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Expression of 92 kD Type IV Collagenase/Gelatinase B in Human Osteoclasts

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ABSTRACT

The digestion of type I collagen is an essential step in bone resorption. It is well established that osteoclasts solubilize the mineral phase of bone during the resorptive process, but the mechanism by which they degrade type I collagen, the major proteinaceous component of bone, is controversial. Differential screening of a human osteoclastoma cDNA library was performed to characterize genes specifically expressed in osteoclasts. A large number of cDNA clones obtained by this procedure were found to represent 92 kD type IV collagenase (gelatinase B; MMP-9, EC 3.4.24.35), as well as tartrate-resistant acid phosphatase. *In situ* hybridization localized mRNA for gelatinase B to multinucleated giant cells in human osteoclastomas. Gelatinase B immunoreactivity was demonstrated in giant cells from eight of eight osteoclastomas, osteoclasts in normal bone, and osteoclasts of Paget's disease by use of a polyclonal antiserum raised against a synthetic gelatinase B peptide. In contrast, no immunoreactivity for 72 kD type IV collagenase (gelatinase A; MMP-2, EC 3.4.24.24), which is the product of a separate gene, was detected in osteoclastomas or normal osteoclasts. We propose that the 92 kD type IV collagenase/gelatinase B plays an important role in the resorption of collagen during bone remodeling.

INTRODUCTION

BONE RESORPTION BY OSTEOCLASTS involves the removal of both the mineral and the organic components of bone.[1] It has been shown that the mineral phase is solubilized by acidification of the subosteoclastic lacuna, thus allowing dissolution of hydroxyapatite.[2,3] However, the mechanism(s) by which type I collagen, the major structural protein of bone, is degraded remains controversial.

Accumulating evidence has implicated cysteine proteinases, in particular the cathepsins, as key enzymes in osteoclastic digestion of type I collagen in bone. Cathepsin B[4] and, in particular, L[5,6] have high activity on native type I collagen at an acidic pH. They act by cleaving the telopeptide extensions of collagen,[7] resulting in depolymerization of the cross-linked fibers[8] and partial degradation. Cathepsin L has been shown to be present in isolated osteoclasts,[9] and specific inhibitors depress bone resorption.[5,10,11]

Interstitial collagenase (MMP-1), a member of the metalloproteinase family, is also capable of degrading native type I collagen to the ¼ and ¾ products TC^A and TC^B, with maximal activity at neutral pH.[12] Most evidence to date has indicated that osteoclasts lack interstitial collagenase,[13] whereas osteoblasts, the bone-forming cell type, secrete this enzyme in response to stimulators of bone resorption, including parathyroid hormone, vitamin D_3, interleukin-1, tumor necrosis factor α, endotoxin, and prostaglandin E_2.[14–16] MMP-1 is associated mainly with the unmineralized bone matrix[17] and was recently identified in the subosteoclastic compartment.[18,19] The *in vitro* resorption of neonatal mouse calvaria was partially inhibited (~50%) by a proteinase inhibitor specific for collagenase and other metalloproteinases.[10] However, this inhibitor failed to inhibit resorption by isolated osteoclasts.[20] These discordant results indicate that osteoblast-derived interstitial collagenase may be necessary for the removal of unmineralized bone (osteoid),[21] which separates resting osteoclasts from mineral-

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ized bone but is not active under the low-pH conditions produced in the resorption lacuna.

In the present study, we demonstrate that another member of the metalloproteinase family, the 92 kD type IV collagenase (gelatinase B; EC 3.4.24.35), is also expressed in high levels in human osteoclasts and multinucleated cells of giant cell tumors of bone. This enzyme was previously shown to be associated primarily with neutrophils and monocytes, in which it facilitates basement membrane degradation and digestion of partially degraded collagen to low-molecular-weight peptides. The possible role for this enzyme in the process of bone resorption is discussed.

MATERIALS AND METHODS

cDNA library, cloning, and sequencing

Poly $(A)^+$ mRNA was isolated from snap-frozen tumor tissue (giant cell tumor of human long bone, Fast-Track; InVitrogen, San Diego, CA). cDNA cloning into a pcDNAII vector was carried out using a commercially available kit (Librarian II; InVitrogen). Clones were screened by differential hybridization using mixed ^{32}P-labeled cDNA probes produced from mRNA isolated from the tumor and from propagated stromal cells (Y.-P. Li, unpublished data).

