Human Osteoblasts Support Human Hematopoietic Progenitor Cells in In Vitro Bone Marrow Cultures

By Russell S. Taichman, Marcelle J. Reilly, and Stephen G. Emerson

Hematopoietic stem cell differentiation occurs in direct proximity to osteoblasts within the bone marrow cavity. Despite this striking affinity, surprisingly little is known about the precise cellular and molecular impact of osteoblasts on the bone marrow microenvironment. Recently, we showed that human osteoblasts produce a variety of cytokine mRNAs including granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and interleukin-6. We examined here the ability of osteoblasts to support the development of hematopoietic colonies from progenitors as well as the ability to maintain long-term culture-initiating cells (LTC-IC) in vitro. Examination of the hematopoietic cells revealed after 2 weeks of culture showed that osteoblasts support the maintenance of immature hematopoietic phenotypes. In methylcellulose assays, osteoblasts stimulate the development of hematopoietic colonies to a level at least 10-fold over controls from progenitor cells. Using limiting dilutional bone marrow cultures, we observed an activity produced by osteoblasts resulting in a threefold to fourfold expansion of human LTC-IC and progenitor cells in vitro. Thus, the presence of hematopoietic stem cells in close proximity to endosteal surfaces may in vivo may be due in part to a requirement for osteoblast-derived products.

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MATERIALS AND METHODS

Human osteoblasts. Enriched human osteoblast cultures were obtained using a modification of methods described by Robey and Termine. Normal human trabecular bone was obtained from patients undergoing orthopedic surgery in accordance with the University of Michigan Human Investigational Review Board. Bone cleaned of loosely adherent tissue was ground to produce a uniform particle size (size <1 mm³; BioComp Minimill, W. Lorenz, Jackson-ville, FL) and incubated in 1 ml/mg of 1% bacterial collagenase (Type P; Boehringer Mannheim Biologicals, Indianapolis, IN). The explants were placed into culture until confluent monolayers were produced in a 1:1 (vol/vol) mixture of F12/Dulbecco’s modified Eagle’s medium (DMEM) medium (Biofluids, Rockville, MD) with low Ca²⁺ and 10% fetal bovine serum. Thereafter, cultures were maintained in calcium replete DMEM/F12 (1:1 vol/vol) medium containing 10% heat-inactivated fetal bovine serum (FBS), antibiotics, 10 mmol/L β-glycerophosphate, and 10 mg/mL L-ascorbate. To verify that the cells expressed an osteoblast phenotype, the cultures were screened for the presence of osteoblast-specific markers, including osteocalcin and osteopontin.

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for the expression of the osteoblast-specific protein osteocalcin (osteocalcin\(^\text{\textregistered}\)), of c-kit ligand (c-kit ligand\(^{\text{\textregistered}}\)) by reverse transcriptase-polymerase chain reaction (RT-PCR) using sense and antisense primers (c-kit ligand: sense, GAAGGAGATCTCCAGGAATCT-GTG; antisense, GCCCTTGAAGACCTGCGTCTC\(^{\text{\textregistered}}\); osteocalcin: sense, GGCAGCGAGGTAGTGAAGAG; antisense, GATGTTGTCACGCAACTGCTG\(^{\text{\textregistered}}\)) as well as in vitro mineralization and expression of alkaline phosphatase, as previously detailed.\(^8\) By these morphologic and RNA criteria, we can easily detect contamination of our osteoblast preparations by normal marrow stromal elements to levels of 1% of the total population. Therefore, the osteoblasts are at least 99% pure of contaminating stromal cells, with a possible maximal contamination of 1% stromal cells.

**Isolation of human CD34\(^{\text{\textregistered}}\) bone marrow cells.** Human bone marrow cells were obtained from healthy adult volunteers by iliac crest puncture and aspiration into preservative-free heparin under a protocol approved by the University of Michigan Human Investigations Review Board. Mononuclear cells were isolated by density separation on Ficoll-Hypaque (specific gravity, 1.077). After two rounds of plastic adherence at 37°C for 1 hour each in Iscove’s modified Dulbecco’s medium (IMDM medium; GIBCO-BRL Laboratories, Grand Island, NY) with 10% FBS (Hyclone, Logan, UT), 10% equine serum (BRL Life Technologies, Grand Island, NY), and 1 \(\mu\)mol/L hydrocortisone (BRL Life Technologies), the nonadherent cells were recovered. CD34\(^{\text{\textregistered}}\) bone marrow cells were isolated from nonadherent cell populations by positive immunoselection (CellPro Inc, Bothwell, WA).

