

## Stromal cells

# *W/W<sup>v</sup>* marrow stromal cells engraft and enhance early erythropoietic progenitors in unconditioned *Sl/Sl<sup>d</sup>* murine recipients

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### Summary:

Transplantation of marrow stromal cells may provide a means of modulating hematopoiesis and serve as a form of cell therapy. We employed a murine transplant model using *Sl/Sl<sup>d</sup>* mice, which have macrocytic anemia due to defective expression of stem cell factor (SCF) on bone marrow stromal cells. Donor cells were derived from the complementary mutant strain *W/W<sup>v</sup>*, which also exhibit anemia, due to mutations in *c-kit*, the SCF receptor expressed on hematopoietic stem cells. The strength of this model is that any correction of the *Sl/Sl<sup>d</sup>* anemia from the infusion of *W/W<sup>v</sup>* stromal cells can be attributed to the effect of the stromal cells and not to contaminating *W/W<sup>v</sup>* hematopoietic stem cells, a major concern in experiments involving wild-type animals. Cultured stromal cells were infused into unconditioned non-splenectomized *Sl/Sl<sup>d</sup>* mice. Engraftment of donor stromal cells reached levels of up to 1.0% of total marrow cells 4 months post transplant. However, stromal engraftment was not detectable in the spleen. Recipients of *W/W<sup>v</sup>* stroma showed a significant increase in the committed erythroid progenitors compared with those receiving *Sl/Sl<sup>d</sup>* stromal cells:  $109 \pm 26$  vs  $68 \pm 5$  CFU-E per  $10^5$  BMC,  $P = 0.002$ ;  $25 \pm 10$  vs  $15 \pm 5$  BFU-E per  $10^5$  BMC,  $P = 0.037$ , for *W/W<sup>v</sup>* and *Sl/Sl<sup>d</sup>* stroma recipients, respectively. Despite this increase in erythroid progenitors, the anemia was not corrected. Our data suggest that in this murine model, splenic erythropoiesis may influence stromal cell therapy, and that higher levels of marrow engraftment may be necessary to obtain a clinically significant effect.

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The hematopoietic microenvironment plays an important role in the support and regulation of hematopoiesis. Stromal cells, one component of this environment, are required for support of long-term hematopoiesis *in vitro*.<sup>1</sup> These cells are an important source of cytokines, adhesion molecules and extracellular matrix components.<sup>2–4</sup>

The importance of a normal hematopoietic microenvironment is illustrated by the *Sl/Sl<sup>d</sup>* mouse. These mice offer a model of abnormal hematopoiesis due to defective production of the cytokine stem cell factor (SCF) by marrow stromal cells.<sup>5,6</sup> In addition to macrocytic anemia, *Sl/Sl<sup>d</sup>* mice experience a deficiency of tissue mast cells, as well as fertility and pigmentation defects. These defects have been attributed to abnormal microenvironments for these cell lineages.<sup>7</sup> The anemia of *Sl/Sl<sup>d</sup>* mice is not corrected by transplantation of hematopoietic cells,<sup>8</sup> unlike the complementary mutant *W/W<sup>v</sup>* mouse, which responds to marrow transplantation.<sup>9,10</sup> The *W/W<sup>v</sup>* mouse has a similar disease phenotype, but in contrast is attributed to a defect in the receptor for SCF, *c-kit*.<sup>7</sup> The anemia in *Sl/Sl<sup>d</sup>* mice responds to transplantation of splenic tissue, which provides an intact normal hematopoietic microenvironment. *In vitro* studies by Dexter and Moore<sup>11</sup> showed that *Sl/Sl<sup>d</sup>* adherent cells support *W/W<sup>v</sup>* hematopoiesis poorly, yet a *W/W<sup>v</sup>* adherent layer supports co-cultured *Sl/Sl<sup>d</sup>* marrow cells, resulting in sustained hematopoietic cells production. Molecular studies reveal that *Sl/Sl<sup>d</sup>* mice have mutations in the gene for SCF, including an intragenic deletion removing the transmembrane and cytoplasmic domains.<sup>12</sup> Stromal cells from these mice are incapable of producing the membrane-bound isoform of SCF.<sup>5,6</sup> It is feasible that transplantation of stromal cells with normal SCF expression into *Sl/Sl<sup>d</sup>* mice may provide an attractive and convincing model for treating an abnormal hematopoietic microenvironment.

