Improved Hematopoiesis in Anemic SI/Si² Mice by Splenectomy and Therapeutic Transplantation of a Hematopoietic Microenvironment

By Pervin Anklesaria, T.J. FitzGerald, Kenneth Kase, Akira Ohara, and Joel S. Greenberger

The ability of a clonal hematopoiesis-supportive bone-marrow stromal cell line GB1neo' to engraft and alter the microenvironment-induced anemia of SI/Si² mice was studied. Prior to stromal cell transplantation, SI/Si² mice received 1 Gy total body irradiation (TBI) and 13 Gy to the right hind limb. Two months after intravenous (IV) injection of 5 x 10⁶ GB1neo' cells, 54.4% ± 17.0% donor origin (G418') colony-forming cells were recovered from the right hind limb of SI/Si² mice. Long-term bone marrow cultures (LTBMCs) established from GB1neo'-transplanted mice produced 189.5 CFU-GEMM-forming progenitors/flask over 10 weeks compared with 53.7 ± 6.2 CFU-GEMM forming progenitors/flask from irradiated nontransplanted SI/Si² mice. A partial correction of macrocytic anemia was detected 2 months after GB1neo' transplantation in splenectomized, irradiated SI/Si² mice (HgB 7.2 ± 0.4 g/dL; MCV 86.3 ± 7.0 fl) compared to splenectomized, irradiated, nontransplanted SI/Si² mice (HgB 8.5 ± 1.1 g/dL; MCV 76.9 ± 8.5 fl) or control SI/Si² mice (HgB 5.4 ± 0.5 g/dL; MCV 82.4 ± 1.3 fl). Mean RBC volume distribution analysis showed a 2.5-fold increase in percentage of peripheral blood RBCs with MCV ≥ 45 fl and confirmed the reduction of the MCV in splenectomized-GB1neo'-transplanted mice compared to control SI/Si² mice. A hematopoiesis-suppressive clonal stromal cell line derived from LTBMCs of SI/Si² mice (SI²neo') engrafted as effectively (43.5% ± 1.2% G418' CFU-F/limb) as did GB1neo' cells (38.3% ± 0.16% G418' CFU-F/limb) to the irradiated right hind limbs of C57Bl/6 mice. LTBMCs established after 2 or 6 months from SI²neo'-transplanted mice showed decreased hematopoiesis (182 ± 12 [2 months] and 3494.3 ± 408.1 [6 months] CFU-GEMM progenitors/flask over 10 weeks) compared to those established from GB1neo'-transplanted mice (5980 ± 530 [2 months] and 7728 ± 607.6 [6 months] CFU-GEMM progenitors forming/flask). Thus, transplantation of clonal bone-marrow stromal cell lines in vivo can stably transfer their physiologic properties to normal or mutant mice.

MATERIALS AND METHODS

Mice. Adult, 5- to 8-week-old, severely anemic WCB6F1 SI/Si² mice and C57Bl/6 mice were obtained from Jackson Laboratories, Bar Harbor, ME. B6Cast-GPI-1 transgenic mice, obtained from Jackson Laboratories, were bred at the University of Massachusetts Medical Center.

Bone-marrow stromal cell lines. Derivation and characterization of clonal-marrow stromal cell lines (GBl/6, GB1neo', SI²³, +/+ 2.4 and D2XR1I) have been described previously.13,16 The embryo fibroblast cell line Bl/6embC was provided by Dr S. Aaronson, NCI, Bethesda, MD. Neomycin (G418')-resistant subclones of SI² and D2XR1I and Bl/6embC were established by retroviral vector-mediated gene transfer.13 The subclones are designated SI²neo', D2Xneo', and Bl/6embneoC, respectively.

Total body and hind-limb boost irradiation. Adult recipient SI/Si² or C57Bl/6 mice received 1 to 2 Gy TBI and 10.0 to 20.0 Gy
to the right hind limb (RHL) or both hind limbs (BHL) delivered by a linear accelerator as described. We used 1 Gy TBI and 10 Gy to the RHL due to the relative sensitivity of S/I/Sf mice to TBI. Irradiated mice were transplanted with 5 x 10^6 stromal cells of each line (GB1neo', SI/Sfneo', B1/6 emboeoC or D2Xneo') by IV injection (single schedule). For sequential boost irradiation-translation studies with S/I/Sf mice, 1 Gy TBI and 10 Gy to the RHL was delivered by linear accelerator on day 0. A single-cell suspension of the GB1neo' cell line was injected IV 48 hours later. Two months after the first irradiation schedule, the same group of mice received 1 Gy TBI and 10 Gy to the left hind limb (LHL). Another injection of the GB1neo' cell line was administered 48 hours later (multiple transplant schedule). Control irradiated nontransplanted mice received 2 Gy TBI and 10 Gy to both hind limbs (BHL).

