Identification of Stromal Cell Precursors in Human Bone Marrow by a Novel Monoclonal Antibody, STRO-1

By Paul J. Simmons and Beverly Torok-Storb

Murine IgM monoclonal antibody STRO-1 identifies a cell surface antigen expressed by stromal elements in human bone marrow (BM). STRO-1 binds to approximately 10% of BM mononuclear cells, greater than 95% of which are nucleated erythroid precursors, but does not react with committed progenitor cells (colony-forming unit granulocyte-macrophage [CFU-GM], erythroid bursts [BFU-E], and mixed colonies [CFU-Mix]). Fibroblast colony-forming cells (CFU-F) are present exclusively in the STRO-1+ population. Dual-color cell sorting using STRO-1 in combination with antibody to glycophorin A yields a population approximately 100-fold enriched in CFU-F in the STRO-1+/glycophorin A- population. When plated under long-term BM culture (LTBMC) conditions, STRO-1+ cells generate adherent cell layers containing multiple stromal cell types, including adipocytes, smooth muscle cells, and fibroblastic elements. STRO-1+ cells isolated from LTBMC at later times retain the capacity to generate adherent layers with a cellular composition identical to that of the parent cultures. The STRO-1-selected adherent layers are able to support the generation of clonogenic cells and mature hematopoietic cells from a population of CD34+ cells highly enriched in so-called long-term culture-initiating cells. We conclude that antibody STRO-1 binds to BM stromal elements with the capacity to transfer the hematopoietic microenvironment in vitro.

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THE SUSTAINED PRODUCTION of hematopoietic cells in vitro is critically dependent on cellular interactions between primitive hematopoietic progenitors and the stromal tissue of the bone marrow (BM). Within the marrow, various mesenchymal stromal elements establish a complex reticulum consisting of cells, assorted extracellular matrix glycoproteins, and growth factors produced by the stroma, collectively termed the hematopoietic microenvironment (HM). In vitro studies of the HM, using long-term BM cultures (LTBMC), have identified several cell types as components of the HM, including fibroblasts, smooth muscle cells, adipocytes, endothelial cells, osteogenic cells, and macrophages.

This heterogeneity of stromal elements within a functional LTBMC has complicated attempts both to define the contribution of each component to the regulation of stem cell self-renewal, proliferation, and differentiation, and also to determine the ontologic relationship between the various stromal cell types. Here we report on the development of a murine IgM monoclonal antibody (MoAb) (STRO-1) that binds to human BM fibroblast-like cells and to various nonhematopoietic cellular components of the adherent cell layer in LTBMC. STRO-1 antibody can also be used to isolate stromal precursors in freshly aspirated BM cell suspensions.

MATERIALS AND METHODS

BM samples. BM aspirates were obtained from normal donors after informed consent as defined by the Internal Review Board at the Fred Hutchinson Cancer Research Center (FHCRC). Mononuclear cells of density less than 1.077 g/mL were obtained by centrifugation over Ficoll (Lymphoprep, Nygaard, Oslo), filtered through Nytex to remove large aggregates, and then passed repeatedly through a 21-gauge needle.

Production of antibody STRO-1. Antibody STRO-1 was the product of a lymphocyte hybrid fusion between NSI-Ag4-1 murine myeloma cells and BALB/c spleen cells from an animal immunized with a population of CD34 positive (CD34+) BM cells. The rationale for this immunization was based on the observation that CD34+ cells contain many different cell types in addition to early progenitors and stem cells. Therefore, we hypothesized that antibodies that could further divide the CD34+ population into minor subpopulations may be of interest for identifying specific types of precursor cells. Owing to limitations in cell number, animals were immunized intraperitoneally with 10^7 cells in the presence of muramyl dipeptide (Sigma, St Louis, MO) as adjuvant. Animals were boosted four times at 3-week intervals with between 4 to 6 x 10^7 cells injected in the footpad. Fusion of splenocytes and myeloma cells using polyethylene glycol and selection of hybridomas in medium containing hypoxanthine-aminopterin-thymidine (HAT) was performed according to standard procedures.