The stromal cells were expanded in Dulbecco's modified Eagle's medium (high glucose; Sigma) supplemented with 10% newborn calf serum (MA Bioproducts), gentamycin (0.5 mg/ml), L-glutamine (2 mM), and nonessential amino acids (0.1 mM, GIBCO). The stromal cell population was passaged at least five times and showed a homogenous, fibroblastic appearing cell population that contained no multinucleated cells. The cells tested negative for acid phosphatase, but approximately 70% were positive for alkaline phosphatase. It was anticipated that clones reactive with the mixed tumor probe but unreactive with the stromal cell probe should contain either osteoclast-related or *in vivo* "activated" stromal cell-related gene products. cDNA clones that hybridized to tumor cell but not to stromal cell RNA were sequenced by the dideoxy chain termination method of Sanger et al.[22] using Sequenase (U.S. Biochemical). The DNASIS (Hitatchi) program was used to carry out sequence analysis and homology search in GenBank/EMBL.

In situ hybridization

For generation of the probes, gelatinase B cDNA was subcloned into BlueScript II SK, and the 3′ terminus containing the poly(A) tail was removed by endonuclease digestion. This deletion resulted in a 1.1 kb HindIII/XbaI gelatinase B cDNA fragment, which did not indiscriminately cross-react with unrelated poly$(A)^+$ mRNA. The orientation of inserts was determined by restriction analysis of subclones. With ^{35}S labeling (^{35}S-UTP, 850 Ci/mmol; Amersham, Arlington Heights, IL), the T7 promoter was used to generate a collagenase antisense RNA probe; the sense control RNA was generated from the T3 promoter.

In situ hybridization was carried out on 7 μm cryostat sections of a human osteoclastoma, as described previously.[23] Briefly, tissue was fixed in 4% paraformaldehyde and embedded in OCT (Miles, Inc., Kankakee, IL). The sections were rehydrated, postfixed in 4% paraformaldehyde, washed, and pretreated with 10 mM dithiothreitol (DTT), 10 mM iodoacetamide, 10 mM *N*-ethylmaleimide, and 0.1% triethanolamine-HCl. Prehybridization was done with 50% deionized formamide, 10 mM Tris-HCl, pH 7.0, Denhardt's 500 μg/ml of tRNA, 80 μg/ml of salmon sperm DNA, 0.3 M NaCl, 1 mM EDTA, and 100 mM DTT at 45°C for 2 h. Fresh hybridization solution containing 10% dextran sulfate and 1.5 ng/ml of ^{35}S-labeled RNA probe was applied after heat denaturation. Sections were coverslipped and incubated in a moistened chamber at 45–50°C overnight. Hybridized sections were washed four times with 50% formamide and twofold SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0) containing 10 mM DTT and 0.5% Triton X-100 at 45°C. Sections were treated with RNase A and RNase T1 to digest single-stranded RNA, washed four times in twofold SSC and 10 mM DTT, dehydrated, dried, and coated with Kodak NTB-2 emulsion. The duplicate slides were split, and each set was placed in a black box with desiccant, sealed, and incubated at 4°C for 2 days. The slides were developed (4 minutes) and fixed (5 minutes) using Kodak developer D19 and Kodak fixer. Hematoxilin and eosin were used as counterstain.

Immunohistochemistry

Immunohistochemical staining was done on frozen and paraffin-embedded tissues as well as on cytospin preparations, as indicated (Table 1). The following antibodies (Ab) were used: polyclonal rabbit antihuman gelatinase antibodies Ab110 for gelatinase B; Ab31 and 45 for 72 kD type IV procollagenase and collagenase (gelatinase A; EC 3.4.24.24), respectively (provided by Dr. Stetler-Stevenson, Laboratory of Pathology, National Cancer Institute, National Institutes of Health)[24]; monoclonal mouse antihuman CD68 antibody (clone KP1; DAKO, Denmark); Mo1 (CD11b) and Mo2 (CD14) derived from American Type Culture Collection; cell lines HB CRL 8026 and TIB 228/HB44. The antihuman gelatinase B antibody Ab110 was raised against a synthetic peptide with the amino acid sequence EALMYPMYRFTEGPPLHK, which is specific for the human gelatinase B.[24] Ab31 was raised against the synthetic peptide APSPIIKFPGDVAPKTDK, corresponding to the first 18 amino acids of the proenzyme,[25] and Ab45 against NPDVANYNFFPRKPKWDKNQ, which overlaps the cleavage site of the proenzyme. Homology of the amino acid sequence to the gelatinase B is 45% for Ab31 and 25% for Ab45.[26]

