The Cell-surface Form of Colony-stimulating Factor-1 Is Regulated by Osteotropic Agents and Supports Formation of Multinucleated Osteoclast-like Cells*

(Received for publication, August 12, 1997, and in revised form, November 17, 1997)

Gang-Qing Yao‡, Ben-hua Sun§, Elizabeth E. Hammond‡, Elizabeth N. Spencer‡, Mark C. Horowitz‡, Karl L. Insogna§, and Eleanor C. Weir‡

From the ‡Section of Comparative Medicine, the §Department of Internal Medicine, and the ¶Department of Orthopedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut 06520

Colony-stimulating factor-1 (CSF-1) is a hematopoietic growth factor that is released by osteoblasts and is recognized to play a critical role in bone remodeling in vivo and in vitro. CSF-1 is synthesized as a soluble or cell-surface protein. It is unclear, however, whether human osteoblasts express both molecular forms of CSF-1, and whether these isoforms can independently mediate osteoclastogenesis. In the present study, using a combination of quantitative reverse transcriptase polymerase chain reaction, flow cytometry, and Western immunoblot analysis, we have demonstrated that human osteoblast-like cells as well as primary human osteoblasts express the cell-surface form of CSF-1 both constitutively and in response to parathyroid hormone and tumor necrosis factor. Furthermore, using an in vitro coculture system, we have shown that cell-surface CSF-1 alone is sufficient to support osteoclast formation. These findings may be especially significant in view of evidence that direct cell-to-cell contact is critical for osteoclast formation, and suggest that differential regulation of expression of the CSF-1 isoforms may influence osteoclast function modulated by osteotropic hormones.

The precise mechanism whereby osteoblasts mediate osteoclastic bone resorption is unclear. One widely held hypothesis is that activated osteoblasts secrete cytokines that directly or indirectly influence osteoclast formation or function (1). Although the exact nature of all of these cytokines is unknown, compelling in vivo and in vitro data have emerged to support a role for colony-stimulating factor-1 (CSF-1) as an osteoblast-derived factor involved in osteoclast formation. Thus, in vivo, deficiency of CSF-1 in the op/op osteopetrotic mouse causes a failure of osteoclast formation and bone resorption (2–4), while in vitro studies have demonstrated that CSF-1 is critical for the proliferation and differentiation of osteoclast progenitors (5, 6), that CSF-1 stimulates bone resorption in the fetal mouse metacarpal assay (7), and that CSF-1 receptors are present on osteoclasts (8, 9). Additionally, we have reported that CSF-1 is the principal colony-stimulating activity released from osteoblasts constitutively and in response to PTH and parathyroid hormone-related protein (8). In support of a role for CSF-1 in bone remodeling in humans, Sarma et al. (10) have recently reported that, consistent with studies in mice, recombinant human CSF-1 induces osteoclastogenesis and bone resorption in human marrow cultures.

Multiple human CSF-1 mRNA species (4.0, 3.0, 2.3, 1.9, and 1.6 kb) are expressed by the CSF-1 gene (11–15), and molecular cloning of cDNAs derived from these transcripts has demonstrated that the size differences are due to alternative splicing in exon 6 and the alternative use of the 3’-end exons 9 or 10 (11–13). A combination of nucleotide sequence analysis and transfection studies indicates that two distinct CSF-1 protein products are encoded by these transcripts. Both primary translation products are membrane-bound glycoproteins that are released by proteolysis (16). The 1.6- and 3.0-kb CSF-1 cDNAs, however, give rise by alternative splicing to a short exon 6, yielding a cell-surface or membrane-bound form of CSF-1, which lacks a proteolytic cleavage site and is therefore slowly and inefficiently released by extracellular proteolysis (15, 17), whereas the products of the 1.9-, 2.3-, and 4.0-kb cDNAs are rapidly secreted as soluble growth factors (16, 18). Both soluble and cell-surface CSF-1 support macrophage proliferation in vitro (16, 19), and mRNAs encoding both forms are expressed in human endometrial glands and placenta (15, 20). Whether the molecular forms of CSF-1 differ in their physiologic effects is, however, still unclear.

It is well recognized that osteoblasts synthesize and release the soluble form of CSF-1 both constitutively and in response to osteotropic agents, (9, 21–24) and that this form of CSF-1 stimulates osteoclast formation in vivo and in vitro (5, 6, 25). Recent studies indicate that murine osteoblasts also express the cell-surface form of CSF-1 (26, 27), but the physiologic relevance of these observations is unclear. Specifically, it is unknown whether primary human osteoblasts or osteoblast-like cells express cell-surface CSF-1. Additionally, although PTH and TNF have been shown to increase expression of the soluble form of CSF-1 in osteoblasts (9, 24), it is unclear whether they regulate expression of the cell-surface form of CSF-1. Finally, it is unknown whether cell-surface CSF-1 expressed in osteoblasts has any physiologic role in bone remodeling.

In the present study, using a combination of quantitative reverse transcriptase PCR and flow cytometry, we have shown...
that primary human osteoblasts and osteoblast-like cells express mRNA and protein species consistent with the cell-surface form of CSF-1, and that expression is regulated by PTH and TNF. In addition, we report that the cell-surface form of CSF-1 supports the formation of multinucleated osteoclast-like cells in an in vitro co-culture system.

**EXPERIMENTAL PROCEDURES**

**Hormones, Antibodies, and Reagents**—Human recombinant TNFα was purchased from Sigma, Human and bovine PTH-(1–34), PTH-(1–84), and PTH-(7–34) were purchased from Bachem (Torrance, CA). A neutralizing antibody against recombinant human CSF-1 was purchased from R&D Systems (Minneapolis, MN), and an antibody raised in rabbits against partially purified human CSF-1 was kindly provided by Dr. E. R. Stanley (Albert Einstein College of Medicine, New York, NY). Prostaglandin E2 was purchased from Sigma and 1,25-dihydroxy vitamin D3 (1,25-(OH)2D3) from Wako, Richmond, VA.

**Cell Culture**—Normal primary human osteoblasts were obtained as described previously from patients who were undergoing surgery after accidental injury or for spinal correction or hip replacement (28). Primary human osteoblasts and the osteosarcoma cell line MG63 were maintained in Eagle’s α-minimum essential medium (Sigma) containing 10% fetal bovine serum (FBS, Life Technologies, Inc.). Saos-2 human osteosarcoma cells were maintained in RPMI 1640 (Sigma) containing 10% FBS, penicillin, and streptomycin. Osteoblast cell lines were passages with trypsin every week and studied at least 3 days post-confluence unless otherwise indicated. Primary osteoblasts were passaged with trypsin when confluent and were used after the second passage. NIH3T3 cells transfected with the 1.6- and 4.0-kb cDNAs encoding the cell-surface and soluble forms of CSF-1, respectively (18), were kindly provided by Dr. Carl Rettenmier (Children’s Hospital, Los Angeles, CA). These cells were maintained in Dulbecco’s modified Eagle’s medium containing 7% FBS, 1% L-glutamine, and penicillin/streptomycin as above. All cells were cultured at 37 °C in 95% air and 5% CO2.

