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Engraftment of a clonal bone marrow stromal cell line *in vivo* stimulates hematopoietic recovery from total body irradiation

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ABSTRACT **Whether bone marrow stromal cells of donors contribute physiologically to hematopoietic stem cell reconstitution after marrow transplantation is unknown. To determine the transplantability of nonhematopoietic marrow stromal cells, stable clonal stromal cell line (GB1/6) expressing the a isoenzyme of glucose-6-phosphate isomerase (Glu6*PI*-a, D-glucose-6-phosphate ketol-isomerase; EC 5.3.1.9) was derived from murine long-term bone marrow cultures and made resistant to neomycin analogue G418 by retroviral gene transfer. GB1/6 cells were fibronectin$^+$, laminin$^+$, and collagen-type IV$^+$ and collagen type I$^-$; these GB1/6 cells supported *in vitro* growth of hematopoietic stem cells forming colony-forming units of spleen cells (CFU-S) and of granulocytes, erythrocytes, and macrophage/megakarocytes (CFU-GEMM) in the absence of detectable growth factors interleukin 3 (multi-colony-stimulating factor), granulocyte/macrophage colony-stimulating factor, granulocyte-stimulating factor, or their poly(A)$^+$ mRNAs. The GB1/6 cells produced macrophage colony-stimulating factor constitutively. Recipient C57BL/6J (glucose-6-phosphate isomerase b) mice that received 3-Gy total-body irradiation and 13 Gy to the right hind limb were injected i.v. with GB1/6 cells. Engrafted mice demonstrated donor-originating Glu6*PI*-a$^+$ stromal cells in marrow sinuses *in situ* 2 mo after transplantation and a significantly enhanced hematopoietic recovery compared with control irradiated nontransplanted mice. Continuous (over numerous passages) marrow cultures derived from transplanted mice demonstrated G418-resistant, Glu6*PI*-a$^+$ stromal colony-forming cells and greater cumulative production of multipotential stem cells of recipient origin compared with cultures established from irradiated, nontransplanted control mice. These data are evidence for physiological function *in vivo* of a transplanted bone marrow stromal cell line.**

Successful transplantation of bone marrow stem cells relies on their ability to proliferate and differentiate in contact with stromal cells of the microenvironment. Total-body irradiation (TBI) and/or treatment with chemotherapeutic alkylating agents before transplantation of autologous or allogeneic stem cells does not always allow hematopoietic recovery due to functional change in the recipient's marrow stromal cells (1). Correcting the defective marrow stroma by transplantation of marrow stromal cells is one theoretical therapy. Several earlier reports suggest that marrow stromal cells can be transplanted *in vivo*. Werts *et al.* (2) reported that irradiated mouse limbs were reconstituted by stromal cell progenitors migrating through the circulation. Donor-originating marrow fibroblasts have been detected in lethally irradiated mice after i.v. bone marrow transplantation (2–4). Long-term marrow cultures (LBMCs) established from human marrow transplant patients, revealed donor-originating fibroblastic and endothelial cells (5). In contrast, other studies have failed to show donor-originating stromal cells after bone marrow transplantation (6–8).

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MATERIALS AND METHODS

Derivation and Characterization of Stable Marrow Stromal Cell Lines and Test of Physiological Function *in Vitro*. To establish a stromal cell line with a dominant selectable marker, the neomycin-resistance gene was transferred by a retroviral vector (10) to a cell line GB1/6 established from the adherent layer of LBMCs from B6Cast (Glu6*PI*-a) mice (9) as described (11). A subclone GB1neo^r containing the *neo*^{r} gene was selected in G418 (500 μg/ml) and expanded *in vitro*. Staining for alkaline phosphatase, acid phosphatase, peroxidase, α-naphthyl esterase, and lysozyme was done as described (12). Antisera to extracellular matrix proteins laminin, fibronectin (Collaborative Research, Waltham, MA), collagen types I and IV, and the alloenzyme marker Glu6*PI*-a (13) identified each protein in the GB1/6 cell line using each specific antiserum and the immunoperoxidase technique (14). For immunohistological studies, proximal tibiae were collected, split longitudinally, and fixed for 18 hr in 2% paraformaldehyde/0.1 M cacodylate buffer, pH 7.4. Bones were decalcified in 0.3 M EDTA/0.1 M cacodylate buffer, pH 7.4, for 4 days. Paraffin-embedded bones were sectioned at 5 μm and stained for reactivity with rabbit antiserum against mouse Glu6*PI*-a and indirect immunoperoxidase staining (14). Total cellular RNA isolated from the GB1neo^r stromal cell line was used to prepare poly(A)$^+$ mRNA by a modification of the guanidine hydrochloride extraction method (15). Specific message for interleukin 3 (IL-3) (16), granulocyte/macrophage colony-stimulating factor (GM-CSF) (17), macrophage colony-stimulating factor (M-CSF) (18), IL-1 (19), and granulocyte colony-stimulating factor (G-CSF) (20) was identified by hybridization with specific cDNA probes ($>10^8$ cpm/μg) as described (15). Methods for marrow transplantation *in vitro* and hematopoietic cell assays have also been described (11, 21–22).