For detection, a goat antirabbit glucose oxidase kit (Vector Laboratories, Burlingame, CA) was used according to the manufacturer's directions. Briefly, the sections were rehydrated and pretreated with either acetone or 0.1% trypsin. Normal goat serum was used to block nonspecific binding. Incubation with the primary antibody for 2 h or overnight (Ab110, 1:500 dilution; Ab31 and Ab45, 1:200 dilution) was followed by either a glucose oxidase-labeled secondary antirabbit serum, or in the case of the mouse monoclonals, these were reacted with purified rabbit antimouse Ig before incubation with the secondary antibody.

RESULTS

Characterization of osteoclastoma cells

A cDNA library was produced from mRNA isolated from a human giant cell tumor of long bone ("osteoclastoma"). These

TABLE 1. IMMUNOHISTOCHEMICAL LOCALIZATION OF GELATINASE A AND B IN HUMAN TISSUE[a]

Samples	Antibodies tested: *Ab 110; gelatinase B*	*Ab 31; gelatinase A*	*Ab 45; gelatinase A*
I. Bone giant cell tumors			
Osteoclastoma (long bone, frozen; $n = 2$)			
Giant cells	++	−	−
Stromal cells	−	−	−
Osteoclastoma (long bone, paraffin; $n = 6$)			
Giant cells	++	−	+/−
Stromal cells	−	−	− (5 of 6)
Central GCC (mandible, $n = 4$)			
Giant cells	++ (3 of 4)		
Stromal cells	−		
II. Soft tissue giant cell tumors			
Peripheral GCT ($n = 4$)			
Giant cells	−		
Stromal cells	−		
III. Paget's disease ($n = 3$)			
Osteoclasts	++	−	−
Osteoblasts	−	−	−
IV. normal bone ($n = 3$)			
Osteoclasts	++	−	−
Osteoblasts	−	−	−
V. Monocytes (cytospin)	+	−	−

[a]Distribution of gelatinase A and B in multinucleated giant cells, osteoclasts, osteoblasts, and stromal cells in various tissues. In general, paraffin-embedded tissues were used; exceptions are indicated. GCG, giant cell granuloma, GCT, giant cell tumor. ++, strong staining; +, positive staining; +/−, weak staining; −, no staining.

tumors are actively resorptive and locally invasive but are usually nonmetastatic. In sections, osteoclastomas consist of ~30% multinucleated cells positive for tartrate: resistant acid phosphatase (TRAP), a widely utilized phenotypic marker for osteoclasts,[26] and ~70% "stromal" cells, a mixture of cell types with fibroblastic and mesenchymal morphology. Furthermore, monoclonal antibody reagents were used to characterize partially the surface phenotype of the multinucleated cells in the giant cell tumors of long bone. In frozen sections, all multinucleated cells expressed CD68 (not shown), which was previously reported to define an antigen specific for both osteoclasts and macrophages.[27] In contrast, no staining of giant cells was observed for CD11b or CD14 surface antigens, which are present on monocyte-macrophages and granulocytes.[28,29] Cytocentrifuge preparations of human peripheral blood monocytes were positive for CD68, CD11b, and CD14. These results demonstrate that the multinucleated giant cells of osteoclastomas have a phenotype distinct from that of macrophages and consistent with that of osteoclasts. Although controversial, it is generally held that the osteoclasts in these tumors are nontransformed and are activated to resorb bone *in vivo* by substance(s) produced by the stromal cell element.[30]

Differential screening of a human osteoclastoma library

A total of 12,000 clones drawn from this library were screened by differential hybridization using mixed ^{32}P-labeled probes derived from (1) giant cell tumor mRNA and (2) stromal cells cultivated from the same tumor. Of these clones, 195 gave a positive hybridization signal with tumor mRNA but not with stromal cell mRNA. Among the tumor-specific cDNA clones, 14 of 195 were identified as containing inserts with a sequence identical to TRAP (GenBank Accession Number J04430 M19534). The high representation of TRAP-positive clones indicates the effectiveness of the screening procedure in enriching for clones that contain osteoclast-specific cDNA sequences.