Stromal cell transplantation has been achieved using non-hematopoietic mesenchymal cells derived from long-term bone marrow cultures,<sup>13</sup> as well as stromal cell lines.<sup>14</sup> Anklesaria *et al*<sup>15</sup> showed that infusion of a transformed stromal cell line into irradiated and splenectomized *Sl/Sl<sup>d</sup>* mice could partially correct their anemia. The aim of our study is to extend this observation by transplanting normal stromal cells, derived from long-term bone marrow cultures, into unconditioned *Sl/Sl<sup>d</sup>* recipients as a model for human cell therapy.

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## Materials and methods

### *Stromal cell transplantation*

Stromal cells were harvested from the adherent layer of long-term bone marrow cultures initiated from femoral marrow of male WBB6F1-W/W<sup>v</sup> (W/W<sup>v</sup>) and WCB6F1-SI/SI<sup>d</sup> (SI/SI<sup>d</sup>) (Jackson Laboratory, Bar Harbor, ME, USA). The cells were maintained in  $\alpha$ -medium (Gibco BRL, Burlington, ON, Canada) with 10% horse serum, 10% fetal bovine serum (HyClone Laboratories, Logan, UT, USA), 10<sup>-6</sup> M hydrocortisone (Sigma, St Louis, MO, USA), L-glutamine, and antibiotics (Gibco BRL) and incubated at 37°C in 5% CO<sub>2</sub>. Stromal cells were used for transplantation after five or six serial passages *in vitro*. A sex-mismatch transplant model was employed, in which male donor stromal cells were infused into unconditioned female SI/SI<sup>d</sup> recipient mice according to a local IRB-approved protocol. The cells were injected intravenously via the lateral tail vein, and recipients were monitored for changes in hematological parameters over 4 months until death post transplant.

### *Stromal cell phenotype*

Expression of Mac-1 and CD45 on stromal cells was assayed by flow cytometry using a FACScan analyzer and CELLQuest software (Becton Dickinson, Mississauga, ON, Canada). Stromal cells were washed with phosphate buffered saline (PBS) and incubated with either FITC-conjugated rat anti-mouse Mac-1 antibody (Serotec, Oxford, UK) or FITC-conjugated rat anti-mouse CD45 antibody (PharMingen, San Diego, CA, USA) at room temperature. Appropriate isotype antibodies were used as controls. Analyses were done in triplicate on 10 000–20 000 cells. To assess collagen IV expression, stromal cells were grown on Lab-Tek chamber slides and fixed with paraformaldehyde prior to staining (Nalge Nunc, Naperville, IL, USA). Cells were incubated with rabbit anti-mouse collagen IV antibody (Biodesign, Kennebunk, ME, USA) followed by FITC-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories) at room temperature. To assess both positive and negative staining cells, a DAPI counterstain was used (4',6-diamidino-2'-phenylindole dihydrochloride) (Oncor, Gaithersburg, MD, USA). Collagen IV-positive stromal cells were viewed using a fluorescence microscope. Analyses were done in duplicate on 200–700 cells.

### *Engraftment of male stromal cells into female recipients*

Fluorescence *in situ* hybridization (FISH) was used to identify the frequency of male cells in the marrow of female recipient mice. Bone marrow suspensions were fixed in acetic acid:methanol and cells dropped on to microscope slides. The slides were treated with 100  $\mu$ g/ml RNase, washed and dehydrated before denaturation at 75°C, in 50% formamide in 2  $\times$  SSC. A biotinylated Y chromosome paint (Cambio, Cambridge, UK) was mixed with Hybrisol VI (Oncor) and denatured at 75°C, prior to hybridization with the slides overnight at 37°C. The hybridized probe was detected by incubation with FITC-labeled avidin, fol-

lowed by an amplification step involving incubation with anti-avidin antibody and another round of FITC-avidin (Oncor). The slides were counterstained with DAPI and propidium iodide (PI). To determine the percentage of donor cells, 1000 to 1500 nuclei per slide were counted using a fluorescence microscope.