Supernatants from antibody-producing HAT-resistant hybridomas were firstly screened by means of an alkaline phosphatase-based enzyme-linked immunosorbent assay (ELISA) against a panel of T- and B-cell lines to eliminate hybridomas producing antibodies against the relatively common human leukocyte antigens (HLA) and any T- or B-cell-lineage markers. The remainder were screened by indirect immunofluorescence on unfraccionated BM mononuclear cells (BMMC) to identify antibodies reactive with ≤ 10% of cells. Selected supernatants were then used to stain CD34+ cells by two-color immunofluorescence to identify antibodies reactive with subpopulations of CD34 expressing cells. The CD34+ cells used for screening were isolated by avidin-biotin immunoadsorption using antibody MY-10 and were generously provided by Dr Ron Berenson (FHCRC). Hybridomas secreting antibodies that fulfilled these criteria were cloned three times by limiting dilution and adapted to growth in serum-free medium (Nutridoma NS; Boehringer-Mannheim). STRO-1 was identified as an antibody that reacted with stromal cells in the adherent layer of LTBMC. The antibody was isolated as an IgM by means of a peroxidase ELISA (Boehringer-Mannheim, Indianapolis, IN).

Assay of hematopoietic progenitor cells. Committed granulocyte/macrophage progenitor cells (colony-forming unit granulocyte-macrophage [CFU-GM]) were assayed in Iscove’s Modified Dulbecco’s Medium (IMDM) supplemented with 20% fetal calf serum (FCS), 10% human placenta-conditioned medium, 0.05 mmol/L 2-mercaptoethanol, and 0.3% agar (Bacto Agar; Difco, Detroit, MI).

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M1). Primitive erythroid colony-forming cells (burst-forming unit-erythroid [BFU-E]) and multipotential colony-forming cell (CFU-Mix) were assayed in 1 mol/L of medium consisting of IMDM, 30% FCS, 0.9% bovine serum albumin, 4% phytohemagglutinin-stimulated leukocyte-conditioned medium, 1 unit of recombinant human erythropoietin (Amgen Biologics, Thousand Oaks, CA), 0.05 mmol/L 2-mercaptoethanol, and 1.2% methylcellulose. Colonies were scored on day 14 according to standard criteria.

BM fibroblast colony-forming unit assay (CFU-F). Details of this procedure have been described elsewhere. Briefly, unseparated cells and fluorescence-activated cell sorter (FACS)-purified populations were plated in triplicate at 5 × 10⁴ to 10⁵/mL in 35-mm plastic dishes (LUX Lab-Tech Division, Miles Laboratories, Naperville, IL) in a minimal essential medium (αMEM) supplemented with 20% FCS (Hyclone, Logan, UT), penicillin (100 U/mL), and streptomycin (100 μg/mL). Cultures were gassed with 5% CO₂ in air and incubated at 37°C. The growth medium was totally renewed on day 7 of culture. Cultures were terminated on day 10 to day 12 and fibroblast colonies were stained with Wright-Giemsa and counted with an inverted microscope at 25X. Cell clusters comprising greater than 50 fibroblasts were scored as a CFU-F colony.

Establishment of LTBM C. Unsorted BM cells and aliquots of the various populations isolated by FACS were cultured under conditions necessary to establish a permissive stromal microenvironment in vitro, as previously described. Cells were plated either in 35-mm dishes or 8-chamber slide cultures (LUX Lab-Tech Division, Miles Laboratories) in IMDM at 365 mOsmol/L supplemented with 12.5% FCS (Hyclone Laboratories), 12.5% horse serum (Flow Laboratories, McLean, VA), 100 U/mL penicillin, 100 μg/mL streptomyacin, 0.4 mg/mL of L-glutamine (GIBCO, Grand Island, NY), 0.01 mg/mL folic acid, 0.04 mg/mL myoinositol, 0.01 mmol/L 2-mercaptoethanol, and 1 μg/mL hydrocortisone sodium succinate (Sigma). Cultures were maintained at 37°C in an atmosphere of 5% CO₂ and air for 3 to 4 days and switched to 33°C thereafter. LTBM Cs were fed on a weekly basis by demiepidermolysis, as described. A quantitative assessment of the number of cells detectable in LTBM C supernatants was obtained by pooling half of the nonadherent cell population from quadruplicate cultures, concentrating these cells 10-fold, performing cell counts using a standard hemocytometer, and then correcting for the number of cultures and cell concentration.

