Stromal Cells in Myeloid and Lymphoid Long-Term Bone Marrow Cultures Can Support Multiple Hemopoietic Lineages and Modulate Their Production of Hemopoietic Growth Factors

By Anne Johnson and Kenneth Dorshkind

Hemopoiesis in long-term bone marrow cultures (LTBMC) is dependent on adherent stromal cells that form an in vitro hemopoietic microenvironment. Myeloid bone marrow cultures (MBMC) are optimal for myelopoiesis, while lymphoid bone marrow cultures (LBMC) only support B lymphopoiesis. The experiments reported here have made a comparative analysis of the two cultures to determine whether the stromal cells that establish in vitro are restricted to the support of myelopoiesis or lymphopoiesis, respectively, and to examine how the different culture conditions affect stromal cell physiology. In order to facilitate this analysis, purified populations of MBMC and LBMC stroma were prepared by treating the LTBMC with the antibiotic mycophenolic acid; this results in the elimination of hemopoietic cells while retaining purified populations of functional stroma. Stromal cell cultures prepared and maintained under MBMC conditions secreted myeloid growth factors that stimulated the growth of granulocyte-macrophage colonies, while no such activity was detected from purified LBMC stromal cultures. However, this was not due to the inability of LBMC stroma to mediate this function. Transfer of LBMC stromal cultures to MBMC conditions resulted in an induction of myeloid growth factor secretion. When seeded under these conditions with stromal cell-depleted populations of hemopoietic cells, obtained by passing marrow through nylon wool columns, the LBMC stromal cells could support long-term myelopoiesis. Conversely, transfer of MBMC stroma to LBMC conditions resulted in a cessation of myeloid growth factor secretion; on seeding these cultures with nylon wool-passed marrow, B lymphopoiesis, but not myelopoiesis, initiated. These findings indicate that the stroma in the different LTBMC are not restricted in their hemopoietic support capacity but are sensitive to culture conditions in a manner that may affect the type of microenvironment formed.

RESULTS

From a number of experimental studies have suggested that the development of various hemopoietic lineages and maturational states in the bone marrow does not occur randomly in the medullary cavity, but is localized in distinct microenvironments.1-3 Considerable attention has focused on a population of supporting elements known as stromal cells in the formation of these niches.4 These are a presumably nonhemopoietic population of cells on which active blood cell formation is dependent (reviewed in reference 5). The precise means by which stromal cells could form a particular microenvironment is unknown. One possibility is that subpopulations of stromal cells exist that specifically support a particular lineage or maturational state. This would necessitate that considerable stromal cell heterogeneity exists. Morphological examination of in situ bone marrow has identified a variety of stromal cell types that include reticular cells, endothelial cells, fibroblasts, and fat cells,6,7 and in vitro analysis of stromal cell clones has provided additional evidence of heterogeneity by describing various subpopulations based on morphology or extracellular matrix secretion.8,9 Whether or not all these populations are functional in vivo is unknown. Alternatively, the number of functional stromal cell populations that exist may be limited, and one stromal cell type may be able to support multiple hemopoietic lineages and maturational states.

Blood, Vol 68, No 6 (December), 1986; pp 1348–1354
the culture conditions used affect stromal cell function, and this appears to contribute to the type of microenvironment that is created.

MATERIALS AND METHODS

Mice. BALB/cAn mice were obtained from Life Sciences, St Petersburg, FL, or bred in the vivarium of the Division of Biomedical Sciences, University of California, Riverside. Severe combined immunodeficient (SCID) mice were bred and maintained under sterile, laminar flow conditions in the divisional vivarium from homozygous breeding stock obtained from the Ontario Cancer Institute, Canada (a gift of Drs RA Phillips and MJ Bosma). The genetics of this mutant and its characterization have been previously described.22 Male or female mice were used at 4 to 6 weeks of age. Briefly, SCID mice have an autosomal recessive defect that results in the absence of lymphocytes in their hemopoietic tissues. However, the microenvironment in these mutants is normal and lymphocytes can be reconstituted without prior irradiation with grafts of normal stem cells.23

Preparation of cell suspensions. Animals were sacrificed by cervical dislocation, and spleens, femurs, and tibiae removed and placed in α-minimal essential medium (α-MEM; Gibco, Grand Island, NY). Bone marrow cells were flushed from the bones with a syringe and needle and single-cell suspensions prepared by gentle pipetting. Cell viability was determined by eosine dye exclusion, and all cell counts were performed in a hemocytometer.