Total Body and Hind Limb Irradiation, Transplantation, and Measurement of Hematopoietic Recovery *in Vivo*. Adult recipient C57B1/6 (Glu6*PI*-b) mice received TBI (3.0–8.5 Gy) and in addition right-hind-limb (RHL) irradiation of 10.0–12.5 Gy, delivered by a ^{137}Cs γ cell 40 irradiator. Dose rate for the TBI ranged from 0.25 to 1.0 Gy/min, while dose

Abbreviations: Glu6*PI*-a, glucose-6-phosphate isomerase a; IL, interleukin; LBMCs, long-term bone marrow cultures; CFU, colony-forming unit; CFU-S, CFU of spleen cells; CFU-GEMM, CFU of granulocytes, erythrocytes, and macrophage/megakaryocytes; CSF, colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; M-CSF, macrophage CSF; G-CSF, granulocyte CSF; TBI, total-body irradiation; RHL, right hind limb.

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rate to the exposed RHL ranged from 1.0 to 1.15 Gy/min. A single-cell suspension of stromal cell line GB1/6 or GB1neo^r was injected i.v. 48 hr after irradiation. Peripheral blood from mice transplanted with GB1/6 cell line and from control irradiated nontransplanted mice was analyzed weekly for at least three mice per group. No mice were bled more than once every 3 weeks. Red and white blood cells, hematocrit, and platelets were counted using an automated TOA-II Sysmex counter (American Scientific Products, Stone Mountain, GA).

RESULTS

Characterization of the GB1/6 Stromal Cell Line. Analysis of confluent cultures by phase microscopy often showed large binucleate cells. The presence of the Glu6*P*I-a isoenzyme was identified in 100% of the GB1/6 cells using specific antiserum. GB1/6 stromal cells were also assayed for expression of cytoplasmic enzymes and were positive for α-naphthyl acetate esterase and acid phosphatase and negative for peroxidase and alkaline phosphatase (data not shown). Extracellular matrix components produced by GB1/6 cells included fibronectin, laminin, and collagen type IV, with no detectable collagen type I (data not shown). When these data for cell line GB1/6 were compared with several other stromal cell lines including MBA-1, MBA-13, 14F-1 (23), and D2XRII (10), the cell line was classified as endothelial-like based on published criteria (23). GB1neo^r cells had cytochemical properties and extracellular matrix proteins indistinguishable from those of GB1/6 cells. Newborn C57BL/6J and sublethally irradiated adult C57BL/6J mice injected with 5×10^6 to 1×10^7 GB1/6 cells did not show detectable tumors after 6 mo (data not shown).

GB1/6 cells were tested for support of purified hematopoietic progenitor cells *in vitro*. Fig. 1 shows that hematopoietic progenitor cells were supported over 4 weeks. The cumulative number of CFU-S-forming progenitors produced was 23.1 ± 6.1 per flask and colony-forming unit of granulocyte, erythrocyte, macrophage/megakaryocytes (CFU-GEMM)-forming progenitors was 81.4 ± 36.2 × 10^2 per flask (Fig. 1). Further, the GB1/6 cell line supported growth of IL-3-dependent multipotential hematopoietic progenitor cell line B6SUtA (17) without added IL-3 (data not shown).