**Splenectomy and irradiation of S/I/Sf mice.** S/I/Sf mice (5 to 6 weeks old) were anesthetized using an inhalation anesthetic methoxyflurane (metofane, Pitman Moore, Inc, Washington Crossing, NJ). A small incision was made in the flank region, and the spleen was gently removed and cauterized as described. The incision was sutured with a 5.0 chromic gut and the skin flaps held together with wound clips using Mikron auto clip (Clay Adams). Two weeks after splenectomy, mice were irradiated (1 Gy TBI and 10 Gy BHL) and transplanted (single schedule) with the GB1neo' marrow stromal cell line as described above. Transplanted and control animals were followed for clinical improvement of their sampled blood Hgb concentration, hematocrit, and mean corpuscular volume (MCV), as described below.

**Measurement of stromal cell engraftment.** The CFU-F assay was carried out as described. Briefly, control-irradiated–nontransplanted or irradiated-transplanted mice were sacrificed at time intervals indicated, and the harvested bone-marrow cells from each hind limb were counted and plated at different cell concentrations in 60-mm dishes (Falcon, Lincoln Park, NJ) in the absence or presence of 200 μg/mL G418 (Gibco). CFU-F colonies (>25 cells) were counted at days 7 and 14.

**Measurement of hematopoietic recovery in vivo and in vitro.** Peripheral blood from transplanted and control-irradiated–nontransplanted mice was analyzed monthly. Blood from the tail vein (80 μL) was collected in heparinized microhematocrit capillary tubes (Fisher Scientific, Springfield, NJ) and mixed with 20 mL of diluent (NaCl [6.38 g/L], Boric acid [1.0 g/L], sodium tetraborate [0.2 g/L], and EDTA-2K [0.2 g/L]). RBCs, WBCs, platelets, Hgb concentration, hematocrit, and MCV were measured using an automated TOA-II Sysmex Counter (American Scientific Products, Stone Mountain, GA). Mean volume distribution studies of RBCs obtained from control-irradiated–splenectomized and transplanted mice were studied using a Coulter Counter with a Channelizer (Coulter, Hialeah, FL). Peripheral blood smears were made and stained with Wright's-Giemsa stain.

The functional integrity of transplanted stromal cells was evaluated by establishing LTBMCS from each hind limb as described. Hematopoietic recovery was quantitated by measuring cumulative nonadherent CFU-GE-MM–forming progenitor cells removed weekly for over 10 weeks in culture.

**In vitro hematopoietic progenitor cell engraftment to clonal stromal cell lines.** Plateau-phase cultures of each stromal cell line (GB1neo' or SI/Sfneo') were individually established by plating cells in 25 cm² flasks (Corning Plastics, New York). The confluent stromal cell cultures were engrafted with 2.5 to 3.0 x 10^6 washed, nonadherent hematopoietic progenitor cells harvested from 4- to 6-week-old LTBMCS established from C57Bl/6 mice. At weekly intervals after engraftment, nonadherent cells were harvested and assayed for CFU-GE-MM–forming progenitor colonies. Colony assays were performed using pokeweed-mitogen–stimulated spleen cell-conditioned medium (PWM-SCCM) and erythropoietin (EPO) as described.

In other experiments, different ratios of the two stromal cell lines were added to same flasks to test for dominance of biologic properties.

**Statistical evaluation.** The differences between the means was determined by standard Student's test and chi-square analysis.

**RESULTS**

**Recovery of bone marrow stromal cells in vitro after TBI and isolated hind-limb boost irradiation in vivo.** The effects of TBI (1 to 2 Gy) and high-dose irradiation to the RHL (13 Gy) on regeneration of normal C57Bl/6 and SI/Sf mouse-marrow stromal cells in vivo were studied first. Two days following 1 Gy TBI and 13 Gy to the RHL, the femoral CFU-F from normal C57Bl/6 mice decreased to 53% of the level detected in control-nonirradiated mice (Fig 1). By 60 days after irradiation, a further decrease (26% of control) in the colony-forming potential of marrow stromal cells from irradiated C57Bl/6 mice was observed (P < .05). Improvement in the colony-forming ability of stromal cells from irradiated mice at 180 days after irradiation was not significant (Fig 1).