Interestingly, an even larger proportion of the tumor-specific cDNA clones (77 of 195; 39.5%) hybridized to a probe for human gelatinase B (macrophage-derived gelatinase),[31] again indicating high-level expression of this enzyme by osteoclasts. Of the putative gelatinase B clones 25 were subjected to dideoxy sequence analysis; all showed 100% sequence homology to the published gelatinase B sequence (GenBank Accession Number J05070). The portions of the gelatinase B cDNA sequence covered by these clones is shown in Fig. 1.

In situ hybridization of human osteoclastomas

To establish the cell type that expressed gelatinase B mRNA, *in situ* hybridization was carried out on frozen sections of an osteoclastoma. ^{35}S-labeled antisense and sense riboprobes were utilized. As shown in Fig. 2a, reaction of the tumor with the antisense probe resulted in a strong accumulation of silver grains over all multinucleated giant cells and only minimal background labeling over the surrounding stromal cells. In contrast, the sense probe produced only minimal background signal, which was not localized (Fig. 2b). *In situ* hybridization was carried out on a second giant cell tumor with an identical result, indicating the reproducibility of the finding (not shown).

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   1 AGACACCTCT GCCCTCACCA TGAGCCTCTG GCAGCCCCTG GTCCTGGTGC TCCTGGTGCT
  61 GGGCTGCTGC TTTGCTGCCC CCAGACAGCG CCAGTCCACC CTTGTGCTCT TCCCTGGAGA
 121 CCTGAGAACC AATCTCACCG ACAGGCAGCT GGCAGAGGAA TACCTGTACC GCTATGGTTA
 181 CACTCGGGTG GCAGAGATGC GTGGAGAGTC GAAATCTCTG GGGCCTGCGC TGCTGCTTCT
 241 CCAGAAGCAA CTGTCCCTGC CCGAGACCGG TGAGCTGGAT AGCGCCACGC TGAAGGCCAT
 301 GCGAACCCCA CGGTGCGGGG TCCCAGACCT GGGCAGATTC CAAACCTTTG AGGGCGACCT
 361 CAAGTGGCAC CACCACAACA TCACCTATTG GATCCAAAAC TACTCGGAAG ACTTGCCGCG
 421 GGCGGTGATT GACGACGCCT TTGCCCGCGC CTTCGCACTG TGGAGCGCGG TGACGCCGCT
 481 CACCTTCACT CGCGTGTACA GCCGGGACGC AGACATCGTC ATCCAGTTTG GTGTCGCGGA
 541 GCACGGAGAC GGGTATCCCT TCGACGGGAA GGACGGGCTC CTGGCACACG CCTTTCCTCC
 601 TGGCCCCGGC ATTCAGGGAG ACGCCCATTT CGACGATGAC GAGTTGTGGT CCCTGGGCAA
 661 GGGCGTCGTG GTTCCAACTC GGTTTGGAAA CGCAGATGGC GCGGCCTGCC ACTTCCCCTT
 721 CATCTTCGAG GGCCGCTCCT ACTCTGCCTG CACCACCGAC GGTCGCTCCG ACGGCTTGCC
