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OSTEOCLAST FEATURES OF CELLS THAT RESORB DEMINERALIZED AND MINERAL-CONTAINING BONE IMPLANTS IN RATS

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Abstract

The comparative temporal tissue response to demineralized bone matrix powder (DBP) and devitalized mineral containing bone powder (MCP) implanted subcutaneously in rats was studied. The tissue response to implanted DBP followed the well described endochondral osteogenic pathway and included the appearance of osteoblasts and osteoclasts. On the other hand, implanted MCP resulted in the appearance of a large population of giant cells that resorbed the implants. At later times (3-4 weeks), most of the cells in the MCP implants appeared as typical foreign body giant cells with extensive membrane foldings, usually away from bone surface. Some cells did, however, have the histological appearance of osteoclasts, although this could not be completely confirmed by transmission electron microscopy. Scanning electron microscopy of anorganic preparations of the MCP following implantation showed resorption pits covering most of the surface, providing additional evidence that the resorption of bone by osteoclasts and giant cells may involve similar mechanisms. The observations suggest that both osteoclasts and giant cells may be involved with the resorption of ectopic MCP.

Key Words: Osteoclasts, giant cells, bone, bone matrix, implants, bone resorption, scanning electron microscopy.

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Introduction

The tissue response to demineralized or undemineralized osseous tissue implants is substantially different. Subcutaneous [30] or intramuscular [37] implantation of demineralized bone matrix in allogeneic rats results in a sequence of cellular and biochemical events reminiscent of endochondral bone formation. In brief, after a transient inflammatory response (day 1) the implanted bone matrix is surrounded by numerous mesenchymal cells (day 3). Proliferation of the cells is followed by their differentiation to chondrocytes (day 7) and vascular invasion and mineralization of the hypertrophic cartilage (day 10). Osteogenesis begins on days 11-13 with the appearance of osteoblasts and is immediately followed by the appearance of osteoclasts (days 14-20). After about 22 days, the implanted matrix is partially replaced with new bone, containing osteoblasts, osteocytes and osteoclasts and developing hematopoietic tissue [14,29].

In contrast to the highly predictable osteogenic events associated with demineralized implants, the subcutaneous implantation of devitalized mineral-containing bone powder results in an initial inflammatory reaction followed by the appearance, within two weeks, of numerous giant cells [6,9] which subsequently resorb the implant. The resorption of these implants has some similarities to the normal resorption of bone by osteoclasts. This has led to some debate on the true nature of the giant cells that are elicited by mineralized implants. Some investigators have noted that the cells have many features of typical giant cells (e.g., foreign body giant cells), while others have noted that they have characteristics of the osteoclast phenotype. Some of the osteoclast attributes noted in these cells include tartrate-resistant acid phosphatase activity, contact-mediated resorption of particles, and morphological specializations including ruffled borders and clear zones at the cell to bone interface [7,9,29]. Furthermore, resorption of bone particles appears to be slowed by calcitonin [10], and the presence of calcitonin receptors have been reported in these cells [11]. These findings have led to the suggestion that the mineralized tissue might promote the expression of the osteoclast phenotype in multinucleated giant cells.

The purpose of this study was to investigate by light and electron microscopy the comparative features of bone-resorbing cells which are present in demineralized and mineralized subcutaneous bone powder implants at different times after implantation in the same animal. This study also examined, using scanning electron microscopy, the areas resorbed on mineralized implants by giant cells,

and compared these areas to those described by osteoclastic bone resorption.

Materials and Methods

Preparation of demineralized and mineralized bone matrix

Compact bone from the femoral and tibial diaphyses of 3 month old Sprague-Dawley male rats was cleaned free of adhering tissues, cut into cylinders approximately 1 cm long, and rinsed 3 times with distilled water to remove blood and bone marrow. The cylinders were washed 1 h in cold distilled water with gentle stirring, defatted in cold acetone and ether (1:1; v/v) for one h, lyophylized and crushed to powder (50 - 300 μm). Some of the bone powder was demineralized in cold 0.5 N HCl for 12 h with slow stirring at a solid-to-solution ratio of 1 g fresh bone powder to 100 ml acid. The demineralized powder was washed repeatedly with cold distilled water for 2 hours, lyophilized and stored at -70° C until the time of implantation. The remaining mineralized bone powder was subjected to three freeze-thaw cycles (-70° C and thawed to +37° C), lyophilized and stored at -70° C until implantation.

Approximately 0.5 cm^3 of demineralized bone matrix powder (DBP) and the same volume of devitalized mineral containing powder (MCP) was implanted subcutaneously in the pectoral region of 3 month old Sprague-Dawley male rats. The DBP was implanted on the right side and the MPC on the left side of each rat. At 5 day intervals, extending from 5 to 30 days, rats were killed by exsanguination under ether anesthesia, implants removed and fixed in buffered formalin for 24 h and prepared for light and electron microscopy.

Light microscopy

DBP and MPC implants were decalcified in 10% EDTA (pH 7.3) for 7 days, dehydrated in ethanol and embedded in paraffin or methyl methacrylate. The tissues were sectioned at 4 μm in thickness, stained with hematoxylin and eosin or toluidine blue and evaluated by light microscopy.