The number of colony-forming CFU-F progenitors/hind limb obtained from nonirradiated S/I/Sf mice was 53% lower than that of control nonirradiated C57Bl/6 mice. At two
The C57BL/6 mice received 3 Gy TBI and 13 Gy to the RHL; S1/S14 mice received 1 Gy TBI and 11 Gy to the RHL. Results are expressed as mean ± SD for three to five animals/group.

†The values in parenthesis are % of the values obtained in cultures without G418.

Table 2. Recovery of Donor Origin Cells From Hind Limb Explants of Mice Transplanted With Clonal Stromal Cell Lines

<table>
<thead>
<tr>
<th>Group*</th>
<th>Total No. of Stomatal Cell Colonies Per Hind Limb</th>
<th>Total No. G418-Resistant Donor Origin Stromal Cell Colonies Per Hind Limb†</th>
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<tbody>
<tr>
<td></td>
<td>2 mos Right</td>
<td>Left</td>
</tr>
<tr>
<td>1. C57BL/6 mice transplanted with GBlneo' 8.1 cells</td>
<td>78.0 ± 13.0</td>
<td>98.7 ± 13.3</td>
</tr>
<tr>
<td>2. S1/S14 mice transplanted with GBlneo' 8.1 cells</td>
<td>182.4 ± 9.1</td>
<td>126.0 ± 47.0</td>
</tr>
<tr>
<td>3. C57BL/6 mice transplanted with S1'neo' 3.3 cells</td>
<td>88.5 ± 7.0</td>
<td>90.0 ± 20.3</td>
</tr>
<tr>
<td>4. Control-irradiated–nontransplanted</td>
<td>65.5 ± 4.1</td>
<td>110.1 ± 10.3</td>
</tr>
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</table>

*The C57BL/6 mice received 3 Gy TBI and 13 Gy to the RHL; S1/S14 mice received 1 Gy TBI and 11 Gy to the RHL. Results are expressed as mean ± SD for three to five animals/group.

†The values in parenthesis are % of the values obtained in cultures without G418.
(Table 2, group 2). In contrast, at 6 months, 22.5% G418
CFU-F colonies were still detected in GBlneo-transplanted
C57Bl/6 mice (Table 2, group 1).

The in vivo hematopoietic recovery of GBlneo-transplanted 
$S/Si^d$ mice was next studied. Peripheral blood
counts from control-irradiated (1 Gy TBI and 10 Gy RHL)–
nontransplanted $S/Si^d$ mice were similar to those of nonirradiated
$S/Si^d$ mice (Table 3, group II, III). Irradiated $S/Si^d$
transplanted with GBlneo cells (single schedule)
showed no significant recovery of the peripheral blood counts
at 60 days (Table 3, group IVa). $S/Si^d$ mice receiving
sequential bone irradiation of both hind limbs and multiple
transplantation schedule also showed no significant hematopoietic
recovery in vivo at 120 days (Table 2, group IVb).

To determine whether the failure to achieve detectable hematopoietic recovery in the GBlneo-transplanted mice was attributable to the inability of these cells to overcome the suppressive microenvironment of the $S/Si^d$ anemia, we next
repeated the transplant studies in splecnecotomized $S/Si^d$
mice. A group of $S/Si^d$ mice were splecnecotomized 15 days
before TBI and hind-limb irradiation (10 Gy BHL) and were
then transplanted with the GBlneo cell line. Splecnecotomized,
irradiated, GBlneo-transplanted mice had significantly
increased WBC counts and Hgb concentration (9.5 ± 1.4 x
$10^9/\mu$L and 7.2 ± 0.4 g/dL, respectively [Table 3, group V])
at 2 months, compared with nonirradiated $S/Si^d$ mice
(WBC counts: 4.6 ± 0.6 x $10^9/\mu$L; Hgb concentration:
5.4 ± 0.5 g/dL. Table 3, group III P < .05). The splecnecotomized GBlneo-transplanted mice at 2 months had reduced
MCV (68.3 ± 7.0 fl) compared with that from control mice
(82.4 ± 1.3 fl; P < .05, Table 3, group II). RBC volume
distribution analysis demonstrated clear differences in RBC
volumes comparing $S/Si^d$ mice to normal C57Bl/6 mice,
with only a small overlap between the two populations (Fig
2). The median MCV for normal RBCs from C57Bl/6 mice
was 30 fl (range 16 to 45 fl) and that for $S/Si^d$ mice was 78 fl
(range 52 to 83 fl; Fig 2). In the peripheral blood RBC
population obtained from splecnecotomized GBlneo-transplanted
$S/Si^d$ mice, there was a 2.5- and 1.5-fold increase in the
percentage of cells with MCV of 45 fl and 52 fl
respectively as compared with those obtained from $S/Si^d$
mice (Fig 2). This shift in red cell volume to normal levels
suggests a detectable partial correction of the macrocytic
anemia in vivo in GBlneo-transplanted mice.