 781 CTGGTGCAGT ACCACGGCCA ACTACGACAC CGACGACCGG TTTGGCTTCT GCCCCAGCGA
 841 GAGACTCTAC ACCCGGGACG GCAATGCTGA TGGGAAACCC TGCCAGTTTC CATTCATCTT
 901 CCAAGGCCAA TCCTACTCCG CCTGCACCAC GGACGGTCGC TCCGACGGCT ACCGCTGGTG
 961 CGCCACCACC GCCAACTACG ACCGGGACAA GCTCTTCGGC TTCTGCCCGA CCCGAGCTGA
1021 CTCGACGGTG ATGGGGGGCA ACTCGGCGGG GGAGCTGTGC GTCTTCCCCT TCACTTTCCT
1081 GGGTAAGGAG TACTCGACCT GTACCAGCGA GGGCCGCGGA GATGGGCGCC TCTGGTGCGC
1141 TACCACCTCG AACTTTGACA GCGACAAGAA GTGGGGCTTC TGCCCGGACC AAGGATACAG
1201 TTTGTTCCTC GTGGCGGCGC ATGAGTTCGG CCACGCGCTG GGCTTAGATC ATTCCTCAGT
1261 GCCGGAGGCG CTCATGTACC CTATGTACCG CTTCACTGAG GGGCCCCCCT TGCATAAGGA
1321 CGACGTGAAT GGCATCCGGC ACCTCTATGG TCCTCGCCCT GAACCTGAGC CACGGCCTCC
1381 AACCACCACC ACACCGCAGC CCACGGCTCC CCCGACGGTC TGCCCCACCG GACCCCCCAC
1441 TGTCCACCCC TCAGAGCGCC CCACAGCTGG CCCCACAGGT CCCCCCTCAG CTGGCCCCAC
1501 AGGTCCCCCC ACTGCTGGCC CTTCTACGGC CACTACTGTG CCTTTGAGTC CGGTGGACGA
1561 TGCCTGCAAC GTGAACATCT TCGACGCCAT CGCGGAGATT GGGAACCAGC TGTATTTGTT
1621 CAAGGATGGG AAGTACTGGC GATTCTCTGA GGGCAGGGGG AGCCGGCCGC AGGGCCCCTT
1681 CCTTATCGCC GACAAGTGGC CCGCGCTGCC CCGCAAGCTG GACTCGGTCT TTGAGGAGCC
1741 GCTCTCCAAG AAGCTTTTCT TCTTCTCTGG GCGCCAGGTG TGGGTGTACA CAGGCGCGTC
1801 GGTGCTGGGC CCGAGGCGTC TGGACAAGCT GGGCCTGGGA GCCGACGTGG CCCAGGTGAC
1861 CGGGGCCCTC CGGAGTGGCA GGGGGAAGAT GCTGCTGTTC AGCGGGCGGC GCCTCTGGAG
1921 GTTCGACGTG AAGGCGCAGA TGGTGGATCC CCGGAGCGCC AGCGAGGTGG ACCGGATGTT
1981 CCCCGGGGTG CCTTTGGACA CGCACGACGT CTTCCAGTAC CGAGAGAAAG CCTATTTCTG
2041 CCAGGACCGC TTCTACTGGC GCGTGAGTTC CCGGAGTGAG TTGAACCAGG TGGACCAAGT
2101 GGGCTACGTG ACCTATGACA TCCTGCAGTG CCCTGAGGAC TAGGGCTCCC GTCCTGCTTT
2161 GCAGTGCCAT GTAAATCCCC ACTGGGACCA ACCCTGGGGA AGGAGCCAGT TTGCCCGATA
2221 CAAACTGGTA TTCTGTTCTG GAGGAAAGGG AGGAGTGGAG GTGGGCTGGG CCCTCTCTTC
2281 TCACCTTTGT TTTTTGTTGG AGTGTTTCTA ATAAACTTGG ATTCTCTAAC CTTT
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FIG. 1. A large fraction of cDNA clones obtained by differential screening of a human osteoclastoma library represent the sequence of gelatinase B. The portions of gelatinase B identified in different cDNA clones are marked on the published nucleotide sequence of gelatinase B. These sequence data are available from GenBank, Accession Number J05070.