Presence of donor cells in spleen samples was determined using a duplex PCR method. This allowed identification of a 292 bp portion of the testis determining region on the Y chromosome (*Tdy*),<sup>16</sup> along with a 147 bp portion of SCF exon 5, a region unaffected by the *Sl<sup>d</sup>* mutation, as an internal positive control.<sup>12,17</sup> The oligonucleotide primers used had the following sequences: TDY1 5'-GACTG GTGACAATTGTCTAG-3', TDY2 5'-TAAAATGCCACT CCTCTGTG-3', SCF1 5'-AAAGAATCTCCGAAGAG GCC-3', SCF2 5'-CTCGGGACCTAATGTTGAAG-3'. PCR involved 30 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. The reaction mixture consisted of 500 ng sample DNA, 4 ng/ $\mu$ l of each TDY primer, 2 ng/ $\mu$ l of each SCF primer, 1.25 units *Taq* polymerase (ID Labs Biotechnology, London, ON, Canada), 0.2 mM nucleotides, 1.5 mM MgCl<sub>2</sub>, 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67 mM Tris-HCl and 0.01% Tween 20. With this methodology, at least 0.5% male DNA can be detected in a mixture of female genomic DNA spiked with male DNA.

### *Hematological parameters*

Erythrocyte indices were monitored on a monthly basis from peripheral blood samples obtained from the retro-orbital sinus while mice were anesthetized by inhalation of isoflurane (Schein Pharmaceutical, Etobicoke, ON, Canada). Littermates of SI/SI<sup>d</sup> mice, which did not exhibit the mutant phenotype, were used as controls for hematological parameters. Samples were processed by a CELL-DYN 3000 automated blood cell analyzer to determine erythrocyte (RBC) number, mean cell volume (MCV), hemoglobin (HGB), and leukocyte count (WBC). The mean erythrocyte size distribution was also assessed for each treatment group, and statistical analysis performed using a Wilcoxon rank sum nonparametric test.

Marrow hematopoietic progenitor levels were determined by methylcellulose colony assays.<sup>18</sup> Briefly, 1  $\times$  10<sup>5</sup> femoral bone marrow cells were cultured in  $\alpha$ -medium containing 1% methylcellulose (StemCell Technologies, Vancouver, BC), 30% fetal bovine serum, 1% BSA (Boehringer Mannheim, Laval, QC, Canada), 10<sup>-4</sup> 2-mercaptoethanol (Sigma), 14% pokeweed mitogen-spleen conditioned medium (Gibco BRL), 2 U/ml recombinant human erythropoietin (Ortho Pharmaceutical, Raritan, NJ, USA), L-glutamine and antibiotics and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub>. Colony numbers (BFU-E, CFU-E, CFU-GM) were counted between days 8 and 11 of culture. Statistical comparisons were done using a Student's *t*-test.

## Results

Serial passage *in vitro* was performed to increase the number of stromal cells, as well as deplete contaminating hematopoietic cells. The phenotype of stromal cells in the adher-

**Table 1** Phenotype of stromal cells derived from adherent layers of long-term marrow cultures

Stroma	Collagen IV	Mac-1	CD45
<i>Sl/Sl<sup>d</sup></i>	100%	0.1 ± 0.1%	0.9 ± 1.4%
<i>W/W<sup>v</sup></i>	100%	0.2 ± 0.1%	0.9 ± 0.8%

Data are means of two analyses for collagen type IV (200–700 cells) and mean ± s.d. for Mac-1 and CD45 (*n* = 3, 10 000–20 000 cells).

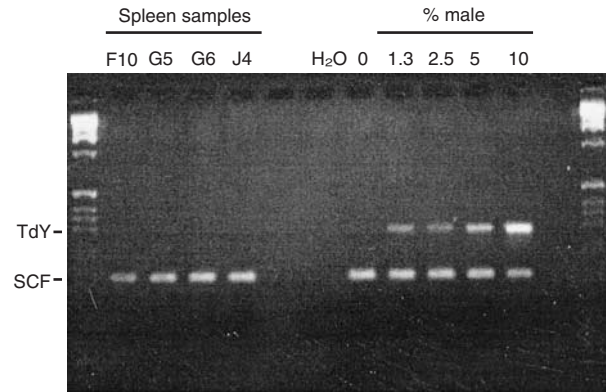
ent layer was assessed after five or six passages. Marrow stromal cells were predominantly non-hematopoietic, with a small percentage of cells expressing hematopoietic markers (0.9% CD45<sup>+</sup>, less than 0.2% Mac-1<sup>+</sup>) (Table 1). Furthermore, as previously reported for stromal cells,<sup>5</sup> virtually all of the cells produced collagen IV. This level of enrichment was necessary for transplantation experiments.