The physiologic function of GBlneo cells in different
groups of transplanted $S/Si^d$ mice was next evaluated by in
vitro culture of marrow. The first group consisted of mice
receiving 1Gy TBI and 13 Gy to the RHL. Marrow cultures
established from irradiated-nontransplanted $S/Si^d$ mice
showed increased cumulative progenitor cell production per
flask (RHL, 10 Gy; 52 ± 10 progenitors/flask) compared to
those from nonirradiated control mice (16 ± 4 progenitors/
flask; P < .01; Fig 3). The cumulative number of nonadherent
CFU-GEMM–forming progenitors produced in
LTBMCs from RHL (10 Gy) of GBlneo-transplanted $S/Si^d$
mice was 189.5 progenitors/flask. This was higher than that
produced either in LTBMCs from control-nonirradiated or
control-irradiated–nontransplanted mice. In contrast,
LTBMCs established from the left hind limb of transplanted
mice (1 Gy) supported multipotential progenitor cell production
less efficiently (46.5 CFU-GEMM forming progenitors/flask; Fig
3). In the next two groups tested, we attempted to replace a dominant fraction of the marrow microenvironment of $S/Si^d$ mice by preparing the mice with either 2 Gy TBI and 20 Gy to both hind limbs (BHL, single
schedule) or by sequential irradiation transplantation (multipl
schedule), as described in Materials and Methods. In
both experiments none of the control-irradiated–nontransplanted
mice survived. LTBMCs established from GBlneo-
transplanted mice (2 Gy TBI and 20 Gy BHL) at 4 months
showed increased cumulative CFU-GEMM–forming progenitors/flask in both right (298.8 ± 32.7) and left
(415.8 ± 36.5) hind limb cultures compared to nonirradiated
$S/Si^d$ mice (P < .05; Fig 4). In separate experiments, 2
months after mice received the second irradiation transplantation, the cumulative number of CFU-GEMM–forming progenitors obtained per flask was higher in LTBMCs
established from engrafted RHL (136.1 ± 32) and LHL
(78.6 ± 15.4) of GBlneo-transplanted mice compared with
those from control-nonirradiated $S/Si^d$ mice (P < .05; Fig