Transmission electron microscopy

DBP and MPC implants were fixed in 0.1 M phosphate buffered formalin (ph 7.4) for at least 1 day. Portions were post-fixed in 0.1 M sodium cacodylate buffered 1% OsO_4 and embedded undecalcified in epoxy resin. Other portions of the retrieved implants were decalcified in 10% EDTA for 7 days, post-fixed OsO_4 and embedded in epoxy resin. Sections for electron microscopy were stained with uranyl acetate and lead citrate prior to viewing in a JEOL 100S electron microscope at an accelerating voltage of 60 kV.

Scanning electron microscopy

Portions of the mineralized implants were fixed for several hours in buffered formalin and then rendered anorganic by treatment with 5% sodium hypochlorite (commercial Chlorox). The specimens were then dehydrated in ethanol, critical point dried, coated with gold and viewed in a JEOL JSM-35 scanning electron microscope at an accelerating voltage of 25 kV.

Results

Demineralized Bone Implants

Five days after implantation of the DMP, connective tissue had developed around the implant. Fibroblasts and differentiating cells were present between the bone particles as well as surrounding the implant. By day 10 after implantation, the majority of the connective tissue was replaced with hyaline cartilage. The cartilage appeared well developed with chondrocytes in all stages of differentiation. Connective tissues encapsulated the implant and were still present in a few spaces among the bone particles and cartilage. Vascular invasion and the appearance bone cells were observed by day 15 post-implantation. Most of the cartilage had been resorbed and blood vessels were present between the bone particles and in the surrounding connective tissue. The implanted bone particles had osteoblasts lining much of the surface with osteoclasts prominent in some areas (Fig. 1). The osteoclasts were usually small, had rounded profiles and usually contained 2 to 4 nuclei. The bone cells were especially abundant in areas of greater vascularization.

After 20 days, the implant surfaces were lined with mature osteoblasts. Osteoclasts were present, especially conspicuous in areas of resorption. The osteoclasts were generally larger than those observed at 15 days and had areas of cytoplasmic vacuolation adjacent to the bone surface. Electron microscopy of these osteoclasts showed an extensive ruffled border associated with the bone-cell interface surrounded by an organelle-free region or 'clear zone' (Fig. 2). The implants appeared similar to the 20 day time period at 25 and 30 days post-implantation. The majority of the bone surfaces continued to be lined with osteoblasts. There are still some osteoclasts present, but the resorption areas are not as prevalent as the formation surfaces.

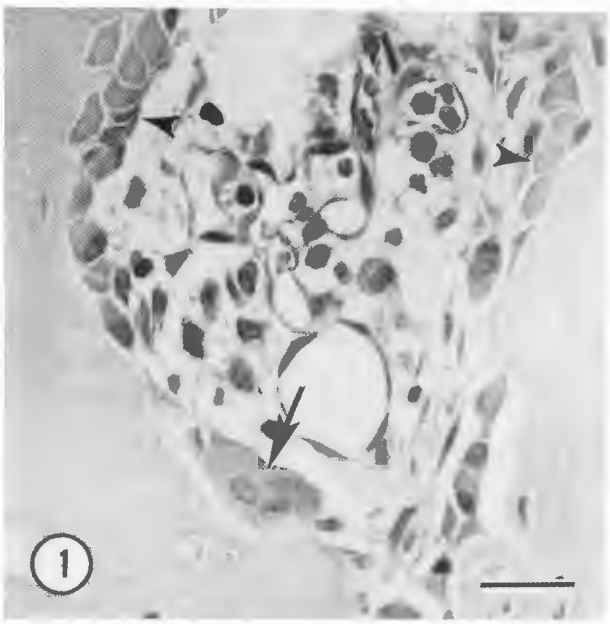
Mineralized Implants

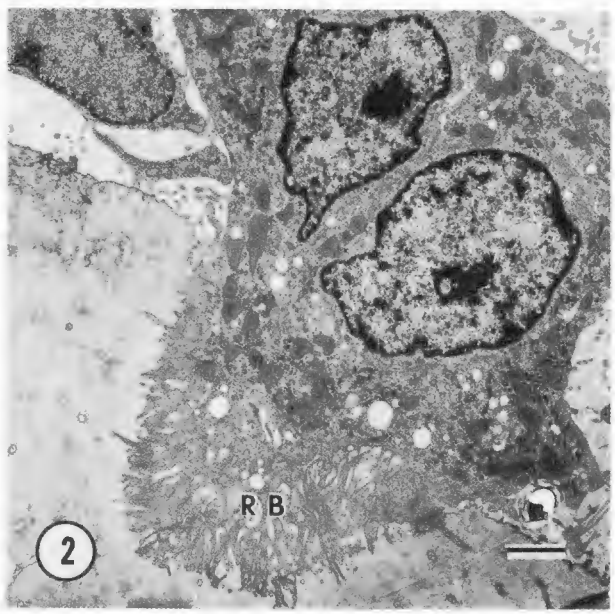
After 5 days, the MCP were surrounded by connective tissues. Some multinucleated cells were also seen in the connective tissues, but many were not adjacent to implant surfaces. At 10 days after implantation, the surrounding connective tissue was more compact and had developed a vascular system. From 10-30 days after implantation, the multinucleated cells increased in size, became more numerous and were usually elongated and extended over the implant surface (Fig. 3). The cytoplasm of these cells had a homogeneous appearance containing many mitochondria and the oval nuclei are linearly arranged within the cell. The cell membranes of these giant cells have numerous invaginations and convolutions at 20 days post implantation, but in contrast to the ruffled borders of osteoclasts, they occur most often on the portion of the cell not adjacent to the bone (Fig. 4). The osseous matrix beneath the giant cells appeared to be undergoing resorption as the surface often had a frayed appearance.

