Augmented Production of Interleukin-6 by Normal Human Osteoblasts in Response to CD34+ Hematopoietic Bone Marrow Cells In Vitro

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

Based on anatomic and developmental findings characterizing hematopoietic cells in close approximation with endosteal cells, we have begun an analysis of osteoblast/hematopoietic cell interactions. We explore here the functional interdependence between these two cell types from the standpoint of de novo cytokine secretion. We determined that, over a 96-hour period, CD34+ bone marrow cells had no significant effect on osteoblast secretion of granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, or transforming growth factor-beta, but in some experiments minor increases in leukemia inhibitory factor levels were observed. However, when CD34+ bone marrow cells were cocultured in direct contact with osteoblasts, a 222% ± 55% (range, 153% to 288%) augmentation in interleukin-6 (IL-6) synthesis was observed. The accumulation of IL-6 protein was most rapid during the initial 24-hour period, accounting for nearly 55% of the total IL-6 produced by osteoblasts in the absence of blood cells and 77% of the total in the presence of the CD34+ cells. Cell-to-cell contact does not appear to be required for this activity, as determined by coculturing the two cell types separated by porous micromembranes. The identity of the soluble activity produced by the CD34+ cells remains unknown, but is not likely due to IL-1beta or tumor necrosis factor-alpha, as determined with neutralizing antibodies. To our knowledge, these data represent the first demonstration that early hematopoietic cells induce the production of molecules required for the function of normal bone marrow microenvironments, in this case through the induction of hematopoietic cytokine (IL-6) secretion by osteoblasts.

© 1997 by The American Society of Hematology.

MATERIALS AND METHODS

**Human osteoblasts.** Enriched human osteoblast cultures were established using modifications 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’s Investigational Review Board. Bone cleaned of loosely adherent tissue was ground to produce a uniform particle size (size, ±1 mm²; BioComp Minimmill, W. Lorenz, Jacksonville, FL) and incubated in 1 mg/mL 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 Ham’s F12/Dulbecco’s minimal essential medium (DMEM; Biofluids, Rockville, MD) with low Ca²⁺ and 10% fetal bovine serum. Thereafter, cultures were maintained in calcium replete Ham’s F12/DMEM (1:1 vol/vol) medium containing 10% heat-inactivated fetal bovine serum (FBS; Life Technologies, Grand Island, NY). 5

**Bone marrow cells.** Bone marrow cells were harvested from normal adult bone marrow donors by aspirate using a standard 18-gauge needle. Bone marrow cells were obtained from patients undergoing orthopedic surgery in accordance with the University of Michigan’s Institutional Review Board. Bone marrow cells were aspirated into heparinized tubes and washed twice in phosphate-buffered saline. Bone marrow cells were then incubated with anti-CD34 magnetic beads (Miltenyi Biotec, Auburn, CA) under the manufacturer’s recommendations. After incubation, the mature hematopoietic cell population was collected as the CD34+ population by magnetic separation with a MACS Separator (Miltenyi Biotec). The purity of the CD34+ population was confirmed by FACS analysis. The CD34+ population was then resuspended in RPMI 1640 media supplemented with 10% fetal bovine serum.

**Coculture conditions.** Enriched human osteoblast cultures were set up in 6-well plates at 30,000 cells/cm². Bone marrow cells were added to osteoblast cultures at a ratio of 1:1 (vol/vol) at various densities. The cocultures were then incubated at 37°C in a humidified atmosphere containing 5% CO₂. At various time points, the cultures were harvested and the IL-6 protein content of the supernatants was measured by ELISA. The results were expressed as the percentage of the total IL-6 produced by osteoblasts in the absence of blood cells.

**RESULTS**

We report here that IL-6 and, to a lesser degree, LIF, but not G-CSF, GM-CSF, and transforming growth factor-beta (TGF-beta) are specifically secreted by osteoblasts in response to hematopoietic progenitor cells. The maximal accumulation of secreted IL-6 occurs during the initial 24-hour period of coculture and is correlated with human CD34+ bone marrow cell number. Furthermore, cell-to-cell contact does not appear to be required for this activity. The identity of the soluble activity produced by the CD34+ cells remains unknown, but is not likely due to IL-1beta or tumor necrosis factor-alpha, as determined with neutralizing antibodies. To our knowledge, these data represent the first demonstration that normal hematopoietic progenitor cells induce the production of molecules required for the function of normal bone marrow microenvironments, in this case through the induction of hematopoietic cytokine (IL-6) secretion by osteoblasts.