**RNA Preparation**—Cells were grown in T-25 tissue culture flasks and in aliquots at 20 °C. To control for differences in quantity of RNA in untreated and treated samples, endogenous GAPDH and amylase (Molecular Dynamics Personal Densitometer, SI). For quantitative analysis of samples, the amount of competitive template added to the sample before amplification was plotted against the target to competitor ratio. An unknown amount of specific mRNA can be calculated according to the equation: 

$$ D^0 = D / C \times C^0 $$

where $D^0$ is the initial amount of target cDNA, $D$ is the amount of target DNA obtained from cPCR, and $C$ and $C^0$ are, respectively, the initial amount of competitor and the amount obtained from cPCR (31). Thus, when $D/C = 1$, then $D^0 = C^0$, i.e. when target DNA and competitor obtained from cPCR are equally amplified, as indicated by equal band intensity, the initial amount of target cDNA in the sample is equal to the amount of input competitor. Since a known amount of competitor is added to each reaction, the amount of cDNA in each sample can be calculated.

**Preparation of Competitive Templates**—To control for differences in amplification efficiency between treated and untreated samples, in each amplification reaction, we included a competitive external standard that consisted of a shortened CSF-1 DNA fragment that competes for and is amplified by the same primer pair as the target sequence. This competitive template was prepared by amplifying the target CSF-1 cDNA sequence with the same 5’ primer but a recombinant 3’ primer to produce a shortened template (30). To prepare a competitive template for the cell-surface form of CSF-1, a 42-bp primer P6 was designed to include 21 bp of P2 sequence added to the 5’ end of a sequence corresponding to bases 666–686 of CSF-1 cDNA (Fig. 1). Thus, using P1 as the 5’ primer and P6 as the 3’ primer, 121 bp of the CSF-1 sequence was amplified. With the appended 21 bp of primer P2, this resulted in a 142-bp amplicon that included the P1 and P2 binding sequences at its 5’ and 3’ ends, respectively (Fig. 1). To prepare a competitor for the soluble form, primer P7 was designed to include 21 bp of primer P3 sequence added to the sequence corresponding to nucleotides 907–927. Thus, primers P1 and P7 generated a 383-bp competitor for the soluble form. Similarly, primers P4 and P8 were used to generate a 205-bp competitive template for GAPDH cDNA (Fig. 1B).

To prepare the competitive templates, total RNA prepared from MG63 cells was reverse-transcribed (RT) and amplified using the GeneAmp RT-PCR kit from Perkin-Elmer according to the recommendations of the manufacturer. Briefly, the reverse transcription reaction was performed at 37 °C for 60 min. and reverse transcriptase was inactivated by heating to 99 °C for 5 min, followed by cooling at 4 °C for 5 min. The PCR products were separated by electrophoresis in a 1.8% agarose gel. The 142-, 205-, and 383-bp amplicons were excised from the gel, purified with a QIAquick gel extraction kit (QIAGEN), and stored in aliquots at −20 °C.

**Competitive RT-PCR and Quantitation**—Total RNA prepared from untreated or PTH/TNF-treated primary osteoblasts or osteoblast-like cells was reverse-transcribed as described above, and, for each sample, serial dilutions of competitive template were added to cDNA prepared from 100 ng of total RNA. PCR was performed at a final concentration of 1 × PCR buffer, 3 mM MgCl2, 2.5 units of Taq DNA polymerase (Perkin-Elmer), 5 mM dNTP, and 100 pmol each of 32P-end-labeled or unlabeled 5’ and 3’ primers in a total volume of 50 μl. If unlabeled primers were used, 0.1 μl (10 μCi) of a [32P]dCTP (6000 Ci/mmol, Amersham Life Science, Inc.) was added to the reaction mixture. The reaction mixtures were heated for 2 min at 94 °C and amplified in a DNA Thermal Cycler (Perkin-Elmer). The amplification profile included denaturation at 94 °C for 1 min, primer annealing at 60 °C for 2 min, and extension at 72 °C for 3 min for 35 cycles, followed by a final 7 min of extension at 72 °C. The competitive PCR products were separated by electrophoresis through 12% polyacrylamide gel. Photographic copies were made by exposing the gel to Kodak film and band intensity for target and competitor bands was determined by densitometric scanning (Molecular Dynamics Personal Densitometer, SI). For quantitative analysis of samples, the amount of competitive template added to the sample before amplification was plotted against the target to competitor ratio. An unknown amount of specific mRNA can be calculated according to the equation: 

$$ D^0 = D / C \times C^0 $$

where $D^0$ is the initial amount of target cDNA, $D$ is the amount of target DNA obtained from cPCR, and $C$ and $C^0$ are, respectively, the initial amount of competitor and the amount obtained from cPCR (31). Thus, when $D/C = 1$, then $D^0 = C^0$, i.e. when target DNA and competitor obtained from cPCR are equally amplified, as indicated by equal band intensity, the initial amount of target cDNA in the sample is equal to the amount of input competitor. Since a known amount of competitor is added to each reaction, the amount of cDNA in each sample can be calculated.

**Flow Cytometry Analysis**—Cells grown in six-well plates were treated with/without PTH or TNF for 24 h. The cells were washed twice with cold PBS and harvested with a rubber policeman. After incubation with anti-CSF-1 antiserum at a dilution of 1:100 (20 min at 4 °C) the cells were washed twice with cold PBS and incubated for 20 min at 4 °C in a 1:100 dilution of fluoresceinated secondary antibody. Flow cytometry was performed using a fluorescence-activated cell sorting Vantage flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA).
The excitation wavelength was 488 nm, and the fluorescence was collected through a 530/30-nm band pass filter. At least 10,000 cells were analyzed for each sample.

Preparation of Plasma Membranes—Plasma membranes for immunoblot analysis were extracted as described previously (32). Briefly, treated and untreated cells in the exponential phase of growth were washed with cold PBS and incubated with lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml pepstatin A, and 0.5 μg/ml leupeptin) and allowed to swell for 15 min on ice. The cells were disrupted using a ground glass homogenizer in an ice bath, and the homogenate was centrifuged at 1000 g for 10 min to remove nuclear and cellular debris. The supernatant was overlaid on a 35% sucrose solution and centrifuged for 60 min at 20,000 g. The plasma membranes, which were found in a single band at the interface of the supernatant and sucrose, were collected and centrifuged for 60 min at 100,000 g. The pellets were resuspended and stored at −270 °C until used. The concentration of plasma membrane protein was determined using a commercially available kit by the manufacturer's recommended protocol (Bio-Rad protein assay kit).