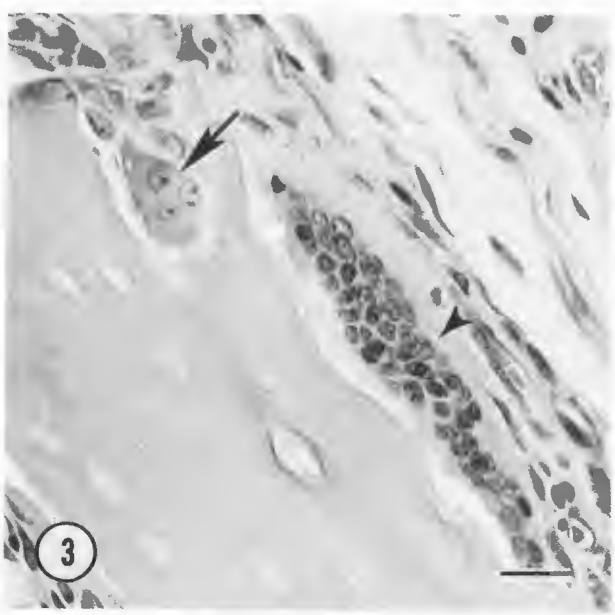
After 20 days of implantation, the majority of the cells adjacent to the remaining bone had the histological appearance of foreign body giant cells, but there was a population of smaller, more rounded, multinucleated cells present (Fig. 3). These cells tended to have larger, more euchromatic-appearing nuclei than those seen in the larger giant cells (Fig. 3). The smaller cells appeared to have a membrane formation, somewhat similar (Fig. 5), but not identical, to ruffled borders typically seen on active osteoclasts (Fig. 2). The cytoplasm of these cells was rich in vacuoles, mitochondria and lysosomes.

Observations with the scanning electron microscope showed the surface of the 20 day mineralized implant to be covered with areas containing resorption pits (Figs. 6 and 7). The fractured surfaces of the particles examined prior to implantation did not have any structures that resembled resorption pits (Fig. 8).

The MCP implants are almost entirely resorbed after four weeks. At this stage of resorption, the huge giant cells, many containing more than 20 nuclei in a profile,







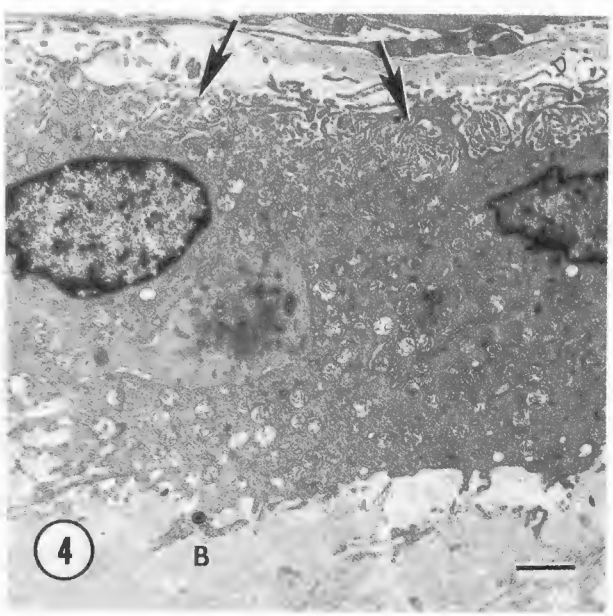


Fig. 1. DBP implant after 15 days. Osteoblasts (arrowheads) and osteoclasts (arrow) are evident on bone surfaces. Bar = 20 μm.

Fig. 2. Transmission electron micrograph of an osteoclast from DBP implant after 15 days. The osteoclast is located in a resorption pit and has a well developed ruffled border (RB). Bar = 2 μm.

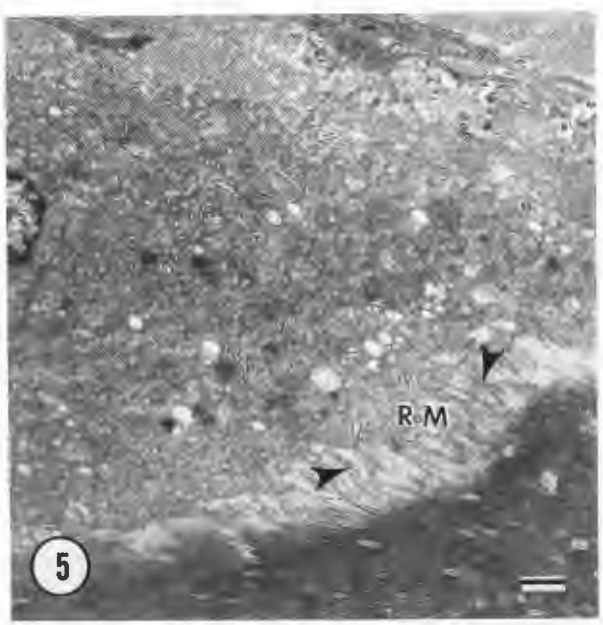
Fig. 3. MCP implant at 28 days. Large giant cells (arrowhead) are found on most of the surface but smaller cells that resemble osteoclasts (arrow) can also be found. Bar = 20 μm.