Antibody-dependent complement-mediated cytotoxicity. Light-density BM cells were resuspended in RPMI medium containing 20% heat-activated FCS at 10⁶ cells/mL. Aliquots of cells were incubated for 45 minutes at 4°C with antibody STRO-1, antibody H12C12 (an isotype-matched control antibody), or antibody P5.1 (anti-HLA-A2, used as a positive control). After washing twice at 4°C in HHF (Hank's balanced salts solution without Ca++, Mg++, 0.01 mg/mL HEPES, and 2% FCS), endotoxin-free baby rabbit serum was added as the complement source at a final dilution of 1/3, and the cells were incubated for an additional 30 minutes at 37°C. The cells were then pelleted and resuspended in αMEM with 10% FCS and aliquots plated in both hematopoietic progenitor cell and CFU-GM assays.

Immunofluorescence and flow cytometric analysis. Following Ficoll separation, mononuclear BM and peripheral blood (PB) cells were resuspended in wash buffer (Hank's balanced salts solution, 0.01 mol/L HEPES buffer, pH 7.3, 2% FCS, 'HHF') and washed twice at 4°C. To block potential Fc receptor-mediated binding, the cells were incubated in HHF supplemented with 2% human AB serum before antibody labeling. The reactivity of STRO-1 antibody with various hematopoietic lineages in the blood and marrow was analyzed by two-color indirect immunofluorescence using a panel of IgG MoAbs that define various cluster designations (CD) as reported in the Third International Work-

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STRO-1 failed to inhibit the growth of CFU-GM, BFU-E, or CFU-Mix when compared with the growth obtained with the non-binding isotype-matched antibody H12C12. Plated in triplicate, the mean ± SE number of colonies obtained from 10^5 control cells in two experiments was 54 ± 2, 55 ± 2 CFU-GM; 61 ± 5, 58 ± 3 BFU-E; and 3 ± 1, 4 ± 0.3 CFU-Mix. STRO-1–treated cells gave comparable growth: 53 ± 3, 47 ± 5 CFU-GM; 58 ± 3, 45 ± 8 BFU-E; and 2 ± 1.3, 5 ± 1.3 CFU-Mix. In contrast, the anti-HLA-A2 antibody, P5.1, used as a positive control for cell lysis, led to the complete elimination of all colony growth.

To control for the possibility that STRO-1 was weak in its ability to fix complement, additional experiments were performed using FACS to separate BMNC into STRO-1–positive and –negative fractions according to the gates shown in Fig 1. Data from one such experiment indicated that unsorted cells plated at 10^5 gave 50 ± 1 CFU-GM, 34 ± 4.3 BFU-E, and 1 ± 1 CFU-Mix. STRO-1–negative cells gave 123 ± 13 CFU-GM, 57 ± 12 BFU-E, and 1 ± 1 CFU-Mix, whereas no colony growth was detected in the STRO-1–positive population.

Distribution of STRO-1 on PB, BM, and leukemic cell lines.

To determine which hematopoietic lineages express the STRO-1 antigen, mononuclear cells from the PB and BM were stained with STRO-1 in combination with a panel of IgG MoAbs of defined CD. The results of this dual-fluorescence analysis are shown in Table 1. All hematopoietic lineages in the PB were nonreactive with STRO-1 with the exception of low-level binding to a minor subpopulation of CD20-positive cells. A similar lack of reactivity was seen in the BM, where STRO-1 failed to bind to T cells, B cells, myeloid cells, macrophages, or megakaryocytic cells. STRO-1 did bind to subpopulations of cells expressing CD34, CD44, and CD29, but greater than 95% of STRO-1–positive cells in the BM were nucleated erythroid cells, as demonstrated by expression of glycophorin A (Table 1, Fig 2), and by examination of the morphology of sorted STRO-1–/glycophorin A+ cells.