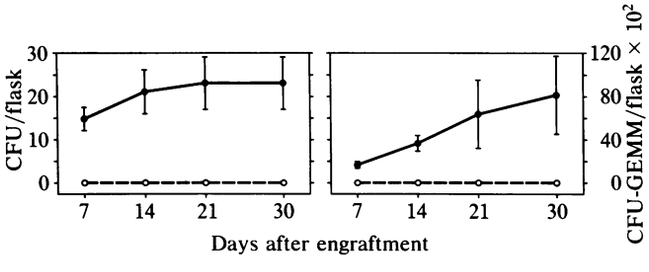

FIG. 1. *In vitro* support and maintenance of hematopoietic progenitor cells by the GB1/6 cell line. To confluent plateau-phase GB1/6 stromal cell line 2×10^7 purified day-40 hematopoietic cells harvested from donor C57BL/6J LBMCs were engrafted. Control donor hematopoietic progenitor cells plated without GB1/6 cell line did not form any stromal cell colonies and were not viable at day 7 (○). Weekly nonadherent cells produced from engrafted cultures (●) were harvested and assayed for the following: (*i*) CFU-S-forming progenitor cells (27). CFU-S colonies were scored on day 14. Results are expressed as cumulative mean ± SEM of colonies per flask from 10 mice per week. Control mice had less than 0.1 ± 0.03 CFU-S. (*ii*) Multilineage progenitor cells forming CFU-GEMM colonies in response to 10% medium conditioned by pokeweed mitogen-stimulated spleen cells and 2.5 units of erythropoietin per ml. Results are expressed as cumulative mean ± SEM of colonies per flask. The donor cell inoculum initially contained 166 ± 70 CFU-S-forming progenitors and 49.5 ± 0.5 × 10^2 CFU-GEMM progenitors per flask.

To determine whether the hematopoietic support capacity of GB1/6 and GB1neo^r cells was due to production of a known colony-stimulating factor (CSF), and in particular IL-3, poly(A)$^+$ mRNA from GB1neo^r cells was analyzed by RNA blot hybridization, for mRNA of known growth factors. GB1neo^r cells had no detectable poly(A)$^+$ mRNA for IL-3, GM-CSF, G-CSF, or IL-1; but the cells had detectable poly(A)$^+$ mRNA for M-CSF (data not shown). Thus, the hematopoietic support capacity of GB1/6 cell line *in vitro* could not be attributed to the synthesis of detectable quantities of any known hematopoietin with multi-CSF activity.

Homing and Function of GB1/6 Cells in Transplanted Mice. The ability of injected stromal cells to "home" and stably seed into marrow sinuses *in vivo* was first evaluated by *in vivo* immunohistochemical technique. Glu6*P*I-a$^+$ stromal cells were identified *in situ* 2 mo after transplant in the RHL marrow sinuses of transplanted mice. Two months after transplantation neither engrafted nor irradiated nonengrafted control mice demonstrated detectable donor-originating Glu6*P*I-a cells in spleen, liver, lung, or peritoneal washings (data not shown). As shown in Table 1 and Fig. 2 *Upper*, 1 mo after transplantation 26.2 ± 3.2% of the adherent stromal cells in marrow cultures explanted from transplanted mice were of donor origin. The highest percentage of donor-originating cells was 82.5 ± 0.5% and 62.5 ± 12.5% of total adherent cells in marrow explants 2 and 3 mo, respectively, after transplantation (Table 1). Glu6*P*I-a$^+$ cells composed 78.0% of adherent cells in LBMCs established from RHLs (13-Gy irradiated) of transplanted mice (Table 1). In contrast, nonadherent hematopoietic progenitor cells harvested from these same LBMCs had no detectable Glu6*P*I-a$^+$ cells. No detectable Glu6*P*I-a$^+$ stromal cells were identified *in situ* or in LBMCs from control-irradiated nontransplanted mice (Fig. 2 *Lower*, Table 1).

Explanted marrow cells from control-irradiated nontransplanted mice and mice transplanted with GB1neo^r cells were selected for growth in G418 (500 μg/ml). Table 2 shows that G418-resistant stromal cell colonies were found in the explanted RHL marrow of transplanted, but not of control-irradiated mice.