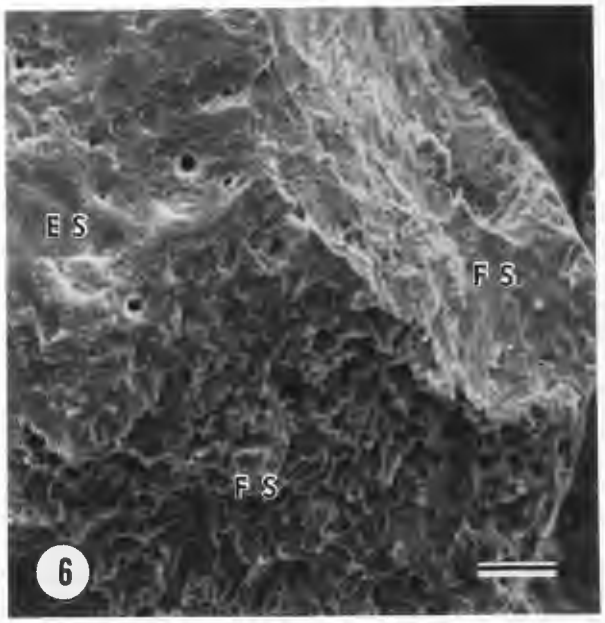
Fig. 4. Transmission electron micrograph of a giant cell adjacent to the bone (B) surface in a MCP implant after 28 days. Some ruffling of the membrane is evident adjacent to the bone surface but extensive ruffling is usually seen on the opposite sides of the cell (arrows). Bar = 2 μm.

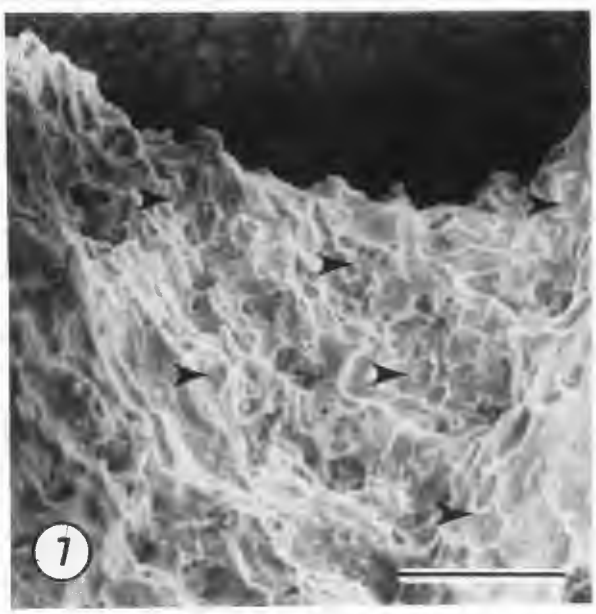
were surrounding the remaining implant particles.

Discussion

This study compared the histological and ultrastructural features of the cellular responses, as a function of time, to demineralized bone matrix particles (DBP) and devitalized, mineral-containing bone particles (MCP). Our experimental system which involves implantation of both demineralized and mineralized bone powder in the same animal, gave us an opportunity to compare two opposite processes; e.g., bone formation







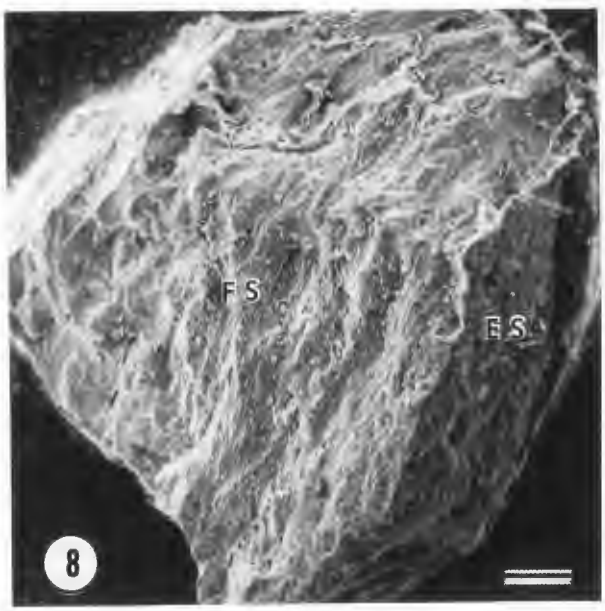


Fig. 5. Transmission electron micrograph illustrating some membrane ruffling (arrowheads) of a smaller giant cell at 28 days after implantation of MCP. While breakdown of the bone particles is evident, the ruffled membranes (RM) do not appear entirely similar to those observed on osteoclasts (compare with Fig. 2). Bar = 1 μm.

Fig. 7. Higher magnfication of MCP retreived after being implanted for 20 days illustrating in greater detail the resorption pits (arrowheads) found on the implant surfaces. Bar = 0.1 mm.

Fig. 6. Scanning electron micrographs of MCP retrieved after being implanted for 20 days. The anorganic surfaces, including the previously existing surface (ES) as well as the fractured surfaces (FS) exhibit resorption pits typical of Howship's lacunae. Bar = 0.1 mm

Fig. 8. Scanning electron micrograph of MCP before implantation. The previously existing bone surface (ES) can be distinguished from the fractured surfaces (FS). Structures resembling resorption pits are not evident on these surfaces. Bar = 0.1 mm.

induced by demineralized bone powder and bone resorption induced by mineral-containing bone powder implants. The cellular events associated with implantation of DBP has been well documented and includes the appearance of osteoblasts and osteoclasts. The osteoclasts induced by the DBP have generally small profiles, containing several nuclei, similar to those found in the skeletal tissues of the

rat. The ultrastructural features of these cells, as noted in this study, are also typical of the osteoclast phenotype.

