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Interleukin-6: A Potential Mediator of the Massive Osteolysis in Patients with Gorham-Stout Disease

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ABSTRACT

Gorham-Stout disease (GSD) or massive osteolysis, is an extremely rare osteolytic condition that involves extensive locally aggressive resorption of bone. The etiology and pathophysiology are unknown, and the role of the osteoclast in GSD is unclear. We studied a patient with GSD who had massive resorption of his mandible, which extended to his maxilla, zygoma, right parietal region, and cranium. To investigate the cause of the extensive resorption, we tested the effects of the patient's serum, sampled early in the course of treatment and later after the osteolysis was stabilized, on the formation of osteoclast-like multinucleated cells (MNC) in cultures of normal human marrow. GSD serum (10%, vol/vol) markedly increased the number of MNC formed in these cultures compared to that in normal serum as well as stimulated the formation of resorption pits by these MNC on dentine slices. GSD serum, collected after further therapy, did not enhance the number of MNC formed in marrow cultures compared to that in normal serum. Elevated levels of interleukin-6 (IL-6) were detected in the earlier GSD serum that were 7 times the upper limit of the normal range, and after further treatment, IL-6 levels fell to one quarter the pretreatment value. The levels of IL-1β, tumor necrosis factor-α, transforming growth factor-α, PTH, and PTH-related peptide in pretreatment GSD serum were not increased. Moreover, the addition of neutralizing antibodies to IL-6 to the normal human bone marrow cultures effectively blocked the increase in MNC formation induced by active GSD serum. These data suggest that bone resorption in GSD patients is due to enhanced osteoclast activity, and that IL-6 may play a role in the increased bone resorption in GSD. (*J Clin Endocrinol Metab* **81:** 1893–1897, 1996)

GORHAM-STOUT DISEASE (GSD) or massive osteolysis (1), also known as disappearing bone disease, vanishing bone disease, or phantom bone (2), is characterized by spontaneous bone resorption frequently following minor trauma. It is exceedingly rare, with fewer than 100 cases reported in the literature since it was first described by Jackson (3) in 1838. The etiology and pathophysiology are unknown. It can be discrete or multifocal, with the most commonly affected sites being the shoulder, upper arm, pelvis, jaw, thorax, and spine (4, 5). Gorham and Stout (1) established the association between massive osteolysis and the presence of hemangiomatous or lymphangiomatous tissue. Despite extensive bone resorption, they did not find osteoclasts in the areas of bone resorption. However, other investigators have described prominent osteoclasts (6–12). Careful examination of the lytic front may be a crucial point for the location of osteoclasts.

We recently had the opportunity to study a case of GSD and investigate the case from the standpoint of known mediators of osteolysis, particularly interleukin-6 (IL-6).

Case report

The patient suffered a blow to the chin at age 9 yr and subsequently developed mandibular osteolysis. He was initially treated for suspected osteomyelitis, but there was no response to antibiotics, and his entire mandible eventually resorbed. In addition, his maxilla, zygoma, right parietal bone, and cranium underwent extensive osteolysis (Fig. 1). The patient, then age 10 yr, was referred to the oral surgery clinic at Henry Ford Hospital. GSD was suspected, and a biopsy of the right zygoma was consistent with that diagnosis. Daily sc injections of calcitonin were administered, which resulted in a decline in the rate of bone resorption, as indicated by serial computerized tomography. The patient then received infusions of pamidronate every 3–4 months, and there was no further enlargement of the lytic lesions. Due to deformation of the softened skull base, he was placed on cervical traction with halo apparatus, and radiation therapy was administered. Peripheral serum and plasma were obtained for bone marrow culture studies at two time points in the patient's treatment course. The first specimen was drawn after the calcitonin therapy and initial pamidronate treatments. The second specimen was obtained about 9 months later, after additional courses of pamidronate and the radiation therapy. A year after radiation therapy, a successful cervical fusion was performed. Unfortunately, the clinical biopsy specimens were unsuitable for immunohistology due to fixation in acid-formalin, and additional bone biopsy specimens could not be obtained for study because of concern about reactivating the osteolytic process.