The physiological function *in vitro* of transplanted GB1/6 stromal cells was next evaluated. At monthly intervals LBMCs were established individually from each hind limb of GB1/6-transplanted and -irradiated nonengrafted control

Table 1. Identification of donor-originating stromal cells in bone marrow explanted from transplanted mice

Time, mo	GB1/6 cells injected per mouse	Donor-originating Glu6*P*I-a$^+$ cells, %		
		Adherent stromal cell explants* per limb, %		Adherent† stromal cells from right limb LBMCs
		Right	Left	
1	0	<0.01	<0.01	Not tested
	1×10^6	26.2 ± 3.2	28.4 ± 4.4	Not tested
2	0	<0.01	<0.01	<0.01
	1×10^5	75.5 ± 9.5	65.0 ± 2	76.7 ± 15.7
	5×10^5	82.5 ± 0.5	32.5 ± 3.5	78.0 ± 0
	1×10^6	59.0 ± 14.4	16 ± 0	Not tested
3	0	<0.01	<0.01	<0.01
	1×10^6	62.5 ± 12.5	<0.01	Not tested

*Adherent stromal cell explants were established, and donor-originating stromal cells were identified at day 18 using specific antiserum against Glu6*P*I-a alloenzyme marker (13), and immunoperoxidase staining (PAP).

†Adherent stromal cells from day-70 LBMCs were trypsinized, replated on coverslips, and processed for PAP. The percentage of donor-originating Glu6*P*I-a$^+$ cells was <0.01% in the nonadherent cells and in individual CFU-GEMM colonies derived from these LBMCs.

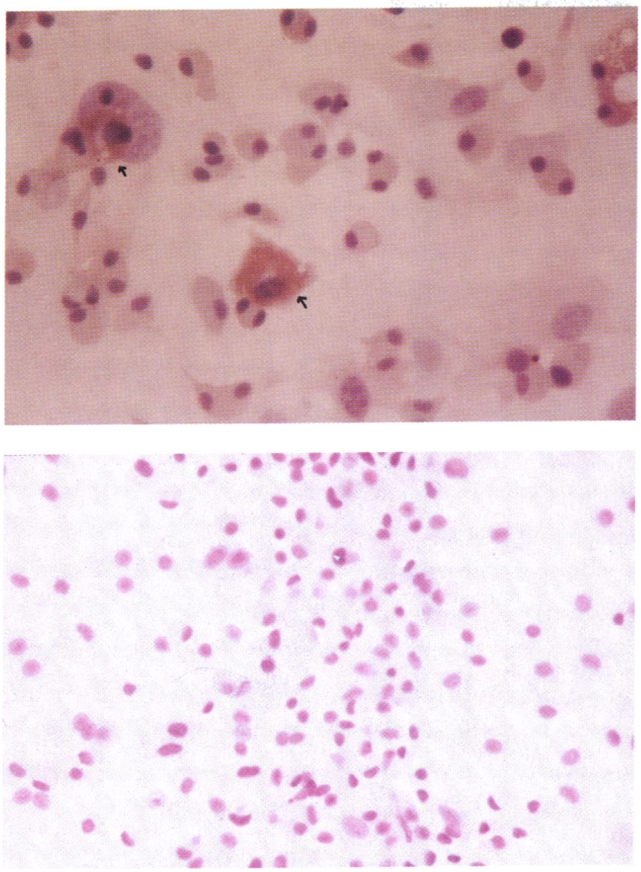

FIG. 2. Detection of GB1/6 stromal cell line in adherent cells explanted *in vitro*. (*Upper*) Adherent marrow cells from the RHL of transplanted mice at 1 mo after transplantation. (*Lower*) RHL marrow cells explanted *in vitro* from control irradiated but nontransplanted mice at 1 mo. Donor-originating cells were identified *in vitro* using specific rabbit antiserum against murine Glu6*PI*-a and immunoperoxidase staining. (×260.) Arrows, Glu6*PI*-a-containing stromal cells.

