57BL/6 recipient mice were used as indicated in the tables, and all transfusions were between isogeneic males and/or females of the same strain, except for CBA/HT6J donors. This strain, though originally derived from CBA, is no longer syngeneic with CBA/J in that parabiosis intoxication results from joining CBA/J and CBA/HT6J mice of the same sex.

Results. The results of isogeneic marrow transfusion into previously irradiated mice are summarized in Table I. When the recipients were female, cells from either male or female second donors proliferated in the marrow (8.2 and 7.8%) of the hematologically restored mice without reirradiation. However in male recipients only male donor cells proliferated to any extent (14.0%). Proliferation of female cells in the male host (1.4%) was not significantly different from the minimal proliferation in nonirradiated controls. It should be noted that the cells from the second donor could be identified unequivocally only when female mice were restored with female marrow or males with male marrow. In that case the first donor was identical with the host and any male cells in the female recipient or female cells in the male must have been derived from the second donor. However, when female recipients were initially restored with male cells or male recipients with female cells in order to test the acceptance of cells from second donors of the same sex as the host, surviving host cells may be present and obscure the results. Fortunately residual host cells seldom exceed 2%. Specifically, in appropriate controls given no second transfusions (lines 3 and 6 of Table I), the residual host cells numbered 2.0 and 0.6%, indicating that the bulk of female cells in the female host and male cells in the male host came from the second donor.

To test this interpretation further we used T6 marrow for the initial restoration of ir-

TABLE I. BONE MARROW TRANSPLANTATION: MALE-FEM

Recipient	First bone marrow transfusion	Second bone marrow transfusion	Time between transfusions in days	Percentage second donor cells (Mean, SE)	No. of mice
Male	Male	Fem	33–63	1.4 0.5	9
Fem	Male	Fem	36–50	7.8 2.1	7
Fem	Male	—	43–48	2.0[a] 2.0	3
Fem	Fem	Male	31–86	8.2 2.1	11
Male	Fem	Male	31–48	14.0 2.7	4
Male	Fem	—	30–56	0.6[a] 0.3	7

Note. About half the animals in each group were C57B1, and half CBA.
[a] Residual host cells.

TABLE II. BONE MARROW TRANSPLANTATION: CBA/J—CBA/HT6J

Recipient	First bone marrow transfusion	Second bone marrow transfusion	Percentage of second donor cells
Fem CBA	Fem T6	Fem CBA	27,33
Fem CBA	Fem T6	— —	0,0[a]
Fem CBA	Fem CBA	Fem T6	0,0
Male CBA	Male T6	Male CBA	6,10
Male CBA	Male T6		0,0[a]
Male CBA	Male CBA	Male T6	0,0,1
Fem CBA	Fem CBA	Male T6	0,0,0,0

Note. Time between transfusions comparable to those in Table I.
[a] Residual host cells in animals restored with T6 marrow of the same sex and not given a second transfusion.

radiated CBA mice. As already noted, the particular CBA and T6 lines available to us were no longer histocompatible in parabiosis. However, T6 marrow readily proliferated in CBA mice when transfused immediately after irradiation, with complete return to hematologic normalcy of the recipient by the criteria outlined under Materials and Methods. In such animals the entire marrow was of T6 origin as indicated by the absence of host cells (lines 2 and 5, Table II). It could readily be demonstrated that CBA marrow proliferated in recipients of the same line and sex (Table II). Cells of T6 origin, however, did not proliferate in CBA animals of the same sex restored with CBA marrow. Nor did male T6 proliferate in female CBA restored with female CBA marrow, indicating that any male "superiority" that might be invoked to explain the proliferation of male cells in irradiated females of the same strain cannot overcome the histocompatibility barrier between the T6 and CBA lines of mice available to us.

Finally we explored the possibility that proliferation of marrow of the same strain might be prevented by filling the putative empty proliferative sites or niches with a prior transfusion given 1 day earlier. The first experiment in Table III indicates that proliferation of male cells in CBA females restored with CBA female marrow proceeded similarly, whether the prior transfusion intended to fill the niches was from a compatible CBA or an incompatible T6 female. In the second experiment of this series, we restored CBA females with male T6 marrow. After hematologic recovery, we gave two transfusions of CBA marrow 1 day apart, one from a male and one from a female donor. Regardless of the order in which these two transfusions were given, the cells from both donors proliferated suggesting that no filling of empty proliferative sites had taken place.

Discussion. Transfusion of marrow into nonirradiated, hematologically normal recipients results in minimal or no seeding

TABLE III. BONE MARROW TRANSPLANTATION ATTEMPT TO FILL EMPTY NICHES

First bone marrow transfusion	Second bone marrow transfusion	Third bone marrow transfusion	Percentage of second donor cells	Percentage of third donor cells
Fem CBA	Fem CBA	Male CBA	—	5,10,10,13
Fem CBA	Fem T6	Male CBA	0,0	11,14
Male T6	Fem CBA	Male CBA	3,26	25,10
Male T6	Male CBA	Fem CBA	16,6	34,10
Male T6	Fem CBA	—	19,29	
Male T6	Male CBA	—	4,5	

Note. All recipients were CBA females. The first transfusion was given immediately after irradiation. The second transfusion was given after full hematologic recovery, intervals between first and second transfusion similar to those in Table I. Interval between second and third transfusion 24 hr.