**Adherent bone marrow stromal cell layers.** Mononuclear bone marrow cells were separated by density centrifugation over Ficoll-Hypaque and the stromal cells separated by adherence to plastic. The cells were grown to confluence in IMDM medium with 10% FBS, 10% horse serum, and 1 \(\mu\)mol/L hydrocortisone with the nonadherent layers removed after 48 hours. For most experiments, the adherent heterogeneous bone marrow stromal cell layers were passages at confluence 2 or 3 times and irradiated before culture initiation (2,000 R).

**Liquid culture of CD34\(^{\text{\textregistered}}\) bone marrow cells and adherent cells.** CD34\(^{\text{\textregistered}}\) bone marrow cells were seeded directly onto osteocalcin\(^{\text{\textregistered}}\), c-kit ligand\(^{\text{\textregistered}}\) confluent osteoblast monolayers at a final density of 1 \(\times\) 10\(^7\) cells/cm\(^2\) for 14 days in DMEM/F12 (1:1 vol/vol) containing 10% heat-inactivated FBS, antibiotics, 10 \(\mu\)mol/L \(\beta\)-glycerol phosphate, and 10 mg/mL L-ascorbate. Because the intent of these experiments was to test whether osteoblasts play a role in hematopoiesis, we compared osteoblasts to the standard, generally most favored conditions for stromal fibroblast-supported hematopoiesis. Therefore, CD34\(^{\text{\textregistered}}\) bone marrow cells were seeded directly onto irradiated stromal cells in IMDM medium with 10% fetal calf serum (FCS), 10% horse serum, and 1 \(\mu\)mol/L hydrocortisone. Where indicated, exogenous c-kit ligand (50 ng/mL; Genzyme, Cambridge, MA), recombinant human G-CSF (rhG-CSF; 20 ng/mL; R&D Systems, Minneapolis, MN), GM-CSF (20 ng/mL), IL-3 (10 ng/mL; GIBCO-BRL), neutralizing murine antihuman transforming growth factor (TGF)-\(\beta\) antibody (IgG); 40 \(\mu\)g/mL added daily; Genzyme), or MOPC31 (murine IgG; Sigma Chemical Corp, St Louis, MO) was added to the cultures. At 2 weeks, all cultures were harvested by trypsinization (20 minutes at 37°C, 0.05% trypsin/0.5 mmol/L EDTA; BRL) and prepared for either light microscopy in which cell number and chain reaction was determined for progenitor and LTC-IC. Trypsinization was performed to recover the tightly adherent hematopoietic cells from the osteoblasts. Using these conditions, trypsin had no discernable effect on the ability of the hematopoietic cells to form colonies in methylcellulose.

**Progenitor cell-derived colony formation in methylcellulose on adherent osteoblast or bone marrow stromal cell layers.** Confluent human heterogeneous bone marrow stromal cells or osteocalcin\(^{\text{\textregistered}}\), c-kit ligand\(^{\text{\textregistered}}\) osteoblast monolayers were established by plating 4 \(\times\) 10\(^3\) cells/cm\(^2\) in triplicate-gridded 35-mm dishes (Nunc, Naperville, IL). After 48 hours of culture, the adherent cell layers were washed (3\(\times\)) in phosphate-buffered saline (PBS; 37°C) and overlayed with 1 mL methylcellulose (Stem Cell Technologies, Vancouver, British Columbia, Canada) containing 3,000 human CD34\(^{\text{\textregistered}}\) bone marrow cells in the presence or absence of recombinant IL-3 (10 ng/mL), GM-CSF (20 ng/mL; Life Technologies), and erythropoietin (Epo; 2 U/mL; Stem Cell Technologies) and returned to culture under fully humidified conditions in an atmosphere of 5% CO\(_2\) and 5% O\(_2\) at 37°C. These conditions were chosen because they are permissive for the formation of hematopoietic colonies from progenitor cells that were determined in preliminary experiments (data not presented). The clonogenic cells were scored at 14 days as either colony-forming unit–granulocyte-macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E), or colony-forming unit granulocyte, erythroid, monocyte, megakaryocyte (CFU-GEMM).