Western Blot Analysis—Western analysis was performed as described previously, with minor modifications (33). Briefly, 50–80 μg of plasma membrane protein in sample buffer were heated at 100 °C for 5 min in the presence of 100 mM dithiothreitol to reduce homodimeric proteins, followed by separation on a 10% SDS-polyacrylamide gel. Proteins were then transferred to nitrocellulose and incubated at 4 °C overnight with rabbit anti-human CSF-1 antiserum (1:10,000) in TBST buffer (20 mM Tris, 137 mM sodium chloride, and 0.1% Tween 20) with 3% nonfat milk. After washing three times with TBST, the blots were incubated with peroxidase-labeled goat anti-rabbit antiserum (1:20,000) for 60 min. After washing three times, the blots were developed by electrochemiluminescence (Amersham Corp.).

Co-culture System—To examine whether the cell-surface form of CSF-1 supports formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells, an in vitro co-culture system was established in which osteoblasts, prepared from op/op mice, were co-cultured with murine bone marrow cells. To supply the osteoblast-derived CSF-1 otherwise missing from these co-cultures, unfixed or glutaraldehyde-fixed NIH 3T3 cells that had been transfected with cDNAs encoding the various isoforms of CSF-1 were also added.

Preparation of Primary Murine Osteoblasts—Heterozygous op/+ male and female mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and op/op progeny derived from these matings were identified by radiographing long bones (Faxitron cabinet x-ray system, Hewlett Packard, Norwalk, CT). Osteoblasts were prepared from op/op and wild-type calvariae by collagenase-dispase digestion as described previously (1). Cells were grown in α-minimum essential medium supplemented with 10% FBS, penicillin, streptomycin, 1-glutamine, and 20 mM HEPES (co-culture medium). Five days later, these cells were trypsinized and used for co-culture experiments.

**Glutaraldehyde Fixation of Transfected NIH 3T3 Cells—**Nontransfected NIH 3T3 cells or cells transfected with cDNA encoding the

---

**Table:**

| Primer | Direction of Extension | Sequence (5′-3′) | Corresponding Nucleotides |
|--------|------------------------|-----------------|--------------------------|
| P₁₅   | 3′-5′                  | AGCAGTGCGAACAGACGACTCC GCCGTGAGCATTCAGCAAGCC | 666-868 |
| P₁₅   | < primer 3 sequence | > < binding sequence | 908-927 |
| P₁₅   | < primer 5 sequence | > < binding sequence | 507-524 |

**Fig. 1.** A, scheme for the preparation of competitive templates used as external standards in each amplification reaction. The cell-surface form of CSF-1 is illustrated as an example. This competitive template was prepared by amplifying the target CSF-1 cDNA sequence with the same 5′ primer (P₁) but a recombinant 3′ primer (P₆), that included 21 bp of P₂ sequence (P₂ add.) addended to the binding sequence, b. Thus, using P₁ as the 5′ primer and P₆ as the 3′ primer, 121 bp of the CSF-1 sequence was amplified, which, with the appended 21 bp of primer P₂, resulted in a 142-bp amplicon that included the P₁ and P₂ binding sequences at its 5′ and 3′ ends, respectively. Competitive templates for the soluble form of CSF-1 and for GAPDH were prepared by a similar strategy (see “Experimental Procedures”). B, primers employed for preparation of competitive templates.
or transfected cells. In both cases, osteoblasts were allowed to attach
op/op
pared as described above from
culture medium in a 12-well tissue culture dish. In subsequent exper-
tration in well, 6 nM).
mice, with and without added recombinant human CSF-1 (final concen-
type mice and bone marrow co-cultured with osteoblasts from
fixed, dipped in NTB-2 photoemulsion (Eastman Kodak Corp., Roches-
ticles in co-culture were
8 M).
Cell-surface CSF-1 in Human Osteoblasts
Effect of PTH and TNF on Soluble and Cell-surface CSF-1 mRNA—We next examined whether PTH regulates osteoblast expression of cell-surface and soluble forms of CSF-1 by competitive RT-PCR analysis of total RNA prepared from untreated and PTH-treated Saos-2 cells. In these experiments, as decreasing amounts of competitor were added to a fixed amount of sample RNA, there was an increase in amplification of the endogenous product because of diminished competition between the competitor DNA and the sample DNA. As shown in Fig. 3A, for both soluble and cell-surface forms, equal amplification, as indicated by equal band intensity, was achieved at a higher concentration of competitor in the PTH-treated osteoblasts than in the untreated cells, indicating that expression of soluble and cell-surface CSF-1 mRNAs is higher in PTH-treated osteoblasts than in untreated cells. While GAPDH expression was slightly higher in PTH-treated than in untreated cells, indicating slight inequality in RNA amount between treated and untreated samples, this increase was substantially less than the PTH-induced up-regulation of CSF-1 expression (Fig. 3A). Densitometric analysis of these results and correction for RNA quantity by analysis of GAPDH expression indicated that, as shown in a representative experiment (Fig. 3B), PTH caused an approximately 12-fold increase in expression of the soluble and cell-surface forms of CSF-1.

To further examine PTH-induced expression of cell-surface CSF-1 mRNA, Saos-2 cells were treated with several concentrations of either PTH-(1–84) or PTH-(7–34) (10−8 m), and mRNA levels examined by quantitative RT-PCR as described above. As shown in Fig. 3C, we found that PTH-(1–84) caused a dose-dependent increase in expression, whereas PTH-(7–34) had no significant effect. Although PTH-(7–34) binds to the PTH receptor with high affinity, it is a weak stimulator of adenylate cyclase, thus suggesting that PTH-induced expression of cell-surface CSF-1 is a cyclic AMP-dependent (36).

Using the same techniques, we examined the effect of TNF on osteoblast expression of both forms of CSF-1. As shown in a representative experiment (Fig. 3D), TNF treatment of MG63 cells caused an approximately 7-fold increase in mRNA encoding both the soluble and cell-surface forms of CSF-1.