© 1997 by The American Society of Hematology.

From the Department of Periodontics, Prevention, Geriatrics, University of Michigan School of Dentistry, Ann Arbor, MI; and the Departments of Internal Medicine and Pediatrics, University of Pennsylvania, Philadelphia, PA.

Submitted March 13, 1996; accepted September 26, 1996.

Supported in part by National Institutes of Health Grants. S.G.E. is supported by a Scholar Award from the Leukemia Society of America.

Address reprint requests to Russell S. Taichman, DMD, DMSc, Department of Periodontics, Prevention, Geriatrics, University of Michigan School of Dentistry, 1011 N University Ave, Ann Arbor, MI 48109-1078.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1997 by The American Society of Hematology.

0006-4971/97/8904-0022$3.00/0

land, NY), antibiotics, 10 mmol/L β-glycerol phosphate, and 10 mg/mL L-ascorbate. To verify that the cells expressed an osteoblast phenotype, the cultures were screened for the expression of the osteoblast-specific protein osteocalcin (osteocalcin5) and the absence of c-kit ligand (c-kit ligand7) by reverse transcriptase polymerase chain reaction (RT-PCR), as previously detailed.4 Using morphologic and RNA criteria, we can detect contamination of our osteoblast preparations by marrow stromal elements to levels of 1% of the total population (data not presented).

Isolation of human CD34+ 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’s Investigational Review Board. Mononuclear cells were isolated by density separation on Ficoll-Hypaque (specific gravity, 1.077). Plastic adherence

Tris, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl2, and 0.01% gelatin, Hialeah, FL). size, 548 bp 18), as previously detailed.4 The samples underwent ther-

in situ with 2% paraformaldehyde in phosphate-buffered saline for G-CSF (sensitivity, 7.2 pg/mL; range, 39 to 2,500 pg/mL), GM-CSF (sensitivity, 1.5 pg/mL; range, 7.8 to 5,000 pg/mL; R&D Systems), IL-6 (sensitivity, 0.1 pg/mL; range, 3.9 to 250 pg/mL; R&D Systems), TGF-

of all osteoblast/blood cell coculture. Where indicated, serum concentrations were reduced from 10% to 1% (vol/vol).

To determine whether viable osteoblasts were required for the production of cytokines, osteoblasts grown to confluence were fixed in situ with 2% paraformaldehyde in phosphate-buffered saline for 10 minutes at 25°C. Subsequently, the osteoblast cell monolayers were washed four times with medium before the initiation of coculture experiments. For direct cell-cell contact investigations, CD34+ bone marrow cells were seeded onto the osteoblast monolayers. Thereafter, conditioned medium was collected over the first 96 hours of the coculture experiments. For direct cell-cell contact investigations, CD34+ bone marrow cells that stimulates IL-6 production by human osteoblasts, IL-1β and TNF-α neutralizing monoclonal antibodies (murine IgG monoclonal; Genzyme Corp, Cambridge, MA), isotype-matched control (MOPC-21; Sigma, St Louis, MO), or vehicle were added daily to a final concentration of 10 µg/mL.

Production of bone marrow CD34+ cell-conditioned medium. CD34+ bone marrow cells (1 × 105) were seeded into the top chamber of TransWell (Corning Costar Corp, Cambridge, MA) dual-chambered 24-well plates (0.4-µm pore size), with confluent primary human osteoblasts in the bottom chamber. To determine whether IL-1β or TNF-α was responsible for the soluble activity produced by the CD34+ cells that stimulates IL-6 production by human osteoblasts, IL-1β and TNF-α neutralizing monoclonal antibodies (murine IgG monoclonal; Genzyme Corp, Cambridge, MA), isotype-matched control (MOPC-21; Sigma, St Louis, MO), or vehicle were added daily to a final concentration of 10 µg/mL.

Production of bone marrow CD34+ cell-conditioned medium. CD34+ bone marrow cells (1 × 105) were seeded into the top chamber of TransWell (Corning Costar Corp) dual-chambered 24-well plates (0.4-µm pore size) with 0.7 mL total of a 1:1 mixture of DMEM/F12 medium supplemented with 10% fetal calf serum, Pen/Strep, 10 mmol/L L-ascorbate, and 10 mg/mL β-glycerol phosphate in 24-multiwell culture plates. At 24, 48, and 72 hours, the conditioned medium was harvested, spun at 12,000 rpm at 4°C for 15 minutes, and frozen at −80°C.