mice. The functional integrity of the adherent stromal cells was quantitated by measuring the longevity of hematopoiesis as cumulative number of total nonadherent cells and multipotential progenitor cells produced over 70 days *in vitro*. Fig. 3*A* shows the cumulative number of viable nonadherent cells produced per culture of marrow established at 1, 2, and 3 mo after transplant from the RHL of mice transplanted with GB1/6 cells was higher than that produced by cultures from irradiated, nontransplanted control mice. The cumulative number of multipotential hematopoietic progenitor cells forming mixed CFU-GEMM colonies per RHL culture (13 Gy), established at 1, 2, and 3 mo from transplanted mice was $30.5 \pm 3.7 \times 10^2$, $45.6 \pm 2.5 \times 10^2$, and $34.7 \pm 4.2 \times 10^2$, respectively, compared with $5.13 \pm 2.2 \times 10^2$, $7.3 \pm 0.9 \times 10^2$, and $6.04 \pm 0.13 \times 10^2$ for control irradiated, nontransplanted mouse marrow cultures ($P < 0.05$ for each timepoint; Fig. 3*B*).

The effect of donor stromal cell number injected on recovery of hematopoiesis in irradiated mice was next tested. Fig. 4 shows that LBMCs from the RHL (13 Gy) of irradiated nontransplanted control mice produced $11.45 \pm 4.8 \times 10^5$ cells per flask (Fig. 4*A*) and $7.41 \pm 0.8 \times 10^2$ CFU-GEMM progenitors per flask (Fig. 4*C*). The cumulative production of nonadherent cells as well as the CFU-GEMM progenitor cells in LBMCs from RHL marrow cultures of transplanted mice increased with the number of stromal cells injected per mouse over the range of 1×10^5–1×10^6 cells (Fig. 4 *A* and *C*). Production of hematopoietic progenitor cells by RHL cultures from transplanted mice reached 48% of the level seen in cultures from nonirradiated mice compared with 5% by marrow cultures from irradiated nontransplanted mice (Fig.

Table 2. Recovery of donor-originating GB1neo^r cells in cultures explanted from transplanted mice

Group	Stromal colonies per hind limb*, no.		G418-resistant stromal colonies per hind limb†, no.	
	Right	Left	Right	Left
Control-irradiated	65.5 ± 4.1	110.1 ± 10.3	0	0
Transplanted	78.0 ± 13.0	98.7 ± 13.3	30 ± 2.0 (39 ± 3%)	10.3 ± 2.0 (11 ± 2%)

Mice were transplanted with 5×10^5 GB1neo^r cells per mouse as described.

*Two months after transplantation the total number of cells recovered were as follows: 5.8×10^6 per RHL (13 Gy) and 8.6×10^6 per left hind limb (3 Gy) from control irradiated nontransplanted mice; 6.5×10^6 per RHL (13 Gy) and 6.9×10^6 per left hind limb (3 Gy) from transplanted mice. Adherent stromal cell explants were established with 5×10^6 cells per dish (60 × 10 mm). Some were fed biweekly with G418 (500 μg/ml).

†Number of G418-resistant colonies were scored 17 days after cultures were established *in vitro*. Values in parentheses represent percent control G418-resistant colonies calculated as the number of G418-resistant stromal cell colonies per 5×10^6 cells by the total number of stromal cell colonies per 5×10^6 cells × 100.