A sex-mismatch transplant model was employed to allow identification of engrafted male donor stromal cells in unconditioned female recipients. The frequency of donor stromal cells in recipient marrow 4 months after infusion was determined by FISH using a Y-chromosome paint. Recipients of a dose of under 2 million cells per mouse showed negligible levels of marrow engraftment of either *W/W<sup>v</sup>* or *Sl/Sl<sup>d</sup>* stroma (Table 2). However, recipients of higher doses of cells (approximately 2.5 million per mouse) did have appreciable engraftment in the marrow. Donor cells were found in similar ranges of frequencies in both stromal transplant groups, with levels up to 1% of the total marrow nucleated cell population. FISH analysis was specific because female marrow cells gave a false positive rate of only 0.1%. The mean engraftment level of *W/W<sup>v</sup>* stroma was 0.4 ± 0.3% and 0.6 ± 0.4% for *Sl/Sl<sup>d</sup>* stroma, with probabilities of 0.015 and 0.0005, respectively, based on a Poisson distribution of FISH-positive events in control female marrow cells. A control group receiving 6 million *W/W<sup>v</sup>* whole marrow cells had low levels of hematopoietic cell engraftment (0.4 ± 0.3%), suggesting that potential hematopoietic contamination in the stromal grafts would not have any advantage over donor stromal cells. Engraftment of donor stromal cells in recipient spleens was not evident by PCR for the *Tdy* gene sequence (Figure 1). Attempts at infusing 4 million or more stromal cells in a single dose resulted in a high incidence of death in *Sl/Sl<sup>d</sup>* recipients, despite having successfully infused up to 6 million stromal cells into Balb/c mice.<sup>19</sup>

**Table 2** Engraftment of male stromal cells into the bone marrow of female recipient *Sl/Sl<sup>d</sup>* mice 4 months post transplant as assayed by FISH using a Y-chromosome paint

Stromal cell dose	Type of stromal graft	<i>n</i>	Percentage FISH-positive cells <sup>a</sup>
≤2 million	<i>W/W<sup>v</sup></i> stroma	9	0.1 ± 0.1% (0–0.3%)
	<i>Sl/Sl<sup>d</sup></i> stroma	4	0.1 ± 0.1% (0–0.2%)
2.5 million	<i>W/W<sup>v</sup></i> stroma	9	0.4 ± 0.3% (0.1–1.0%)
	<i>Sl/Sl<sup>d</sup></i> stroma	3	0.6 ± 0.4% (0.1–0.9%)
	<i>W/W<sup>v</sup></i> BMC	3	0.4 ± 0.3% (0.1–0.7%)

<sup>a</sup>Mean ± s.d. with the range in parentheses.



**Figure 1** Representative PCR analysis of spleen for engraftment of male donor cells. Lanes: spleen samples from transplant mice F10, G5, G6 and J4, respectively; H<sub>2</sub>O PCR control; mixtures of female genomic DNA spiked with male DNA ranging from 0 to 10% male. The PCR products are a 292 bp region of *TdY* gene on the Y-chromosome and a 147 bp region of the *SCF* gene as an internal control. A 1-kb marker was run in the first and last lane.