Immunohistology of gelatinase B

Paraffin-embedded and frozen sections from osteoclastomas (giant cell tumors) were reacted with a rabbit antiserum against gelatinase B (antibody 110),[24] followed by color development with glucose oxidase-linked reagents. As shown in Fig. 3a, all osteoclasts of a giant cell tumor were strongly positive for gelatinase B, whereas the stromal cells were unreactive. Control sections reacted with rabbit preimmune serum were negative (Fig. 3b). Identical findings were obtained for all eight long bone giant cell tumors tested (Table 1). The osteoclasts present in three of four central giant cell granulomas of the mandible were also positive for gelatinase B expression. These neoplasms are similar but not identical to the long bone giant cell tumors, apart from their location in the jaws.[32] In contrast, the multinucleated cells from a peripheral giant cell tumor, which is a generally nonresorptive tumor of oral soft tissue, were unreactive.[32]

Antibody 110 was also utilized to assess the presence of gelatinase B in normal bone ($n = 3$) and in Paget's disease

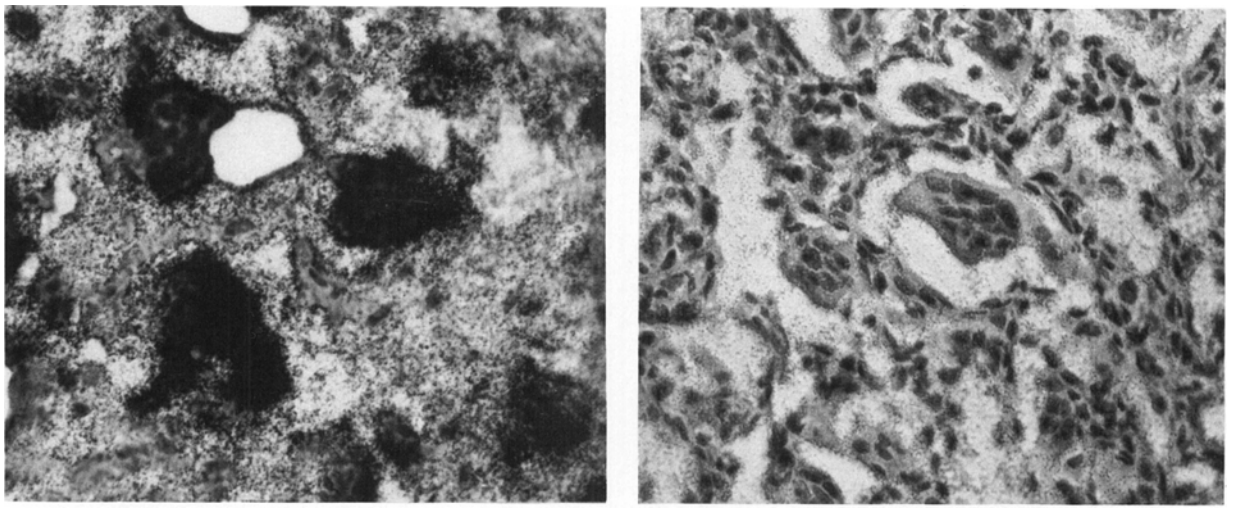

FIG. 2. Gelatinase B mRNA is localized in multinucleated giant cells of a human osteoclastoma but not in the stromal cell population. *In situ* hybridization of frozen sections of a giant cell tumor with ^{35}S-labeled antisense (A) and sense (B) probes for gelatinase B. Original magnification ×440.

(n = 3), in which there is elevated bone remodeling and increased osteoclastic activity. Strong staining for gelatinase B was observed in osteoclasts both in normal bone (mandible of a 2 year old, Fig. 4; hip replacements, not shown) and in Paget's disease (Fig. 5). The uniformly cytoplasmic staining pattern of this secreted enzyme is probably caused by the abundance of gelatinase B and leakage during tissue preparation. Staining was again absent in controls incubated with preimmune serum (not shown). Osteoblasts did not stain in any of the tissue sections, indicating that gelatinase B expression is limited to osteoclasts in bone. Finally, peripheral blood monocytes were also reactive with antibody 110 (Table 1).

Expression of gelatinase A

In contrast to gelatinase B, the gelatinase A enzyme is primarily fibroblast rather than macrophage derived. Its expression has been linked to tumor invasion and metastasis.[30] It was therefore of interest to determine whether gelatinase A was also expressed in osteoclasts and osteoblasts. Two antisera with distinct specificities were used. Antiserum 31 was directed against a synthetic peptide corresponding to the first 18 amino acids of gelatinase A and was previously shown to immunoprecipitate this enzyme and recognize it on western blots.[25] As shown in Table 1, this antiserum did not stain any of the different