Preparation of mycophenolic acid. Mycophenolic acid (MPA), a gift of the Eli Lilly Co, Indianapolis, was prepared by dissolving 10 mg of the drug in 400 μL of 95% ethanol at room temperature. Once in solution, a 1 mg/mL stock was obtained by adding phosphate-buffered saline to a final volume of 10 mL. The solution was sterile filtered and stored at 4 °C. Fresh stock solutions were prepared every 3 weeks, as loss of activity was noted with older preparations.

Preparation of stromal cell cultures. Stromal cell cultures were generated by establishing long-term bone marrow cultures under myeloid or lymphoid conditions and subsequently treating these with MPA. Cultures were initiated with marrow from either BALB/cAn or SCID mice. The stromal cells in these latter mice are normal, and no differences were observed between experiments using SCID or BALB/c stem cells. MBMC were established by flushing the contents of one femur and tibia into a 25-mm tissue culture flask (#3013, Falcon, Oxnard, CA) containing 8 mL of α-MEM supplemented with 20% horse serum (Gibco) and 10−4 mol/L hydrocortisone sodium succinate (Upjohn, Kalamazoo, MI). The cultures were then gassed with a 5% CO2, 95% air mixture, tightly sealed, and placed in a dry 33 °C incubator. Cultures were fed after 1 week, and by 2 weeks a confluent, adherent layer of cells had been established. MBMC were usually rechallenged at this time by adding fresh bone marrow cells to the cultures. Instead, MBMC were treated with MPA in order to prepare purified stromal cell layers. All culture medium was removed from the flasks and replaced with α-MEM supplemented with 20% horse serum, 10−4 mol/L hydrocortisone, and 5 μg/mL MPA. Three days later all the MPA-containing medium was replaced with fresh medium. After an additional three-day incubation, the cycle was repeated. In most cases, two cycles of drug treatment were sufficient to eliminate hemopoietic cells. It has been shown that no hemopoietic colonies can be detected among the cells harvested from MPA-treated cultures, indicating that the procedure does eliminate hemopoietic cells.24 All cultures were maintained at least 2 weeks after the last drug treatment before use.

LBMC were established by plating 13.5 mL of a single-cell suspension of bone marrow cells at 1×106 cells per milliliter in RPMI-1640 medium supplemented with 5% fetal calf serum (Gibco) and 5 × 10−3 mol/L 2-mercaptoethanol (2-ME; Sigma Chemical Co, St Louis) in 10-cm2 tissue culture dishes (Corning Plastics, Santa Clara, CA). Cultures were fed twice a week as described previously.19 At the end of 2 weeks, a confluent, adherent layer was established and cultures were treated with MPA according to the protocol noted earlier.

In some experiments, adherent cells were harvested from LBMC by removing all the culture medium and replacing it with 2 mL of a 0.15% collagenase solution in phosphate-buffered saline supplemented with 1 μg/mL DNase (Sigma). After incubation for 15 minutes at 37 °C, adherent cells were gently removed by scraping with a rubber policeman. Viability of the harvested cells was always 95% to 100%.

Separation of cells on nylon wool column. Cells from fresh bone marrow were passed through nylon wool columns prepared according to the method of Julia et al.25 in order to deplete stromal cells. A maximum of 5 × 107 fresh bone marrow cells in a 2-mL volume was loaded onto a column packed in a 12-mL syringe and washed into the nylon wool with 1 mL of 37°C medium. The nonadherent cells were eluted from the column by washing with 20 mL of the medium. The eluted cells were then passed through a second column as described earlier. Cells were incubated for 45 minutes on each column. Approximately 7% of the initial cell input remained after passage through the second nylon wool column. To confirm the efficacy of the separation procedure, aliquots of 5 × 105 of the twice-nylon wool-passed cells were cultured alone in the appropriate medium and observed throughout the course of the experiment for growth of stromal or hemopoietic populations.