During the course of the study, erythropoiesis was monitored after stromal cell transplantation including the assessment of erythroid progenitor levels in the bone marrow at death. There was a significant difference in marrow erythroid progenitors in mice receiving  $2.5 \times 10^6$  *W/W<sup>v</sup>* stromal cells (Table 3). Both CFU-E and BFU-E were elevated in recipients of *W/W<sup>v</sup>* stroma compared with those receiving  $2.5 \times 10^6$  *Sl/Sl<sup>d</sup>* stroma (CFU-E:  $109 \pm 26$  vs  $68 \pm 5$  per  $10^5$  BMC, *P* = 0.002; BFU-E:  $25 \pm 10$  vs  $15 \pm 5$  per  $10^5$  BMC, *P* = 0.037, for *W/W<sup>v</sup>* and *Sl/Sl<sup>d</sup>* stromal recipients, respectively). Levels of CFU-GM were not significantly different between these two groups (*P* = 0.087). Contamination of the stromal grafts by hematopoietic cells is not a likely source of the increased number of erythroid progenitors. Because hematopoiesis was assessed in recipient mice 4 months after transplant, only contamination by stem cells could account for this increase. Hematopoietic contamination of the stromal graft is at a low level (Table 1), and thus not likely to contain a significant number of stem cells, which would have an even lower frequency. We have shown that  $3 \times 10^6$  culture-derived stromal cells had no detectable CFU-S.<sup>20</sup> In addition, whole marrow from *W/W<sup>v</sup>* donors did not engraft well in unconditioned *Sl/Sl<sup>d</sup>* mice (Table 2). Furthermore, transplantation of a much higher number of *W/W<sup>v</sup>* hematopoietic cells was incapable of correcting the *Sl/Sl<sup>d</sup>* anemia.<sup>8</sup> Thus, any contaminating *W/W<sup>v</sup>* stem cells in the stromal cell graft

**Table 3** Hematopoietic colony number assayed 4 months post transplant of high dose of stromal cells (colonies per  $10^5$  BMC)

Type of stromal graft	<i>n</i>	CFU-E	BFU-E	CFU-GM
<i>W/W<sup>v</sup></i> stroma	10	$109 \pm 26^a$	$25 \pm 10^a$	$109 \pm 40$
<i>Sl/Sl<sup>d</sup></i> stroma	5	$68 \pm 5$	$15 \pm 5$	$82 \pm 18$

<sup>a</sup>Statistically different by *t*-test (*P* ≤ 0.05), compared to the *Sl/Sl<sup>d</sup>* stroma group.

can be discounted as a source of the increase in erythroid progenitors.

The mice were monitored to determine whether there was any effect on mature erythrocytes. No change in red cell indices (RBC number, MCV, hemoglobin) was observed during the course of the experiment, nor were WBC levels affected, for all stromal cell dose groups (Table 4). A correction in the macrocytic anemia would require an increase in erythrocyte number and a reduction in the mean cell volume, however both indices remained unchanged. Transplantation of  $W/W^v$  whole marrow also had no effect on erythrocyte parameters, consistent with previous studies.<sup>8</sup> Analysis of the mean RBC size distribution was performed for recipients receiving the higher dose of stromal cells to determine if more subtle changes in erythropoiesis were occurring that were not reflected in the MCV (Figure 2). Although a slight shift towards smaller erythrocytes in recipients of  $W/W^v$  stroma was noted, the overall distribution was not significantly different from the erythrocytes of  $Sl/Sl^d$  stromal cell recipients ( $P = 0.195$ ).

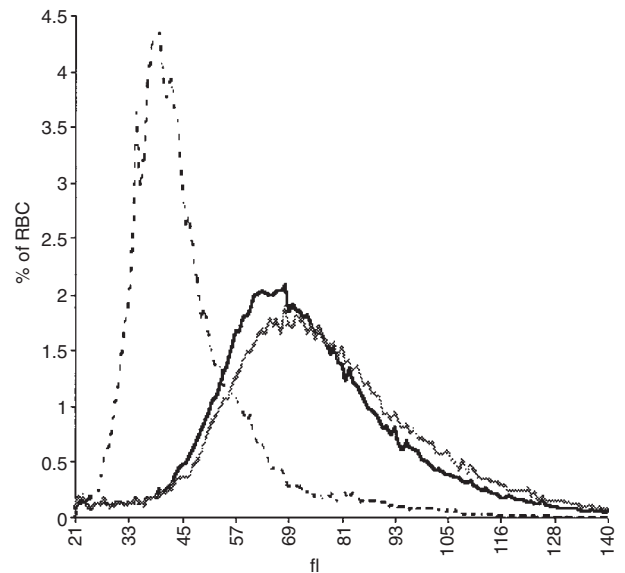
## Discussion

The ability of stromal cells to engraft in unconditioned recipients has important implications for the clinical applications that do not require myeloablation. The present study demonstrates stromal engraftment in the marrow of unconditioned  $Sl/Sl^d$  mice. Although engraftment did not exceed 1% of the total nucleated cell population, it should be noted that endogenous stromal cells constitute only a minor population and thus, the donor cells may represent a doubling of the original number.<sup>21,22</sup> Donor cell engraftment must be due to non-hematopoietic stromal cells because hematopoietic cell contamination was negligible. Although donor