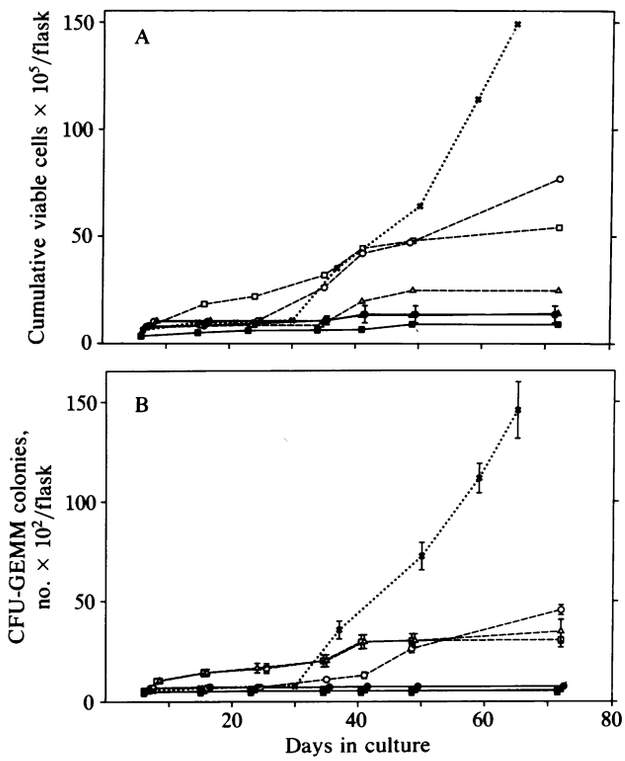

FIG. 3. Hematopoiesis in LBMCs established at 1, 2, and 3 mo after mice were irradiated and transplanted with GB1/6 cell line as described. At monthly intervals at least five mice were sacrificed, and LBMCs were established from the femur and tibia of each right hind limb. Weekly viable nonadherent cells were counted and plated at 5×10^4 cells per ml in the CFU-GEMM assay (28). Results are expressed as follows: (*A*) the cumulative mean number of viable cells produced per flask over 70 days; and (*B*) cumulative mean ± SEM of mixed and erythroid colony-forming progenitor cells produced in those same flasks. Irradiated, nonengrafted control mice at 1 mo (■), 2 mo (●), and 3 mo (▲); GB1/6 transplanted mice at 1 mo (□), 2 mo (○), or 3 mo (△); nonirradiated control mice (✕).

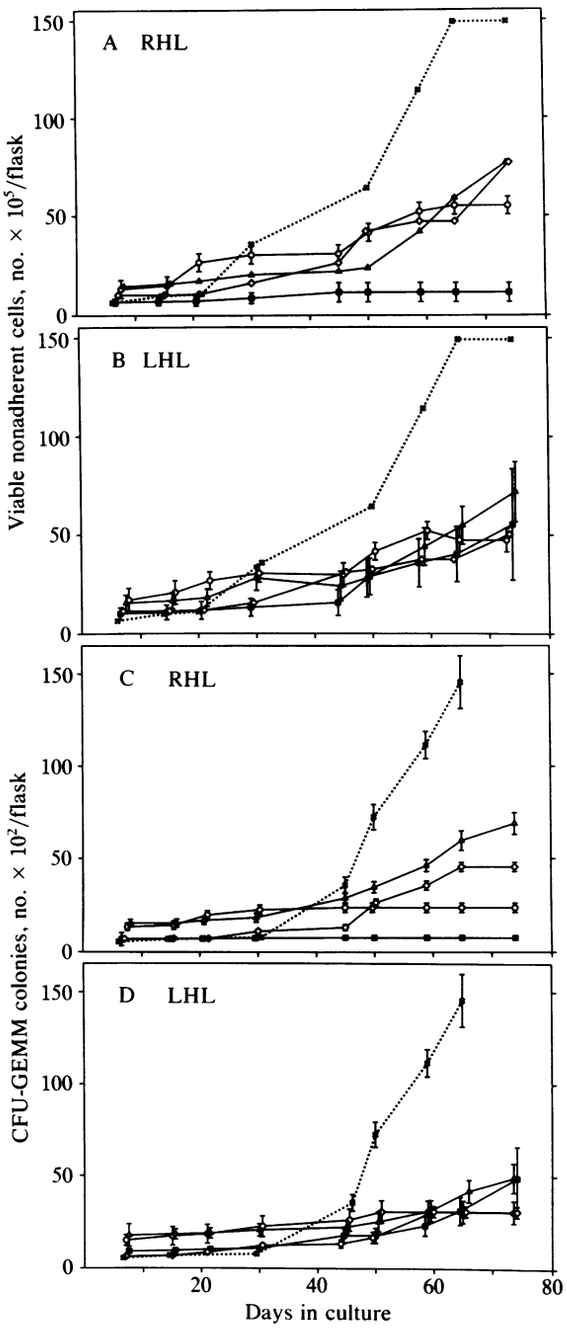

FIG. 4. Hematopoiesis in LBMCs established from mice transplanted with different numbers of GB1/6 cells. LBMCs were established 2 mo after GB1/6 cell transplantation as described for Fig. 3. Results are expressed as cumulative mean ± SEM of viable cells produced per flask over 70 days, from each RHL (13 Gy) (*A*) and left hind limb [LHL (3 Gy); *B*] cultures and cumulative mean ± SEM of mixed and erythroid CFU-GEMM colony-forming progenitor cells per flask from RHL (13 Gy) (*C*) and LHL (3 Gy) (*D*) cultures. Mice inoculated with 1×10^6 (◇), 5×10^5 (△), or 1×10^5 (○) cells; or irradiated nonengrafted controls (■); control nonirradiated mice (x).