RT-PCR. Total cellular RNA was recovered by lysing cells directly in Stat-60 according to the directions of the manufacturer (Tel-Test Inc, Friendswood, TX). RNA integrity and purity was evaluated by electrophoresis with ethidium bromide and absorbance at A260/A280. RNA (1.0 µg), 10 × RT buffer (1 × RT buffer: 50 mmol/L Tris HCl, pH 8.3, 50 mmol/L KCl, 8.0 mmol/L MgCl2, and 10 mmol/L dithiothreitol), 25 mmol/L dXTP mix (25 mmol/L of each dXTP [adenosine, cytosine, thymidine, guanine (ACGT)], 3.0 µg oligo d(T), and 2.5 U reverse transcriptase (M-MLV Reverse Transcribe-

scriptase; Life Technologies, Grand Island, NY) were incubated to-
gather at 38°C for 1 hour. One-fifth of the double-stranded product was mixed with 10× Taq buffer (1 × Taq/RT buffer: 10 mmol/L Tris, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.01% gelatin, and 2.0 mmol/L dithiothreitol), 1 mmol/L dXTP mix, 500 ng of each sense and antisense oligonucleotide, and 2.5 U Taq polymerase (Taq polymerase; Gibco-BRL). Sense and antisense primers were prepared by the oligonucleotide synthesis core at the University of Michigan and were designed to cross intron/exon boundaries: c-kit ligand (sense, GAGGGGATCTGGAGAAATCGTGTG; antisense, GCCCTTTGAACACCTGCGTCTC; expected size, 665 bp), osteocalcin (sense, GGCGACGGAGTARGAAGAG; antisense, GATGTTGTCGCCAACCTGT; expected size, 137 bp), IL-6 (sense, GGATTTGAGTCTTCACACGGCCCTGGTCACA; antisense, AAGGTTGTTCCTCTACTACTCTCAAATGTGTTCTG; expected size, 398 bp), actin (sense, GTCGGCGGCCCTGAGCA-CCA; antisense, CTCCCCATGTCACGGACAGTCTTC; expected size, 548 bp), as previously detailed.4 The samples underwent ther-
mals at 94°C for 1 minute and at 72°C for 3 minutes for 35 cycles, followed by 10 minutes of extension at 72°C (Perkin Elmer Cetus DNA thermal cycler; Perkin Elmer Cetus, Norwalk, CT). The products were electrophoresed in 3% agarose and visualized using ethidium bromide. To control for false-positives due to overamplification or DNA contamination, reverse transcriptase was omitted from the reaction (data not presented) and primers were designed to cross intron/exon boundaries.

Cytokine enzyme-linked immunosorbent assays (ELISAs). Conditioned medium was collected at the times indicated and stored at −80°C until assayed for cytokine levels by double-antibody sandwich method with commercially available ELISA kits according to the directions of the manufacturer: IL-1β (sensitivity, 0.1 pg/mL; range, 3.9 to 250 pg/mL; R&D Systems, Minneapolis, MN), IL-6 (sensitivity, 0.7 pg/mL; range, 3.33 to 300 pg/mL; R&D Systems), G-CSF (sensitivity, 7.2 pg/mL; range, 39 to 2,500 pg/mL), GM-CSF (sensitivity, 1.5 pg/mL; range, 7.8 to 5,000 pg/mL; R&D Systems), LIF (sensitivity, 2.0 pg/mL; range, 31 to 2,000 pg/mL), R&D Systems), TGF-β1 (sensitivity, 0.05 ng/mL; range, 0.1 to 4 ng/mL; Genzyme), and TNF-α (sensitivity, 3 pg/mL; range, 6 to 1,024 pg/mL; Genzyme). Cytokine levels are presented as the mean ± standard error cytokine per milliliter for triplicate determinations.

Statistical analyses. Each experiment was repeated a minimum of three times. Analysis of variance (ANOVA) was used to determine statistical significance.