(1–4). In contrast, the present data indicate that seeding of normal marrow can take place in hematologically normal recipients that had been previously irradiated and restored with either histocompatible or incompatible marrow. Only strain- and sex-matched donor cells proliferated in the previously irradiated and restored animals with one exception. Females accepted male cells of the same strain. Female cells transfused into similarly restored and hematologically normal males failed to proliferate (Tables I and II).

The reasons for the proliferation of transfused syngeneic marrow in previously irradiated recipients and the lack of such proliferation in nonirradiated mice is not at once apparent. The effect does not appear to be restricted to the immediate postirradiation period, since preliminary experiments (not listed under results) demonstrated proliferation of matched donor cells transfused 14 months after irradiation and restoration of the recipients. Since all recipients were hematologically normal by criteria of peripheral blood examination for numbers of white cells, red cells, platelets, and proportion of granulocytes and lymphocytes in the differential, a putative "need" for additional stem cells in the irradiated animals appears to be ruled out.

The suggestion of Micklem *et al.* (1) that special "proliferative sites" exist for CFU-S which are filled in the normal animal appeared to offer an explanation. Vos has shown that lethally irradiated and restored animals increase their CFU-S rapidly during the first 3 weeks postirradiation, but closely approach an asymptote at 30 to 40 days with a deficit in the number of CFU-S compared with normal 10–15% (7). One might conclude that this deficit represents empty proliferate sites which can now be occupied by the transfused cells. However, the failure of syngeneic female or male marrow cells to fill these sites and thus prevent subsequent seeding and proliferation of isogeneic male or female cells (Table III) militates against this interpretation.

The niche theory assumes that circulating CFU-S enter the marrow and thus identify empty proliferative sites. An alternative explanation may be that few transfused CFU-S enter or are retained in normal marrow. By the same token, few circulating stem cells originating from the animals' own marrow or spleen would reenter the normal marrow. This thesis is supported by the data of Dorie *et al.* (8), who parabiosed female and male CBA to study the kinetics of circulating stem cells. They assayed CFU-S in spleen and marrow after 12 days of parabiosis. In contrast we identified all dividing donor cells 14 or 16 days after transfusion which included both CFU-S and their progeny. Dorie *et al.* found 26.2% of splenic CFU-S to have originated in the joined animal of opposite sex, while the percentage of such CFU-S in the marrow was 2.8% and increased to only 4.4% in splenectomized animals. In a preliminary experiment in which we assayed donor and recipient CFU-S 24 hr after transfusion into a normal recipient, we found similar values of 4% in the marrow and 40% in the spleen. The data suggest that both transfused CFU-S of marrow origin and circulating CFU-S are retained in the normal marrow only to a minimal extent, though in large numbers in the spleen. The nonretention of normal marrow cells after transfusion appears to be abrogated in irradiated, hematologically normal recipients in which an average of 10% of marrow cells are of donor origin 2 weeks after transfusion.

Neither the niche theory nor the normally almost complete exclusion of transfused stem cells from the marrow can explain the proliferation of male marrow cells in irradiated and marrow restored syngeneic females, while no proliferation of female marrow takes place in similarly irradiated and restored syngeneic males. In preliminary experiments neither ovariectomy of adult females nor castration of adult males altered the observed phenomenon. The explanation could be that transfused female cells are exposed to a vast excess of H-Y antigen to which they respond, resulting in the elimination of all transfused female cells. When only the hemopoietic cells are male, due to a prior male marrow transfusion into female recipient, a subsequent transfusion of female cells are not elimi-

nated because the H-Y antigen excess is not so overwhelming. The general concept on which this possible interpretation of our results is based is taken from Gorer and Boyse (9), who quote instances of rapid elimination of donor cells due to a foreign antigen excess before the transfused cells can react against the recipient's antigen. They have termed the event allergic death.

It is recognized that there are wide variations in the percentage of donor cells between individual animals in each group. Nevertheless, the difference between irradiated and nonirradiated recipients are consistent. For example, in multiple experiments the average number of compatible donor cells in the marrow of irradiated and restored recipients was 10–12%, while the percentage of nonirradiated recipients never exceeded 2%. The replacement of an average close to 10% of endogeneous marrow was observed also in pairs of mice examined 4, 8, and 14 months after irradiation and restoration by bone marrow cells given within hours of irradiation. The extreme swings in percentages of donor cells regularly noted when very small numbers of donor CFU-S are involved in repopulating the marrows of acutely irradiated animals was not observed (12). It may be concluded that at least moderate numbers of donor cells were involved in establishing the proliferation of transfused cells reported here and that the observed major differences in the percentages of donor cells in the recipients' marrow are meaningful.

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