Interleukin 6

A Potential Autocrine/Paracrine Factor in Paget's Disease of Bone

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Abstract

Pagetic osteoclasts are greatly increased in number and size and have increased numbers of nuclei per cell compared to normal osteoclasts. The mechanisms responsible for enhanced osteoclast formation in Paget's disease are unknown. We have used our recently described model system for pagetic osteoclast formation to evaluate culture media conditioned by these atypical multinucleated cells (MNC) to determine if pagetic osteoclasts produce an autocrine or paracrine factor that enhances osteoclast formation. Conditioned media from long-term bone marrow cultures from patients with Paget's disease stimulated osteoclast-like MNC formation in normal marrow cultures. At least part of this activity could be ascribed to interleukin 6 (IL-6). In contrast, conditioned media from normal marrow cultures contained lower levels of IL-6 and did not stimulate formation of osteoclast-like MNC. 7 of 8 bone marrow plasma samples taken from involved bones and 18 of 27 peripheral blood serum samples from Paget's patients had high levels of IL-6. Normal marrow plasma and peripheral blood serum had no or very low levels of IL-6. These results suggest that IL-6 produced by marrow and/or bone cells in patients with Paget's disease may be an autocrine/paracrine factor for pagetic osteoclasts. (*J. Clin. Invest.* 1992. 89:46–52.) Key words: interleukin 6 • osteoclasts • Paget's disease • precursors

Introduction

Paget's disease of bone is characterized by grossly distorted bone remodeling with the primary abnormality residing in the osteoclast (1–7). The pagetic osteoclasts are greatly increased in number and size and have increased numbers of nuclei compared with normal osteoclasts (5–7). The pagetic osteoclasts have viral-like nuclear and cytoplasmic inclusions and express viral antigens and viral transcripts for paramyxoviruses (8–14), which suggests a possible viral etiology for Paget's disease. However, the mechanisms responsible for the enhanced osteoclast formation seen in this disease are unknown. Possibly the viral infection enhances the fusion of osteoclast precursors to form osteoclasts. Alternatively, or in addition, pagetic osteoclasts or other cells present in the pagetic bone may produce a stimulatory factor that enhances osteoclast formation and/or activity.

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To determine if pagetic osteoclasts produced an autocrine or paracrine factor that stimulates osteoclast formation, we used our recently described model system of pagetic osteoclast formation in which atypical multinucleated cells (MNC)[1] that resemble pagetic osteoclasts form (15). In these long-term cultures of marrow mononuclear cells from patients with Paget's disease, the MNC are increased in number and size, have more nuclei per cell, form large resorption lacunae on calcified matrices, and have plasma membrane antigens that cross-react with monoclonal antibodies that preferentially bind to osteoclasts (16, 17). We used this model system to test media conditioned by these atypical MNC for osteoclast stimulatory activities.

In this report we demonstrate that conditioned media from long-term bone marrow cultures of patients with Paget's disease stimulate normal osteoclast-like MNC formation in vitro. At least part of this activity could be ascribed to interleukin 6 (IL-6). Conditioned media from normal marrow cultures contained lower levels of IL-6 and did not stimulate formation of osteoclast-like MNC. Bone marrow plasma taken from involved bone and peripheral blood serum samples from patients with Paget's disease had high levels of IL-6. Marrow plasma from normal marrow and normal peripheral blood serum did not have detectable levels or had very low levels of IL-6. These results suggest that IL-6 produced by marrow and/or bone cells in patients with Paget's disease may be an autocrine/paracrine factor for pagetic osteoclasts and may in part, be responsible for the increased osteoclast formation seen in Paget's disease.

Methods

Recombinant human IL-6 was prepared by expressing a cDNA for BSF-2 in *Escherichia coli* followed by further purification as previously described (18) and was generously provided by Dr. Akiyama (Ajinomoto, Inc., Tokyo, Japan). The specific activity of the recombinant human IL-6 was 3.9×10^9 U/mg as determined by the SKW6-CL4 assay, a BSF-2–responsive human B lymphoblastoid cell line. 1,25 dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] was a generous gift of Dr. Ishizuka (Teijin Institute for Biomedical Research, Tokyo, Japan). Monospecific rabbit anti–human IL-6 was purchased from Genzyme Corp., Boston, MA. The anti–IL-6 did not cross-react with IL-1, tumor necrosis factor-α (TNF-α), granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon-α, or interferon-γ and did not block the stimulatory effects of IL-1, TNF, or $1,25(OH)_2D_3$ on MNC formation in human marrow cultures. 1 mg of anti–IL-6 was capable of neutralizing 10,000 U of recombinant human IL-6 in the CESS cell immunoglobulin production assay. Polyclonal antibody to recombinant human TNF-α was generously provided by Cetus Corp., Emeryville, CA. A 1:100 dilution of the anti-TNF neutralized 4,000 U of TNF per ml.