On the other hand, there is considerable debate on the nature of the giant cells that appear with implants of MCP. Previous studies have documented that these giant cells are generally very large and often have histological features typical of foreign body giant cells and Langerhans cells. They generally lacked ruffled borders and clear zones adjacent to bone surfaces, although they had extensive membrane foldings, often away from the bone surface. These features have led some investigators to conclude that these cells were not osteoclasts [13,28,39]. Contrary to this view, there are reports that giant cells induced by MCP have osteoclast features, including tartrate-resistant acid phosphatase activity, contact mediated resorption of bone powder, membrane specializations structures similar to the osteoclast ruffled border and inhibition of resorption activity by calcitonin treatment [6-10]. Most recently, Goldring et al., [11] have shown that multinucleated cells elicited in response to implants of devitalized bone particles possess receptors for calcitonin. It is possible that the different phenotypes described in these various studies are due to differences in procedures used to prepare the mineral-containing particles.

While the present study does not entirely resolve this issue, there are several findings that are of interest. By 20 days post-implantation, the remaining MCP were surrounded by what appeared to be two types of multinucleated cells. One type had histologic and ultrastructural features typical of multinucleated giant cells [4] including folded or smooth membranes, organelle free areas of cytoplasm, or sometimes these cells exhibited an abundance of mitochondria and lysosomes as well as vacuolated cytoplasm which did not correlate with membrane foldings. Although these cells appeared to be typical giant cells, there was ultrastructural evidence of bone degradation beneath these cells, indicated by the frayed appearance of the matrix.

The other type of multinucleated cell present after 20 days in the MCP implants was smaller and had the histologic appearance of osteoclasts. At the ultrastructural level, these cells had organelles similar to osteoclasts and occasionally a region similar, but not identical, to a ruffled border. While these cells could not be definitively identified as osteoclasts in this study, it does lend some support to the intriguing suggestion made by Glowacki [10] that mineralized implants may promote the expression of the osteoclast phenotype in multinucleated cells. The results from this study also leave open the possibility that at later times after implantation, both osteoclasts and giant cells may be present, but this remains to be confirmed.

When the mineralized bone particles were removed from the animal, cleaned of all organic materials and examined by SEM, numerous resorption bays (Howship's lacunae) were present on the implants. It is already well established that mineralized implants are readily resorbed (but the nature of the cells responsible for the resorption is debated), and this study demonstrates that the resorption surfaces are quite similar in appearance to those in normal bone [16,23]. This lends support to the suggestion that resorption of the bone by either giant cells or osteoclasts may occur in a similar manner.

While osteoclasts and other foreign body giant cells differ both in function and some morphological characteristics, they may share some commonalty in their origin. Current evidence suggests that the initial pathway of osteoclast differentiation is identical to that of mononuclear phagocytes which give rise to foreign body giant cells, but the final pathway is divergent for osteoclasts and mononuclear phagocytes [2,3,20,25,36]. According to this view, the granulocyte-macrophage stem cell is the common progenitor of both the osteoclast and monocyte [38], but at some stage along the differentiation pathway, a committed osteoclast progenitor develops [15,17,27,36]. Alternatively, the noncommitted progenitor may differentiate irreversibly toward the monocyte which after vascular dissemination gives rise to the tissue macrophage. There is little present evidence to indicate that monocytes and macrophages are able to form osteoclasts [2,3,32] or to resorb intact skeletal bone surfaces after their fusion into multinucleated giant cells [1,3,5]. They may, however, resorb mineralized bone powder [3,9,18,31,35].

Bone resorption involves the degradation and removal of both - the mineral and organic components of the bone matrix. Physiologic bone resorption which occurs in normal skeletal bone development and remodeling is considered to be the primary responsibility of the osteoclasts [13,22]. Also, the other bone cells, particularly the osteoblasts or the osteoblast-derived bone lining cells [24] appear now to play an important role in bone resorption by controlling the activity of the osteoclasts. Moreover, other neighboring cells, such as monocytes/macrophages, lymphocytes, fibroblast type cells, or cancer cells, have also been identified as important potential regulators of localized bone resorption through their production of cytokines, prostaglandins, or other mediators. Observations that macrophages and monocytes accumulate near areas of bone resorption *in vivo* [31,33], respond chemotactically to the products of normal bone resorption and components of bone matrix [22,28] and appear capable of bone resorption *in vitro* [12,18] have prompted speculation that mononuclear phagocytes may play a role in normal bone resorption. Also, macrophages are known to secrete collagenase, lysosomal enzymes, and prostaglandins, all of which are believed to be fundamental to bone resorption [34]. In the adult mouse, osteoclast progenitors are found only apart from the bone mesenchyme in tissues with a large mononuclear phagocyte population, the bone marrow and lymphoid organs [36], from which they may be transported by the blood to sites of bone resorption. The suggestion has been made [3] that they may accumulate together with other blood-derived inflammatory bone losses. Also, chemotactic factors might be involved in the invasion of the bone tissue by osteoclast precursor.