RESULTS

The effect of human bone marrow CD34+ cells on cytokine synthesis by human osteoblasts. In previous investigations, we have shown that normal human osteoblasts constitutively produce a variety of cytokine mRNAs under basal conditions.4 In the present investigations, we examined whether untransformed human osteoblast-like cells would respond to normal hematopoietic cells by augmenting their secretion of soluble G-CSF, GM-CSF, IL-6, LIF, and TGF-β1. We chose
these particular cytokines as representatives of proteins with largely stimulatory (IL-6, GM-CSF, and G-CSF) and inhibitory (LIF and TGF-β) activity on hematopoietic cells.\textsuperscript{10,11} Explant-derived osteoblast monolayers were seeded with CD34\textsuperscript{+} bone marrow cells for 4 days in Ca\textsuperscript{2+} replete Ham’s F12/DMEM (1:1 vol/vol) containing 10% heat-inactivated FBS, antibiotics, 10 mmol/L β-glycerol phosphate, and 10 mg/mL L-ascorbate. Under these conditions, we have previously determined that osteoblasts maintain hematopoietic progenitors and LTC-IC activity for at least 2 weeks in vitro.\textsuperscript{12}

Over a 96-hour period, we observed that CD34\textsuperscript{+} cells had no significant effect on the production of osteoblast-derived, soluble G-CSF to the limits of the assay (7.2 pg/mL; Fig 1). During the same period, no significant alterations in the levels of GM-CSF produced were noted, and the production of TGF-β\textsubscript{1} was also not significantly altered (Fig 1). In contrast, the presence of CD34\textsuperscript{+} cells induced osteoblasts in some experiments to secrete enhanced levels of LIF. However, more striking was the effect that CD34\textsuperscript{+} cells had on IL-6 levels. Under basal conditions (no CD34\textsuperscript{+} cells), osteoblasts produced levels of IL-6 that were easily detected by the ELISA, ranging from 67 to 137 pg/mL under the 10% serum conditions. When CD34\textsuperscript{+} cells were placed in direct contact with the osteoblasts, an average increase in IL-6 production of 222% ± 55% (range, 135% to 288%) was observed (Fig 1). To verify that CD34\textsuperscript{+} bone marrow cells and not a small number of contaminating cells were responsible for the IL-6 stimulation, CD34\textsuperscript{+} cells were isolated by positive immunomagnetic selection and further purified by FACS using a phycoerythrin conjugate of the anti-CD34 antibody, HPCA-2 (Becton Dickinson). Under these conditions, the FACS-sorted CD34\textsuperscript{+} cells exhibit the same IL-6 stimulatory activity on human osteoblasts as do the immunoselected cells (Table 1).

To determine the time frame in which IL-6 synthesis oc-
continued to accumulate but were not significantly different from the initial 24-hour period. The inclusion of CD34+ bone marrow cells significantly increased the levels of IL-6 detected at all time points relative to the osteoblasts alone (Fig 2). Curiously, after the initial 24 hours, the IL-6 levels plateaued rather than continuing to accumulate, possibly representing consumption by the CD34+ cells. In addition, IL-6 levels in vitro correlated with increasing CD34+ cell numbers (Fig 3).

**Cell-to-cell contact between CD34 cells is not required for increased IL-6 synthesis.** To evaluate whether direct contact between osteoblasts and hematopoietic cells is required for the augmented production of IL-6, CD34+ bone marrow cells were seeded either directly onto osteoblast monolayers or into the top chamber of dual-chambered culture plates that facilitate the free exchange of soluble molecules but physically separate the heterologous populations. As reported previously, significant increases in IL-6 production were observed when osteoblasts were cultured with CD34+ cells (Fig 4). Under conditions in which osteoblasts and hematopoietic cells were separated, a significant, albeit somewhat smaller increase in IL-6 production was observed relative to controls. These results suggest that either the osteoblasts or the hematopoietic cells produce soluble factor(s) that mediates the production of IL-6 by human osteoblasts (Fig 4).

**Osteoblasts are primarily responsible for IL-6 synthesis in coculture with CD34+ bone marrow cells.** Alone, osteoblasts constitutively produce IL-6, but in coculture with CD34+ bone marrow cells, increased levels of IL-6 are observed. We next wanted to determine which of the cell populations were responsible for the enhanced IL-6 synthesis. We therefore determined whether viable osteoblasts were required for the augmented IL-6 levels. For these experiments, 2% paraformaldehyde was used to fix the osteoblasts before the initiation of the cocultures. As shown in Fig 4, alone or in the presence of fixed osteoblasts. CD34+ bone marrow cell-conditioned medium contained no immunodetectable IL-6.