### Table 3. Hematopoietic Recovery at Two Months in $S/Si^d$ Mice Transplanted With GBlneo Cells

<table>
<thead>
<tr>
<th>Peripheral Blood Analysis</th>
<th>WBC x 10^9/µL</th>
<th>RBC x 10^9/µL</th>
<th>PLT x 10^9/µL</th>
<th>Hgb g/dL</th>
<th>HCT %</th>
<th>MCV (FL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td></td>
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<tr>
<td>I. Normal C57Bl/6</td>
<td>7.5 ± 1.7</td>
<td>8.1 ± 0.2</td>
<td>514 ± 145</td>
<td>12.1 ± 0.4</td>
<td>36.4 ± 6.6</td>
<td>36.6 ± 4.4</td>
</tr>
<tr>
<td>II. Nonirradiated S/Si^d</td>
<td>4.6 ± 0.67</td>
<td>2.1 ± 0.2</td>
<td>234.9 ± 70.2</td>
<td>5.4 ± 0.5</td>
<td>17.5 ± 1.2</td>
<td>82.4 ± 1.3</td>
</tr>
<tr>
<td>III. Irradiated-nontransplanted S/Si^d</td>
<td>4.5 ± 0.7</td>
<td>2.9 ± 0.1</td>
<td>142 ± 5</td>
<td>6.6 ± 0.1</td>
<td>20.8 ± 0.15</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>IV. S/Si^d transplanted with GBlneo</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) At 60 days (RHL)</td>
<td>4.3 ± 0.5</td>
<td>2.57 ± 0.37</td>
<td>411 ± 200</td>
<td>6.0 ± 1.1</td>
<td>18.1 ± 3.4</td>
<td>70 ± 2.9</td>
</tr>
<tr>
<td>b) At 120 days (BHL)†</td>
<td>3.26 ± 0.5</td>
<td>1.9 ± 0.57</td>
<td>153.3 ± 53</td>
<td>4.9 ± 1.6</td>
<td>17.0 ± 6.0</td>
<td>86.6 ± 7.5</td>
</tr>
<tr>
<td>V. S/Si^d spleenectomized and transplanted with GBlneo (BHL)</td>
<td>9.5 ± 1.4‡</td>
<td>3.15 ± 0.2</td>
<td>152.6 ± 43.4</td>
<td>7.2 ± 0.4‡</td>
<td>19.1 ± 3.8</td>
<td>68.3 ± 7.0‡</td>
</tr>
<tr>
<td>VI. S/Si^d spleenectomized irradiated nontransplanted</td>
<td>7.6 ± 1.9</td>
<td>2.24 ± 0.6</td>
<td>201.3 ± 118.0</td>
<td>5.5 ± 1.1</td>
<td>16.6 ± 3.5</td>
<td>76 ± 8.5</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SD for at least three to five mice per group.
†In group (b) mice received sequential irradiation transplantation (multiple schedule), and all control-irradiated–nontransplanted mice died within 15 days after second irradiation.
‡P < .05 as compared to control-nontransplanted S/Si^d mice.
4). Thus the transplanted GBlneo⁻ cells functioned both in vivo in splenectomized SI/SI² mice to support hematopoiesis and in vitro after explant to LTBMC.

Transplantation of the SI'neo⁺ stromal cell line to irradiated C57BL/6 mice. Normal C57Bl/6 mice that received TBI and RHL irradiation (1 Gy TBI and 10 Gy RHL) were transplanted with the SI'neo⁺ stromal cell line. At 2 and 6 months after transplantation, adherent stromal cells explanted from femurs and tibias of transplanted mice were selected in G418, and the percentage of donor-origin stromal cells recovered was 43.5% ± 1.2% and 8% ± 4.5% respectively (Table 2, group 3). Thus the SI'neo⁺ cell line engrafted in vivo to Bl/6 mice as efficiently as the Gbneo⁺ cell line (Table 2, group 1). Marrow cultures established from mice transplanted with the GBlneo⁻ Stromal cell line had significantly higher CFU-GEMM-forming progenitors per flask (2 months 5980 ± 530; 6 months 7728 ± 607, P < .05) compared with those from either control-irradiated–nontransplanted or SI'neo⁻ transplanted mice (Fig 5). In contrast, the cumulative number of hematopoietic progenitors supported by LTBMCs established at 2 and 6 months post-transplantation from SI'neo⁺ transplanted mice was 182 ± 12 and 3494 ± 408 CFU-GEMM progenitors/flask, respectively, and was no higher than that detected with marrow cultures
Fig 4. Support of hematopoietic progenitors harvested from LTBMCs established from $S^4/Si^4$ mice after 2 Gy TBI and 20 Gy to BHL (single schedule) or 1 Gy TBI and 10 Gy RHL followed by a second irradiation dose of 1 Gy TBI and 10 Gy LHL 2 months later (multiple transplant schedule). At 4 months after irradiation-transplantation, LTBMCs were established from three mice/group. Control, nonirradiated $S^4/Si^4$ mice (—□—), $G^4$ neo-transplanted RHL-20 Gy (—○—), LHL-20 Gy (—■—), sequentially irradiated $G^4$ neo-transplanted RHL-10 Gy (—●—), and LHL-10 Gy (—□—). Results are expressed as mean ± SD of cumulative, nonadherent CFU-GEMM progenitors/flask.