The binding of STRO-1 to leukemia cell lines representing a variety of hematopoietic lineages was also studied. Consistent with the observations made with BM and PBMC, STRO-1 bound only to the human erythroleukemia cell line HEL-DR" and to Epstein-Barr virus (EBV)-immortalized B-cell lines. The leukemic B-cell lines RAJI and DAUDI were STRO-1 negative, as were myeloid cells KG1, KG1a, HL60, U937, and the T-cell lines CEM and Jurkat.

STRO-1 binds to BM CFU-F.

In some preliminary experiments in which we examined the growth of STRO-1–positive cells isolated by FACS in liquid culture, we routinely observed the development of colonies of fibroblastic stromal cells similar in many respects to those assayed as CFU-F. To determine whether STRO-1 bound to CFU-F, we performed complement-mediated lysis using fresh aspirates of BM. The results of a representative experiment (one of six are summarized in Table 2. In the presence of rabbit complement, STRO-1 consistently inhibited CFU-F growth by more than 70% (range 50% to 85%) compared with a control antibody H12C12. Antibody 6.19, which was previously shown to be lytic for CFU-F, was used as a positive control in these experiments and reduced CFU-F growth by more than 90% in all assays.

To confirm these data, we attempted to positively select

Table 1. Reactivity of STRO-1 With Hematopoietic Lineages in the PB and BM as Defined by Dual-Color Flow Cytometry

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Antibody</th>
<th>Binding of STRO-1</th>
<th>PB</th>
<th>BM</th>
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<tbody>
<tr>
<td>T cells</td>
<td>CD2</td>
<td>–</td>
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<td></td>
<td>CD3</td>
<td>–</td>
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<td>CD4</td>
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<td>CD7</td>
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<td></td>
<td>CD8</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>B cells</td>
<td>CD10</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>CD19</td>
<td>ND</td>
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<td></td>
<td>CD20</td>
<td>+*</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Myeloid cells</td>
<td>CD13</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>CD33</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Macrophages</td>
<td>CD14</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>CDW41</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Erythroid cells</td>
<td>Glycophorin A</td>
<td>–</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Progenitor cells</td>
<td>CD34</td>
<td>ND</td>
<td>+*</td>
<td>–</td>
</tr>
<tr>
<td>Multiple</td>
<td>HLA-DR</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CD11a</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>CD11b</td>
<td>ND</td>
<td>–</td>
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<td></td>
<td>CD11c</td>
<td>ND</td>
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<td></td>
<td>CD29</td>
<td>ND</td>
<td>+*</td>
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Abbreviation: ND, not determined.  
*Denotes binding of STRO-1 to a subpopulation of cells experiencing the specified antigen.

Fig 2. Two-parameter fluorescence histogram showing binding of antibody STRO-1/antimouse IgM-FITC and anti-glycophorin A antibody R10/antimouse IgG-PE to BMNC. The vertical and horizontal lines represent staining obtained with the isotype-matched control antibodies H12C12 and 1A14, respectively. STRO-1+ cells are shown in panels 2 and 4; the STRO-1+/glycophorin A- cells isolated as a source of stromal precursors are shown in panel 4. The cells were not gated for their forward and perpendicular light scattering properties. Histogram is based on 20,000 events collected as list mode data.
for CFU-F by isolating the STRO-1-positive cells from BM-MNC by means of FACS. As shown in Table 3, CFU-F were only recovered in the STRO-1-positive fraction. “Add-back” experiments, in which STRO-1+ cells were mixed with STRO-1− or unfractionated, BM cells at known ratios did not show the presence of the capacity either to inhibit or enhance CFU-F colony formation in the STRO-1−-negative fraction (data not shown).