Effect of PTH and TNF on Osteoblast Expression of Cell-surface CSF-1 Protein—To examine whether PTH and TNF regulate expression of cell-surface CSF-1 protein, flow cytometry was performed on untreated osteoblasts and osteoblasts treated with PTH or TNF. To confirm that our antibody recognized the cell-surface form of CSF-1, we first examined NIH 3T3 cells transfected with cDNAs encoding the soluble and cell-surface forms of CSF-1. As expected, nontransfected NIH 3T3 cells showed no increase in fluorescence in the

**RESULTS**

**Osteoblast Expression of mRNAs Encoding the Soluble and Cell-surface Forms of CSF-1**—To examine whether osteoblast-like cells express mRNAs encoding the cell-surface and soluble forms of CSF-1, RT-PCR was performed on total RNA prepared from MG63 cells and PCR products were examined on an agarose ethidium bromide gel. As shown in Fig. 2, primer pair P1 and P3 resulted in a 528-bp ampiclon (lane 1), consistent with the soluble form of CSF-1, and primer pair P1 and P2 yielded a 186-bp product (lane 5), consistent with the cell-surface form. Although theoretically P1 and P2 would also be expected to give rise to a 1026-bp ampclon encoding the soluble form, this was not detected under these PCR conditions because of preferential amplification of the shorter (186-bp) se-
sequence (15, 20). GAPDH-specific primers P4 and P5 yielded a 256-bp ampclon (lane 3). As indicated above, to control for differences in amplification efficiency, the same primer pairs were used to amplify competitive templates, which were added to target DNA in each PCR reaction. Amplification of these competitive templates resulted in ampclons that represented shortened forms of each target DNA. Thus, in Fig. 2, lanes 2 and 6 show 382- and 141-bp products resulting from the amplification of competitor DNAs for the soluble and cell-surface forms of CSF-1, respectively, and lane 4 shows the 205-bp ampclon resulting from amplification of the GAPDH competi-
tor. The same experiment performed on RNA prepared from Saos-2 cells yielded identical results (data not shown). The sequence of each ampclon was confirmed by performing at least two restriction digests (data not shown).

**Effect of PTH and TNF on Soluble and Cell-surface CSF-1**

**Fig. 2. Amplification of the soluble and cell-surface forms of CSF-1, GAPDH, and their competitors by RT-PCR.** cDNA was prepared from MG63 total RNA by RT and subjected to PCR using the primers described in Table I and Fig. 1B. *Lanes 1* and *2* show the 528-bp product derived from the soluble form, and its 382-bp competitor, respectively, and *lanes 5* and *6* show the 186-bp cell-surface form and its 141-bp competitor. Amplification of GAPDH and competitor yield 256- and 205-bp products, respectively (*lanes 3* and *4*). PCR products were separated in a 1.8% agarose gel and stained with ethidium bromide.

Cell-surface form of CSF-1 or a truncated form of cell-surface CSF-1 were grown to 50% confluence in a 12-well tissue culture plate. For glutaraldehyde fixation, cells were washed with Hank’s balanced salt solution, fixed in 2.5% glutaraldehyde for 1 min, followed by the addi-
tion of 1.5% glycine. Cells were washed with Hank’s solution and incubated overnight in co-culture medium. Medium was aspirated and

**Effect of PTH and TNF on Osteoblast Expression of Cell-surface CSF-1 mRNA**—We next examined whether PTH regulates osteoblast expression of cell-surface and soluble forms of CSF-1 by competitive RT-PCR analysis of total RNA prepared from untreated and PTH-treated Saos-2 cells. In these experiments, as decreasing amounts of competitor were added to a fixed amount of sample RNA, there was an increase in amplification of the endogenous product because of diminished competition between the competitor DNA and the sample DNA. As shown in Fig. 3A, for both soluble and cell-surface forms, equal amplification, as indicated by equal band intensity, was achieved at a higher concentration of competitor in the PTH-treated osteoblasts than in the untreated cells, indicating that expression of soluble and cell-surface CSF-1 mRNAs is higher in PTH-treated osteoblasts than in untreated cells. While GAPDH expression was slightly higher in PTH-treated than in untreated cells, indicating slight inequality in RNA amount between treated and untreated samples, this increase was substantially less than the PTH-induced up-regulation of CSF-1 expression (Fig. 3A). Densitometric analysis of these results and correction for RNA quantity by analysis of GAPDH expression indicated that, as shown in a representative experiment (Fig. 3B), PTH caused an approximately 12-fold increase in expression of the soluble and cell-surface forms of CSF-1.

To further examine PTH-induced expression of cell-surface CSF-1 mRNA, Saos-2 cells were treated with several concentrations of either PTH-(1–84) or PTH-(7–34) (10−8 m), and mRNA levels examined by quantitative RT-PCR as described above. As shown in Fig. 3C, we found that PTH-(1–84) caused a dose-dependent increase in expression, whereas PTH-(7–34) had no significant effect. Although PTH-(7–34) binds to the PTH receptor with high affinity, it is a weak stimulator of adenylate cyclase, thus suggesting that PTH-induced expression of cell-surface CSF-1 is a cyclic AMP-dependent (36).

Using the same techniques, we examined the effect of TNF on osteoblast expression of both forms of CSF-1. As shown in a representative experiment (Fig. 3D), TNF treatment of MG63 cells caused an approximately 7-fold increase in mRNA encoding both the soluble and cell-surface forms of CSF-1.

**Effect of PTH and TNF on Osteoblast Expression of Cell-surface CSF-1 Protein**—To examine whether PTH and TNF regulate expression of cell-surface CSF-1 protein, flow cytometry was performed on untreated osteoblasts and osteoblasts treated with PTH or TNF. To confirm that our antibody recognized the cell-surface form of CSF-1, we first examined NIH 3T3 cells transfected with cDNAs encoding the soluble and cell-surface forms of CSF-1 (16–18). As expected, nontransfected NIH 3T3 cells showed no increase in fluorescence in the

**Cell-surface CSF-1 mRNA—** We next examined whether PTH regulates osteoblast expression of cell-surface and soluble forms of CSF-1 by competitive RT-PCR analysis of total RNA prepared from untreated and PTH-treated Saos-2 cells. In these experiments, as decreasing amounts of competitor were added to a fixed amount of sample RNA, there was an increase in amplification of the endogenous product because of diminished competition between the competitor DNA and the sample DNA. As shown in Fig. 3A, for both soluble and cell-surface forms, equal amplification, as indicated by equal band intensity, was achieved at a higher concentration of competitor in the PTH-treated osteoblasts than in the untreated cells, indicating that expression of soluble and cell-surface CSF-1 mRNAs is higher in PTH-treated osteoblasts than in untreated cells. While GAPDH expression was slightly higher in PTH-treated than in untreated cells, indicating slight inequality in RNA amount between treated and untreated samples, this increase was substantially less than the PTH-induced up-regulation of CSF-1 expression (Fig. 3A). Densitometric analysis of these results and correction for RNA quantity by analysis of GAPDH expression indicated that, as shown in a representative experiment (Fig. 3B), PTH caused an approximately 12-fold increase in expression of the soluble and cell-surface forms of CSF-1.

To further examine PTH-induced expression of cell-surface CSF-1 mRNA, Saos-2 cells were treated with several concentrations of either PTH-(1–84) or PTH-(7–34) (10−8 m), and mRNA levels examined by quantitative RT-PCR as described above. As shown in Fig. 3C, we found that PTH-(1–84) caused a dose-dependent increase in expression, whereas PTH-(7–34) had no significant effect. Although PTH-(7–34) binds to the PTH receptor with high affinity, it is a weak stimulator of adenylate cyclase, thus suggesting that PTH-induced expression of cell-surface CSF-1 is a cyclic AMP-dependent (36).