4 *A* and *C*). An x-ray dose of 3 Gy to the left hind limb decreased hematopoietic stem cell production in marrow cultures from irradiated nontransplanted mice to 35% compared with cultures from nonirradiated mice. However, GB1/6 cell engraftment did not detectably increase cell production in LBMCs from limbs irradiated at this dose (Fig. 4 *B* and *D*).

The efficiency of repopulation of marrow sinuses of the RHL (13 Gy) by endogenous CFU-S and the recovery of peripheral blood counts was next measured in GB1/6 cell-transplanted and in irradiated nontransplanted control mice. Groups of C57BL/6J mice were irradiated in 2-Gy increments from 3 Gy to 7 Gy with the RHL receiving between 10 and 12.5 Gy (Table 3). A subgroup from each irradiation dose group was injected with 5×10^5 GB1/6 cells per mouse (optimal cell inoculum based on data in Fig. 4 *A* and *C*). Six weeks after irradiation and transplantation, the RHL from each animal in each dose group was assayed for the number of multipotential stem cells forming CFU-S. As shown in Table 3 at lower TBI doses of 3 and 5 Gy, the number of CFU-S-forming multipotential stem cells per RHL was similar in GB1/6 transplanted and in control irradiated nontransplanted mice. In contrast, sublethally irradiated (7-Gy TBI) mice transplanted with GB1/6 cells showed a significantly higher number of stem cells forming CFU-S in the RHL compared with the number recovered from the RHL of irradiated nontransplanted control mice ($P < 0.01$).

The kinetics of recovery of peripheral blood counts in mice after TBI doses of 3 or 5 Gy were similar in transplanted and control irradiated nontransplanted mice (Table 3). In contrast, a significant recovery of peripheral blood white blood cell count ($6.9 \pm 1.0 \times 10^3$ per mm^3) and platelet count ($112.5 \pm 2.5 \times 10^3$ per mm^3) was seen in 7-Gy-irradiated GB1/6-transplanted mice compared with irradiated nontransplanted controls (white blood cell count: $4 \pm 0.05 \times 10^3$ cells per mm^3; platelet count 50 ± 3 platelets per mm^3; $P < 0.05$; Table 3).

DISCUSSION

The function of donor-originating stromal cells in the marrow-transplant recipient is unknown. The data show a stable murine stromal cell line that provides a favorable microenvironment for hematopoietic stem cells *in vitro*, engrafts *in vivo* in irradiated recipients, and stimulates recovery of recipient hematopoietic stem cells *in vivo*. Using specific antiserum against the Glu6*P*I-a isoenzyme, stromal cells of donor origin were identified in marrow sinuses *in situ* as well as in adherent cells explanted *in vitro* several months after transplant. Thus, donor-originating stromal cells can infil-

Table 3. Hematopoietic recovery in C57BL/6J mice transplanted with GB1/6 stromal cell line

Total* body dose, Gy	CFU-S per right hind limb,† no.	Peripheral blood analysis on day 42 after irradiation‡ WBC, × 10³/mm³	PLT, × 10³/mm³	RBC, × 10⁶/mm³
3.0	92.0 ± 24.5	8.3 ± 1.5	156.5 ± 6.5	7.7 ± 0.03
	(104.6 ± 26.0)	(7.2 ± 0.3)	(126.5 ± 5.5)	(8.2 ± 0.02)
5.2	108.3 ± 9.2	6.8 ± 0.3	131.5 ± 10.5	8.2 ± 0.05
	(132.5 ± 24.4)	(5.7 ± 0.6)	(108.0 ± 1.0)	(7.4 ± 0.12)
7.0	101.8 ± 11.7§	6.9 ± 1.0¶	112.5 ± 2.5¶	7.5 ± 0.5
	(49.1 ± 9.5)	(4.0 ± 0.05)	(50.0 ± 3.0)	(5.9 ± 1.3)

*Each irradiation group had 5–10 mice per group. All mice received an additional 10–12.5 Gy to the RHL. One group was injected with 5×10^5 GB1/6 stromal cells per mouse. Values in parentheses are from the group that received no cells but were control irradiated.