1. *Abbreviations used in this paper:* GM-CSF, granulocyte/macrophage colony-stimulating factor; IMDM, Iscove's modified Dulbecco's media; MNC, multinucleated cells; TNF, tumor necrosis factor.

Anti-human IL-1 was purchased from Cistron, Pine Brook, NJ. 1 ml of the anti-human IL-1 neutralized 500 half-maximal units of human monocyte IL-1 in the C3H thymidine proliferation assay. Monospecific polyclonal antibody to human GM-CSF was purchased from Genzyme Corp. 1 mg of anti-GM-CSF was capable of neutralizing 10,000 U of recombinant human GM-CSF. Horse serum and fetal calf serum were purchased from Hyclone Laboratories Inc., Logan, UT, and a single lot of serum was used in all experiments. α-Minimum essential medium (αMEM) and Iscove's modified Dubelcco's medium (IMDM) were purchased from Gibco Laboratories, Grand Island, NY. 96- and 24-well tissue culture plates, T-25 flasks, and 35-mm tissue culture plates were purchased from Corning Glass, Inc., Corning, NY. Bovine serum albumin was purchased from Sigma Chemical Co., St. Louis, MO.

Bone marrow cultures. After obtaining informed consent, bone marrow was aspirated from the posterior superior iliac crest of normal volunteers or from clinically involved sites or uninvolved sites from patients with Paget's disease. The degree of iliac crest involvement had been documented previously by X-ray or bone scans. All Paget's disease patients had elevated serum alkaline phosphatases and had not received treatment for Paget's disease for at least 3 mo. These studies were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio and the Cedars-Sinai Hospital. Plasma samples from the bone marrow aspirates were separated and assayed immediately or were stored at −70°C, and the bone marrow was then processed as previously described (19). Nonadherent marrow mononuclear cells were resuspended in αMEM–20% horse serum at 10^6 cells/ml and plated (0.5 ml per well) in 24-well tissue culture plates. The cells were cultured in a humidified atmosphere of 4% CO_2–air at 37°C in the presence or absence of $1,25(OH)_2D_3$. The cultures were fed twice per week by replacing half of the media. After 3 wk of culture the cells were fixed and stained with Wright's Geimsa stain. In addition, the cells were tested for expression of the osteoclast phenotype by determining their cross-reactivity with the monoclonal antibody 23c6, which identifies osteoclasts (16) (generously provided by Dr. M. Horton, St. Bartholomew's Hospital, London, UK). Reactivity with 23c6 monoclonal antibody was determined by using biotin-conjugated rabbit anti-mouse IgG coupled to alkaline phosphatase (Vector Laboratories, Burlingame, CA), and then counterstaining the cells with methyl green as described (17). Cells that contained three or more nuclei were counted as MNC.

Preparation of Paget's disease and normal bone marrow conditioned media. Nonadherent bone marrow mononuclear cells were cultured in T25 tissue culture flasks at 10^6 cells/ml for 3 wk as described above in the presence of $1,25(OH)_2D_3$ (10^{-8} M). At the end of the culture period, the cultures were washed three times with αMEM, then αMEM containing 2% fetal calf serum but not containing $1,25(OH)_2D_3$ was added to the cultures, and the cultures were allowed to incubate 24–48 h. At the end of this second culture period, supernatants were collected, filtered, and either used immediately or frozen at −70°C. In selected experiments, varying concentrations of the conditioned media were added to normal bone marrow cultures lacking $1,25(OH)_2D_3$. In some of the experiments with pagetic bone marrow conditioned media, neutralizing antibodies to IL-1, GM-CSF, TNF-α, or recombinant human IL-6 were added at dilutions of 1:10,000 to 1:100 at the start of the cultures and with each media change. The cultures were maintained as described above. Control cultures were incubated in the presence or absence of 10^{-8} M $1,25(OH)_2D_3$.