B lymphocyte colony-forming assay. Colony-forming B cells (CFU-B) were assayed as described by Kincade et al.26 Nine parts of lsoeve’s modified Dulbecco’s medium supplemented with 15% fetal calf serum, 0.4% nonessential amino acids, 0.8% essential amino acids, 0.4% vitamin mixture (all from Gibco), 0.6% NaHCO3, and 5 × 10−5 mol/L 2-ME were mixed with one part 3% agar (Difco, Detroit) dissolved by boiling in double distilled H2O. In addition, the medium contained 0.05% sheep red blood cells (Colorado Serum, Denver) that had been washed four times before use and 10 μg/mL Escherichia coli lipopolysaccharide (LPS, Difco). The medium was mixed with 1 × 106 cells in 0.2 mL and plated in 1-mL volumes in 35-mm plastic Petri dishes (Falcon). After the agar had gelled, cultures were placed in a humidified 5% CO2 and 95% air incubator at 37 °C. After six days of incubation, red cells were lysed by adding 1 mL of 3.0% glacial acetic acid in distilled H2O to allow visualization of B cell colonies.

Antibody labeling of cells and fluorescence microscopy. Surface IgM-bearing cells were identified with an affinity-purified, fluorescein-conjugated goat antimouse IgM (Southern Biotechnology, Birmingham, AL). This reagent was diluted 1:5 in α-MEM supplemented with 5% fetal calf serum and 1 mg/mL sodium azide. Aliquots of 1 to 2×105 cells harvested from cultures or fresh bone marrow were suspended in 100 μL of the diluted antisera and incubated for 45 minutes on ice. Cells were then washed twice at 4°C in α-MEM without serum and resuspended in 0.2 mL of α-MEM. To this was added an equivalent volume of 2% paraformaldehyde in phosphate-buffered saline. Cells were stored at 4°C until cell counts were performed on wet mount preparations using a E. Leitz (Rockleigh, NJ) Laborlux microscope equipped for epifluorescence. At least 500 cells were counted per sample.

Myeloid colony assay. Myeloid progenitor cells capable of forming colonies in semisolid medium were assayed by culturing 5 × 105 cells in 35-mm plastic Petri dishes containing 1 mL of methylcellulose medium.27 The latter contained 0.8% methylcellulose, 30% fetal calf serum, 5 × 10−2 mol/L 2-ME, and 10% medium conditioned by WEHI-3B (D−) cells. This was prepared by culturing WEHI cells at 106 cells per milliliter in 40 mL of α-MEM supple-
mented with 1% fetal calf serum. After a 24-hour incubation, supernatants were collected, centrifuged, and stored at 4°C until used. Colonies were enumerated on day 8.

Collection of conditioned medium. Medium conditioned by MPA-treated stromal cell cultures was harvested and tested for the presence of colony-stimulating factors in some experiments. On day 0 all medium was removed from the cultures and replaced with either fresh α-MEM supplemented with 20% horse serum and 10^-4 mol/L steroids or RPMI-1640 supplemented with 5% fetal calf serum and 5 x 10^-5 mol/L 2-ME. At various time points thereafter, medium from three replicate flasks was collected and pooled. A separate set of cultures was used for collection of medium at different time points. Cultures initiated under MBMC or LBMC conditions were switched to LBMC or MBMC conditions, respectively, in some experiments, and conditioned medium was collected as noted. Cultures were allowed to adapt to the new culture conditions for 2 weeks before collection of medium.