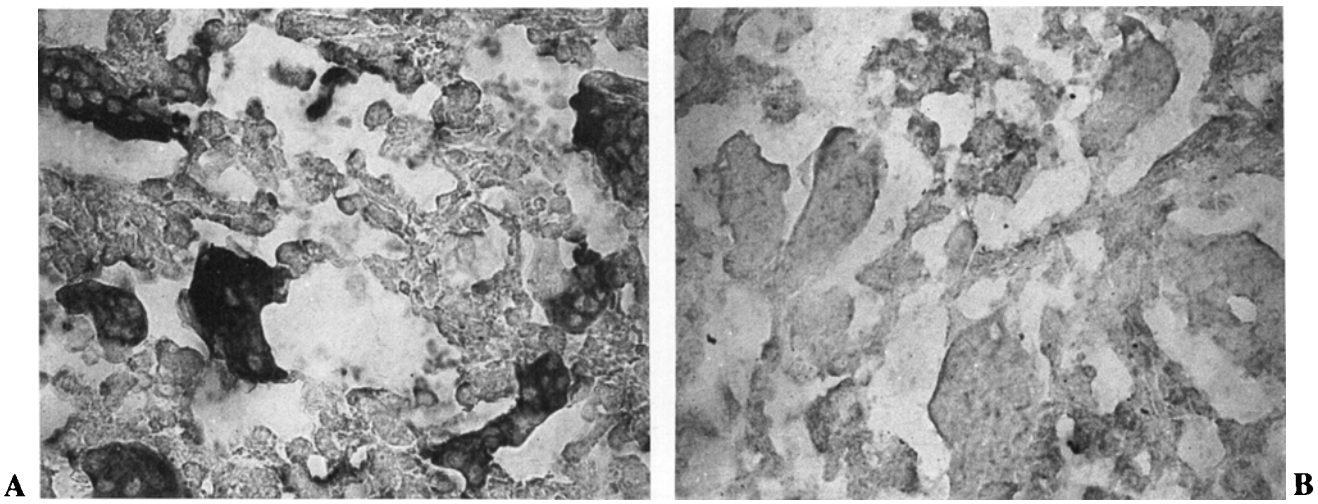

FIG. 3. A polyclonal antibody specific for gelatinase B labels the cytoplasm of multinucleated cells in giant cell tumors. Immunohistochemical staining of frozen sections from a giant cell tumor with the antigelatinase B antibody (A) or with rabbit preimmune serum (B). Original magnification ×440.

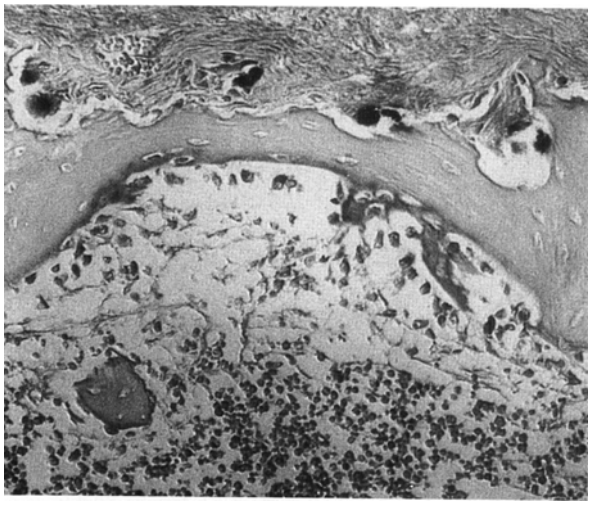

FIG. 4. Gelatinase B is expressed by osteoclasts in normal human bone. Immunohistochemical staining of a section from the mandibular bone of a 2 year old with the antigelatinase B antibody. Original magnification ×110. Insert shows high-power enlargement of marked area. Original magnification ×440.

cell types in tissue sections from giant cell tumors, Paget's disease, or normal bone. Antiserum 45, raised against a peptide that spans the cleavage site, produced trace reactivity with osteoclasts in giant cell tumors, whereas there was no staining of osteoclasts in Paget's or normal bone. No staining of other cell types was observed, with the single exception of reactivity of moderate intensity with a subpopulation of stromal cells (~75%) in one of the paraffin-embedded giant cell tumors. Taken together, these results suggest that, in contrast to gelatinase B, expression of gelatinase A is absent or very weak in human osteoclasts.

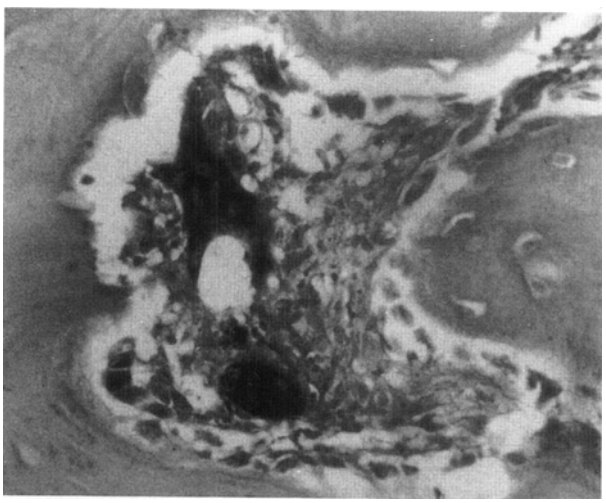

FIG. 5. Gelatinase B immunoreactivity in osteoclasts of a case with Paget's disease. Immunohistochemical staining of bone sections with the antigelatinase B antibody. Original magnification ×440.