Previous phenotype data demonstrated that the majority of STRO-1+ cells in the BM coexpressed glycophorin A (Table 1). However, we consistently observed a minor subpopulation (approximately 5%) of STRO-1+ cells that lacked glycophorin A. Expression of glycophorin A is tightly restricted to erythroid cells and has not been reported on BM stromal elements, suggesting the possibility that CFU-F would have the phenotype STRO-1+/glycophorin A−. To investigate this hypothesis we performed FACS on BM cells double-labeled with both antibody reagents by dual-color indirect immunofluorescence. As shown in Table 3, virtually all CFU-F that were recovered were detected in the STRO-1+/glycophorin A− subpopulation. Notably, the incidence of CFU-F in this population was approximately one in 100 cells plated, which compares with a frequency of approximately 1/10^4 cells in the unfractionated marrow.

STRO-1+ cells give rise to fibroblasts, smooth muscle cells, and adipocytes in LTBMSC. The successful maintenance of hematopoiesis in vitro is associated with a more phenotypically diverse population of adherent stromal elements than is generated under the CFU-F assay conditions, including adipocytes, smooth muscle cells, endothelial cells, and macrophages. To determine whether STRO-1+ BM cells contain the precursors to any of these stromal cell types, STRO-1+ cells isolated by FACS were plated in medium containing horse serum, FCS hydrocortisone (LTBMSC medium), fed weekly, and maintained at 33°C, as previously reported.13

Over the course of 3 to 4 weeks, these STRO-1+ cells generated an adherent layer of cells morphologically indistinguishable from those generated from unfractionated BM. Phenotypic analysis of these adherent layers demonstrated the presence of fibroblastic cells, smooth muscle cells, and adipocytes (Table 4). F VIII rAg+ endothelial cells, or CD14-positive macrophages, were not detected in cultures established from STRO-1+ cells, although they were present in LTBMSC generated from unfractionated BM cells. Significantly, in cultures established from STRO-1− sorted cells, granulocytic cells or hematopoietic progenitors (CFU-GM, BFU-E, CFU-Mix) were not detected over 8 weeks in culture in three separate experiments, either in the adherent or nonadherent layers (data not shown).

Analysis of the expression of the STRO-1 antigen in LTBMSC. To extend these observations, we examined the pattern of binding of STRO-1 to the adherent cells in LTBMSC established from unfractionated BM by means of in situ dual-color indirect immunofluorescence using the antibody reagents listed in Table 4. STRO-1 bound to fibroblastic cells expressing 6.19, CD10, CD13, and TE7 (Fig 3A and B) and to 50% to 75% of the cells that bound the smooth muscle actin-specific antibodies 1A4 and HHF35 (Fig 3C and D). The STRO-1 antigen was brightly expressed by the majority of cells showing early evidence of lipid accumulation (“pre-adipocytes”) but was absent from mature adipose cells (Fig 3E and F). Endothelial cells (F VIII rAg+) and macrophages (CD14+) failed to bind to STRO-1 (Fig 3G, H, and I).

STRO-1 binds to a trypsin-insensitive epitope on stromal cells. We therefore investigated the expression of the STRO-1 antigen in the LTBMSC by means of flow cytometry using trypsin-detached stromal cells after various periods of

<table>
<thead>
<tr>
<th>Table 2. Binding of Antibody STRO-1 to CFU-F Detected by Means of Complement-Mediated Lysis</th>
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</thead>
<tbody>
<tr>
<td>Antibody</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>H12C12</td>
</tr>
<tr>
<td>STRO-1</td>
</tr>
<tr>
<td>6.19</td>
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</table>