Assay of IL-6 in bone marrow plasma, peripheral blood sera, and conditioned media from long-term marrow cultures. The B9 hybridoma cell line (generously provided by Dr. M. Horowitz, New Haven, CT) was used to detect IL-6 activity in conditioned media, marrow plasma, and peripheral blood sera. The B9 cell line is an IL-6–dependent cell line that requires IL-6 for growth (20). This cell line has a maximal response to IL-6 between 5 and 10 pg/ml and can detect concentrations as low as 0.3 pg/ml in samples of marrow plasma, peripheral blood sera, and conditioned media. B9 cells were cultured at 2×10^5 cells per ml in IMDM containing 10% fetal calf serum in the presence of 0.01% T24 human bladder carcinoma cell conditioned media, which contains high levels of IL-6. The B9 cells were harvested and washed three times with 50 ml of culture media to remove the IL-6, and the cell concentration was adjusted to 4×10^5 cells per ml. 50 μl of the cell suspension was plated in each well of a 96-well plate, and then 50 μl of serially diluted test samples was added in duplicate, and the cultures were incubated for 42 h in the presence or absence of anti–IL-6 (1:500 dilution). Control incubations contained only IMDM-10% fetal calf serum or varying concentrations of recombinant human IL-6 to generate a standard IL-6 dose-response curve. After incubating for 42 h, 1 μCi of tritiated thymidine was added to each well (New England Nuclear, Boston, MA, sp act, 20 C/mmol) for 4 h. The cells were washed, and the incorporation of tritiated thymidine into acid-insoluble product was determined as previously described (21). Results obtained from the B9 assay were confirmed using an enzyme-linked immunosorbent assay (ELISA) for measuring IL-6 (22) that was generously provided by Dr. Ida (Toray Industries, Kanagawa, Japan). The false positive rate for the B9 assay was 7%.

Testing for IL-1, TNF-α, or GM-CSF activity in Paget's disease conditioned media. Conditioned media prepared from long-term bone marrow cultures from patients with Paget's disease were tested for the presence of IL-1α and β as previously described (23) using a commercial ELISA kit for IL-1α or IL-1β (Otsuka Pharmaceutical, Tokyo, Japan) that was generously supplied by Oncomembrane, Inc., Seattle, WA. A standard curve for IL-1α or β was run in each plate to determine the concentration of IL-1α or β in the unknown samples. These assays detect as little as 8 pg of IL-1α and 16 pg of IL-1β. Conditioned media were also tested for TNF-α using the L929 assay (24). This assay detects TNF concentrations as low as 2.0 pg/ml. GM-CSF was assayed in semisolid cultures of human bone marrow mononuclear cells (25). Colonies were counted at days 7 and 14 of culture, and the colony numbers were compared to the number of colonies formed in the presence of known concentrations of GM-CSF (0.01–1 ng/ml). This assay detects GM-CSF at concentrations as low as 25–50 pg/ml.

Dentine resorption studies. Bone marrow cells from normals were cultured in Lab Tek slides (Lab-Tek Products, Naperville, IL) for 3 wk in the presence of 15% (vol/vol) Paget's normal marrow–conditioned media. Sperm whale dentine (generously provided by the U.S. Fish and Wildlife Commission) was placed over the cultures, and the cultures were maintained for an additional week. The dentine slices were removed, fixed, and then stained with toluidine blue to score the number of MNC. The cells were then removed, the dentine slices sputter-coated with gold-palladium and then processed for scanning electron microscopy to detect resorption lacunae as previously described (15).

Statistical analysis. Results are reported as the mean±SEM for four cultures. Results from marrow cultures were compared using a two-way analysis variance for repeated measures. The levels of IL-6 in marrow plasma and peripheral blood sera were compared by using the Mann-Whitney nonparametric test. Levels of IL-6 detected by the B9 hybridoma bioassay and the IL-6 ELISA were compared using linear regression analysis. Results were considered significantly different for $P < 0.05$.

Results

The effects of adding varying concentrations of Paget's disease conditioned media or normal marrow conditioned media to long-term cultures of normal marrow mononuclear cells were examined (Fig. 1). Concentrations between 5% and 30% (vol/vol) of the Paget's-conditioned media stimulated both total and 23c6-positive MNC formation. In contrast, normal marrow conditioned media did not significantly increase MNC formation at concentrations between 5% and 30% (vol/vol). At concentrations as high as 60% (vol/vol), slight but significant enhancement of total and 23c6-positive MNC formation was observed (data not shown). Conditioned media were obtained

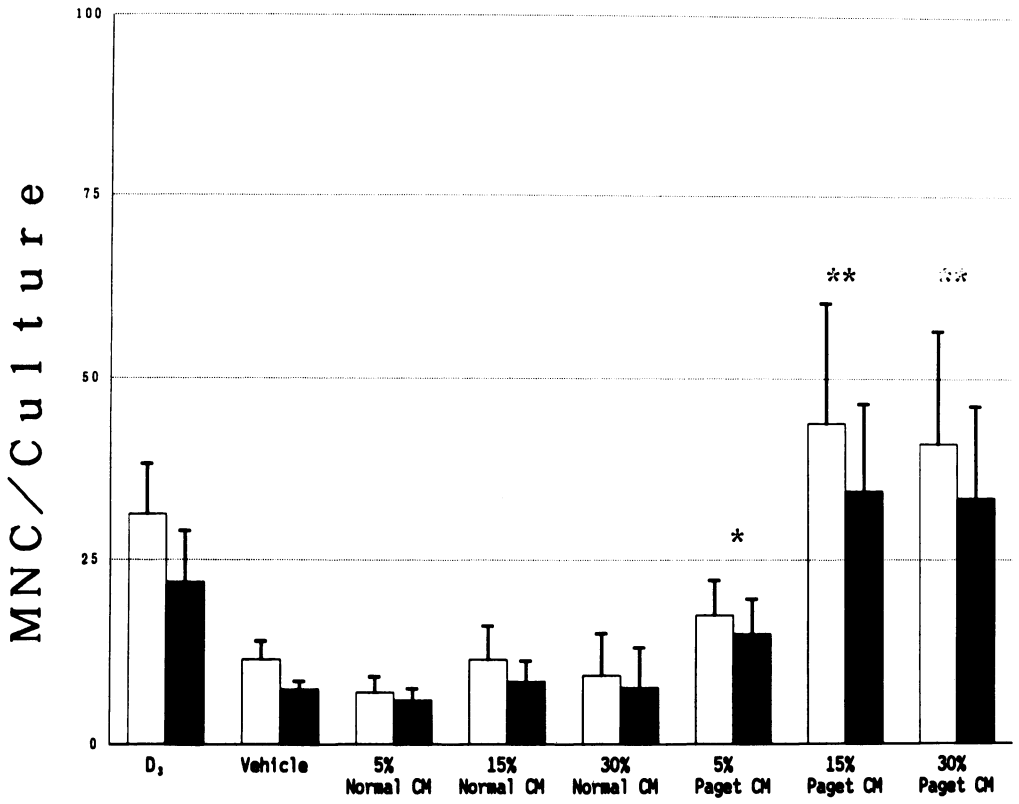

Figure 1. Effects of conditioned media from long-term marrow cultures derived from Paget's or normal patients on MNC formation in long-term normal human marrow cultures. Conditioned media were obtained from long-term marrow cultures of Paget's patients and normals after 3 wk of culture as described in Methods. Varying concentrations (5–30% vol/vol) of conditioned media (*CM*) were added to long-term cultures of normal human marrow in the absence of $1,25(OH)_2D_3$ (D_3). At the end of 3 wk, the cells were fixed and the number of MNC cells (total, shown in the open bars, and those reacting with the 23c6 monoclonal antibody that identifies osteoclasts, shown in the solid bars) determined. Results represent the mean±SEM for four replicates from a typical experiment. Similar results were seen in three independent experiments. $*P < 0.05$ compared to control cultures without $1,25(OH)_2D_3$. $**P < 0.01$ compared to control cultures without $1,25(OH)_2D_3$.

from Paget's marrow cultures after the first week, second week, or third week of culture, and tested for stimulation of total and 23c6-positive MNC cell formation in normal marrow mononuclear cell cultures. Conditioned media obtained after 1, 2, or 3 wk of culture increased MNC formation (Fig. 2). Normal marrow cultures stimulated with Paget's conditioned media or $1,25(OH)_2D_3$ but not normal marrow conditioned media induced MNC that formed resorption lacunae on sperm whale dentine (Fig. 3). Approximately 10–20% of the MNC formed resorption lacunae.

Our previous data have shown that cytokines such as IL-6, IL-1, TNF-α, and GM-CSF increase formation of MNC in human marrow cultures (26–28). Therefore, Paget's and normal conditioned media were assayed for IL-1, TNF-α, GM-CSF, and IL-6 after 3 wk of culture. TNF-α, IL-1, or GM-CSF activity was not detected in these media. Addition of neutralizing antibodies to TNF-α or GM-CSF did not block the stimulatory effects of the Paget's conditioned media on normal MNC formation, and only high concentrations of anti–IL-1 (1:100) significantly decreased MNC formation (data not shown). In contrast, increased concentrations of IL-6 were present in conditioned media from long-term cultures of Paget's marrow (Table I). This IL-6 activity detected in the B9 hybridoma bioassay was completely neutralized by anti–IL-6 (1:500) but not by anti–IL-4 or anti-TNF (data not shown). Addition of neutralizing antibodies to human IL-6 to normal marrow cultures treated with Paget's conditioned media completely blocked the stimulatory effects of the Paget's conditioned media on normal MNC formation (Fig. 4, *top*) but had no effect on $1,25(OH)_2D_3$ or TNF-stimulated MNC formation (Fig. 4, *bottom*).

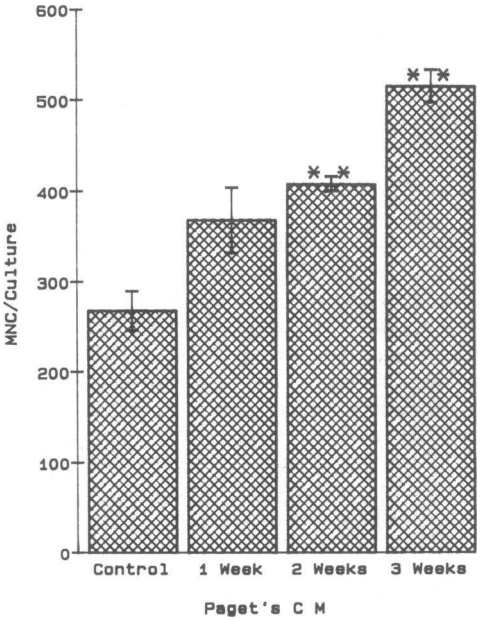

Figure 2. Time course for the production of osteoclast-like cell stimulatory activity in conditioned media from Paget's marrow cultures. Conditioned media (*CM*) were obtained from long-term marrow cultures from patients with Paget's disease after 1, 2, or 3 wk of culture. The conditioned media were tested at 15% (vol/vol) in long-term normal marrow cultures lacking $1,25(OH)_2D_3$. The normal marrow cultures were maintained for 3 wk, then the cells were fixed, and the number of total MNC was determined. Results represent the mean±SEM of four cultures from a typical experiment. Similar results were seen in two separate experiments. $**P < 0.01$ compared to control cultures lacking Paget's conditioned media.

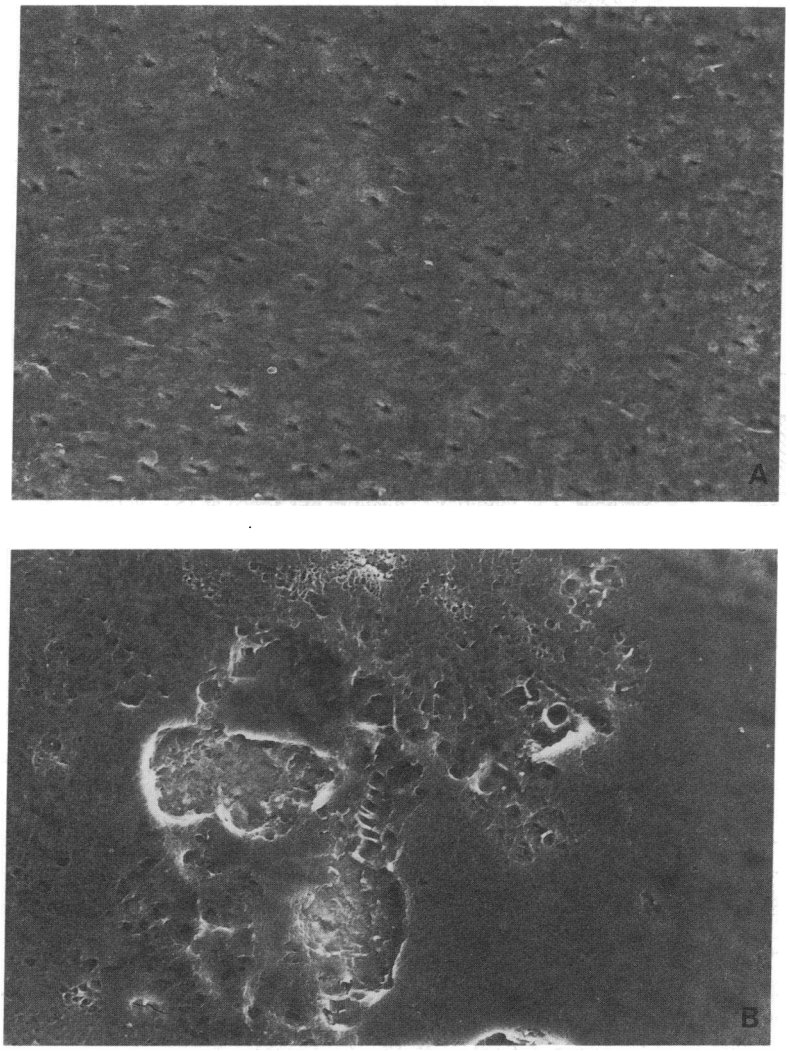

Figure 3. Formation of resorption lacunae on sperm whale dentine by MNC formed in normal marrow cultures treated with Paget's conditioned media. Paget's or normal marrow conditioned media (15% vol/vol) were added to normal bone marrow cultures. After 2 wk of culture, the cells were overlaid with dentine slices and the cultures were continued for an additional week. At the end of the culture period, the dentine slices were processed for viewing by scanning electron microscopy. (*A*) Dentine slice from normal marrow culture treated with normal marrow conditioned media. No MNC formed in these cultures. ×750. (*B*) Dentine slice from normal marrow culture treated with Paget's conditioned media. Note the presence of resorption lacunae. ×750.

Normal marrow culture conditioned media also contained IL-6, but at lower concentrations than Paget's conditioned media (Table I). The concentration of IL-6 present in the normal marrow conditioned media was within the range of concentrations of recombinant human IL-6 that can stimulate MNC formation in normal marrow cultures (27). Therefore, to determine if normal marrow conditioned media contained an inhibitor that was blocking the effects of IL-6 on MNC formation, equal proportions of Paget's and normal marrow conditioned media were added to the normal marrow cultures. As seen in Fig. 5, addition of normal marrow conditioned media did not inhibit the stimulatory effects of Paget's marrow conditioned media on MNC formation.

Table I. Detection of IL-6 in Conditioned Media from Long-Term Cultures from Paget's Patients and Normals

Source of media	IL-6 activity
	pg/ml
Paget's (n = 4)	103±3*
Normal (n = 3)	70±7

Conditioned media were prepared as described in Methods and then were tested in duplicate in the B9 assay for IL-6 activity. All IL-6 activity detected by the B9 assay was completely neutralized by an anti-IL-6 antibody. Results represent the mean±SEM for all samples tested. Similar results were seen in three independent experiments. The number of culture media tested from different donors is shown in parenthesis.

* $P < 0.01$ compared to normal.

To determine if IL-6 levels were also increased in pagetic lesions in vivo, IL-6 levels were measured in plasma from marrow aspirates of patients with Paget's disease or from normal individuals by the B9 hybridoma bioassay and/or by the IL-6 ELISA. Marrow plasma samples from active lesions from seven of eight patients with Paget's disease had elevated IL-6 levels with a range of 21–300 pg/ml by the ELISA (Table II), whereas marrow plasma from eight normal donors had little or no detectable IL-6 activity. Peripheral blood serum samples from 18 of 27 patients with active Paget's disease had elevated levels of IL-6 with a range of 11–710 pg/ml by the ELISA (Table II). Normal serum had little or no detectable IL-6 activity. The levels of IL-6 detected by the B9 hybridoma bioassay were highly correlated to the levels of IL-6 detected by the IL-6 ELISA (Fig. 6, $r = 0.9808$, $P < 0.0001$). There was no correlation between the peripheral blood levels of IL-6 and the serum

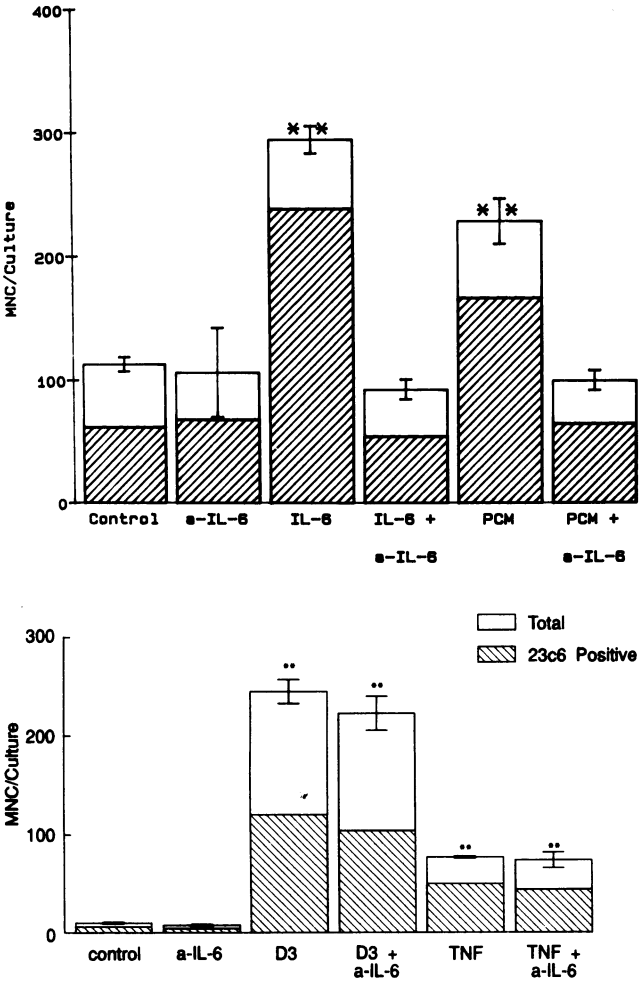

Figure 4. Effects of a neutralizing antibody to IL-6 on MNC formation in long-term marrow cultures treated with Paget's conditioned media. Conditioned media from pagetic marrow cultures were prepared as described in Methods. The pagetic conditioned medium (*PCM* 15% vol/vol, *top*), $1,25(OH)_2D_3$ (10^{-8} M), or TNF-α, (*TNF*, 10^{-9} M) (*bottom*) was added to long-term cultures of normal human marrow in the presence or absence of a 1:500 dilution of a neutralizing antibody to IL-6 (*a-IL-6*). After 3 weeks the cells were fixed and stained, and the number of total and 23c6-positive MNC was determined. Results represent the mean±SEM of four determinations. Similar results were seen in three independent experiments. $^{**}P < 0.01$ compared to control cultures treated with vehicle alone.

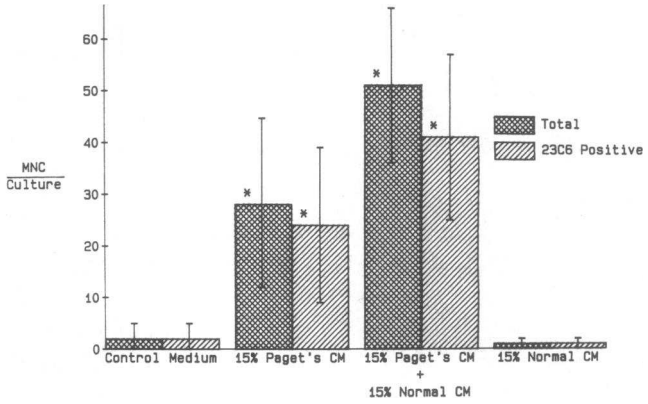

Figure 5. Effects of combinations of Paget's and normal marrow conditioned media on MNC formation. Conditioned media from pagetic and normal marrow cultures were prepared as described in Methods. The conditioned medium was added at 15% (vol/vol) alone or in combination with normal marrow conditioned medium, and the cultures incubated for 3 wk. Results represent the mean±SD for a typical experiment done in quadruplicate. Similar results were seen in two independent experiments. $^{*}P < 0.05$ compared to control cultures lacking conditioned media.

alkaline phosphatase levels in these patients. In a preliminary study of two Paget's patients, marrow plasma obtained from bone not clinically involved with Paget's disease did not have detectable levels of IL-6.

Discussion

In this report we demonstrate that conditioned media produced by long-term marrow cultures from patients with Paget's disease stimulated total and 23c6-positive MNC formation in normal marrow cultures, and that the MNC formed resorption lacunae on sperm whale dentine. IL-6, present in the Paget's disease conditioned media, appeared to be a major mediator responsible for this increase in normal MNC formation. The MNC stimulatory activity in the Paget's disease conditioned media was neutralized by anti-IL-6 and elevated levels of IL-6 were detected by the B9 hybridoma bioassay. The IL-6 activity detected by B9 hybridoma assay was neutralized by a monospecific anti-IL-6 antibody and the levels of IL-6 detected in the bioassay were highly correlated to IL-6 levels detected by the IL-6 ELISA. Further, the marrow plasma obtained from bones involved with Paget's disease contained increased amounts of IL-6. In contrast, normal peripheral blood sera or normal marrow plasma did not contain elevated levels of IL-6. These data suggest that IL-6 may be an autocrine or paracrine factor that enhances osteoclast formation and bone turnover in active lesions in patients with Paget's disease.

IL-6 is an important regulator of bone cell function. Osteoblasts produce IL-6 as do a variety of other cells in the marrow

Table II. Serum and Marrow IL-6 Levels in Paget's Patients and Normals

Source of media	Serum	Marrow
	pg/ml	
Paget's	94.7±35.2* (n = 27)	87.6±38.3* (n = 8)
Normal	5.7±5.7 (n = 10)	6.1±6.1 (n = 8)

Marrow plasma was separated immediately from marrow aspirates taken from involved bones of Paget's patients and from normals. The marrow plasma was serially diluted and assayed for IL-6 activity using an IL-6 immunosorbent assay. The number of patient samples studied is shown in parentheses. Results represent the mean±SEM for IL-6 values for all samples tested. All samples were assayed on at least two separate occasions in duplicate. Results were compared using the Mann-Whitney nonparametric test. 18 of 27 Paget's peripheral serum samples had significant IL-6 levels (range 11–710 pg/ml) and 7 of 8 Paget's marrow samples had elevated IL-6 levels (range 21–300 pg/ml). Only one normal marrow and peripheral blood sample had detectable IL-6 levels.
* $P < 0.05$ compared to normal.

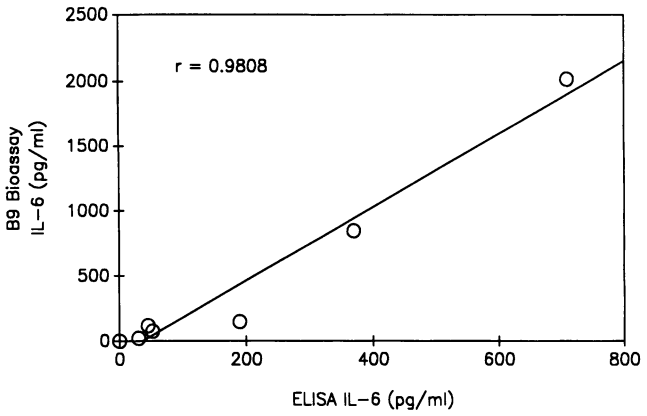

Figure 6. Correlation of IL-6 levels detected by the B9 hybridoma bioassay to those detected by the IL-6 ELISA in marrow and peripheral blood samples from Paget's patients. Seven individual marrow plasma or peripheral blood serum samples were assayed for IL-6 activity with the B9 hybridoma bioassay and the IL-6 ELISA. Results were compared by linear regression analysis and were significantly correlated ($r = 0.9808$, $P < 0.0001$).

microenvironment (29–33). We reported recently that recombinant human IL-6 is a potent stimulator of osteoclast-like cell formation in long-term human marrow cultures at concentrations of 10–100 pg/ml. IL-6 induced IL-1 production in these marrow cultures during the first 24 h after addition of IL-6 to the cultures, and preferentially stimulated the formation of MNC expressing the osteoclast phenotype (28). Addition of anti-IL-1 to marrow cultures treated with IL-6, blocked the increase in MNC (28). Consistent with these observations, high concentrations of anti-IL-1, added at the start of the cultures and with each feeding, blocked the effects of Paget's disease conditioned media in normal marrow cultures, although IL-1 was not detected in the Paget's disease conditioned media. It is not surprising that IL-1 was not present in the Paget's conditioned media although anti-IL-1 blocked the effects of Paget's disease conditioned media. The Paget's disease conditioned media were harvested after 3 wk of culture, and IL-6 stimulates IL-1 production only during the first 24 h after addition of IL-6 in the marrow cultures. Similarly, Lowik et al. (33) and Ishimi et al. (34) also reported that IL-6 induced osteoclast formation in fetal mouse metacarpals and calvaria, respectively.

In that IL-6 was produced in Paget's disease marrow cultures obtained from active pagetic lesions, it is possible that the pagetic osteoclast is a major source of the IL-6 present in the marrow plasma from pagetic bones. However, inasmuch as osteoblasts are increased in Paget's patients and also can produce IL-6 (34), it is possible that osteoblasts are also releasing IL-6. We have previously shown that the majority of MNC (70–80%) formed in marrow cultures from patients with Paget's disease are 23c6 positive and express the osteoclast phenotype (15). Although our data are consistent with the hypothesis that 23c6-positive MNC produce IL-6 in vitro, other cells present in these cultures can also produce IL-6.

Normal marrow conditioned media had similar levels of IL-6 to those present in pagetic marrow conditioned media. However, the normal marrow conditioned media did not increase MNC formation. In contrast, pagetic marrow conditioned media stimulated normal MNC formation at concentrations as low as 5% (vol/vol). These data suggest conditioned media from Paget's marrow cultures may contain other factors that enhance the effects of IL-6 on osteoclast-like cell formation in normal marrow, since the relative concentrations of IL-6 present in normal marrow conditioned media were within the range of recombinant human IL-6 that stimulate MNC formation in normal marrow cultures (28). The identity of this factor(s) is presently unknown.

The presence of high levels of IL-6 in pagetic marrow plasma may reflect the high bone turnover rates associated with Paget's disease. It is possible that the elevated levels of IL-6 present in Paget's media and marrow plasma reflect the greater number of osteoclasts and osteoclast-like MNC present in these samples as compared to normal. We have previously shown that between 10- and 100-fold more MNC form in Paget's marrow cultures treated with $1,25(OH)_2D_3$ than in normals (15), and pagetic bone has increased numbers of osteoclasts. IL-6 may act to amplify the already increased osteoclast formation and activity in Paget's disease and thus, may in part be responsible for the enhanced bone remodeling present in Paget's disease. It is unknown if the initial pathological event that induces Paget's disease directly induces IL-6 production or simply increases the number of activated osteoclasts that in turn produce IL-6. The finding that peripheral blood levels of IL-6 did not correlate with alkaline phosphatase activity in the Paget's sera may result from the multiple causes of increased plasma levels of IL-6. Other cytokines released in response to the rapid bone turnover may induce IL-6 production. In this regard, marrow plasma levels should more accurately reflect the pagetic process because of the localized nature of this disease.

These data further suggest that IL-6 may be important in the pathogenesis of disease processes associated with increased osteoclast formation. It is conceivable that therapeutic interventions to block the effects of IL-6 may be beneficial for patients with Paget's disease and other diseases associated with high bone turnover rates.

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