Using the same techniques, we examined the effect of TNF on osteoblast expression of both forms of CSF-1. As shown in a representative experiment (Fig. 3D), TNF treatment of MG63 cells caused an approximately 7-fold increase in mRNA encoding both the soluble and cell-surface forms of CSF-1.
presence of anti-CSF-1. Similarly, cells expressing the soluble form of CSF-1 or cells expressing a truncated form of cell-surface CSF-1, which lacks the membrane spanning domain and is rapidly secreted, showed no significant increase in fluorescence in the presence of anti-CSF-1 as compared with the same cells in nonimmune serum (Fig. 4A). By contrast, NIH3T3 cells expressing the cell-surface form of CSF-1 showed a significant increase in fluorescence compared with nonimmune serum (Fig. 4A).

Having shown that cell-surface CSF-1 was detectable using this technique, we examined untreated and PTH and TNF-treated osteoblasts by flow cytometry. As shown in Fig. 4B, untreated MG63 cells had higher fluorescence in the presence of anti-CSF-1 as compared with fluorescence in the presence of nonimmune serum, indicating constitutive expression of cell-surface CSF-1 in MG63 cells. Furthermore, TNF resulted in a 1.8-fold increase in fluorescence compared with untreated cells. While untreated Saos-2 cells showed only modest constitutive expression of cell-surface CSF-1, treatment with PTH caused a 2.6-fold increase in fluorescence (Fig. 4B). These findings indicate that, consistent with results of RT-PCR analysis, TNF and PTH increase expression of cell-surface CSF-1 protein in osteoblasts.

**Regulated Expression of Soluble and Cell-surface CSF-1 in Human Osteoblasts**

**Fig. 3.** Competitive PCR of reverse-transcribed RNA prepared from untreated and PTH-treated Saos-2 cells. A, cells were treated with vehicle or hPTH(1–84) (10⁻¹² M) for 24 h and harvested for RNA. RNA was reverse-transcribed, and, for each sample, serial 3-fold dilutions of competitive template were added to cDNA prepared from 100 ng total RNA. PCR was performed as described under “Experimental Procedures,” and products were separated on a 12% polyacrylamide gel. As decreasing amounts of competitor were added to the fixed amount of sample, there was an increase in amplification of the endogenous product. B, densitometric analysis of a representative experiment after correction for RNA quantity. Results are expressed as percentage of soluble and cell-surface CSF-1 mRNA levels in untreated cells. C, PTH causes a dose-dependent increase in cell-surface CSF-1 mRNA expression in Saos-2 cells. Cells were treated with vehicle alone, hPTH(1–84) (10⁻¹² to 10⁻⁸ M) or hPTH(7–34) (10⁻⁸ M) for 24 h and harvested for RNA. Quantitative RT-PCR and densitometry were performed as above. Results shown are the mean of three independent experiments, and are expressed as percentage of cell-surface CSF-1 mRNA levels in untreated cells. D, effect of TNF on expression of soluble and cell-surface CSF-1 mRNA levels in MG63 cells. Cells were treated with vehicle alone or TNF (20 ng/ml) for 24 h and harvested for RNA. Quantitative RT-PCR was performed as described above. Densitometric analysis of a representative experiment after correction for RNA quantity is shown. Results are expressed as percentage of soluble and cell-surface CSF-1 mRNA levels in untreated cells.
Primary Human Osteoblasts—Since MG63 and Saos-2 are transformed cell lines, we next examined nontransformed primary human osteoblasts for expression of soluble and cell-surface CSF-1. For these studies, we used primary human osteoblasts that have been well characterized for their osteoblastic phenotype (28). Consistent with our findings in MG63 and Saos-2 cells, competitive RT-PCR analysis of mRNA prepared from primary osteoblasts indicated that these cells constitutively express both forms of CSF-1 (Fig. 6A). Furthermore, RT-PCR analysis of untreated and TNF-treated cells revealed that, after correction for RNA quantitation, TNF induced an 8- and 4-fold increase in expression of the soluble and cell-surface forms of CSF-1, respectively. By contrast, PTH treatment of primary osteoblasts cells resulted in no increase in expression of either form (Fig. 6B).

Effect of Cell-surface CSF-1 on Formation of OCLs in Vitro—In preliminary experiments, we found that, after 7–14 days, bone marrow cells co-cultured with osteoblasts from wild-type mice in the presence of 1,25-(OH)2D3 and prostaglandin E2 formed TRAP-positive multinucleated cells. Similarly, in the same time period and under the same conditions, osteoblasts from op/op mice supported formation of TRAP-positive cells in the presence of recombinant human CSF-1 (6 nM) (data not shown).

We then co-cultured NIH3T3 cells expressing the cell-surface form of CSF-1 with mouse bone marrow cells, with and without 1,25-(OH)2D3 and prostaglandin E2, and found that, after 7–14 days, these cells failed to support OCL formation. We therefore reasoned that an osteoblast-derived moiety in addition to CSF-1 might be necessary for OCL formation. Using osteoblasts derived from op/op mice, which do not secrete CSF-1, as a source of this osteoblast-derived moiety, we found that NIH3T3 cells expressing the cell-surface form of CSF-1, when

**Fig. 4.** Flow cytometric analysis of CSF-1-transfected NIH3T3 cells and of untreated PTH/TNF-treated osteoblasts. A, parental NIH3T3 cells and the same cells transfected with cDNA encoding the soluble form of CSF-1, a truncated form of cell-surface CSF-1 that is rapidly secreted, and the cell-surface form of CSF-1 were harvested and incubated with anti-CSF-1 (1:100), followed by fluoresceinated secondary antibody (1:100). Only the cells expressing the cell-surface form of CSF-1 showed a significant increase in fluorescence compared with preimmune serum. B, MG63 cells were treated with vehicle alone or TNF (20 ng/ml) for 24 h and Saos-2 cells with vehicle or PTH (10−8 M) for 24 h. Cells were harvested and prepared for flow cytometric analysis as described above. Treatment with TNF and PTH was associated with an increase in fluorescence as compared with untreated cells.