To rule out the possibility that osteoblasts furnish signal(s) to the hematopoietic cells that indirectly stimulates the hematopoietic cells to produce IL-6, two types of experiments were performed. First, RT-PCR was performed to detect IL-6 mRNA on cells recovered from dual-chambered plates in which IL-6 protein levels are significantly elevated relative to osteoblasts cultured alone (Fig 4). As shown in Fig 5, IL-6 message was observed (1) in osteoblasts alone, (2) in osteoblasts and CD34+ cultured in direct contact, and (3) in osteoblasts isolated from the bottom of the dual-chambered culture wells. IL-6 mRNA was not observed in CD34+ bone marrow cells isolated from the top of the dual-chambered culture wells in which enhanced IL-6 protein synthesis was observed (Fig 4). In the second type of experiment, CD34+ cell-conditioned medium was collected and placed on osteoblasts. Under these conditions, CD34+ cell-conditioned medium alone stimulated augmented IL-6 production by the human osteoblasts (Fig 6).

**IL-1β and TNF-α production by CD34+ cells is not responsible for increased IL-6 synthesis by human osteoblasts.** We next hypothesized that IL-1β or TNF-α was responsible for the soluble activity produced by the CD34+ cells that stimulates IL-6 production by human osteoblasts. To address this issue, conditioned CD34+ cell medium was assayed by ELISA to determine to what level IL-1β and TNF-α were present in the culture supernatants. IL-1β levels were below the level of detection under the conditions of the assay (assay range, 3.9 to 250 pg/mL). Similarly, we found that TNF-α levels were less than 6 pg/mL (assay range, 6 to 1,024 pg/
**CD34+ CELLS STIMULATE OSTEOBLAST IL-6 SYNTHESIS**

Fig 5. RT-PCR detection of IL-6 mRNA. RT-PCR was performed (35 cycles) to detect mRNA for β-actin and IL-6 from cells recovered from dual-chambered plates at 96 hours in which IL-6 protein levels were known. Primers were designed to cross intron/exon boundaries. Negative controls included omitting reverse transcriptase (not shown) from the reverse transcription reaction or using H2O. Results from two independent experiments are presented. mRNA from osteoblasts only (HOB), mRNA from osteoblasts and CD34+ cells in coculture (contact), mRNA from osteoblasts recovered from dual-chambered coculture (HOB noncontact), and mRNA from CD34+ cells recovered from dual-chambered coculture (CD34+ non-contact).

![Graph showing IL-6 production](image)

**DISCUSSION**

Because events localized to endosteal surfaces are probably critical for the maintenance of early hematopoietic cells in vivo, we have begun to explore this unique tissue compartment at the cellular and molecular level with several models of endosteal hematopoiesis. Based on the knowledge that hematopoiesis is facilitated by close associations with cells of the stromal cell microenvironment and that osteoblasts are present on endosteal surfaces, we hypothesized that affiliations between osteoblasts and hematopoietic cells might stimulate production of factors required for normal hematopoiesis. In the present study, we examined the ability of normal human CD34+ bone marrow progenitor cells for their ability to stimulate the production of cytokines by primary human osteoblasts.

We determined that human CD34+ hematopoietic bone...
marrow progenitors stimulate human osteoblasts to produce IL-6 but not soluble levels of G-CSF, GM-CSF, or TGF-β1 to the limits of the assays. The augmented production of IL-6 over basal levels correlated well with the CD34+ cell number where the most rapid rate in protein accumulation occurred during the initial 24-hour period. Based on RT-PCR and in situ fixation, it seems unlikely that the blood cells themselves were directly responsible for the augmented IL-6 production. However, we determined that CD34+ cells are metabolically engaged in the production of IL-6, because CD34+ cells that are prevented from direct contact with osteoblasts stimulate the secretion of IL-6 to levels roughly comparable to those in which direct contact is permitted. The nature of the molecules/signals produced by CD34+ cells that stimulate osteoblast-derived IL-6 remains to be determined.

Previous studies have shown that osteoblasts produce IL-6, but its role in mineralized tissue metabolism remains uncertain. Under basal conditions, most human osteoblasts examined produce IL-6 levels that can be enhanced by steroids or proinflammatory mediators, including IL-1 or TNF with marked synergism. For these reasons, we evaluated whether CD34+ cells stimulate IL-6 production by IL-1β or TNF-α-dependent pathways. We found that the activity produced by CD34+ cells is not likely due to IL-1β or TNF-α. Because IL-6 is believed to uncouple bone resorption from bone formation by activating osteoclast activity while inhibiting osteoblastic activity, determining the identity of the CD34+ cell-derived activities may be of great clinical importance.