RESULTS

Effects of culture conditions on stromal cells. Investigations on stromal cell lines have demonstrated that these cells are sensitive to the culture conditions under which they are maintained. This suggests that the different culture conditions in MBMC and LBMC might also affect stromal cells, and this in turn could influence hemopoiesis in the respective cultures.

As a simple, reproducible assay of stromal cell function, the ability of stromal cells in the LTBM, to condition medium with growth factors that could stimulate the development of granulocytemacrophage progenitors was used. Because hemopoietic cells in intact cultures could possibly bind to the growth factors and decrease their concentration in the supernatants, the analysis was conducted using purified MBMC and LBMC stromal cell cultures. This was accomplished by treating the cultures with MPA as described in Materials and Methods. The data in Table 1 indicate that there are differences with regard to growth factor secretion by MPA-treated MBMC and LBMC stromal cell cultures. Myeloid colony-stimulating activity was present in supernatants conditioned by MBMC stroma, with the highest activity at one day after feeding of the cultures. Minimal activity was present in supernatants conditioned by MBMC stroma. However, this was not due to an inability of LBMC stromal cells to produce this substance; after transfer to MBMC conditions, stromal cells initiated under LBMC conditions could secrete myeloid growth factors. Conversely, a marked decline in their production by MBMC stroma was observed after transfer to MBMC conditions. Minimal CFU-GM growth was stimulated by horse or fetal calf serum used to grow the cultures (Table 1 footnote). The molecular species of the secreted colony-stimulating factors has not been determined.

The photomicrographs in Fig 1 demonstrate that stromal cell morphology is also affected by the culture conditions. MPA-treated stroma was used to eliminate hemopoietic cells that might obscure the stroma. Fat cells are a prominent feature of the adherent layer in MBMC (Fig 1A), but these disappear almost entirely after transfer to LBMC conditions (Fig 1B). Fat cells are not readily observed in LBMC (Fig 1C); after transfer to MBMC conditions, these appeared and the stroma took on an appearance characteristic of a primary MBMC (Fig 1D).

LBMC stroma can support myelopoiesis after transfer to MBMC conditions. Because LBMC stromal cells were capable of myeloid growth factor production, it was possible that they could support long-term myelopoiesis in vitro. This was tested by treating LBMC with MPA, seeding the cultures with 5 x 10^5 nylon wool-passed marrow cells in MBMC medium, and transferring them to 33°C. The nylon wool passage removes stromal cells from the marrow that can form an adherent stromal cell layer in vitro, thus ensuring that precursors in the reseeded population are dependent for growth on the preestablished LBMC stroma. The addition of nylon wool-passed hematopoietic cells to LBMC stroma was essential in order to supply a source of myeloid precursors, since the latter are not detectable in LBMC. The rationale for using MPA-treated stroma was to eliminate any

Table 1. Culture Conditions Affect Production of Colony-Stimulating Factors by Stromal Cells

<table>
<thead>
<tr>
<th>Day Supernatant Collected</th>
<th>No. of CFU-GM/5 x 10^4 Stimulated by Supernatants From</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBMC</td>
</tr>
<tr>
<td>Experiment 1*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>91 ± 13</td>
</tr>
<tr>
<td>4</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>7</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Experiment 2†</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>59 ± 8</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

Supernatants were collected from primary cultures 2 weeks after the last MPA treatment. Cultures transferred to alternate conditions were allowed to adapt to the different medium for 2 weeks before collection of supernatants. Values are expressed as mean ± SD.

Abbreviation: ND, not determined.

*Frequency of CFU-GM/5 x 10^4 BALB/c bone marrow cells stimulated by WEHI-3 conditioned medium was 63 ± 10. A 10% mixture of culture medium containing 5% fetal calf serum or 20% horse serum only as stimulator instead of WEHI-conditioned medium stimulated the growth of 0.5 ± 0.7 and 1.5 ± 0.7 CFU-GM/5 x 10^4 marrow cells, respectively, in methylcellulose cultures.