** Colony formation/differentiation and LTC-IC assays.** Progenitor cell-derived differentiation in methylcellulose was used to assay osteoblast-derived activity on progenitors in vitro. CD34\(^{\text{\textregistered}}\) bone marrow cells were cultured for 2 weeks in the presence or absence of human osteoblasts, whole bone marrow stroma, or recombinant IL-3 (10 ng/mL), GM-CSF (20 ng/mL; Life Technologies), and Epo (2 U/mL; Stem Cell Technologies). Aliquots of recovered hematopoietic progenitors were plated in triplicate-gridded 35-mm tissue culture dishes (Nunc) under fully humidified conditions in an atmosphere of 5% CO\(_2\) and 5% O\(_2\) at 37°C in 1 mL methylcellulose (Stem Cell Technologies) containing IL-3, GM-CSF, and Epo. Clonogenic cells were scored at 14 days as CFU-GM, BFU-E, or CFU-GEMM. Although carry-over osteoblasts are present in the progenitor assays resulting from the difficulty in differential recovery of the hematopoietic cells and osteoblasts after trypsinization, we have determined in methylcellulose assays that osteoblasts have no significant negative effect on the formation of hematopoietic colonies.

The effect of osteoblasts or fibroblast layers on the survival and proliferation of LTC-IC was determined by measuring LTC-IC numbers before and after a period of 2 weeks. To measure LTC-IC content before and after culture, increasing dilutions (5,000, 1,000, 500, 100, and 50) of bone marrow cells are cultured for 5 weeks on an irradiated (2,000 R) bone marrow fibroblast monolayers with 10 replicates per cell dilution for three experiments. After 5 weeks (week 7), each well was harvested, and the cells are replated in colony-forming methylcellulose assays. By enumerating each secondary colony assay as positive or negative for hematopoietic colony growth, a statistical calculation was derived for frequency of cells in the undiluted starting population capable of initiating a (5-week) culture producing progenitor cells. This assay enumerates the most primitive human hematopoietic stem cell yet identifiable by any in vitro culture technique.\(^9\)

**Statistical analyses.** Each experiment was repeated a minimum of three times. The unpaired Student’s \(t\)-test was used to determine statistical significance at a level of \(P < .05\).

**RESULTS**

**Maintenance of an immature phenotype for hematopoietic cells by osteoblasts in vitro.** In previous investigations, we observed that primary human osteocalcin\(^{\text{\textregistered}}\), c-kit ligand\(^{\text{\textregistered}}\) osteoblasts produce G-CSF in an cell-associated form, as well as several other cytokines, including mRNAs for GM-CSF and IL-6, which have activity on hematopoietic cells. In addition, osteoblasts were capable of stimulating the limited proliferation of human CD34\(^{\text{\textregistered}}\) bone marrow cells in coculture, to which the hematopoietic cells were tightly adherent.
We therefore wanted to determine the maturational status of the hematopoietic cells recovered from osteoblast cocultures.

For these investigations, human CD34+ hematopoietic bone marrow cells were seeded directly onto osteoblast monolayers or a mixed population of bone marrow stromal cells or in the presence or absence of recombinant cytokines for 2 weeks. At the conclusion of the culture period, the hematopoietic cells were recovered by trypsinization and prepared for light microscopy. Trypsinization was performed because the hematopoietic cells were tightly adhered to the underlying osteoblasts. Under these conditions, hematopoietic cells grown in the presence of exogenously supplied GM-CSF and IL-3 differentiated along the granulocytic pathway into bands/polymorphonuclear neutrophils (Fig 1B), whereas the majority of the hematopoietic cells grown in the absence of the cytokines died. However, in the presence of osteoblasts, the majority of the recovered hematopoietic cells maintained an immature morphology (Fig 1D). Similar results were observed for CD34+ bone marrow cells grown in coculture with stromal cells over the two week period (Fig 1C).