*Mean number of CFU-F ± SE obtained from a starting population of 10^6 mononuclear BM cells per group.
†Inhibition calculated relative to the number of CFU-F obtained following treatment with control antibody H12C12 and C′.
Expression of STRO-1 antigen by phenotypically distinct stromal elements in the adherent layer of LTBMC demonstrated by dual-color indirect immunofluorescence. (A) Cell surface staining of fibroblast-like cells with STRO-1/FITC and (B) corresponding image showing cytoplasmic staining with TE7/Texas Red. (C) STRO-1/FITC and (D) staining with the smooth muscle actin-specific antibody, 1A4/Texas Red. (E) Binding of STRO-1/FITC antibody to a region of the adherent layer containing numerous adipocytes. (F) Corresponding phase contrast image of the field in (E). Note STRO-1 antigen expression by adipocytes at early stages of lipid accumulation (open arrow) and lack of staining with STRO-1 on mature adipocytes (closed arrows). (G) Staining of fibroblast-like stromal elements with STRO-1/FITC and (H) macrophages with LeuM3/Texas Red. (I) Phase contrast image of field depicted in (G) and (H). Note mutually exclusive pattern of staining with these two reagents. All micrographs, original magnification × 40.

time in culture. As shown in Table 5, the number of STRO-1-positive cells increases over the first 2 weeks in culture, corresponding to the period of adherent layer establishment. Thereafter, the number of STRO-1-positive cells progressively declines to approximately 15% of their maximum frequency by week 6 in culture. Significantly, isolation of STRO-1-positive cells from LTBMC by means of FACS at or beyond 6 weeks in culture, followed by plating in fresh LTC medium, resulted in the generation of stromal cell layers with identical cellular composition to those produced by STRO-1+ cells isolated from fresh aspirates of BM.

Adherent layers generated from STRO-1+ cells support hematopoietic cell development in vitro. Adherent cell layers were established from the same normal donor from either unfractionated BMMC or from STRO-1-positive cells selected by FACS. At week 6, a portion of the LTBMC established from unfractionated marrow were killed, the adherent cells detached by trypsin, and STRO-1-expressing cells selected by FACS were plated in fresh LTC medium. When the latter cells had formed a confluent layer (week 4), cultures from each of the three groups were assayed for their ability to support the production of progenitors and mature hematopoietic cells from a population of BM cells highly enriched in LTC-initiating cells. The latter cells were selected on the basis of high-level expression of CD34 antigen, low uptake of the fluorescent vital mitochondrial dye Rhodamine 123, and lack of binding of soybean agglutinin. As shown in Fig 4, adherent layers

<table>
<thead>
<tr>
<th>Time (wk)</th>
<th>Percentage of STRO-1+ Cells*</th>
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<tbody>
<tr>
<td>1</td>
<td>14.7 ± 3.1</td>
</tr>
<tr>
<td>2</td>
<td>58.3 ± 8.2</td>
</tr>
<tr>
<td>3</td>
<td>30.8 ± 11.6</td>
</tr>
<tr>
<td>6</td>
<td>8.5 ± 3.5</td>
</tr>
</tbody>
</table>

*Percentages calculated using trypsin-detached adherent layers simultaneously labeled with STRO-1/anti-IgM-FITC and Leu M3-PE to exclude autofluorescent macrophages from the analysis. Data represent mean ± SE of the percentage of STRO-1+ cells in LTC established from at least three different normal donors.
Aglutinin [SBA]-/Rhodamine-l23 dull) were added to each culture.

Weekly intervals. Data are presented as cumulative production over 8 weeks in culture, and represent one of four comparable experiments. A potentially significant in three additional experiments. A potential explanation for the apparent difference in the hematopoietic supportive ability of STR0-1 versus unseparated marrow-derived adherent layers may have been the need to irradiate the latter cultures to eliminate endogenous hematopoietic cells before seeding with the progenitor cells. To control for this, a portion of the cultures derived from sorted STR0-1-positive LTBM C cells were also irradiated (160 Gy 357Cs, 39.4 Gy/min) before addition of cells. As shown in Fig 4, there was no significant difference in production of nonadherent cells or maintenance of progenitors between the irradiated or unirradiated STR0-1-positive cultures.

DISCUSSION

Defining the nature of the cellular interactions that occur between primitive hematopoietic stem cells and the various stromal components of the BM is fundamental to our understanding of the regulation of hematopoiesis. Although considerable progress in the analysis of these stromal components was made following the introduction of the LTBM C system, subsequent studies of this in vitro HM have mainly contributed to our appreciation of the degree of heterogeneity that exists between stromal elements. Consequently, the relative contribution of each stromal cell type to the support of hematopoiesis in LTBM C remains to be determined.