**Table 4** Erythrocyte parameters in peripheral blood of recipients of high doses of stromal cells (approximately 2.5 million cells)

Type of stromal graft	n	Prior to transplant	Three months post transplant
(1) RBC ( $\times 10^6/\mu\text{l}$ )			
$W/W^v$ stroma	10	$3.71 \pm 1.09$	$4.06 \pm 0.77$
$W/W^v$ BMC	5	$4.40 \pm 0.70$	$4.16 \pm 0.85$
$Sl/Sl^d$ stroma	5	$3.50 \pm 1.47$	$3.55 \pm 1.28$
(2) MCV (fl)			
$W/W^v$ stroma	10	$74.2 \pm 10.8$	$74.2 \pm 7.9$
$W/W^v$ BMC	5	$71.8 \pm 9.4$	$72.7 \pm 8.0$
$Sl/Sl^d$ stroma	5	$70.3 \pm 6.8$	$79.0 \pm 11.3$
(3) Hemoglobin ( $\mu\text{g}/\mu\text{l}$ )			
$W/W^v$ stroma	10	$82.9 \pm 18.7$	$91.1 \pm 12.3$
$W/W^v$ BMC	5	$98.5 \pm 9.8$	$90.9 \pm 13.4$
$Sl/Sl^d$ stroma	5	$76.0 \pm 29.7$	$81.9 \pm 23.6$
(4) WBC ( $\times 10^3/\mu\text{l}$ )			
$W/W^v$ stroma	10	$7.60 \pm 2.46$	$7.66 \pm 2.55$
$W/W^v$ BMC	5	$12.16 \pm 4.36$	$7.39 \pm 2.85$
$Sl/Sl^d$ stroma	5	$5.17 \pm 1.17$	$5.28 \pm 3.23$

Indices measured during the course of the transplants were: (1) Erythrocyte number (RBC), (2) mean erythrocyte volume (MCV), (3) hemoglobin and (4) total white blood cell counts (WBC). Mice were the recipients of  $W/W^v$  stromal cells,  $W/W^v$  BMC or  $Sl/Sl^d$  stromal cells



**Figure 2** Mean erythrocyte size distribution for stromal treatment groups at 3 months post transplant. Plots show RBC distribution of  $W/W^v$  stromal recipients (solid line),  $Sl/Sl^d$  stromal recipients (grey line) and control Steel littermate mice (dashed line).

analysis by FISH was limited to detection of male cells in the whole marrow, the levels found were similar to those of previous studies from our laboratory.<sup>19</sup> We have also shown that donor cells present in whole marrow preparations are detected by FISH at higher levels among the cultured stromal cells derived from the same transplant recipients.<sup>19</sup> Although the present study did not analyze CFU-F chimerism, Anklesaria *et al*<sup>15</sup> showed that over half of the CFU-F were donor-derived after transplantation of a stromal cell line.

While hematopoietic cell transplantation is unable to correct the macrocytic anemia of  $Sl/Sl^d$  mice,<sup>8</sup> the transplantation of a normal hematopoietic microenvironment in the form of an intact spleen can treat these mice effectively.<sup>23</sup> Thus, it could be inferred that infusion of stromal cells with normal SCF expression into  $Sl/Sl^d$  mice may alleviate the anemia, in contrast to  $Sl/Sl^d$  stromal cells which support *in vitro* hematopoiesis poorly.<sup>11</sup> Anklesaria *et al*<sup>15</sup> showed that infusion of a transformed stromal cell line into irradiated and splenectomized  $Sl/Sl^d$  mice could yield a small improvement in erythropoiesis. The aim of our study was to investigate the role of non-transformed marrow stromal cells in correcting the disorder in unconditioned recipients. The use of  $W/W^v$  stromal cells has the advantage that any contaminating hematopoietic cells are defective and would not correct the  $Sl/Sl^d$  anemia. Although transplantation of  $W/W^v$  stromal cells resulted in a significant increase in erythroid progenitors (CFU-E, BFU-E) over the levels found in recipients of  $Sl/Sl^d$  stroma, no improvement in the anemia was observed. This increase in erythroid progenitors is noteworthy given that fetal  $Sl/Sl^d$  mice have reduced liver CFU-E compared with normal littermates.<sup>24</sup> The authors speculate that the defect in these mice may arise at the BFU-E/CFU-E transition. Other studies have also shown the importance of SCF in regulating early erythroid progenitors. The receptor for SCF has been identified on