Materials and Methods

Plasma concentrations of osteotropic cytokines were measured using enzyme-linked immunosorbent assay kits; IL-6 and tumor necrosis-α (TNFα) kits were purchased from Genzyme Corp. (Cambridge, MA), IL-1β from R&D Systems (Minneapolis, MN), and transforming growth factor-α (TGFα) from Oncogene Science (Uniondale, NY). The normal plasma reference range for IL-6 was 0.2–11.5 pg/mL, that for TNFα was 0.5–5.5 pg/mL, and that for IL-1β was 0.1–10.3 pg/mL, as kindly provided by R&D Systems. IL-6 monoclonal murine antihuman IL-6 was purchased from R&D Systems. 1,25-Dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] was a generous gift from Dr. M. Uskokovic (Hoffman La-Roche, Nutley, NJ). FCS was purchased from Hyclone Laboratories (Logan, UT). αMEM was purchased from Life Technologies (Grand Island, NY). Plasma intact PTH (iPTH) was measured using the Allegro two-site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), with an assay sensitivity of 1.0 pg/mL. The

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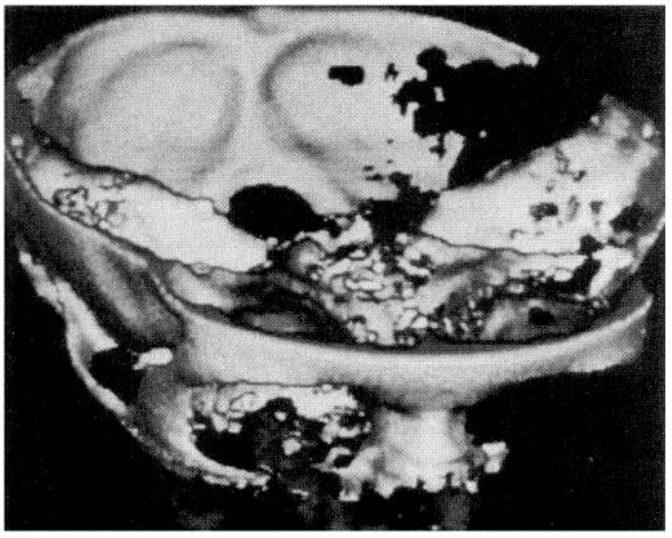


FIG. 1. Three-dimensional computer reconstruction of CT scans (mirror image) of the lower half of the GSD patient's skull before treatment. The calvaria is not shown in this representation. The mandible and right maxilla are totally resorbed, and the zygoma, right parietal bone, and cranium have progressively resorbed.

reference range for iPTH was 10–65 pg/mL. PTH-related peptide (PTHrP) levels were also measured using a two-site immunoradiometric assay with a calculated assay sensitivity of 0.3 pg/mL.

Bone marrow cultures

After obtaining informed consent, bone marrow was aspirated from the posterior superior iliac crest of normal volunteers. These studies were approved by the institutional review board of the University of Texas Health Science Center at San Antonio. The bone marrow was then processed as described previously (13). Nonadherent marrow mononuclear cells were resuspended in αMEM-20% FCS at 10^6 cells/mL and plated (0.5 mL/well) in 24-well tissue culture plates. Cultures were maintained in a humidified atmosphere of 4% CO_2 and air at 37 C for 3 weeks and fed weekly by removing half the medium and replacing it with an equal volume of fresh medium. To test the effect of either serum from the patient with GSD or that from normal volunteers on the formation of multinucleated cells (MNC) in normal human bone marrow cultures, serum at concentrations ranging from 1–10% (vol/vol) was added on the first day of culture and with each medium change. At the end of the culture period, the cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using a commercially available kit (Sigma Chemical Co., St. Louis, MO). TRAP-positive MNC (three or more nuclei per cell) were quantified manually by light microscopy. To test the effect of the patient's serum, at a concentration of 10% (vol/vol), on the formation of osteoclast-like cells in normal human bone marrow culture, the cells were fixed with 2% formaldehyde in PBS. The cells were then tested for expression of the osteoclast phenotype by determining their cross-reactivity with the 23c6 monoclonal antibody, which identifies osteoclasts (14) (generously provided by Dr. Michael Horton, St. Bartholomew's Hospital, London, UK). Reactivity with the 23c6 monoclonal antibody was determined by using biotin-conjugated rabbit antimouse IgG coupled to alkaline phosphatase (Vector Laboratories, Burlingame, CA) and then counterstaining the cells with methyl green, as described previously (15). Cells that contained three or more nuclei were counted as MNC. The 23c6 monoclonal antibody reacts with MNC that express calcitonin receptors and form resorption lacunae on calcified matrices (16).