MoAbs provide a means of separating, characterizing, and studying a defined population of cells in isolation from other components in a heterogeneous system. In this report we have described the generation and specificity of a murine IgM MoAb we have termed STR0-1 by virtue of its reactivity with BM stromal elements in vivo and in vitro. The antibody was shown to be nonreactive with CFU-GM, BFU-E, or CFU-Mix progenitors by both complement-mediated lysis and cell sorting, and to bind to approximately 10% of BM cells that comprise mainly glycophorin A-positive nucleated erythroid precursors.

Binding of STR0-1 to CFU-F was demonstrated by suppression of CFU-F growth in the presence of antibody and complement and by the presence of 100% of assayable CFU-F in the STR0-1+ fraction isolated by FACS. This pattern of reactivity suggests that STR0-1 may be used to selectively deplete human BM of stromal cells without affecting hematopoietic progenitors, allowing studies using the latter cells to be performed free of contaminating stroma. Alternatively, STR0-1 can be used to positively select for CFU-F, thereby facilitating further studies of stromal elements. Significantly, we showed that CFU-F can be enriched 100-fold from fresh aspirates of marrow by selecting cells using the FACS with the phenotype STR0-1+/glycophorin A+.

In addition to generating CFU-F, STR0-1+ cells were shown to contain the precursor(s) for many of the different components of the adherent cell layer in LTBM C, including 6.19+, TE7+ fibroblast-like cell synthesizing laminin, fibronectin, collagen type III, IV, and V; smooth muscle actin-containing cells, and adipocytes. Lim et al19 have reported on the growth of stromal cell colonies with a reticular-fibroblastic morphology, designated CFU-RF, which appear to share many of the properties of STR0-1+ stromal precursors in human LTBM C. Macrophages and F VIII rAg-positive endothelial cells were not detected in cultures established from STR0-1+ cells.

Flow cytometric analysis of the expression of STR0-1 antigen on the stromal cells in LTBM C showed increasing numbers of STR0-1+ cells up to week 2 of culture, followed by a progressive decline. It is unclear what is responsible for this progressive loss of STR0-1 antigen at later times in LTBM C. This finding could represent a culture ephenomenon unrelated to the normal regulation of antigen expression observed in vivo, or alternatively, could be due to a
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related to the in vitro maturation of what might be termed stromal precursors into more differentiated stromal cell types. As yet, we cannot distinguish between these possibilities. In support of the latter hypothesis, however, it is perhaps of interest that stromal cells that express low or undetectable levels of STRO-1 antigen tend to demonstrate the highest levels of the fibroblast antigen TE7 or of smooth muscle actin and vice versa. This phenomenon is somewhat analogous to the expression of the CD34 antigen in the hematopoietic system, where highest levels are seen on the most immature cells while the majority of differentiation antigens are expressed concomitant with loss of CD34.24,25 However, more significant is our observation that the small number of STRO-1+ cells seen at later times in culture are able to produce adherent cell layers that in cellular composition and phenotype are indistinguishable from those of the parent culture or from adherent layers generated from STRO-1− cells freshly isolated from the BM. Thus, we suggest that stromal precursors similar, if not identical, to those in vivo24,25 are maintained for extended periods in LTBM, and that the STRO-1 antigen is a differentiation antigen present on these cells.

We also assayed adherent layers derived from STRO-1− cells for their ability to support the growth of what has been termed LTC-initiating cells, a class of primitive progenitors that are believed to share many properties of stem cells with marrow repopulating ability.26 Interestingly, the STRO-1 layers appeared to be superior to the stromal layers established from unseparated BM in their ability to generate progenitor cells and mature granulocytic cells. The nature of this difference is unclear; however, the application of STRO-1 antibody to the experimental dissection of the HM should facilitate our understanding of this process.

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Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1

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