Fig 5. Hematopoietic progenitors produced in LTBMCs established from the RHLs of $S^4$ neo' and $G^4$ neo' transplanted C57BL/6 mice. At 2 and 6 months after transplantation, three to five mice per group were sacrificed and LTBMCs established from RHL. Results are expressed as mean ± SD cumulative, nonadherent CFU-GEMM progenitors/flask from control-irradiated—nontransplanted mice at 2 (—○—) and 6 (—■—) months; and from $S^4$ neo'—transplanted mice at 2 (—●—) and 6 (—□—) months.

established from control-irradiated—nontransplanted mice (219 ± 9 [2 months] and 3225 ± 363 [6 months] CFU-GEMM—forming progenitors/flask; Fig 5). Thus both the $G^4$ neo' and $S^4$ neo' cells transferred their in vitro biologic properties to the in vivo marrow microenvironment of C57BL/6 mice.

RESULTS

The present studies demonstrate that the $G^4$ neo' marrow stromal cell line engrafts in vivo and improves hematopoietic recovery in splenectomized $S^4/Si^4$ mice. Another stromal cell line, $S^4$ neo', does not support hematopoiesis in vitro but engrafts in vivo and transfers its biologic properties to irradiated C57Bl/6 mice. Thus distinct physiologic properties of two clonal stromal cell lines that are expressed in vitro are maintained following transplantation in vivo.

The engrafted $G^4$ neo' cells in splenectomized irradiated $S^4/Si^4$ mice induced an increase in the HgB concentration as well as WBC counts in the peripheral blood of these mice as compared to that of control or splenectomized mice. The present data confirm and extend a previous report that demonstrated a partial stimulation of erythropoiesis in splenectomized $S^4/Si^4$ mice implanted with intact spleens from +/+ mice. A decrease in the average MCV of peripheral blood cells was observed.

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blood RBCs was also observed in splenectomized GBneo' transplanted mice. Furthermore, LTBMCS established at 2 and 6 months from GBneo'-transplanted SI/SI* mice showed increased longevity compared to marrow cultures from control SI/SI* mice. This may be attributable to a growth advantage in vitro of GBneo' stromal cells that further increase the favorable ratio of supportive stromal cells in vitro. LTBMCS established from control-irradiated SI/SI* mice supported hematopoiesis more efficiently than those from nonirradiated-control SI/SI* mice. Thus in SI/SI* mice, high-dose irradiation may help create a "niche" in the narrow cavity to support transplanted stromal cells and may also eliminate endogenous stromal cells that suppress hematopoiesis.

It is not yet known what characteristics are important for stable engraftment of stromal cell lines in vivo. A clonal murine embryo fibroblast (Bl/6embneo' c) and bone-marrow stromal cell line (GBneo') that support hematopoiesis in vitro can engraft stably in vivo for up to 12 months to irradiated marrow sinuses of normal mice (unpublished observations). However, GBneo' cells were not detected by the CFU-F assay in the bone marrow of mutant SI/SI* mice at 6 or 12 months. One possible explanation for these data may be that the number of donor-origin cells was below the level of sensitivity of the assay used (G418*) for detecting donor-origin cells. Another possibility could be that either migration or regeneration of endogenous SI/SI* stromal cells in irradiated marrow sinuses may have eliminated or reduced the numbers GBneo' cells. Results obtained in vitro using stromal cultures made up of different ratios of SI*neo' and GBneo' cells indicated that one tenth the number of SI*neo' cells could suppress support of hematopoiesis by GBneo' cells. While the SI*neo' stromal cell line did not support hematopoiesis in vitro, it did engraft in vivo and stably transferred its suppressive microenvironment. The stromal cell line D2neo', which does support hematopoiesis in vitro, did not engraft in vivo (unpublished observations). Thus the parameter of in vivo engraftment of a stromal cell line does not necessarily correlate with hematopoietic support in vitro and appears to be an independent biologic marker.

The present results indicate improved hematopoiesis in anemic SI/SI* mice after splenectomy and engraftment of a clonal bone-marrow stromal cell line. These data support the theory that the splenic microenvironment may be involved in the pathogenesis of some types of chronic anemia. Our attempts to replace a dominant fraction of the hematopoietic microenvironment involved sequential high-dose irradiation of two marrow niches and removal of the abnormal spleen microenvironment by splenectomy. While the parameters of stromal-cell transplantation need to be optimized, the present results provide evidence that therapeutic stromal cell transplant may be a viable alternative in diseases associated with a defective marrow microenvironment.

ACKNOWLEDGMENT

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Improved hematopoiesis in anemic Sl/Sld mice by splenectomy and therapeutic transplantation of a hematopoietic microenvironment

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