†Frequency of CFU-GM/5 x 10^4 BALB/c bone marrow cells stimulated by WEHI-3-conditioned medium was 32 ± 5. A 10% mixture of culture medium containing 5% fetal calf serum or 20% horse serum only as stimulator instead of WEHI-conditioned medium stimulated the growth of 0 and 1.5 ± 0.7 CFU-GM/5 x 10^4 marrow cells, respectively, in methylcellulose cultures.
hematopoietic cells present in primary LBMC that might potentially occupy niches required by precursors in the reseed population. An additional advantage of adding a known number of cells and progenitors to purified stroma was that cell growth could be quantitated at weekly intervals thereafter relative to that starting population. Separate aliquots of $5 \times 10^5$ nylon wool-passed cells were cultured alone in quadruplicate to ensure that no stromal cells present in that population grew in vitro.

Table 2 shows the results pooled from two experiments in which nylon wool bone marrow was seeded onto MPA-treated stroma initiated under LBMC conditions. The data indicate that this stroma can support myelopoiesis under MBMC conditions. By 2 weeks after seeding, a 27-fold increase in the number of nonadherent cells per flask was observed, and at least a 16-fold increase was maintained through the 7 weeks of observation. Progenitors of granulocytes and macrophages (CFU-GM) were also present. While
confirming the observed in replicate MPA-treated the frequency of these was below the level in the initial reseed conditions, half of nonadherent cells were removed each week passed BALB/c bone marrow cells, and transferred to MBMC culture and cells did not survive past 1 week (data not shown). Layers; hemopoiesis did not initiate in these flasks, adherent to establish stromal cell foci or confluent cells alone, failed to features not reseeded but transferred course of population, their absolute number was always greater than the results demonstrate that LBMC stroma can support myelopoiesis and maintain B cell precursors.

**Table 2. Stroma Grown Under LBMC Culture Conditions Can Support Myelopoiesis on Transfer to MBMC Conditions**

<table>
<thead>
<tr>
<th>Wk of Culture</th>
<th>Cells per Flask Mean No. CFU-GM per Flask</th>
<th>CFU-B/</th>
<th>CFU-GM/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CFU-B/5 x 10⁶</td>
<td>CFU-GM/5 x 10⁶</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>29 ± 17</td>
<td>213 ± 15</td>
</tr>
<tr>
<td>1</td>
<td>2.1 ± 0.6</td>
<td>125 ± 71</td>
<td>5,000</td>
</tr>
<tr>
<td>2</td>
<td>13.9 ± 1.6</td>
<td>20 ± 6</td>
<td>5,560</td>
</tr>
<tr>
<td>3</td>
<td>11.7 ± 3.0</td>
<td>19 ± 6</td>
<td>4,446</td>
</tr>
<tr>
<td>4</td>
<td>11.8 ± 3.0</td>
<td>21 ± 13</td>
<td>4,956</td>
</tr>
<tr>
<td>5</td>
<td>11.2 ± 3.6</td>
<td>15 ± 10</td>
<td>3,584</td>
</tr>
<tr>
<td>6</td>
<td>8.5 ± 3.0</td>
<td>16 ± 12</td>
<td>2,720</td>
</tr>
<tr>
<td>7</td>
<td>8.2 ± 2.0</td>
<td>14 ± 12</td>
<td>2,296</td>
</tr>
</tbody>
</table>

Stroma from SCID bone marrow was grown under LBMC culture conditions, treated with MPA, seeded with 5 x 10⁶ 2 x nylon wool-passed BALB/c bone marrow cells, and transferred to MBMC culture conditions. Half of nonadherent cells were removed each week and the assays noted were performed. Data pooled from two separate experiments in which a total of seven cultures were processed individually. Values are expressed as mean ± SD.

The frequency of these was below the level in the initial reseeded population, their absolute number was always greater through the course of the experiment. No hemopoiesis was observed in replicate MPA-treated LBMC stromal cell cultures not reseeded but transferred to MBMC conditions or in MPA-treated LBMC maintained under LBMC conditions, confirming the efficacy of the MPA treatment. Four separate cultures, each initiated with 5 x 10⁵ nylon wool-passed cells alone, failed to establish stromal cell foci or confluent adherent layers; hemopoiesis did not initiate in these flasks, and cells did not survive past 1 week (data not shown).