We hypothesized that either the deficient production of c-kit ligand or the production of TGF-β1 by osteoblasts might be responsible for the failure of the hematopoietic cells to develop into mature phenotypes in vitro. To directly test these hypotheses, we added exogenous recombinant G-CSF and/or c-kit ligand or neutralizing TGF-β1,2,3 antibody to the osteoblast-hematopoietic or stromal-hematopoietic cultures for 14 days. We observed that, in the presence of osteoblasts, the proliferation of the hematopoietic cells in response to exogenous G-CSF or in combination with c-kit ligand (G-CSF + KL) was significantly diminished relative to hematopoietic cells grown in the presence or absence of stromal cells (Fig 2). Compared with CD34+/stromal cocultures, no significant proliferation was observed for the hematopoietic cells grown in the presence of c-kit ligand and osteoblasts despite the fact that human osteoblasts produce cell-associated G-CSF. The morphology of the hematopoietic cells recovered from coculture with osteoblasts in either G-CSF or c-kit ligand alone or in combination was not significantly altered relative to the stromal cell groups despite the differences observed in proliferation (Fig 2). In the presence of neutralizing TGF-β1,2,3 antibody, although the number of hematopoietic cells recovered from the osteoblast or stromal cell cocultures was enhanced (Fig 2), no significant alterations were observed in the morphology of the hematopoietic cells (Fig 2). Therefore, based on these findings, the maintenance of an immature blood cell phenotype does not appear to be due to the deficient production of c-kit ligand or the excessive production of TGF-β1,2,3 by osteoblasts.

Osteoblasts support colony-forming cells in methylcellulose. We next asked whether human osteoblasts can directly support the development of hematopoietic colonies from CD34+ bone marrow hematopoietic progenitor cells. For these experiments, we directly seeded CD34+ bone marrow cells onto confluent human bone marrow stromal or osteocalcin+, c-kit ligand+ osteoblast monolayers in methylcellulose in the presence or absence of exogenous cytokines (IL-3, GM-CSF, and Epo). These particular cytokines were chosen because they were determined to be permissive for hematopoietic colony formation from progenitor cells in methylcellulose assays (data not presented). After 2 weeks, hematopoietic colonies were scored as CFU-GM, BFU-E, or CFU-GEMM by light microscopy. As expected, a greater than 20-fold increase in hematopoietic colonies was formed in the presence of cytokines compared with the number of colonies formed in their absence (Fig 3). Osteoblast or mixed bone marrow stromal cell monolayers significantly enhanced the formation of colonies when compared with cultures not supplied with exogenous cytokines. The enhanced frequency of colonies developing in the presence of the feeder layers was largely due to an expansion of the CFU-GM compart-
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from untreated (No Tx), no cell layer control (None) group was added daily. After 14 days, all cultures were harvested by trypsinization and the percentage of bands and segmented leukocytes was determined in cytospin preparations by counting a minimum of 58% (osteoblasts) and 80% (bone marrow cells obtained by positive immunoselection were cultured for 14 days either in the presence or absence (B) of enriched human osteoblast monolayers (■) or irradiated mixed bone marrow stroma or (□) at a final density of 1 x 10⁶ cells/well in 96-well tissue culture plates. Where indicated, G-CSF (20 ng/mL) and/or c-kit ligand (KL: 50 ng/mL) were added at culture initiation. Murine neutralizing antihuman TGF-β1 monoclonal antibody (anti-TGFβ AB; 200 μg/mL [IgG]), or an isotype-matched control (control AB; 200 μg/mL MOPC31) was added daily. After 14 days, all cultures were harvested by trypsinization and the percentage of bands and segmented leukocytes was determined in cytoospin preparations by counting a minimum of 200 cells in random high-power fields. Absolute cell numbers were determined by manual hemocytometer counting with trypan blue. Results are reported as mean ± standard deviation (n = 3). (▲) Significant difference from untreated (No Tx), no cell layer control (None) group (P < .05) using unpaired Student’s t-test (not all relationships are shown). (*) Significant difference for osteoblast groups from no cell layer controls (None) within the treatment group (P < .05). (+) Significant difference from osteoblasts with in the treatment group (P < .05).

Fig 2. Hematopoietic cell proliferation and morphology after in vitro coculture with osteoblasts or mixed bone marrow stromal cells. Human CD34⁺ bone marrow cells obtained by positive immunoselection were cultured for 14 days either in the presence or absence (B) of enriched human osteoblast monolayers (■) or irradiated mixed bone marrow stroma or (□) at a final density of 1 x 10⁶ cells/well in 96-well tissue culture plates. Where indicated, G-CSF (20 ng/mL) and/or c-kit ligand (KL: 50 ng/mL) were added at culture initiation. Murine neutralizing antihuman TGF-β1 monoclonal antibody (anti-TGFβ AB; 200 μg/mL [IgG]), or an isotype-matched control (control AB; 200 μg/mL MOPC31) was added daily. After 14 days, all cultures were harvested by trypsinization and the percentage of bands and segmented leukocytes was determined in cytoospin preparations by counting a minimum of 200 cells in random high-power fields. Absolute cell numbers were determined by manual hemocytometer counting with trypan blue. Results are reported as mean ± standard deviation (n = 3). (▲) Significant difference from untreated (No Tx), no cell layer control (None) group (P < .05) using unpaired Student’s t-test (not all relationships are shown). (*) Significant difference for osteoblast groups from no cell layer controls (None) within the treatment group (P < .05). (+) Significant difference from osteoblasts with in the treatment group (P < .05).