**Fig. 5.** Western analysis of partially purified osteoblast cell membranes. Plasma membranes from untreated and PTH-treated Saos-2 cells and untreated and TNF-treated MG63 cells were partially purified and used for immunoblotting with anti-CSF-1 antiserum as described under “Experimental Procedures.” In both cases, CSF-1 species of around 32–35 kDa were detected, which increased in response to PTH/TNF treatment.
Cell-surface CSF-1 in Human Osteoblasts

A

CONTROL  PTH  TNF

cell-surface CSF-1 mRNA

Soluble CSF-1 mRNA

GAPDH

B

CSF-1 mRNA LEVELS (% control)

Soluble  Cell-surface  Soluble  Cell-surface

PTH-TREATED  TNF-TREATED

Fig. 6. Competitive PCR of reverse-transcribed RNA prepared from untreated and PTH/TNF-treated primary human osteoblasts. A, cells were treated with vehicle, hPTH-(1–84) (10⁻⁸ M), or TNF (20 ng/ml) for 24 h and harvested for RNA. RNA was reverse-transcribed and competitive PCR was performed as described in Fig. 3. B, densitometric analysis of a representative experiment after correction for RNA quantity. Results are expressed as percentage of soluble and cell-surface CSF-1 mRNA levels in untreated cells.

DISCUSSION

Although CSF-1 is derived from a single-copy gene, there is considerable heterogeneity in protein size and structure due to a combination of alternative mRNA splicing, glycosylation, and proteolytic processing (37). The multiple CSF-1 mRNA species identified in human and murine tissues arise as a result of alternative splicing in exon 6 and the alternative use of exons 9 and 10 in the 3’-untranslated region of the gene. The exon 9/10 splice variation gives rise to mRNA species that theoretically have varying stability due to the inclusion or exclusion of AU instability motifs (12). Alternative splicing in exon 6 yields membrane-bound precursors that differ in the rate and efficiency of cleavage from the cell membrane (16). Five CSF-1 cDNAs have been cloned, ranging in size from 1.6 to 4.0 kb. All of these share the same exons 1–5, 7, and 8, whereas the 1.6- and 3.0-kb cDNAs have a short version of exon 6 in which the site for proteolytic cleavage of the CSF-1 precursor has been spliced out (11–15). Thus, the product of the 1.6- and 3.0-kb cDNAs are membrane-bound glycoproteins that are slowly and inefficiently cleaved from the cell surface (15, 17). By contrast, the products of the 1.9-, 2.3-, and 4.0-kb cDNAs have intermediate or long versions of exon 6 in which the proteolytic cleavage site is intact, giving rise to a soluble, rapidly secreted growth factor (11, 14, 18). Several human and murine cell lines and tissues have been shown to express a variety of CSF-1 mRNA species, consistent with the soluble and cell-surface forms of CSF-1. Detailed analysis of these transcripts, of the CSF-1 protein isoforms that they encode, and of the factors that regulate their expression has, however, been limited, and their bioactivity and physiologic relevance is unclear.

We have reported previously that mouse and rat osteoblasts express a major 4-kb CSF-1 mRNA species and several smaller, less abundant transcripts (9, 24). While the 4-kb species is consistent with the cDNA encoding the soluble form of CSF-1, the nature of the smaller transcripts is unclear. In the present study, we have shown by RT-PCR analysis that primary human osteoblasts and osteoblast-like cell lines constitutively express mRNA species consistent with the soluble and cell-surface forms of CSF-1. In addition, using quantitative RT-PCR, we have demonstrated that treatment of osteoblastic cells with PTH and TNF increases expression of both isoforms. Since PCR amplification is an exponential process, sample to sample variation in quantity of input RNA, in conversion of RNA to DNA, or in amplification efficiency can affect the yield of the PCR product. We therefore chose a method for quantitative PCR that attempted to deal with each of these potential limitations (30). To control for variability in the amount of input RNA and in the efficiency of its conversion to cDNA, we used human GAPDH as an endogenous standard. To control for variability inherent in the amplification process, we constructed external standards that shared with the target sequence the same primer sites and most of the amplified sequence. Thus, the two templates competed for the same primer pair and amplified at the same rate, ensuring accurate relative quantitation. RT-PCR analysis appears to be required to detect cell-surface CSF-1 mRNA, since Northern analysis using region-specific probes was unable to identify RNA species for this form of CSF-1 (data not shown), indicating that the cell-surface form is present at lower levels than the soluble form. This finding is not unexpected, since the turnover rate of cell-surface CSF-1 protein on the cell membrane is slow, as compared with the soluble form, which is rapidly secreted and thus likely requires a higher rate of transcription (17). Furthermore, since the effector function of cell-surface CSF-1 is mediated locally by direct interaction with adjacent receptor-bearing cells, it is likely that the minimum concentration required for bioactivity is less than that of the soluble form, which is secreted and mediates its effector function(s) at distant sites.

In addition to demonstrating regulated expression of mRNA transcripts encoding the cell-surface form of CSF-1, the present study shows that osteoblasts express cell-surface CSF-1 pro-
tein. Thus, flow cytometric analysis revealed that MG63 cells constitutively express cell-surface CSF-1, and that expression is increased by TNF treatment. While untreated Saos-2 cells showed only modest expression of cell-surface CSF-1 protein, expression was enhanced by PTH. The low constitutive expression of cell-surface CSF-1 protein in these cells appears to conflict with the results of RT-PCR. This may, however, represent differences in the sensitivity of the two techniques. Results of Western analysis also confirmed that both MG63 and Saos-2 cells produce the cell-surface form of CSF-1, both constitutively and in response to PTH and TNF. The 32- and 35-kDa species that were detected in partially purified human osteoblast cell membranes are consistent with the 30-kDa species previously detected in membrane extracts from murine osteoblasts (27), and with the 31- and 34-kDa species detected in cell lysates prepared from NIH3T3 cells transfected with cDNA encoding the cell-surface form of CSF-1 (17). As was shown in the transfected cells, it is likely that the two protein

**Fig. 7. Effect of cell-surface CSF-1 on formation of OCLs in vitro.** A, TRAP staining of multinucleated cells formed when glutaraldehyde-fixed NIH3T3 cells transfected with the cell-surface form of CSF-1 were co-cultured for 7 days with op/op osteoblasts and mouse bone marrow cells. Fixed parental cells (B) and NIH3T3 cells transfected with a truncated form of cell-surface CSF-1 (C) failed to support the formation of OCLs in the presence of op/op osteoblasts. D, autoradiography of 125I salmon calcitonin binding when fixed cell-surface CSF-1-transfected NIH3T3 cells were co-cultured for 14 days with op/op osteoblasts and mouse bone marrow cells (E). Using the same methods, no calcitonin receptor-positive cells were detected with fixed parental cells. In all cases, co-cultures were performed in the presence of 1,25-(OH)2D3 and prostaglandin E2.
species detected in osteoblast membranes exhibit differing degrees of glycosylation. Since the soluble form of CSF-1 is rapidly released from cell membranes, protein species consistent with this form were not detected in osteoblast membranes by Western analysis, further suggesting a role for osteoblastic cell-surface CSF-1 in the immediate bone microenvironment. While the increase in CSF-1 protein levels associated with PTH and TNF treatment appears to be less than the induction of CSF-1 mRNA expression, this is likely to reflect differences in techniques used to evaluate levels of mRNA versus protein. RT-PCR is an exquisitely sensitive technique with a large dynamic range, whereas both flow cytometry and Western analysis are less quantitative, primarily because they are highly dependent on such factors as the affinity and specificity of the antibody. Additionally, we cannot rule out induction of nontranslated mRNA or rapid degradation of mRNA.