It has been shown that some constituents of the bone matrix, such as osteocalcin or type I collagen peptides, are chemotactic for monocytes [21,26], and it has been reported that osteocalcin deficient bone particles implanted subcutaneously in rats were less efficient than normal bone particles [19]. Osteocalcin has not been shown thus far to be chemotactic for true osteoclast progenitors and true osteoclastic nature of multinucleated giant cells elicited around the bone powder implants is still doubtful. Further hormonal and biochemical studies may further distinguish the cell types involved in ectopic bone resorption and relationships between multinucleated giant cells and osteoclasts.

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References

1. Ali NN, Jones SL, Boyde A (1984). Monocyte-enriched cells on calcified tissues. Anat Embryol 170:169-175.

2. Burger EH, Van der Meer JWM, Nijweide PJ (1984). Osteoclast formation form mononuclear phagocytes: Role of bone-forming cells. J Cell Biol 99:1901-1906.

3. Burger EH, Van der Meer JWM, Van de Gevel JS, Gribnau JC, Thesingh CW, Van Furth R (1982). In vitro formation of osteoclasts from long-term cultures of bone marrow mononuclear phagocytes. J Exp Med 156:1604-1614.

4. Carr I (1973). The macrophage: A review of ultrastructure and function. Academic Press, London, New York, pp. 67.

5. Chambers TJ, Horton MA (1984). Failure of cells of mononuclear phagocyte series to resorb bone. Calcif Tissue Int 36:556-558.

6. Glowacki J, Altobelli D, Mulliken JB (1988). Fate of mineralized and demineralized osseus implants in cranial defects. Calcif Tissue Int 33:71-76.

7. Glowacki J, Cox KA (1986). Osteoclast features of cells that resorb bone implants in rats. Calcif Tissue Int 39:97-103.

8. Glowacki J, Goldring SR (1986). Maintenance of osteoclastic features in cells isolated from subcutaneous implants of devitalized bone particles. J Cell Biol 103:104 (abstract).

9. Glowacki J, Jasty M, Goldring SR (1986). Comparison of multinucleated cells elicited in rats by particulate bone, polyethylene, or polymethylmetacrylate. J Bone Miner Res 1:327-337.

10. Glowacki J. (1984). Morphometric analysis in aging bone. In: Genbari C, Segre G (eds.): Proceedings of the First International Congress of Osteoporosis. Excerpta Medica, Amsterdam, pp. 103-108.

11. Goldring SR, Roelke M, Glowacki J (1988). Multinucleated cells elicited in response to implants of devitalized bone particles possess receptors for calcitonin. J Bone Min Res 1:117-120.

12. Holtrop ME, Cox KA, Glowacki J (1982). Cells of the mononuclear phagocytic system resorb implanted bone matrix: A histologic and ultrastructural study. Calcif Tissue Int 34:488-494.

13. Holtrop ME, King GJ (1977). The ultrastructure of osteoclast and its functional implications. Clin Orthop 123:177-196.

14. Howes R, Bowness JM, Grotendorst GR, Martin GR, Reddi AH (1988). Platelet derived growth factor enhances demineralized bone matrix-induced cartilage and bone formation. Calcif Tissue Int 42:34-38.

15. Ibbotson KA, Roodman GD, McManus LM, Mundy GR (1984). Identification and characterization of osteoclast-like cells and their progenitors in cultures of feline marrow mononuclear cells. J Cell Biol 99:471-480.

16. Jones SJ, Boyde A (1970). Experimental studies on the interpretation of bone surfaces studied with the SEM. In: Scanning Electron Microscopy/1970, IIT Research Institute, Chicago, pp. 193-200.

17. Kahn AJ, Simmons DJ, Krukowski M (1981):. Osteoclast precursor cells are present in the blood of preossification chick embryos. Dev Biol 84:230-237.

18. Kahn AJ, Stewart CC, Teitelbaum SL (1978). Contact-mediated bone resorption by human monocytes in vitro. Science 199:988-990.

19. Lian JB, Tissinari M, Glowacki J (1984). Resorption of implanted bone prepared from normal and warfarin-treated rats. J Clin Invest 73:1223-1226.

20. MacDonald BR, Mundy GR, Clark S, Wang EA, Kuehl TJ, Stanley ER, Roodman GD (1986). Effects of human recombinant CSF-GM and highly purified CSF-1 on the formation of multinucleated cells with osteoclast characteristics in long-term bone marow cultures. J Bone Miner Res 1:227-236.

21. Malone JD, Teitelbaum SL, Griffin GL, Senior RM, Kahn AJ (1982). Recruitment of osteoclast precursors by purified bone matrix constituents. J Cell Biol 92:227-230.

22. Marks Jr SC (1983). The origin of osteoclasts: Evidence, clinical implications and investigative challenges for an extra-skeletal source. J Oral Pathol 12:226-256.

23. Miller SC, Jee WSS (1979). The effect of dichloromethylene diphosphonate, a pyrophosphate analog, on bone and bone cell structure in the growing rat. Anat Rec 193:439-461.

24. Miller SC, Jee WSS (1987). The bone lining cell: a distinct phenotype? Calcif Tissue Int 41:1-5.

25. Mundy GR, Roodman GD (1987). Osteoclast ontogeny and function. In: Peck WA (ed.): Bone and Mineral Research, vol. 5; Amsterdam, Elsevier, p. 209-279.

26. Mundy GR, Varani J, Orr W, Gondek MD, Ward PA (1978). Resorbing bone is chemotactic for monocytes. Nature 275:132-135.