DISCUSSION

In this study we demonstrated that the 92 kD type IV collagenase/gelatinase B is expressed in high levels in human osteoclasts. Gelatinase B was present in a large proportion of clones (0.64%) in a human giant cell tumor cDNA library, as identified by differential screening procedures to select clones containing osteoclast-specific cDNA inserts. Gelatinase B mRNA was localized to human osteoclasts in giant cell tumors by *in situ* hybridization and immunohistochemistry and was furthermore shown to be present in osteoclasts from normal bone and Paget's disease. To our knowledge, this report is the first describing the expression of this enzyme in osteoclasts and suggests that gelatinase B may play an important role in bone resorption and remodeling.

Gelatinase B is a member of the metalloproteinase family, which also includes interstitial collagenase, neutrophil collagenase, stromelysin and stromelysin 2, PUMP-1, and the 72 kD type IV collagenase/gelatinase A.[31–33] Gelatinase A and B, although similar in specificity and activity, are the products of separate genes, are immunologically distinguishable, and possess distinct patterns of tissue distribution.[34] Gelatinase B was previously associated with several normal human cell types, including neutrophils,[35] alveolar macrophages,[36] and placental cytotrophoblasts.[37] Its expression has been linked with metastasis in rodent tumor models, but information is lacking with regard to human tumors.[33] Given that gelatinase B is present in osteoclasts from normal bone and Paget's disease as well as giant cell tumors, it appears that the expression of this enzyme in osteoclasts is not merely reflective of a transformed phenotype. Gelatinase A is expressed predominantly in fibroblasts, as well as in many tumor cell types. Elevated expression of gelatinase A has been linked with metastatic potential in carcinomas of the breast and digestive tract, melanomas, hepatomas, fibrosarcomas, and reticulum cell sarcomas.[38] It is of interest that osteoclasts express the "macrophage" rather than the "fibroblast" type of gelatinase, consistent with the common ontogeny of these cell types.[39] Both gelatinase A and B were previously found in rabbit bone,[34] although the cellular expression of these enzymes was not determined. However, only gelatinase A was found in mouse osteoblasts.[40]

Although usually described as a type IV collagenase/gelatinase, the specificity of gelatinase B is in fact somewhat more complex. It acts upon native basement membrane type IV collagen, generating ¼ amino-terminal and ¾ carboxy-terminal fragments.[41] It also degrades native type V (basement membrane) and VII collagens[31] and has some activity against fibronectin and proteoglycans. Gelatinase B can also synergistically degrade type I collagen fragments (gelatin), generated by the action of interstitial collagenase, to low-molecular-weight fragments.[42] Gelatinase B is secreted as a proenzyme and is activated autocatalytically by organomercurials and low pH.[25] Stromelysin 1, a member of the same collagenase family, is also able to activate gelatinase B and has been immunolocalized in osteoclasts.[43] Available data suggest that maximal activity of gelatinase B is exerted at neutral pH.[44] It is inhibited by the tissue inhibitor of metalloproteinase 1 (TIMP-1).[45]

At present, a potential role for gelatinase B in the bone-remodeling process is unknown. Clearly, very limited amounts of its native substrates—types IV, V, and VII collagens—are

present in bone. On the other hand, this enzyme may be important mainly as a gelatinase in completing the degradation to low-molecular-weight peptides of the large type I collagen fragments generated by either interstitial collagenase or cathepsins. With regard to the former, induction of osteoblast-derived collagenase following stimulation with resorptive agents may only partially degrade type I collagen in the osteoid layer to TC^A and TC^B fragments. However, complete removal of osteoid may be effected by the synergistic action of gelatinase B, thereby permitting osteoclastic access to bone mineral.[21,46] Given the neutral pH optimum for gelatinase B, this process may be expected to be extracellular and to precede acidification of the resorption space. Alternatively, gelatinase B could complement cathepsin-mediated collagenolysis, perhaps acting deep in the resorption lacuna, where the pH is likely to be more neutral because of buffering by dissolved bone salts, as suggested by Delaissé and Vaes.[47] This process could also take place intracellularly within vacuoles containing partially degraded collagen.[48] This mechanism presupposes that the pH of such vacuoles is sufficiently elevated, compared with the resorption lacuna, to permit efficient action of gelatinase B. Finally, gelatinase B may also "clean up" the resorption lacuna after detachment of the osteoclast. Further studies are clearly needed to clarify the role of this enzyme in the remodeling process.

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