B lymphocytes are not produced under MBMC conditions, and consistent with this was the fact that no CFU-B were observed in the cultures by 2 weeks postseeding (Table 2). However, a primitive B cell precursor is present in primary MBMC. Whether or not they were present in the reseeded LBMC transferred to MBMC conditions was tested by harvesting cells from the adherent cultures and transplanting them into SCID mice. The data in Table 3 demonstrate that B cells are present in SCID mice that received a graft of the cultured cells. Higher levels of CFU-B and surface IgM-positive cells were present in recipients of cultures seeded 4 weeks previously as compared with 7 weeks earlier; however, because no B cells are present in unreconstituted SCID mice, reconstitution to any level is significant. No evidence of tumor formation was noted in any of the mice. Because they had received adherent layer cells containing both stromal and hemopoietic components, this suggests that both populations are normal. Taken together, these results demonstrate that LBMC stroma can support myelopoiesis and maintain B cell precursors.

**Table 3. Stroma Grown Under LBMC Culture Conditions Can Support Early B Cell Precursors on Transfer to MBMC Conditions**

<table>
<thead>
<tr>
<th>Cells Transplanted Into SCID Mice</th>
<th>Spleen Cell No. x 10⁶</th>
<th>sIgM+ (%)*</th>
<th>CFU-B/10⁶</th>
<th>Absolute No. CFU-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6.3†</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 10⁶ 4 wk post-reseed</td>
<td>32</td>
<td>16.0</td>
<td>205 ± 7</td>
<td>6.6 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>29.8</td>
<td>280 ± 28</td>
<td>1.0 x 10⁵</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁶ 4 wk post-reseed</td>
<td>33</td>
<td>ND</td>
<td>115 ± 35</td>
<td>3.8 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>ND</td>
<td>385 ± 21</td>
<td>8.5 x 10⁴</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁶ 7 wk post-reseed</td>
<td>12</td>
<td>1.2</td>
<td>4 ± 2</td>
<td>4.8 x 10³</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.2</td>
<td>22 ± 8</td>
<td>2.6 x 10³</td>
</tr>
<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁶ 7 wk post-reseed</td>
<td>27</td>
<td>1.4</td>
<td>2 ± 1</td>
<td>5.4 x 10²</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>0</td>
<td>5 x 0.5</td>
<td>9.0 x 10²</td>
</tr>
</tbody>
</table>

Stroma from SCID bone marrow was grown under LBMC conditions, treated with MPA, seeded with 5 x 10⁵ 2 x nylon wool BALB/c bone marrow cells, and transferred to MBMC conditions; cells were harvested for transplantation into SCID mice at times noted. Values are expressed as mean ± SD.

Abbreviation: ND, not determined.

* Based on counts of 500 cells.
† Data were pooled from six unreconstituted SCID mice.
included in all experiments. No hemopoiesis initiated in unseeded, MPA-treated MBMC transferred to LTBMC conditions or maintained under MBMC conditions. Four replicate cultures, each containing 5 x 10^6 nylon wool cells seeded into empty flasks under LTBMC conditions, did not form an adherent layer and cells did not survive past 1 week.

**DISCUSSION**

The availability of different LTBMC that support myelopoiesis or lymphopoiesis in a stromal cell-dependent system offers an in vitro means of investigating the formation of the hemopoietic microenvironment. Using populations of stromal cells depleted from hemopoietic cells grown under MBMC or LTBMC conditions, obtained by treating established cultures with MPA, it has been possible to compare the stromal cell populations present in the two cultures. Results indicate that the different culture conditions affect stromal cell physiology in a manner that may contribute to the pattern of hemopoiesis in the respective cultures.