erythroid progenitors,<sup>25</sup> and marrow progenitors expressing this receptor differentiate into BFU-E in response to SCF.<sup>26</sup> Also, injection of recombinant SCF into baboons increases the number of primitive hematopoietic progenitors, including BFU-E.<sup>27,28</sup> Thus, the increase in erythroid progenitors following transplantation of *W/W<sup>v</sup>* stromal cells into *Sl/Sl<sup>d</sup>* mice is consistent with the effect of SCF on this cell population.

The limited biological effect of stromal cell transplantation in our model may be due to preponderance of erythropoiesis in the spleen and contrasts with the marrow-based erythropoiesis in humans. Transplantation of marrow cells to the spleen results in predominantly erythroid differentiation in the mouse,<sup>29</sup> and in response to phenylhydrazine-induced anemia, most of the erythroid expansion occurs in the spleen and is dependent on SCF/*c-kit* interaction.<sup>30</sup> Most importantly, the spleen has been shown to be a target for correction of the *Sl/Sl<sup>d</sup>* anemia, as demonstrated by the improvement in erythropoiesis following transplantation of a normal spleen.<sup>23</sup> In the study by Anklesaria *et al*,<sup>15</sup> the recipient mice were irradiated and splenectomized prior to stromal cell infusion. Irradiation may have perturbed endogenous marrow hematopoiesis sufficiently to allow transplanted stromal cells to have an effect, while splenectomy effectively removed a competing site of erythropoiesis. Our study used unconditioned host mice with intact splenic erythropoiesis. We failed to detect splenic engraftment of donor stromal cells, although there was marrow engraftment. It may be that the change in the marrow erythroid progenitor level we observed was insufficient to overcome ongoing abnormal erythropoiesis in the spleen. However, this limitation in the murine model is not relevant to the clinical application of stromal cell therapy because the spleen is not normally a site of human hematopoiesis.

Although we observed an increase in marrow erythroid progenitors, the inability to correct the anemia suggests that higher levels of stromal engraftment may be required. A major improvement in erythropoiesis may require the presence of a significant amount of normal hematopoietic microenvironment. Kapur *et al*<sup>31</sup> has shown that breeding a transgenic mouse expressing a membrane-restricted SCF cDNA, on to a *Sl/Sl<sup>d</sup>* background resulted in improved erythrocyte production compared with unmanipulated *Sl/Sl<sup>d</sup>* mice. In addition, the injection of recombinant SCF at pharmacological doses also corrected the anemia, albeit transiently.<sup>32</sup> Anklesaria *et al*<sup>15</sup> found that *Sl/Sl<sup>d</sup>* stromal cells suppress hematopoiesis *in vitro* and extrapolated that up to 80% of the *Sl/Sl<sup>d</sup>* marrow would need to be replaced for a biological effect to be observed. However because *Sl/Sl<sup>d</sup>* mice are particularly small it was difficult to infuse very high numbers of stromal cells in single or even multiple doses.

Our data, and the studies of others, indicate that stromal cell infusion can influence host hematopoiesis<sup>14,15,33,34</sup> and suggest that this approach has possible application in cell and/or gene therapy.<sup>35,36</sup> The use of stromal cells has the added dimension of extensive transdifferentiation potential to osteoblasts, chondroblasts,<sup>37,38</sup> fibroblasts,<sup>39</sup> muscle,<sup>40</sup> astrocytes,<sup>41</sup> and neurons.<sup>42</sup> Also, stromal cells could be used as a vehicle for gene therapy to introduce defective or missing genes.<sup>43</sup> Based on our murine model, our studies

indicate that for stromal cell transplantation to become a feasible form of cell therapy further studies are required, particularly novel approaches that enhance stromal marrow engraftment in unconditioned recipients.

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