In support of the physiologic relevance of our observations in osteoblastic cell lines, we also demonstrated that primary human osteoblasts express mRNA species for cell-surface CSF-1 and that TNF enhances its expression to a degree comparable to that seen in TNF-treated MG63 cells. In contrast to Saos-2 cells, however, PTH caused no up-regulation of cell-surface or soluble CSF-1 in primary osteoblasts. While the cause of this apparent discrepancy is unclear, other investigators have also reported differences in PTH-induced cytokine production among osteoblast cell lines and primary osteoblasts (28). In the present study, the primary reason for the difference in PTH response between the Saos-2 cells and primary osteoblasts is most likely the weak PTH-induced cAMP response in the latter. We have performed adenylate cyclase assays using primary osteoblasts prepared from a number of patients, and have found little induction of cAMP in response to PTH (1–34). Thus since, as shown in Fig. 3C, PTH induction of CSF-1 expression in Saos-2 cells appears to be cAMP-dependent, it is not surprising that osteoblasts that exhibit a weak PTH-induced cAMP response fail to demonstrate PTH-induced CSF-1 expression. While weak PTH-induced cyclase activity in primary human osteoblasts has been reported previously (38, 39), the precise reason is unclear. It is well recognized that the degree of differentiation of osteoblasts influences expression of the PTH receptor (40), and it is likely that the age of patients from which our osteoblasts are prepared (i.e. adults), or the method of cell preparation (i.e. explant culture) may affect the degree of differentiation and PTH receptor expression. Additionally, the osteoblast preparation is a mixed population of cells, most likely representing cells at varying degrees of differentiation and with varying PTH receptor expression. Thus, the specific proportion of each cell type in each preparation affects the PTH response.

While others have reported that mouse osteoblasts express the cell-surface form of CSF-1 (27, 28), our observation that PTH and TNF regulate its expression in human osteoblast-like cells suggests that this isoform of CSF-1 may play a role in TNF-induced, and possibly PTH-induced, bone resorption. We and others have suggested previously that CSF-1 increases osteoclastogenesis in PTH- and TNF-induced resorption (9, 23, 24), but it is unclear from previous studies whether both the soluble and cell-surface forms are involved. Takahashi et al. (41) observed that when osteoblasts from op/op mice were co-cultured with splenocytes, pharmacologic amounts of CSF-1 were required to support osteoclast formation, and other studies have shown that much higher concentrations of soluble CSF-1 are required to induce osteoclastogenesis in metatarsals from op/op mice than in metatarsals/metacarpals from normal mice (7, 42). These findings suggest that cell-surface CSF-1, either alone or in addition to soluble CSF-1, might be required for osteoclastogenesis. Takahashi also observed that, using an osteoblast/splenocyte co-culture system, osteoblasts failed to support osteoclast formation when separated from precursors by a membrane filter, further suggesting that an osteoblast-derived cell-surface moiety is necessary for target cell activation (1). Consistent with these hypotheses, in the present study, we have shown that glutaraldehyde-fixed NIH3T3 cells transfected with cDNA encoding the cell-surface form of CSF-1 support formation of OCLs in an in vitro co-culture system. By contrast, parental NIH3T3 cells, or cells transfected with a truncated cDNA for cell-surface CSF-1 that does not bind to the cell membrane, failed to support formation of OCLs. Interestingly, we also demonstrated that NIH3T3 cells transfected with cell-surface CSF-1 failed to support osteoclast formation in the absence of op/op osteoblasts, indicating that cell-surface CSF-1 alone does not support osteoclast formation, and that other osteoblast-derived factors are required. Consistent with our results, Stein et al. (19) showed that cell-surface CSF-1 supports the proliferation of mouse mononuclear phagocytes in vitro. Bioactivity of this form of CSF-1 in other tissues known to express it, however, including human uterus and placenta, has not been reported.

As in other tissues, the precise roles of the soluble and cell-surface forms of CSF-1 in osteoblasts are unclear. While the absence of osteoclasts in the op/op mouse indicates a key role for CSF-1 in osteoclastogenesis, deficiency of osteoclasts and macrophages reverses with age (43). Although the mechanism is unknown, it has been hypothesized that other cytokines assume the osteoclastogenic function of CSF-1 in mature mice, or that unique, tissue-specific splice variants in the op/op mouse may lead to an incompletely penetrant phenotype and delayed development of the mononuclear system (44). Multiple CSF-1 splice variants are expressed in human and murine tissues, and in many cases their function within a specific tissue is unknown. Likewise, the functional significance of cell-surface CSF-1 in osteoblasts is unclear. Our data, however, which demonstrate regulated expression and bioactivity of cell-surface CSF-1 in bone, suggest that this isoform of CSF-1 interacts via cell-cell contact with adjacent receptor-bearing cells in the bone microenvironment to regulate osteoclast formation or function.

Acknowledgments—We thank Dr. Xiao-Dong Chen and Monica Andreoli for technical assistance and advice.