Dentine resorption studies

Bone marrow cells from normals were cultured as described above for 3 weeks in the presence of serum from either the GSD patient or normal volunteers at a concentration of 10% (vol/vol). Sperm whale dentine was placed over the cultures for the 3-week incubation. The dentine slices were removed, fixed, and stained with 1% toluidine blue to score the number of MNC. The cells were removed by rinsing with tap water, and the dentine was restained with 2% toluidine blue. The number of resorption lacunae and the area resorbed per dentine slice were determined by light microscopy (×25), using a computer-assisted JAVA image analysis system (Jandel Scientific, Corte Madera, CA), as described previously (16).

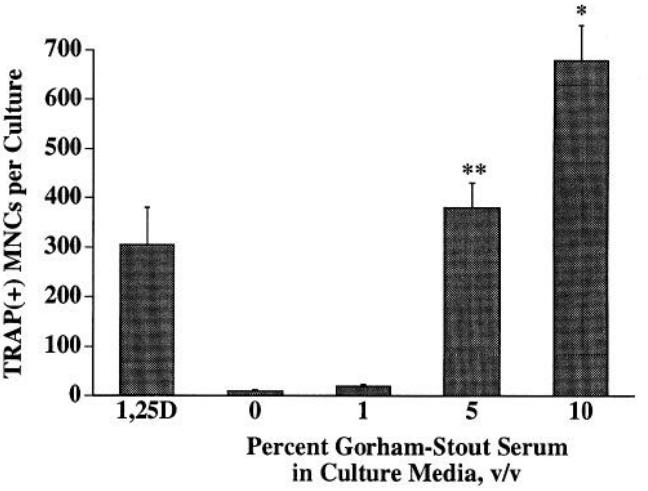


FIG. 2. Effects of serum from an untreated patient with GSD on MNC formation in bone marrow cultures from normal patients. Varying concentrations (1–10%) of the patient's serum were added to cultures of normal human bone marrow in the absence of 1,25-$(OH)_2D_3$ (1, 25D). At the end of 3 weeks, the cells were fixed, and the number of TRAP-positive MNC was determined. Results represent the mean ± SEM for four replicates from a typical experiment. *, $P < 0.02$ compared to control cultures with 1,25-$(OH)_2D_3$ and cultures containing 5% GSD serum. ** $P < 0.01$ compared to control cultures without 1,25-$(OH)_2D_3$.

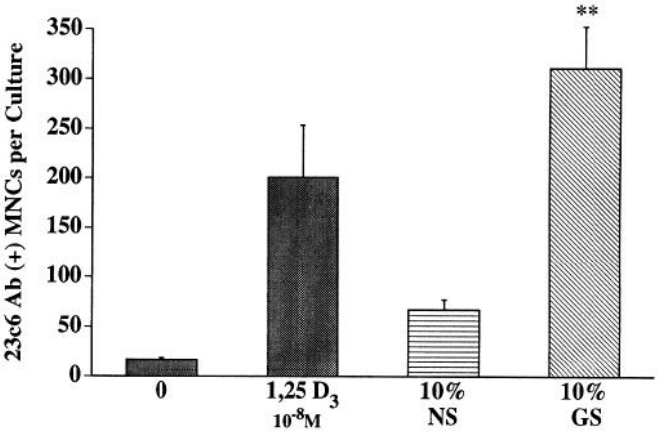
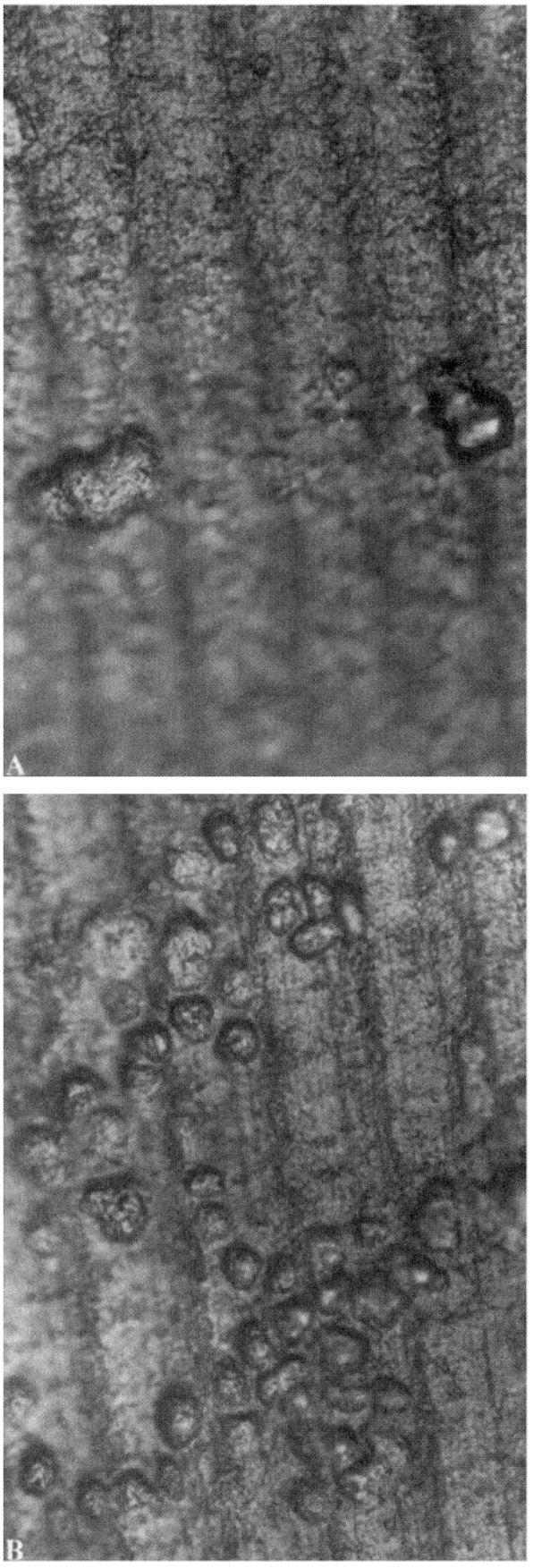


FIG. 3. Effects of serum from an untreated patient with GSD or from normal patients on osteoclast-like MNC formation in normal human bone marrow cultures. Serum, at a concentration of 10%, was added to cultures of normal human bone marrow in the absence of 1,25-$(OH)_2D_3$ (1, 25D). At the end of 3 weeks, the cells were fixed, and the number of MNC reacting with the 23c6 antibody that identifies osteoclasts was determined. Results represent the mean ± SEM for four replicates from a typical experiment. Similar results were seen in three independent experiments. *, $P < 0.01$ compared to control cultures treated with 10% normal serum in the absence of 1,25-$(OH)_2D_3$.



Results

The effects of adding serum from the patient with GSD to cultures of normal marrow mononuclear cells were studied in the absence of any other stimuli. The number of TRAP-positive MNC formed at a concentration of 5% (vol/vol) of the patient's initial serum sample was significantly higher than that in untreated control cultures, reaching a maximum at 10% (vol/vol), which was 1.8 times greater than that in the positive control cultures treated with 10^{-8} mol/L 1,25-$(OH)_2D_3$ (Fig. 2). Normal serum at a concentration of 10% (vol/vol) resulted in higher numbers of TRAP-positive MNC than that in untreated control cultures, but only half that measured in the 1,25-$(OH)_2D_3$-positive control cultures. The number of 23c6 antibody-positive osteoclast-like MNC formed in the presence of 10% patient serum was significantly higher than that found with 10% normal serum and similar to that in the 1,25-$(OH)_2D_3$-positive control cultures (Fig. 3). In addition, the osteoclast-like MNC formed in cultures in the presence of 10% GSD serum formed more resorption lacunae than did those in cultures with 10% normal serum (Fig. 4). The patient's serum, collected after further therapy, was also tested in the normal human bone marrow cultures. There was no difference between the number of 23c6 antibody-positive osteoclast-like MNC formed by the patient's later serum sample and 10% normal serum (Fig. 5).

We reported previously that the cytokines IL-6, IL-1β, TNFα, and TGFα enhance osteoclast-like cell formation (18–20), as do the systemic hormones PTH and PTHrP (13). Therefore, levels of IL-1, IL-6, TNFα, TGFα, PTH, and PTHrP were measured in the GSD patient's earlier and later serum samples. In the earlier specimen, the level of the cytokine IL-6 was elevated to 7 times the upper limit of the normal range, *i.e.* 80 pg/mL (Fig. 6), whereas the levels of IL-1β, TNFα, TGFα, PTH, and PTHrP were not increased. Furthermore, after bisphosphonate and radiation treatments, the level of IL-6 in the patient's serum fell to one quarter of the pretreatment value. Moreover, the addition of neutralizing antibodies to IL-6 to the normal human bone marrow cultures treated with 10% (vol/vol) serum from the GSD patient effectively blocked the increase in formation of osteoclast-like cells compared to that in cultures treated with active serum from the GSD patient (Fig. 7).

Discussion

Although GSD was first reported in 1838 (3), its etiology and pathophysiology have yet to be elucidated. Reports are inconsistent as to the presence or absence of osteoclasts in these lesions. Some investigators have noted osteoclasts in the areas of bone resorption as well as evidence of osteoclas-

FIG. 4. Formation of resorption lacunae on sperm whale dentine by MNC formed in normal marrow cultures treated with serum from patient with GSD. Gorham-Stout or normal serum (10%, vol/vol) was added to normal bone marrow cultures, and the cells were overlaid with dentine slices for 3 weeks of culture. At the end of the culture period, the dentine slices were processed for viewing by light microscopy. A, Dentine slice treated with 10% normal serum. B, Dentine slice from normal marrow culture treated with Gorham-Stout serum. Note that more resorption lacunae formed in Gorham-Stout serum than in 10% normal serum. Magnification ×250.

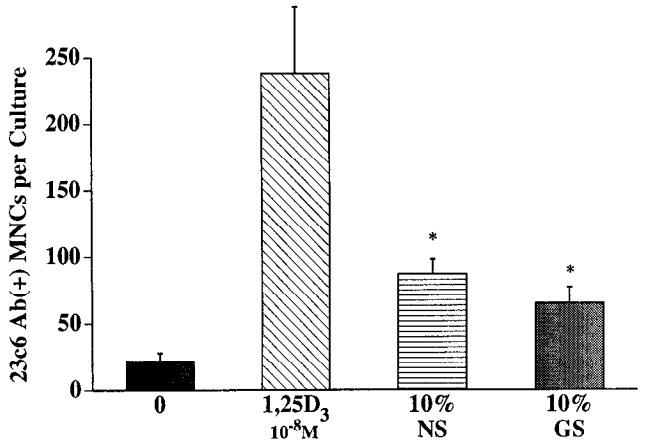


FIG. 5. Effects of serum from a patient with GSD after bisphosphonate and radiation therapy or serum from normal patients on osteoclast-like MNC formation in normal human bone marrow cultures. Serum, at a concentration of 10% (vol/vol), was added to cultures of normal human bone marrow in the absence of 1,25-$(OH)_2D_3$ (1, 25D). At the end of 3 weeks, the cells were fixed, and the number of MNC reacting with the 23c6 antibody that identifies osteoclasts was determined. Results represent the mean ± SEM for four replicates from a typical experiment. *, $P < 0.05$ compared to control cultures treated with 1,25-$(OH)_2D_3$.

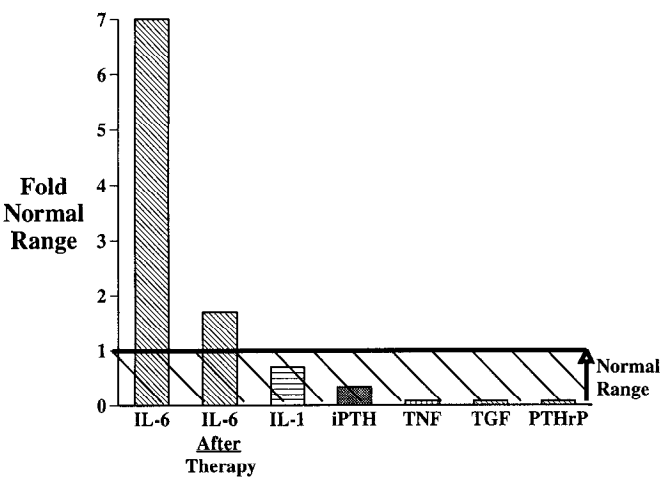


FIG. 6. Plasma levels of the cytokines IL-6, IL-1, iPTH, TNFα, TGFα, and PTHrP in a patient with GSD, assayed before treatment with the bisphosphonate, pamidronate, plus radiation therapy. Results are expressed by dividing each cytokine level by its respective normal range. In addition, the level of IL-6 was assayed after therapy.

tic resorption (6–12), whereas others have been unable to detect any bone-resorbing cells (1, 20–22). However, our studies demonstrated that the serum from the patient with GSD, at a concentration of 10% (vol/vol), stimulated the formation of osteoclast-like MNC in normal human bone marrow cultures (at this concentration, normal serum had no significant effect), strongly suggesting a role for osteoclasts in the massive resorption characteristic of this disease. The demonstration of numerous resorption lacunae at the margins of the lesion is consistent with such a role. Furthermore, treatment of the patient with calcitonin and bisphosphonates, two inhibitors of osteoclastic activity, effectively arrested bone resorption, again implying that osteoclasts are involved in this disease.

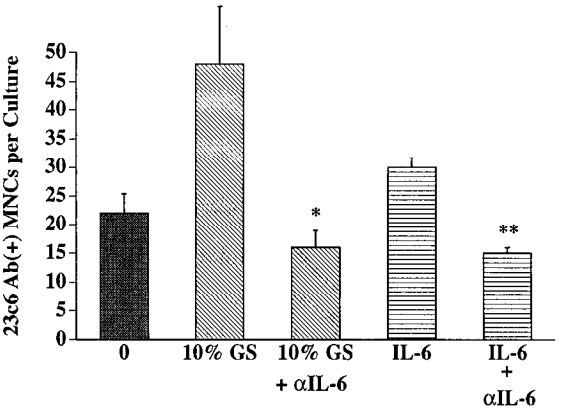


FIG. 7. Effects of neutralizing antibodies to IL-6 on MNC formation in normal human bone marrow cultures treated with 10% serum from GSD patient. The patient's serum was either preincubated with a 1:400 dilution of a neutralizing antibody to IL-6 at 37 C for 30 min before addition (10%, vol/vol) to cultures of normal human bone marrow or added directly to the cultures. Recombinant IL-6, at a concentration of 80 pg/mL, *i.e.* the same concentration as that found in the serum of the GSD patient, was either preincubated with a 1:400 dilution of a neutralizing antibody to IL-6 before adding it (10%, vol/vol) to cultures of normal human bone marrow or added directly to the cultures. After 3 weeks, the cells were fixed and stained, and the number of 23c6 antibody-positive MNC was determined. Results represent the mean ± SEM of four determinations. **, $P < 0.01$, IL-6-treated cultures compared to cultures treated with IL-6 plus neutralizing antibody to IL-6. *, $P < 0.05$, cultures treated with serum from patient with GSD compared to cultures treated with serum plus neutralizing antibody to IL-6.

Serum PTH and calcium levels were normal in the patient with GSD. Previous reports indicate that there may be a transient rise in plasma alkaline phosphatase with pathological fracture (23). In contrast, we found increased levels of the cytokine IL-6, to 7 times normal in the serum of this patient. These data suggest that IL-6, which has been shown to enhance osteoclast formation and bone resorption by human osteoclasts (18, 19), may be produced locally in amounts sufficient to promote osteoclast activity. The elevated levels of IL-6 seen in the patient possibly reflect a spillover of the cytokine from the sites of the lytic lesions into the circulation. Thus, the massive osteolysis observed in this disease would be similar in this respect to that seen in Paget's disease, where there is also a primary focal disorder of osteoclastic activity (24). Furthermore, IL-6 has been implicated in Paget's disease, as essentially all bone marrow plasma samples (9 of 10) had increased IL-6 levels up to 3000 ng/mL, and 19 of 27 peripheral blood samples had elevated IL-6 levels, with a range of 11–710 pg/mL (25). These levels of IL-6 are similar to the level found in the patient with GSD (80 pg/mL). However, it should be noted that although the serum levels were elevated in GSD and Paget's disease, this does not result in generalized osteopenia throughout the skeleton. This suggests that the circulating levels of IL-6 are not sufficient to induce generalized osteopenia, and far higher levels of IL-6 at the site of the lesion may be responsible for the increased localized osteoclastic activity in GSD.

There are several possible sources for high local levels of IL-6 at the site of the lytic lesion. As mentioned previously, the lesions are characterized by an abnormal proliferation of thin-walled endothelial-lined capillaries or sinusoidal chan-

nels of vascular or lymphatic origin. The smooth muscle cells of blood vessels have been shown to produce IL-6 in response to either IL-1 or TNFα (26), so the blood vessels could be a source of the IL-6 in this disease. A second alternative is that dilation of the already vascular marrow, rather than true hypervascularity, could be occurring in GSD, and this could be the source of IL-6. A third alternative is the osteoclast, which produces IL-6 (19) and may be increased in number and activity in this disease, is the source of the increased IL-6 levels. Finally, there are other cells in the bone marrow microenvironment, including monocytes, fibroblasts, and lymphocytes, that are a potential source of IL-6 (27–30).

In summary, IL-6 appears to play a role in the increased bone resorption in GSD, as found in other bone diseases associated with increased bone destruction, including multiple myeloma (31), osteoporosis (32), and Paget's disease (25). These data suggest that therapeutic modalities that affect IL-6 production and/or activity may be of benefit to these patients.

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References

1. **Gorham L, Stout A.** 1955 Massive osteolysis (acute spontaneous absorption of bone, phantom bone, disappearing bone): its relation to hemangiomatosis. J Bone Joint Surg Am. 37:985–1004.
2. **Choma N, Biscotti C, Bauer P, Licata A.** 1981 Gorham's syndrome: a case report and review of the literature. Am J Med. 83:1151–1156.
3. **Jackson J.** 1838 A boneless arm. Boston Med Surg J. 18:368–369.
4. **Heffez L, Doku HC, Carter BL, Feeney JE.** 1983 Perspectives on massive osteolysis. Oral Surg. 55:331–343.
5. **Kulenkampff HA, Richter GM, Hasse WE, Adler CP.** 1990 Massive pelvic osteolysis in the Gorham-Stout syndrome. Int Orthop. 14:361–366.
6. **Jones G, Midgley R, Smith G.** 1958 Massive osteolysis–disappearing bones. J Bone Joint Surg Br. 40:494–501.
7. **Castleman B, McNeely B.** 1964 Case records of the Massachusetts General Hospital: case #16–1964. N Engl J Med. 270:731–736.
8. **Sage M, Allen P.** 1974 Massive osteolysis: report of a case. J Bone Joint Surg. 56:130–135.
9. **Heyden G, Kindblom L, Moller Nielsen J.** 1977 Disappearing Bone Disease. J Bone Joint Surg Am. 59:57–61.
10. **Cannon S.** 1986 Massive osteolysis. J Bone Joint Surg Br. 68:25–28.
11. **Dickson G, Hamilton A, Hayes D, Carr KE, Davis R, Mollan RAB.** 1990 An investigation of vanishing bone disease. Bone. 11:205–210.
12. **Ohya T, Shibata S, Takeda Y.** 1990 Massive osteolysis of the maxillofacial bones. Oral Surg Oral Med Oral Pathol. 70:698–703.
13. **Takahashi N, Kukita T, MacDonald BR, et al.** 1989 Osteoclast-like cells form in long-term human bone marrow but not in peripheral blood cultures. J Clin Invest. 83:543–550.
14. **Horton M, Lewis D, McNulty K, Pringle JAS, Chambers TJ.** 1985 Monoclonal antibodies to osteoclastomas (giant cell bone tumors): definition of osteoclast-specific cellular antigens. Cancer Res. 45:5663–5669.
15. **Kukita T, McManus LM, Miller M, Civin C, Roodman GD.** 1989 Osteoclast-like cells formed in long-term human bone marrow cultures express a similar surface phenotype as authentic osteoclasts. Lab Invest. 60:532–538.
16. **Kurihara N, Chenu C, Miller M, Civin CI, Roodman GD.** 1990 Identification of committed mononuclear precursors for osteoclast-like cells formed in long-term marrow cultures. Endocrinology. 126:2733–2741.
17. Deleted in proof.
18. **Pfeilschifter J, Chenu C, Bird A, Mundy GR, Roodman GD.** 1989 Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclast-like cells *in vitro*. J Bone Miner Res. 4:113–118.
19. **Kurihara N, Bertolini D, Suda T, Akiyama Y, Roodman GD.** 1990 IL-6 stimulates osteoclast-like multinucleated cell formation in long-term marrow cultures by inducing IL-1 release. J Immunol. 144:4226–4230.
20. **Ohsaki Y, Takahashi S, Scarcez T, et al.** 1992 Evidence for an autocrine/paracrine role for interleukin-6 in bone resorption by giant cells from giant cell tumors of bone. Endocrinology. 131:2229–2234.
21. **Branco F, Da Silva Horta J.** 1958 Notes on a rare case of essential osteolysis. J Bone Joint Surg Br. 40:519–527.
22. **Halliday D, Dahlin DC, Pugh DG, Young HH.** 1964 Massive osteolysis and angiomatosis. Radiology. 82:637–644.
23. **Thompson J, Schurman D.** 1974 Massive osteolysis: case report and review of literature. Clin Orthop. 103:206–211.
24. **Singer F, Schiller AL, Pyle EB, Krane SM.** 1978 Paget's disease of bone. In: Avioli L, Krane S, eds. Metabolic bone disease. New York: Academic Press; 489–575.
25. **Roodman GD, Kurihari N, Ohsaki Y, et al.** 1992 Interleukin-6: a potential autocrine/paracrine factor in Paget's disease of bone. J Clin Invest. 89:46–52.
26. **Loppnow H, Libby P.** 1990 Proliferating or interleukin-1-activated human vascular smooth muscle cells secrete copious interleukin-6. J Clin Invest. 85: 731–738.
27. **Hirano T, Yasukawa K, Harada H, et al.** 1986 Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature. 324:73–76.
28. **Zilberstein A, Ruggieri R, Korn JH, Revel M.** 1986 Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth stimulatory cytokines. EMBO J. 5:2529–2537.
29. **Haegeman G, Content J, Volckaert G, Derynck R, Tavernier J, Fiers W.** 1986 Structural analysis of the sequence coding for inducible 26-kDa protein in human fibroblasts. Eur J Biochem. 159:625–632.
30. **Kishimoto T, Hirano T.** 1988 Molecular regulation of B lymphocyte response. Annu Rev Immunol. 6:485–512.
31. **Klein B, Zhang XG, Jourdan M, Portier M, Bataille R.** 1990 Interleukin-6 is a major myeloma cell growth factor *in vitro* and *in vivo* especially in patients with terminal disease. Curr Top Microbiol Immunol. 166:23–31.
32. **Jilka R, Hangoc G, Girasole G, et al.** 1992 Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science. 257:88–91.