27. Nijweide PJ, Burger EH, Feyen HM (1986). Cells of bone: Proliferation, Differentiation, and Hormonal Regulation. Amer Physiol Soc 4:855-886.

28. Popoff SN, Marks SC (1986). Ultrastructure of the giant cell infiltrate of subcutaneously implanted bone particles in rats and mice. Am J Anat 177:491-503.

29. Reddi AH (1984) Extracellular matrix and development. In: Piez KA and Reddi AH (eds.): Extracellular Matrix Biochemistry. New York, Elsevier, p. 375-412.

30. Reddi, AH, Huggins CB (1972). Biochemical sequences inthe transformation of normal fibroblasts in adolescent rats. Proc Natl Acad Sci USA 69:1601-1605.

31. Rifkin BR, Baker RL, Coleman SJ (1979). An ultrastructural study of macrophage-mediated resorption of calcified tissue. Cell Tissue Res 202:125-132.

32. Schneider GB, Brynes JE (1983). Cellular specificity of the cure for neonatal osteopetrosis in the *ia* rat. Exp Cell Biol 51:44-50.

33. Stanka P, Bergsten G (1983). Experimental study on the haematogenous origin of multinucleate osteoclasts in the rat. Cell Tissue Res 233:125-132.

34 Teitelbaum SL, Kahn AJ (1980). Mononuclear phagocytes, osteoclasts and bone resorption. Miner Electrolyte Metab, 3:2-9.

35. Teitelbaum SL, Stewart CC, Kahn AJ (1979). Rodent peritoneal macrophages as bone resorbing cells. Calcif Tissue Int 27:255-261.

36. Thesingh CW (1986). Formation sites and distribution of osteoclast progenitor cells during the ontogeny of the mouse. Dev Biol 117:127-134.

37. Urist MR (1965). Bone formation by autoinduction. Science 150:893-899.

38. Vaes G (1988). Cellular biology and biochemical mechanism of bone resorption. Clin Orthop 231:239-271.

39. Walters LM, Schneider GB (1985). A functional and morphological study of cells adjacent to ectopic bone implants in rats. Am J Anat 173:287-297.

Discussion with Reviewers

M. F. Seifert: How do you reconcile the differences between your findings of resorption lacunae on undemineralized bone particles and those of the authors cited in you paper who have observed no surface resorptive modifications on slices of cortical bone exposed in culture to monocytes, macrophages and multinucleated giant cells? Do you think that differences in particle/matrix size or geometry may contribute to observed differences in phenotypes observed?
Authors: Particle size and geometry and preparative procedures may be the primary determinants governing the cellular phenotypes and cellular reactions that are generated in response to the implants. The observations on cortical bone slices are different from those where devitalized bone particles are resorbed by peritoneal macrophages (Teitelbaum SL, CC Stewart, AJ Kahn, Calcif. Tissue Int. 27:255-261, 1979). Similarly, monocytes will also resorb bone particles in vitro via contact mediated processes and during this process, Howship's lacunae or resorption bays appear to be formed (Kahn AJ, CC Stewart, SL Teitelbaum, Science 199:988, 1978). If monocytes and macrophages can form resorption bays on bone particles in vitro, perhaps we should not have been so surprised to find that giant cells, formed by the fusion of mononuclear phagocytes, could also apparently form resorption bays on bone particles, in vivo.

B. R. Rifkin: It is unclear why formalin fixation was chosen rather than a formaldehyde-glutaraldehyde mixture.
Authors: Glutaraldehyde fixation often causes the connective tissues to become quite brittle, creating greater difficulties for sectioning, particularly in paraffin. In addition, some of the tissues were used for enzyme studies (not presented in this paper) and glutaraldehyde was not recommended because it inhibited the enzymes that we were studying.

B. R. Rifkin: The authors state that resorption pits were observed by scanning electron microscopy and claim that such pits could have been formed by giant cells. Is there evidence that giant cells make resorption pits on devitalized bone chips? Why couldn't all the resorption pits be formed by the osteoclast-type cells?
Authors: The first part of this question has been addressed in the above response to Dr. Seifert. We have not entirely resolved the second issue of whether the resorption pits observed by scanning electron microscopy could have all been formed by the smaller, osteoclast-like cells, rather than the foreign body-type giant cells. Our impression is that the giant cells may form resorption pits as they are often found adjacent to scalloped surfaces, however, we are presently doing a more detailed study looking at different time points to try to resolve this important issue.

G. B. Schneider: You indicate that the resorption surfaces on the particles look similar regardless of the cell involved in the removal of the bone, i.e., osteoclastic bone resorption appears to look like foreign body giant cell bone removal. Can you propose a mechanism by which the foreign body giant cells are removing the bone?
Authors: This is an important question as the mechanisms of osseous tissue resorption by osteoclasts and other cells is not entirely known. The observations made from this study and those cited above in response to Dr. Seifert's question suggest that under these experimental conditions, osteoclasts and cells of the mononuclear phagocyte system may employ similar mechanisms to resorb devitalized, mineral-containing particles.