Like those effects observed on mineralized tissue, IL-6 has multiple activities on hematopoietic cells. Alone, IL-6 has some proliferative activity on early hematopoietic cells, but, in combination with IL-3, IL-1, and c-kit ligand, it exerts synergistic activity by recruiting cells out of G0 and promoting the transition to G1. On other populations, IL-6 facilitates the development of burst-forming unit-erythroid and multipotent colony-forming units (colony-forming unit granulocyte, erythroid, monocyte, megakaryocyte) from hematopoietic progenitors. IL-6 is also a critical regulator of B-cell differentiation into plasma cells. Furthermore, IL-6 is a potent growth factor for plasmacytoma and myeloma cells in which overproduction of IL-6 is associated with progressive disease. Although both autocrine and paracrine IL-6 production has been reported, in most cases it appears that cells of the tumor microenvironment are largely responsible for IL-6 synthesis rather than the tumor cells. For example, Barillé et al. showed that coculture of XG1 and XG6 myeloma cell lines induces the production of IL-6 by several human osteosarcoma cell lines (MG-63 and SaOS-2), possibly through activation of an NF-kB pathway. However, curiously, the production of an osteblast-specific protein (osteocalcin) was reduced in an autocrine-like manner as anti-IL-6 antibodies reversed the decreases in osteocalcin synthesis. That stimulated osteoblast synthesis of IL-6 can be viewed as a generalized feature of osteoblast-hematopoietic metabolism and not restricted to B-cell neoplasms alone is suggested by the demonstration that some human osteosarcomas (HOS cells) secrete IL-6 in response to the adherence of human T cells and/or by crosslinking intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. Thus, the observations that (1) osteoblasts stimulate myeloma cell proliferation by producing IL-6 in response to the tumor cells themselves, while at the same time (2) IL-6 inhibits bone formation in an autocrine fashion suggest that the relationships we have observed among normal cells can be co-opted by tumor cells.

Recent experimentation suggests that close associations between hematopoietic cells and stromal cells are important for maintaining hematopoiesis in vivo, although they are probably not an absolute requirement in vitro. When hematopoietic cells are in direct contact with stromal cells, cell-to-cell adhesive molecules, stromal cell-associated or extracellular matrix-associated factors, and/or soluble growth factors probably all cooperate in stimulating blood cell proliferation and/or maintenance. The importance of this relationship is illustrated by the observation that, if the two tissues are separated by more than a few millimeters in vitro, a decline in LTC-IC and progenitor cell populations may ensue. The basis for stromal cell support of hematopoietic cells that are not in intimate contact with the parenchymal cells of the marrow is not clear but may involve high molecular weight proteoglycans. Alternatively, these observations suggest that molecules critical for hematopoiesis are either short-lived and/or require high local concentrations for function. The nature of those molecules responsible for the enhanced IL-6 production remains to be determined.

In summary, we determined that CD34+ bone marrow cells had no significant effect on osteoblast-secretion of G-CSF, GM-CSF, and TGF-β1. When CD34+ bone marrow cells were cocultured with osteoblasts, augmented IL-6 synthesis was observed. The accumulation of IL-6 was most rapid during the initial 24-hour period and cell-to-cell contact does not appear to be required for this activity. As important as de novo cytokine synthesis by stromal cells or osteoblasts may be after blood cell binding, it will be difficult to differentiate experimentally between these tissue interactions from activities generated from the engagement of receptor-counterreceptor molecules on the hematopoietic cells themselves. Nevertheless, local resident cells responding to the requirements of hematopoietic cells represent hematopoietic regulation of the bone marrow microenvironment. Our finding that CD34+ hematopoietic cells induce IL-6 production by osteoblasts, not necessarily requiring direct cell-cell contact, suggests that the interaction between these two cell types may be similarly coupled as are those between other members of the stromal cell family and blood cells. To our knowledge, these data represent the first demonstration that normal hematopoietic progenitor CD34+ cells induce the production of hematopoietic growth factors, in this case IL-6, by cells of the bone marrow microenvironment.

ACKNOWLEDGMENT

The authors are indebted to Dr. T. Frank and the members of the Surgical Pathology Department for their help in obtaining bone tissues, to Dr. M. Baird and M. Tuck for bone marrow, and to Drs. J.A. D’Errico and G.H. Sam for helpful discussions.

REFERENCES


35. Zheng MH, Wood DJ, Wysocki S, Papadimitriou JM, Wang...