Fig 3. Hematopoietic progenitor cell colony formation on osteoblast or mixed bone marrow stromal cell layers. Human CD34⁺ bone marrow cells were seeded onto confluent human mixed bone marrow stroma or osteocalcin⁺, c-kit ligand⁺ osteoblast monolayers in methylcellulose in the presence or absence of exogenous cytokines (IL-3, GM-CSF, and Epo). After 2 weeks, hematopoietic colonies were scored as (■) CFU-GM, (□) BFU-E, or (□) CFU-GM/M by light microscopy. As shown, osteoblasts produce soluble factors that, without the addition of exogenous cytokines, are able to stimulate the formation of CFUs in vitro. (*) Significant difference from GF control (P < .05) as determined by unpaired Student’s t-test (n = 3).

The ability of osteoblasts to support and expand LTC-IC in liquid culture. We next asked whether human osteoblasts could support long-term myelopoiesis in vitro. To determine whether human osteoblasts can support the maintenance of LTC-IC in vitro, human CD34⁺ bone marrow progenitors were placed on to homogeneous osteoblast, on mixed bone marrow stromal (BMS) cell monolayers, or in the presence or absence of GM-CSF, IL-3, and Epo for 2 weeks of culture. After the coculture period, the hematopoietic cells were recovered, counted, and assayed for the presence of CFU activity (in methylcellulose) or for LTC-ICs (limiting dilutional bone marrow cultures). Over the initial 2-week period, the number of clonogenic progenitors increased several fold over input when cultured on osteoblasts (Fig 4). When the culture period was extended to 4 weeks or when the CD34⁺ bone marrow cells were physically separated from the osteoblasts, this activity was diminished (data not shown). CFUs also increased several fold when cultured in the presence of whole BMS or cytokines, but none was recovered from the cytokine groups. During the same period, a threefold to fourfold expansion in the LTC-IC compartment over input occurred in the presence of osteoblasts, comparing favorably with BMS. No LTC-IC were recovered from the cytokine control groups. Consequently, osteoblasts stimulate the
expansion of hematopoietic progenitors over a 2-week period while supporting the limited expansion and maintenance of the LTC-IC compartment.

**DISCUSSION**

Based on the intriguing anatomic observations of hematopoietic cells in close physical association with osteoblasts, we hypothesized that unique cellular and molecular interactions occur between osteoblasts and hematopoietic cells, which may explain why adult hematopoiesis occurs largely within bones. To test this hypothesis, we used several related assays. In the first assay, we cultured human bone marrow CD34+ hematopoietic bone marrow cells on osteocalcin+, c-kit ligand+ human osteoblasts for 2 weeks. At the conclusion of the culture period, the majority of the recovered hematopoietic cells exhibited an immature morphology. The maintenance of this morphology could not be attributed to a sizable production of TGF-β1, TGF-β2, or TGF-β3 or to the lack of production of c-kit ligand or soluble G-CSF. In fact, osteoblasts may have significant regulatory roles in the bone marrow microenvironment because progenitor cell proliferation was inhibited by osteoblasts compared with controls of recombinant G-CSF and/or c-kit ligand.

Bone marrow stromal cells, in comparison, exhibited similar activities on proliferation only in the presence of G-CSF and c-kit ligand. The nature of these osteoblast and/or stromal cell activities remains to be determined.

To determine whether osteoblasts could inhibit the formation of hematopoietic colonies in methylcellulose from primitive progenitor populations, we directly placed progenitor cells while preserving long-term culture-initiated assays after 2 weeks of culture on osteoblasts. We determined that a threefold to fourfold expansion in the LTC-IC compartment over input occurred in the presence of osteoblasts, which compares favorably or better than bone marrow stroma while stimulating an expansion of the progenitor cell population over a 2-week period. Thus, osteoblasts can stimulate the expansion of hematopoietic progenitors while supporting the limited expansion and maintenance of the LTC-IC compartment. These observations correlate well with the morphologic data suggesting that, in culture, osteoblasts support the maintenance of immature hematopoietic phenotypes.