REFERENCES
1. Takahashi, N., Akatsu, T., Udagawa, N., Sasaki, T., Yamaguchi, A., Moseley, J. M., Martin, T. J., and Suda, T. (1988) Endocrinology 123, 2600–2602
2. Marks, S. C., and Lane, P. W. (1976) J. Hered. 67, 11–18
3. Wiktor-Jedrzejczak, W. A., Ahmed, A., Szylicka, C., and Skelly, R. R. (1982) J. Exp. Med. 156, 1516–1527
4. Wiktor-Jedrzejczak, W., Barteczki, A., Ferrante, A. W. Jr., Ahmed-Ansari, A., Sell, K. W., Pollard, J. W., and Stanley, E. R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4828–4832
5. Takahashi, N., Udagawa, N., Akatsu, T., Tanaka, H., Shinomone, M., and Suda, T. (1991) J. Bone Miner. Res. 6, 977–985
6. Tanaka, S., Takahashi, N., Udagawa, N., Tamura, T., Akatsu, T., Stanley, R. E., Kurokawa, T., and Suda, T. (1993) J. Clin. Invest. 91, 257–263
7. Antonioli-Corbus, V., Cechini, M., Felix, R., Fleisch, H., van der Pluijm, G., and Lowik, C. W. G. M. (1992) Endocrinology 130, 437–442
8. Holstetzer, W., Wettardler, A., Cechini, M., Felix, R., Fleisch, H., and Mueller, C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9637–9641
9. Weir, E., Horowitz, M. B., Centrella, M., Kacinius, B., and Inoue, K. (1993) J. Bone Miner. Res. 8, 1507–1517
10. Sarma, U., and Flanagan, A. M. (1996) Blood 88, 2531–2540
11. Kawasaki, E. S., Ladner, M. B., Wang, A. M., Van Ardsell, J., Warren, M. K., Coyne, M. W., Schweickart, V. L., Lee, M. T., Wilson, K. J., Boosman, A., Stanley, E. R., Ralph, P., and Mark, D. F. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 230, 237–242
12. Ladner, M. B., Martin, G. A., Noble, J. A., Nikoloff, D. M., Tal, T., Kawasaki, E. S., and White, T. J. (1987) EMBO J. 6, 2693–2698
13. Wong, G. G., Temple, P. A., Leary, A. C., Witek-Gianotti, J. S., Yang, Y. C., Chiarletta, A. B., Chung, M., Mutrah, P., Kri, M., Kaufman, R. J., Ferenz, C. B., Sibley, B. S., Turner, K. J., Hewick, R. M., Clark, S. C., Yanai, N., Yokota, H., Yamada, M., Saito, M., Motoyoshi, K., and Takaku, F. (1987) Science 235, 1504–1508
14. Cerretti, D. P., Wignall, J., Anderson, D., Tushinski, R. J., Gallis, B. M., Stya,
M., Gillis, S., Usdal, D. L., and Cosman, D. (1988) Mol. Immunol. 26, 761–770
15. Pampfer, S., Tabibzadeh, S., Chuan, F. C., and Pollard, J. W. (1991) Mol. Endocrinol. 149, 129–141
16. Rettenmier, C. W. (1989) Curr. Top. Microbiol. Immunol. 149, 129–141
17. Rettenmier, C. W., Roussel, M. F., Ashmun, R. A., Ralph, P., Price, K., and Sherr, C. J. (1987) Mol. Cell. Biol. 7, 2378–2387
18. Rettenmier, C. W., Roussel, M. F., Ashmun, R. A., Ralph, P., Price, K., and Sherr, C. J. (1987) Mol. Endocrinol. 1, 2378–2387
19. Stein, J., Borzillo, G. V., and Rettenmier, C. W. (1990) Blood 76, 2378–2387
20. Rettenmier, C. W., and Roussel, M. F. (1988) Mol. Cell. Biol. 8, 5026–5034
21. Shiina-Ishimi, Y., Abe, E., Tanaka, H., and Suda, T. (1986) Biochem. Biophys. Res. Commun. 134, 400–406
22. Elford, P. R., Felix, R., Cecchini, M., Trechse, U., and Fleisch, H. (1987) Calcif. Tissue Int. 41, 151–156
23. Felix, R., Fleisch, H., and Elford, P. R. (1989) Calcif. Tissue Int. 44, 356–360
24. Kaplan, D. L., Eitelson, C. M., Horowitz, M. C., Insogna, K. L., and Weir, E. C. (1996) J. Cell. Physiol. 168, 199–208
25. Hattersley, G., Owens, J., Flanagan, A. M., and Chambers, T. J. (1991) Biochem. Biophys. Res. Commun. 177, 526–531
26. Rubin, J., Fan, X., Thornton, D., Bryant, R., and Biskobing, D. (1996) Calcif. Tissue Int. 59, 291–296
27. Felix, R., Halasy-Nagy, J., Wetterwald, A., Cecchini, M. G., Fleisch, H., and Hofstetter, W. (1990) J. Cell. Physiol. 166, 311–322
28. Elias, J. A., Tang, W., and Horowitz, M. C. (1995) Endocrinology 136, 489–98
29. Chirgwin, J. M., Przybyla, A. E., MacDonald, J. R., and Rutter, W. J. (1979) Biochemistry 18, 5294–5305
30. Jin, C.-F., Mata, M., and Fink, D. J. (1994) PCR Methods Appl. 3, 252–255
31. Diviacco, S., Nito, P., Zentilin, L., Menzo, S., Clementi, M., Biamonti, G., Riva, S., Falaschi, A., and Giacca, M. (1992) Gene (Amst.) 122, 3013–3020
32. Yao, G. Q., Corrias, S., and Cheng, Y. C. (1996) Biochem. Pharmacol. 51, 431–436
33. Yao, G. Q., Tsai, C. H., and Cheng, Y. C. (1995) Virus Genes 9, 247–255
34. Baron, R., Vignery, A., Neff, L., Silvergate, A. and Santa Maria, A. (1983) in Bone Histomorphometry: Techniques and Interpretation (Becker, R. R., ed) pp. 31–32, CRC Press, Boca Raton, FL
35. Perkins, S. L., and Kling, S. J. (1995) Am. J. Physiol. 269, E1024–E1030
36. Mahaffey, J. E., Rosenblatt, M., Shepard, G. L., and Potts, J. T., Jr. (1979) J. Biol. Chem. 254, 6496–6498
37. Shadle, P. J., Aldwin, L., Nitecki, D. E., and Kothes, K. (1989) J. Cell. Biochem. 40, 91–107
38. Gehron Robey, P., and Termine, J. D. (1985) Calcif. Tissue Int. 37, 453–460
39. Civitelli, R., Bacsaki, B. J., Mahaut-Smith, M. P., Adams, S. R., Avioli, L. V., and Teien, R. Y. (1994) J. Bone Miner. Res. 9, 1407–1417
40. Rouleau, M. F., Mitchell, J., and Goltzman, D. (1988) Endocrinology 123, 187–191
41. Takahashi, N., Udagawa, N., Akatsu, T., Tanaka, H., Isogai, Y., and Suda, T. (1991) Endocrinology 128, 1792–1796
42. Morohashi, T., Antonini-Corboz, V., Fleisch, H., Cecchini, M. G., and Felix, R. (1994) J. Bone Miner. Res. 9, 401–407
43. Begg, S. K., Radley, J. M., Pollard, J. W., Chisolm, O. T., Stanley, E. R., and Berthoncello, I. (1993) J. Exp. Med. 177, 237–242
44. Hume, D. A., and Favot, P. (1995) J. Interferon Cytokine Res. 15, 279–284
The Cell-surface Form of Colony-stimulating Factor-1 Is Regulated by Osteotropic Agents and Supports Formation of Multinucleated Osteoclast-like Cells
Gang-Qing Yao, Ben-hua Sun, Elizabeth E. Hammond, Elizabeth N. Spencer, Mark C. Horowitz, Karl L. Insogna and Eleanor C. Weir

*J. Biol. Chem.* 1998, 273:4119-4128.
doi: 10.1074/jbc.273.7.4119

Access the most updated version of this article at [http://www.jbc.org/content/273/7/4119](http://www.jbc.org/content/273/7/4119)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/273/7/4119.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 43 references, 11 of which can be accessed free at [http://www.jbc.org/content/273/7/4119.full.html#ref-list-1](http://www.jbc.org/content/273/7/4119.full.html#ref-list-1)