G. B. Schneider: The phenotypic characteristics of some of the multinucleated giant cells seem to shift toward osteoclast-like features between 3 and 4 weeks after implantation. Do you think there may be a change in the microenvironment at the implantation site at some time after 2 weeks which could account for eliciting osteoclasts or transforming multinucleated cells already present?
Authors: Time may be an important variable in this particular induction system. The microenvironment certainly does change and includes changes in the vascularization and composition of the local connective tissues. Our impression is that with time, more osteoclast-like cells appear around the implants. It is possible that as the particles decrease in size with time that cellular responses might be different as some evidence suggests that smaller particle sizes may favor the osteoclast phenotype (Glowacki J, KA Cox, S Wilcon, Bone Miner. 5:271-278, 1989).

M.F. Seifert: How soon after implantation does one observe these resorption pits and does their appearance and number correspond at all to increases in the numbers of multinucleated giant cells present in the implanted pellet?
Authors: We find some resorption pits on the implants after 10 days, the earliest time that we have examined. Although a semi-quantitative study has not yet been completed, our impression is that the increase in the appearance of the resorption pits coincides with increases in the giant cells.

B. R. Rifkin: Can you estimate the ratio of giant cells to osteoclast-type cells in your MCP implants and, if so, does this ratio change with time? Would this be possible by light microscopy?
Authors: We think the two phenotypes can be recognized by light microscopy and, as noted above, a semi-quantitative study is in progress. Our impression is that the numbers of the smaller, osteoclast-like cells increase with time.

M. F. Seifert: How similar are the resorption pits formed on these implants compared to those produced by osteoclasts on slices of cortical bone or in resorptive areas of intact bone? Are they similar in size, depth, contour?
Authors: They are very similar in size, shape and depth to those described for active bone resorption areas in intact bone.

B. R. Rifkin: Is there any evidence that giant cell lysosomal enzymes are located near the bone surface and secreted from this surface to bone?
Authors: Foreign body giant cells share with osteoclasts the ability to secrete lysosomal enzymes during attempted degradation of extracellular material (bone in the case of osteoclasts). Osteoclasts recognize bone matrix as the appropriate substrate for attack and secrete enzymes at the ruffled border (Miller SC, Calcif. Tissue Int. 37:526-529, 1985). It is an important issue to determine if non-osteoclastic giant cells can also release enzymes to the bone surface. If the resorption pits observed by scanning electron microscopy were indeed formed by giant cells, this would suggest that in this circumstance they share this characteristic with osteoclasts, but further studies are needed to determine the secretion patterns of enzymes in giant cells adjacent to different substrates.

G. B. Schneider: The phenotypic characteristics of some of the multinucleated giant cells seem to shift toward osteoclast-like features between 3 and 4 weeks after implantation. Do you think there may be a change in the microenvironment at the implantation site at some time

after 2 weeks which could account for eliciting osteoclasts or transforming multinucleated cells already present?
Authors: Time may be an important variable in this particular induction system. The microenvironment certainly does change and includes changes in the vascularization and composition of the local connective tissues. Our impression is that with time, more osteoclast-like cells appear around the implants. It is possible that as the particles decrease in size with time that cellular responses might be different as some evidence suggests that smaller particle sizes may favor the osteoclast phenotype (Glowacki J, KA Cox, S Wilcon, Bone Miner. 5:271-278, 1989).

B. R. Rifkin: Cells of the osteoblast phenotype are believed to be absent from the DCP implants. If so, how is osteoclastic activity regulated?
Authors: Current dogma holds that osteoclastic activities are regulated by cells of the osteoblast lineage - a hypothesis that we are not in total agreement with, even among ourselves. We recognize that many tissue and cell culture studies support this contention but in vivo examples can be presented that might argue against this (modeling is an example). It is also possible that in this model of cell induction, the osteoclasts may not be regulated, as they might be in normal skeletal tissues. This raises the important and yet unresolved issue of whether these induced osteoclast-like cells are responsive to systemic calciotrophic influences (e.g., hormones) or local autocrine or paracrine factors.

J. Glowacki: I question the advantage of implanting both types of materials in the same rats. It is not clear why this was done. Could the responses be altered by the contralateral process or its systemic sequelae?
B. R. Rifkin: The purpose of the DBP implant study was not entirely clear. It is to generate true osteoclasts for comparison with cells found in MCP implants?
Authors: Yes. We wished to compare the giants cells in DBP implants with osteoclasts that were also induced in the same animal, at the same time and for the same length of time. This design also allows the comparison with normal skeletal osteoclasts, although this was not presented in this report.

B. R. Rifkin: Might it also be appropriate to examine the MCP undecalcified in order to obtain a clearer view, at the ultrastructural level, of the features of bone degradation?
Authors: The SEM specimens were examined without decalcification, but there would be some advantage to analysis of undecalcified sections by TEM for future studies, although this material is difficult to section.

E. H. Burger: The authors have not fully considered the possibility that the smaller osteoclast-like cells, which appear later, are true osteoclasts, while the large, early cells are fused macrophages, i.e. inflammatory giant cells. All of the available evidence indicates that the prevalence of osteoclast progenitor-precursors in the circulation is very low. Also, osteoclast recruitment seems to depend at least partly on signals originating from bone stromal cells (osteocytes-osteoblasts-lining cells) though the bone powder is devitalized. So, osteoclast development in devitalized bone may take longer than inflammatory giant cell formation.
Authors: The detailed kinetic studies to address these issues have not yet been done. It is entirely possible that the local inductive microenvironment conducive for osteoclasts formation might increase with time, as you suggest. There are a number of possible reasons for this, including changing particle size (decreasing with time), change in local cell types, microvascularization and release of factors from the bone matrix.