The analysis of LTBMC demonstrated that the stroma that establishes in those cultures has the capacity to support long-term myelopoiesis after seeding with nylon wool-passed bone marrow cells and transfer to MBMC conditions. The lack of myelopoiesis in primary LTBMC does not reflect a differentiation block of myeloid stem cells that may be present in the cultures; CFU-S are not present, and the transfer of established LTBMC to MBMC conditions results in a cessation of lymphopoiesis and no initiation of myelopoiesis. The lack of myelopoiesis in LTBMC is also not due to toxic conditions, since the culture medium contains the same basic components used to grow myeloid progenitors in vitro. The simplest explanation would be that culture conditions are suboptimal for the survival and differentiation of myeloid cells. The present findings suggest that this is due in part to the failure of the stroma to form the proper microenvironment and produce myeloid growth factors. The ability of MBMC stroma to support myelopoiesis under MBMC conditions may be due in part to the initiation of myeloid growth factor production by the stroma.

The capacity of MBMC stroma to support B lymphopoiesis was consistent with results that demonstrated that B lymphopoiesis could be induced and maintained in MBMC by transferring an established culture to LTBMC conditions. While the significance of that study was the induction of B lymphopoiesis from early hemopoietic cells in vitro, it also demonstrated that MBMC stroma was not lineage restricted in its support capacity. The high concentration of steroids and horse serum used in MBMC might be inhibitory to B lymphopoiesis. A contributing factor to the lack of lymphopoiesis in the cultures may also be that the stromal cells only secrete myeloid growth factors and not those on which lymphopoiesis is dependent.

The present results support other reports that stromal cells are sensitive to external stimuli. Lanotte et al demonstrated that steroids present in the culture medium resulted in an accumulation of lipids in a stromal cell line. Because steroids are present in MBMC medium, the appearance of lipid in LTBMC stromal cells transferred to MBMC conditions may also be a steroid-mediated effect. Cohen et al also noted effects of steroids on stromal cells; lipid-containing cells were present in steroid-containing cultures, while those established in fetal calf serum without steroids had primarily a fibroblastoid appearance. That study also demonstrated that the stromal cell cultures established without steroids could support myeloid progenitors after seeding with purified populations of hemopoietic cells. The exogenous steroids in LTBMC do not appear to affect growth factor secretion. MBMC stromal cells grown in horse serum-supplemented medium without hydrocortisone secreted myeloid growth factors that stimulated comparable numbers of CFU-GM as in steroid-supplemented cultures. Other components of the culture medium, such as glucose, may also be of importance in affecting stromal cell function.

It is unclear whether the stromal cells initiated under LTBMC or MBMC conditions are identical or different populations. It is possible that at least two distinct stromal cell subpopulations, one for myelopoiesis and one for lymphopoiesis, are present in each of the LTBMC. That population necessary for myelopoiesis would be functional under MBMC but not LTBMC conditions. Those central to lymphopoiesis would be functional under LTBMC but not MBMC conditions. Even if such distinct subpopulations exist in the cultures, their activation into functional cells might still be affected by environmental conditions. Alternatively, the stromal cells in the two cultures may be identical and exhibit a plasticity in response to culture conditions. Under MBMC conditions they form myeloid microenvironments, while under LTBMC conditions they only support lymphopoiesis.
It has been suggested that there are gradients of metabolites and cell products that exist within the medullary cavity, thereby producing intraorgan variability in environmental conditions. If this is the case, then the capacity of stromal cells to modulate their function according to differences in their external milieu could have relevance to the type of microenvironment they form in a particular area. For example, it has been suggested that stem cells are localized to subendosteal regions. In this case the localized conditions in those areas might be optimal not only for stem cells themselves, but also to the function of stromal cell populations that form stem cell niches.

REFERENCES


29. Dorskind K: Unpublished observations, November, 1985


Stromal cells in myeloid and lymphoid long-term bone marrow cultures can support multiple hemopoietic lineages and modulate their production of hemopoietic growth factors

A Johnson and K Dorshkind

Updated information and services can be found at:
http://www.bloodjournal.org/content/68/6/1348.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml