Maintenance of High Levels of Pluripotent Hematopoietic Stem Cells In Vitro: Effect of Stromal Cells and c-kit

By John P. Wineman, Shin-Ichi Nishikawa, and Christa E. Müller-Sieburg

We show here that mouse pluripotent hematopoietic stem cells can be maintained in vitro on stroma for at least 3 weeks at levels close to those found in bone marrow. The extent of stem cell maintenance is affected by the nature of the stromal cells. The stromal cell line S17 supported stem cells significantly better than heterogeneous, primary stromal layers or the stromal cell line Strofl-1. Furthermore, we demonstrate that, while pluripotent stem cells express c-kit, this receptor appears to play only a minor role in stem cell maintenance in vitro. The addition of an antibody that blocks the interaction of c-kit with its ligand essentially abrogated myelopoiesis in cultures. However, the level of stem cells in antibody-treated cultures was similar to that found in untreated cultures. Thus, it seems likely that the maintenance of primitive stem cells in vitro depends on yet unidentified stromal cell-derived factor(s).

A CENTRAL ISSUE in hematopoiesis research has been to find means to maintain pluripotent hematopoietic stem cells in vitro. This would permit analysis of factors that influence stem cell maintenance and could be of importance for therapeutic stem cell transplantation. It is well established that the long-term bone marrow (BM) culture system described by Dexter et al1 can support mouse and human stem cells for several weeks. Indeed, cultured marrow cells have been used as an autologous graft in leukemia patients. In Dexter cultures, a BM-derived stromal layer supports hematopoiesis in the presence of hydrocortisone and high concentrations of horse serum. However, when mouse cells maintained in these conditions were tested for stem cell activity in vivo, it became apparent that the cultured cells had significantly less repopulating capacity than freshly explanted BM stem cells. This suggests that many stem cells are either lost or damaged during culture.

Primary stromal layers are a complex mixture of cells that include macrophages, fibroblastic cells, and endothelial cells. The advent of clonal stromal cell lines has greatly simplified this culture system and has aided in the elucidation of stromal cell-derived signals that affect hematopoiesis. One of these stromal cell lines, S17, derived by Collins and Dorshkind, has been described previously to support myelopoiesis as well as primitive stroma in Dexter cultures. Reportedly, S17 produces high levels of c-kit ligand (also named stem cell factor, mast cell growth factor, or steel factor). This cytokine influences the differentiation of early myeloid and lymphoid precursor cells. Furthermore, mice that are genetically deficient in c-kit or its ligand, or mice in which the interaction of c-kit with its ligand is blocked by injection of an antibody, exhibit severely impaired hematopoiesis, suggesting that c-kit ligand may act directly on pluripotent stem cells. This interpretation was supported by the elegant demonstration that stem cells with extensive repopulation capacity express c-kit, the receptor for c-kit ligand.

The experiments reported here were designed to assess the capacity of different stromal cells to maintain primitive stem cells in vitro and to examine the role of c-kit in stem cell maintenance. We show that the stromal cell line S17 is efficient in supporting maintenance of pluripotent stem cells with extensive repopulation capacity. The receptor c-kit appears to play only a minor role in stem cell maintenance on S17. Thus, the cell line S17 may be a valuable source for identifying the factor(s) that affects stem cell maintenance.

MATERIALS AND METHODS

Mice. CBA/c, C57BL/6, and the Ly5 congenic C57BL-Ly5.1 mice were bred at the Medical Biology Institute (La Jolla, CA). C57BL/6 mice were used at age ≥3 months as hosts for stem cell experiments.

Stromal cells. Primary stromal cell layers were generated essentially as described. Briefly, 1.5×10^7 BM cells derived from 3-month-old C57Bl/6 mice were seeded into T25 tissue culture flasks. Cultures were fed weekly with Iscove's medium supplemented with 20% horse serum and 1.5×10^-6 mol/L hydrocortisone. After reaching confluence, stromal layers were treated with mycophenolic acid as described to remove residual hematopoietic cells. Primary stroma layers were recharged 4 to 6 weeks after initiation. The stromal cell line S17 (the generous gift of Dr K. Dorshkind, University of California-Riverside) was maintained in RPMI 1640 supplemented with 5% fetal calf serum (FCS) and 5×10^-3 mol/L 2-mercaptoethanol (2-ME) as described. Confluent layers of S17 were recharged 1 week after initiation.

The stromal cell line Strofl-1 was derived from the adherent layer of a Dexter culture initiated from the liver of a neonatal CBA/c mouse. Strofl-1 supports growth of the B-lymphoid line pBL-3 in Whittlock-Witte conditions and supports growth and differentiation of MyDex, a myeloid precursor clone in Dexter conditions (data not shown). Strofl-D5 is a subclone of Strofl-1, selected as a particular efficient supporter of myeloid and lymphoid cells. Strofl-1 and Strofl-D5 were maintained in Dexter-type medium, and confluent layers were recharged with bone marrow 1 week after initiation. Stromal cell lines CBA-C1 and COW-A1 were selected from the adherent layer of a Dexter culture initiated from neonatal BM.

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Submitted July 13, 1992; accepted September 15, 1992.

Supported by National Institute of Health Grant No. DK-41214.

C.M.-S. is an American Leukemia Society Scholar.

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0006-4971/93/8102-0003$3.00/0


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Dexter cultures. Dexter cultures were initiated and maintained essentially as described. Confluent layers of primary stroma or clonal stromal cell lines in T25 flasks were simultaneously rechallenged with \(1.5 \times 10^6\) freshly explanted BM cells derived from femurs of 4- to 6-week-old C57BL-Ly5.1 mice. To some cultures we added antibody ACK-2 at a final concentration of 10 \(\mu\)g/mL. This antibody concentration was determined to be saturating, whereas isotype-matched antibody M1/70 did not show an effect in vitro (Ogawa et al. and S-L.N., unpublished data). Antibody ACK-2 is specific for the receptor c-kit and antagonizes the interaction of the receptor with its ligand. Cultures were harvested 3 weeks after rechallenging by a short (2- to 3-minute) trypsin treatment. Cell suspensions consisting of both adherent and nonadherent cells were generated by vigorous pipetting.

Stem cell assays. Two assays were used to assess stem cells: the combined radioprotection and long-term repopulation assay and the competitive repopulation assay. Briefly, for the long-term repopulation assay, lethally irradiated (1,283 rad in two doses) C57BL/6 (Ly5.2) mice were injected with cultured cells derived from Ly5 congenic C57BL-Ly5.1 (Ly5.1) mice. For the competitive repopulation assay, lethally irradiated C57BL/6 mice were injected with cultured or fresh cells derived from C57BL-Ly5.1 mice together with freshly explanted C57BL/6 BM cells. In both assays, surviving mice were bled at various times after reconstitution and the extent of donor-antibody M1/70 did not show an effect in vitro (Ogawa et al. and c-kit and antagonizes the interaction of the receptor with its ligand.

blocks the interaction of c-kit with its ligand, completely suppresses myelopoiesis (Table 1). This indicates that proliferation and/or differentiation of myeloid cells in culture is dependent on c-kit ligand.

Maintenance of stem cells and radioprotecting cells on S17. To assess whether S17 was also more efficient than primary stroma in supporting pluripotent stem cells, cells cultured for 3 weeks on S17 or on primary stroma were injected into lethally irradiated mice. Because most primitive cells are closely associated with the stromal layer in Dexter cultures, cultures were treated briefly with trypsin to harvest the entire culture. To correlate stem cell input with stem cell output after culture, we injected equal fractions of cultures rather than equal cell numbers. For example, having seeded 1.5 \(\times 10^6\) cells per flask, injecting one-fifth of a culture corresponds to 3 \(\times 10^5\) BM cells originally seeded. Cultured cells (Ly5.1) and irradiated mice (Ly5.2) express different forms of the Ly5 antigen. This enabled us to follow the repopulation of WBCs derived from cultured cells by immunofluorescence using an antibody specific for the Ly5.1 antigen.

In agreement with results of Harrison et al., cells cultured on primary stroma had little reconstitution capacity. Even when high cell doses (one-fifth of a culture, corresponding to 3 \(\times 10^5\) BM cells originally seeded) were injected, all mice showed less than 25% donor-type cells in the periphery at 9 months postinjection (Fig 1). Furthermore, reconstitution by cells grown on primary stroma decreased in most mice over the 9-month period that we monitored these mice (Fig 1), suggesting that these cells had limited proliferation capacity. In contrast, mice that received one-fifth of a culture grown on S17 showed high levels (>70%) of donor-type cells, which persisted for the 9-month period, indicating that S17 maintains primitive stem cells with extensive long-term repopulation capacity. In all of these animals (including the single mouse that died between 4 and 5 months postreconstitution), myeloid cells, as well as B and T lymphocytes, were reconstituted (data not shown; but compare Table 2), further strengthening the interpretation that repopulation was due to pluripotent stem cells. Similar results were obtained in two independent experiments in which the capacity of either primary stroma or S17 to support repopulating stem cells was tested (data not shown).

Surprisingly, all animals that had received a 10-fold lower dose of cells cultured on S17 (1/50 of a culture) died within the first 4 weeks posttransplantation. In contrast, four of five
mice injected with 1/50 of a culture maintained on primary stroma survived, albeit showing low and declining levels of donor-type cells (Fig 1). There is increasing evidence that pluripotent stem cells may not convey radioprotection, which is reportedly conferred by different cell types. Thus, it seemed possible that S17 did not effectively maintain radioprotecting cells. This interpretation appears to be correct, as stem cells were readily detected when radioprotecting cells in the form of 1 x 10^5 host-type marrow were provided (Fig 2). Again, cells cultured on S17 repopulated marrow-ablated hosts significantly better than cells cultured on primary stroma.

To estimate the extent of stem cell maintenance, the repopulation capacity of cells cultured on S17 was compared with that of freshly explanted marrow in a competitive repopulation assay. We coinjected cells cultured on S17 (1/10 and 1/100 of a culture) with 1.5 x 10^5 freshly explanted host-type BM cells. Both doses of cells cultured on S17 gave raise to significant levels of donor-type cells (Fig 3). The level of reconstitution in this experiment was lower than that in the experiment depicted in Fig 2, probably because a higher dose of competitor cells was used here. Nevertheless, the cultured cells competed efficiently against the higher dose of freshly explanted BM, indicating that S17 maintains stem cells that resemble BM stem cells in their proliferative capacity. This interpretation is strengthened further when the repopulation capacity of cultured cells was compared with that of freshly explanted BM cells (Fig 3). We injected 1.5 x 10^5 and 1.5 x 10^4 fresh BM cells (corresponding to 1/10 and 1/100 of a culture, respectively) and obtained similar levels of repopulation by fresh and cultured cells. Hamson et al have established the repopulation unit (XRU) as a measure of stem cell content in different grafts. We found that cultured and fresh marrow contained comparable numbers of repopulating units, suggesting that they contained similar numbers of stem cells. In an independent experiment, a similar level of stem cell activity was found. We compared one-fifth of a culture with 3 x 10^3 fresh BM cells in the competitive repopulation assay. Again, 80% of stem cell activity found in fresh marrow was maintained on S17 (data not shown). In these mice (as in all others tested), myeloid cells and B and T lymphocytes were reconstituted in similar ratios by both cultured and fresh BM cells (Table 2), suggesting that the cultured stem cells are qualitatively similar to fresh stem cells.

While S17 was very effective in maintaining stem cells, other stromal cell lines tested did not support significant levels of stem cells (Fig 3). The stromal cell line Strofl-1 was derived from the liver of a newborn CBA/c mouse and, like S17, supports myeloid and lymphoid cells albeit at a lower level than S17 (compare Table 1). Cells cultured on Strofl-1 did not compete well against host-type marrow. A subclone of the stromal cell line Strofl-1, named Strofl-D5, and the stromal cell lines CBA-C1 and Cow-A1, the latter two isolated from neonatal marrow, also failed to support significant levels of stem cells (Table 3). Marrow cells cultured on S17 contained at least six times more repopulating units.
Table 2. Lineage Reconstitution at 5 Months Postinjection in Mice Transplanted With Cultured or Freshly Explanted Cells

<table>
<thead>
<tr>
<th>Cell Source (dose injected)</th>
<th>% Donor-Type Cells in Blood</th>
<th>All PBL*</th>
<th>T Cells*</th>
<th>B Cells*</th>
<th>Myeloid Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured on S17 (1/10 of culture)</td>
<td>58 ± 4</td>
<td>14</td>
<td>21</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>20 ± 1</td>
<td>4</td>
<td>15</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54 ± 1</td>
<td>12</td>
<td>40</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 ± 1</td>
<td>7</td>
<td>34</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23 ± 2</td>
<td>4</td>
<td>16</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freshly explanted (1.5 × 10⁶)</td>
<td>48 ± 1</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>18 ± 1</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 ± 1</td>
<td>8</td>
<td>20</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 ± 2</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 ± 4</td>
<td>12</td>
<td>31</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 ± 1</td>
<td>16</td>
<td>15</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data from individual animals from the experiment depicted in Fig 3 are listed here to demonstrate the extent of repopulation in the lymphoid and myeloid lineages.

* Denotes the percentage of all peripheral WBCs that expressed the donor-type Ly5.1 antigen. All blood samples were split into 3 aliquots for lineage staining. Data depicted are the mean ± SD from these three aliquots. All numbers were rounded to the next integer. The level of reconstitution by fresh and cultured cells is not significantly different (P > .7).

† The percentage of peripheral blood cells that expressed both the donor-type Ly5.1 marker and the lineage markers Thy-1 (for T cells), B220 (for B cells), or Mac-1 and Gr-1 (for myeloid cells) is given.

than cells cultured on the other stromal cells lines (Table 3). This suggests that stromal cell lines can differ significantly in their capacity to support stem cells.

Effect of c-kit–specific antibodies on stem cell maintenance. Because the blockage of c-kit by antibody ACK-2 had a dramatic effect on myelopoiesis in Dexter cultures (Table 1), we assessed the role of this receptor in the maintenance of stem cells in vitro. First, we determined whether pluripotent hematopoietic stem cells express c-kit. Freshly explanted BM cells from C57BL/Ly5.1 mice were stained with antibody ACK-2 and positive (17% of BM) and negative populations were separated and tested in the competitive reconstitution assay for stem cell content. The vast majority of stem cell activity was recovered in the c-kit⁺ population. Injection of 4 × 10⁴ c-kit⁺ cells together with 1 × 10⁵ unseparated host-type cells repopulated lethally irradiated mice at high levels for at least 8 months (Fig 4). In contrast, little if any stem cell activity was recovered in the c-kit⁻ population. These data are in agreement with the elegant demonstration by Okada et al¹² that primitive stem cells can be enriched in the c-kit⁺ population.

Next, we tested whether c-kit ligand was important for stem cell maintenance in vitro on S17. For this, we again used antibody ACK-2, which blocks the interaction of c-kit and its ligand without being cytotoxic.¹¹ After 3 weeks of culture without antibody, hematopoietic cells were found both in the supernatant and closely associated with S17 (cobblestone areas). In contrast, in cultures containing antibody ACK-2, hematopoietic nonadherent cells or cobblestone areas were below the level of detection by microscopic inspection of either the unperturbed cultures or of the harvested cells (compare Table 1). However, primitive stem cells were maintained in these cultures at levels close to those found in untreated cultures (Fig 5). Mice that received cells cultured in the presence of ACK-2 were reconstituted in the myeloid and lymphoid lineages (Table 4), suggesting that primitive stem cells survived in these cultures. At 10 months postinjection, the mean of reconstitution in these mice was 62.1 ± 22.7 as compared with 59.4 ± 26.6 for mice that received cells cultured on S17 without antibody, corresponding to 1.6 and 1.5 repopulation units, respectively. Thus, an antibody
that blocks the interaction of c-kit with its ligand did not significantly \((P > .8)\) affect maintenance of stem cells in culture.

**DISCUSSION**

We show here that the extent to which pluripotent stem cells survive in Dexter cultures is a function of the microenvironment. The clonal stromal cell line S17 was significantly more efficient in maintaining hematopoietic stem cells than primary stroma or the stromal cell lines Strofl-1, CBA-C1, or COW-A1. Stem cells cultured on S17 were capable of repopulating T and B lymphocytes and myeloid cells in marrow-ablated hosts for at least 10 months and competed efficiently against freshly explanted BM. Furthermore, the repopulation capacity of marrow cultured on S17 was close to that of freshly explanted marrow. This indicates that S17 maintains stem cells with extensive repopulating capacity, a hallmark of primitive, pluripotent stem cells.

Antibody ACK-2, which blocks the interaction of c-kit with its ligand,\(^1\) severely suppressed hematopoiesis in culture, indicating that c-kit is important for the proliferation and/or differentiation of myeloid cells in culture. Similar results have been obtained when marrow cells were cultured on primary stroma are found preferentially in close association with the stroma.\(^4\) Therefore, it is possible that the stromal cells would protect stem cells from the antibody. However, antibody ACK-2 did suppress cobblestone areas in culture, indicating that this antibody indeed can reach cells that are in close association with stromal cells. This suggests that even though stem cells express c-kit, this receptor is not crucial for the maintenance

### Table 3. Stromal Cell Lines Differ in Stem Cell Maintenance

<table>
<thead>
<tr>
<th>Cells Cultured on Stromal Cell Line*</th>
<th>XRU at 5 mo Postinjection†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S17</td>
<td>1.2</td>
</tr>
<tr>
<td>Strofl-D5</td>
<td>0.2</td>
</tr>
<tr>
<td>CBA-C1</td>
<td>0.1</td>
</tr>
<tr>
<td>COW-A1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Marrow cells \((1.5 \times 10^6)\) were cultured for 3 weeks on confluent layers of the different stromal cell lines.
† Lethally irradiated mice were injected with 1/10 of a culture together with \(1.5 \times 10^6\) host-type marrow cells. The repopulating units (XRU) were calculated from the percent of donor-type cells in blood as detailed in Materials and Methods.

![Graph](image.png)

**Fig 3.** Comparison of repopulation capacity of fresh and cultured marrow. Freshly explanted BM or cells cultured on S17 or on Strofl-1 were injected together with \(1.5 \times 10^4\) fresh C57BL/6 BM cells. Flasks were initiated with \(1.5 \times 10^4\) cells. Therefore, 1/10 and 1/100 of a culture represent \(1.5 \times 10^3\) and \(1.5 \times 10^2\) cells seeded, respectively. Each block of bars represents a single mouse. Each bar within a block reflects a measurement of donor-type cells. M, months postinjection. Means of reconstitution (± SD) were calculated for each group of mice for the percentage of donor type cells (Ly5.1) at 7 months postreconstitution. Repopulation by cells cultured on S17 and fresh cells was not significantly different at both doses of cells injected \((P > .7\) and \(P > .8)\). XRU, the repopulating unit, was calculated as described by Harrison et al.\(^1\) (see also Materials and Methods).
of stem cells in culture. Our experiments did not address the question of whether stem cells proliferate actively in culture (see below). Therefore, we cannot exclude formally that kit ligand plays a role in stem cell proliferation. However, data from other laboratories support the interpretation that kit ligand does not act on stem cells directly. For example, when highly purified stem cells were exposed in short-term culture to recombinant c-kit ligand, no expansion of pluripotent stem cells was seen and stem cell activity was actually lost. Furthermore, c-kit-deficient WW mice, which have severely impaired levels of many hematopoietic precursors, appear to have significant stem cell activity. Taken together, those data and our results suggest that c-kit ligand affects more mature precursor cells rather than stem cells. If this interpretation is correct, it would suggest that S17 produces a potentially novel factor(s) that promotes stem cell maintenance.

Culture on S17 appears to be the most efficient way yet described to maintain pluripotent stem cells at levels close to that found in fresh marrow for at least 3 weeks in vitro. The mechanism by which S17 maintains stem cells is currently not clear. Stem cells did not expand significantly in culture; the total number of cells per flask increased ninefold, whereas stem cell activity remained close to that originally seeded. It is possible that S17 promotes maintenance of stem cells by keeping them in a suspended, nonactive state. Alternatively, stem cell activity could be maintained by a combination of stem cell self-renewal and death or differentiation. The experiments of Fraser et al using retroviral marking indicate that most stem cells cultured on primary stroma die, whereas a few stem cell clones expand significantly. If this mechanism also operates on S17, it will be interesting to determine whether S17 is more effective than other stromal cells in increasing the number of stem cells able of proliferating or in enhancing proliferation of individual stem cell clones. However, until experiments using clonally marked stem cells are performed, the conservative interpretation of our data is that S17 promotes maintenance of stem cells.
Table 4. Cells Cultured on S17 in the Presence of Antibody ACK-2 Have Reconstituted the Lymphoid and Myeloid Lineages at 10 Months Postinjection