The bone/blood relationship has recently been reevaluated in light of the suggestion that osteoblasts are members of the stromal network supporting normal hematopoiesis. The basis for this view is several fold. First, isolated human osteoblasts constitutively synthesize mRNAs for G-CSF, GM-CSF, IL-1, LT, TGF-β, and tumor necrosis factor-α (TNF-α) as well as leukemia inhibitory factor (LIF) and macrophage colony-stimulating factor (M-CSF). Primary murine osteoblasts have been shown to produce several activities that appear to be G-CSF, M-CSF, GM-CSF, IL-1, and IL-6. Second, cells resembling osteoblast cells have been observed in long-term in vitro bone marrow stromal-dependent cultures that can support limited myelopoiesis. Third, stromal cells share several phenotypic characteristics with osteoblasts. For example, the murine bone marrow stromal cell lines BMS2 and +/+2.4 express high levels of alkaline phosphatase enzyme activity, collagen (I), and bone sialoprotein. Also, human osteoprogenitors cells can be isolated from the STRO-1+ fraction of human bone marrow stromal cells. In addition, mRNA for osteocalcin, an osteoblast-specific protein has been detected in stromal cell lines. In fact, many stromal cell lines possess some osteoblastic features that differ mainly in the degree of expression and can be modulated through the use of a variety of agents, including bone morphogenic protein-2.

In vivo blood formation takes place within the yolk sac, the liver, and the spleen during the early intrauterine period. By month 4 of fetal life, the bone marrow begins to produce

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**Fig 4.** Osteoblast support of hematopoietic progenitors and LTC-ICs in vitro. Human CD34+ bone marrow progenitors were placed on osteoblast and mixed bone marrow stromal (BMS) cell monolayers or in the presence or absence of GM-CSF, IL-3, and Epo. After 2 weeks of coculture, the hematopoietic cells were recovered, counted, and assayed for the frequency of progenitor cells (by CFU assays in methylcellulose) or for LTC-ICs by secondary bone marrow cultures (limiting dilutional bone marrow cultures) and tertiary progenitor assays as described in the Materials and Methods. (*) Significant difference from input (P < .05) as determined by unpaired Student's t-test (n = 3). (+) Significant difference from BMS (P < .05) as determined by unpaired Student's t-test.
blood; from that point on, hematopoiesis occurs almost exclusively within the bone. Earliest attempts to understand this relationship have focused on a protective function that bone serves for the hematopoietic organ. Although initially promising, more recent data call into question this explanation. Specifically, during bone marrow transplantation, donor blood stem cells circulate to all body organs but home specifically to the bone marrow. When marrow cells lodge in other tissues, they may undergo several rounds of division, but hematopoiesis is not sustained in these sites. Furthermore, after the induction of ectopic bone formation in extra-skeletal sites, functional hematopoietic marrow resides outside the protective confines of the skeleton and human fetal bone fragments implanted in immunodeficient scid/scid (SCID) mice sustain active human hematopoiesis. Our data contribute and in part explain these observations in that osteoblasts in vivo may be capable of contributing to the hematopoietic microenvironment by supplying some of the necessary cytokines/growth factors required for the process.

The precise mechanisms whereby osteoblasts support the limited expansion of LTC-IC and progenitor cells are not immediately known. That osteoblasts produce G-CSF as well as IL-6, M-CSF, TGF-β, and TNF-α probably accounts for much of the observed activity. However, the deficient production of c-kit ligand, the apparent inhibition of progenitor proliferation in response to soluble G-CSF and c-kit ligand, and the tight adhesion observed in vitro between CD34+ bone marrow cells and osteoblasts (manuscript in preparation) suggest that osteoblasts play a vital role in hematopoietic stem cell regulation. It is quite possible that osteoblasts are critical for the maintenance and self renewal of hematopoietic stem cells in the bone marrow microenvironment. Thus, the presence of hematopoietic stem cells near endosteal surfaces may in part be due to a need for osteoblast-derived products in the maintenance and expansion of early hematopoietic precursors.

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