†Six weeks after irradiation and transplantation, a subgroup from each group was sacrificed; cells were flushed from each RHL and assayed for CFU-S (21). Results are expressed as mean ± SD of three to five mice. An average of 4.4 ± 2.9 endogenous CFU-S colonies was seen on the spleens of irradiated noninjected mice.

‡Results are expressed as mean ± SD of at least three mice per group. Nonirradiated mice had a white blood cell (WBC) count of 8.8 ± 1.6 × 10/mm³; platelet (PLT) count of 173.7 ± 28.7 × 10³/mm³ and erythrocyte (RBC) count of 7.8 ± 0.05 × 10⁶/mm³.

§$P < 0.01$ compared with values from control irradiated nontransplanted mice.

¶$P < 0.05$ in the same comparison as for §.

trate host marrow sinuses and function *in vivo* to support recovery of primitive hematopoietic stem cells including those forming CFU-S and CFU-GEMM. Whether stimulation of CFU-S recovery by GB1/6 cells in inoculated mice can be attributed to stimulation of CFUs at the irradiation site in proximity to seeded GB1/6 cells, to homing of CFU-S arriving via the circulation from other sites, or to both mechanisms is unknown.

The GB1/6 cell line was chosen for these studies because of its endothelial-like characteristics and *in vitro* support capacity for multipotential hematopoietic progenitor cells. Constitutive production of M-CSF and no detectable production of IL-3, GM-CSF, nor G-CSF suggests that its support capacity for stem cells may be due either to the ability of M-CSF to trigger release of other CSFs from accessory cells *in vivo* and *in vitro* (24) or to another growth factor. Other clonal stromal cell lines demonstrated by us (11) and others (25) do not support multipotential hematopoietic progenitor cells forming CFU-S *in vitro*. Whether these other cell lines also engraft *in vivo* and support or suppress hematopoietic recovery after irradiation is unclear.

Marrow cultures established *in vitro* from the heavily irradiated RHL (13 Gy) of mice transplanted with GB1/6 cells showed stable engraftment of donor stromal cells over 3 mo and enhanced hematopoietic progenitor cell production *in vitro* in LBMCs compared with cultures derived from irradiated nontransplanted control mice. In contrast, hematopoiesis in LBMCs established from the 3-Gy-irradiated left hind limb of transplanted mice was not enhanced compared with cultures from irradiated nontransplanted control mice, and donor-originating stromal cells identified at 1 mo did not persist. The data confirm that previous irradiation damages the stromal cells of the hematopoietic microenvironment (26) and support Brecher *et al.* (27), who suggested that niches, freed of endogenous hematopoietic stem cells by the higher dose, provide more efficient seeding sites for injected donor cells. Our data may explain previous failure to detect donor-originating stromal cells in studies using lower x-ray doses (6, 8).

To establish a detectable chimeric stromal cell population, a minimum of 1×10^5 purified GB1/6 stromal cells were required. Thus, other studies reporting no donor-originating stromal cells in LBMC adherent cell layers derived from transplanted mice using chromosomally marked marrow cells may not have used enough transplanted cells.

The physiological failure of bone marrow has generally been attributed to defects in the hematopoietic stem cells—defects correctable by autologous or allogenic bone marrow-stem cell transplantation (28). However, the pathophysiology of some forms of marrow failure, as in chronic myelogenous leukemia (29) and aplastic anemia (30, 31), may be due to a defect in the stromal microenvironment. Our data revealed evidence for stimulation of hematopoietic recovery *in vivo* in stromal cell line-engrafted mice compared with irradiated nontransplanted controls. Although further studies are required, a therapeutic role for stromal cell infusion in conjunction with hematopoietic stem cell transplantation is suggested in the treatment of some forms of marrow failure.

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