<table>
<thead>
<tr>
<th>Cell Source (dose injected)</th>
<th>% Donor-Type Cells in Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All PBL*</td>
</tr>
<tr>
<td>Without ACK-2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td></td>
<td>37 ± 0</td>
</tr>
<tr>
<td></td>
<td>79 ± 1</td>
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<td></td>
<td>93 ± 1</td>
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<td></td>
<td>60 ± 2</td>
</tr>
<tr>
<td></td>
<td>87 ± 1</td>
</tr>
<tr>
<td>With ACK-2</td>
<td>58 ± 2</td>
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<tr>
<td></td>
<td>35 ± 3</td>
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<td></td>
<td>66 ± 2</td>
</tr>
<tr>
<td></td>
<td>90 ± 2</td>
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</table>

* Denotes the percentage of all peripheral WBCs that expressed the donor-type Ly5.1 antigen. All blood samples were split into three aliquots for lineage staining. Data depicted are the mean ± SD from these three aliquots. All numbers were rounded to the next integer.

† The percentage of peripheral blood cells that expressed both the donor-type Ly5.1 marker and the lineage markers Thy-1 (for T cells), B220 (for B cells), or Mac-1 and Gr-1 (for myeloid cells) is given.

Data from individual animals from the experiment depicted in Fig 5 are listed here to show the extent of repopulation in the lymphoid and myeloid lineages at 10 months postreconstitution. The level of repopulation by cells cultured with and without ACK-2 was not significantly different (P > 0.87).

should be noted that we cultured heterogeneous BM cells. Therefore, we cannot exclude currently that the factor(s) affecting stem cell maintenance is not directly produced by S17, but rather by accessory cells that are efficiently maintained by S17. However, even if the effect by S17 is indirect, it is likely that this culture system will allow the identification of potential accessory cells, thereby making amenable to analysis the cells and/or factors that are important for stem cell maintenance. We are currently addressing this issue by culturing highly enriched Sca-1 'F and 3' stem cells on S17.

What renders S17 a more potent supporter of stem cell maintenance than other stromal cells? In this series of experiments, we compared stromal cells derived from different strains of mice. Strain differences can affect the level of hematopoiesis in long-term marrow cultures. However, it has been demonstrated clearly that the hematopoietic cells and not the stromal cells accounted for the observed variation among mouse strains. Furthermore, preliminary data suggest that stromal cell lines derived from a single strain, C57BL/6, do differ in their capacity to support stem cell maintenance (C.M.-S., unpublished data). The observed heterogeneity in stromal cells could be a reflection of their distinct roles in regulating differentiation and self-renewal in vivo. The primary stroma would then contain, among others, a mixture of S17-like and Strof-1-like stromal cells, raising the possibility that the S17-like stromal cells may lack inhibitory factors or may produce distinct factors important for stem cell maintenance.

While S17 is efficient in maintaining stem cells, our experiments suggest that these cultures may contain reduced levels of radioprotecting cells when compared with cells cultured on primary stroma. It is possible that these cells die due to the lack of an appropriate microenvironment in culture. Alternatively, these cells could be depleted by differentiating and initiating the vigorous myelopoiesis seen in these cultures. This could imply that one of the mechanisms of the factor(s) potentially produced by S17 is to protect stem cell activity by suppressing their differentiation. Regardless of the precise mechanism(s), it is clear that identification of the factors that promote stem cell maintenance and perhaps proliferation could impact on therapeutic stem cell transplantation and that the use of clonal stromal cell lines, such as S17, will facilitate the characterization of these factors. Furthermore, the demonstration that a cloned stromal cell line efficiently maintains stem cells facilitates the search for stromal cell lines that may actually support the expansion of undifferentiated primitive stem cells in vitro.

ACKNOWLEDGMENT

We are grateful to Dr. K. Dorshkind for making available the stromal cell line S17 and for many helpful discussions and suggestions. We thank Drs R. Adkins, C. Cowing, D. Katz, and D. Mosier for critically reviewing this manuscript.

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