FINE STRUCTURAL LOCALIZATION OF ACID PHOSPHATASE IN GIANT CELL TUMORS OF BONE

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The fine structural localization of acid phosphatase activity was studied by Gomori’s method in the four cases of giant cell tumors of bone. In the stromal cells, the final reaction product was deposited over lysosomes. And in the multinucleated giant cells, the final product was deposited over lysosome-like organelles and also over the digestive vacuoles of autophagic or of a little heterophagic origin. These vacuoles were suspected to be secondary lysosome-like organelles in various stages. No deposits of reaction product were observed in giant cells in media containing sodium fluoride, L-(+)-tartrate, or those lacking the substrate.

Degenerating giant cells containing autophagic secondary lysosome-like organelles were sometimes observed. It is suspected that their ultimate fates are cell death or autolysis and that acid phosphatase is excreted in these occasions from the cytoplasm to the extracellular medium.

The giant cell tumor of bone is a neoplasm that develops within the bone. These cells differentiate into stromal components and multinucleated giant cells of the osteoclastic type. Most investigators (11, 20) believe that this tumor is derived from the undifferentiated mesenchymal cells of the bone marrow and that the giant cells are formed by either repeated nuclear division without cytoplasmic cleavage (17) or by fusion of stromal cells (9, 18, 19). However, the genesis of the giant cells still remains obscure. It has been known that the lysosomes in giant cells contain high levels of acid phosphatase. Some investigators (1, 15), however, believe that other regions without lysosomes also contain acid phosphatase. The results of their observation are not established. In this report, the fine structural localization of acid phosphatase in four cases of giant cell tumor of bone and the results of inhibition studies are described.

MATERIALS AND METHODS

Case Presentation;

Case 1: A 19-year-old woman suffered from a pain in the right iliac region for several months. Radiographs revealed a lytic lesion in her right iliac bone. After establishing the lytic lesion as giant cell tumor by biopsy the lesion was curetted. The patient is well without any recurrence for one and half year after the treatment.

Parts of the paper were read at US-Japan Histochemistry and Cytochemistry Congress (1982) at the University of British Columbia in Vancouver, Canada.
Case 2: A 26-year-old woman presented herself with a pain in the left knee. Radiographs showed a lytic lesion in the distal portion of her left femur. A biopsy showed a giant cell tumor and the lesion was curetted. However, the lesion recurred after 7 months and the left leg was amputated 20 cm above the knee joint. Lung metastasis was found half a year later and the lesion was removed by surgery. Since then the patient has been well for more than two years.

Case 3: A 17-year-old girl had a pain in the left knee for several months. Radiographs revealed a destructive lesion in the distal portion of her left femur. A biopsy of the lesion was found to be a malignant giant cell tumor and the left leg was amputated. The patient has been well without recurrence or metastasis for a year after surgery.

Case 4: A 75-year-old man had a pain in the right elbow for several months. Radiographs showed a lytic lesion in the distal end of his humerus. Since the biopsy revealed the lytic lesion to be a giant cell tumor, the right arm was amputated although the lesion recurred two years later. The right shoulder joint was disarticulated. The patient has been well without recurrence or metastasis for more than nine months after that operation.

Experiment; Fresh tumor tissue samples were obtained immediately after removal in the operating room. The samples of cases 1, 2 and 3 were obtained at biopsy. The sample of case 4 was obtained at the time of disarticulation of the right shoulder joint. For diagnostic, clinicopathologic purposes, small pieces of tumor tissue were fixed in buffered. Sections cut at 3–5 µm were stained with hematoxylin and eosin. For electron microscopy, samples were prefixed in 2% glutaraldehyde in cacodylate buffer solution (0.1 M, pH 7.4) at 4°C for 24 hr. Postfixation was performed in cacodylate buffered 1% OsO₄ solution. Dehydration and embedding in Epon 812, 1–3 µm thick sections were prepared and stained with alkaline toluidine blue and examined for orientation. After trimming a suitable area of the embedded material, ultrathin sections of silver or gold interference color were cut on a Porter Blum ultratome, double-stained with uranyl acetate and lead citrate and examined by a Hitachi 300 electron microscope.

For both light and electron microscopic histochemical study, the specimens were cut into 2 mm thick fragments and immediately fixed in 2% glutaraldehyde in cacodylate buffer solution (0.1 M, pH 7.4) at 4°C. For light microscopic histochemistry, these small blocks were fixed for 3 hr, and rinsed in cacodylate buffer (0.1 M pH 7.4) containing 0.1 M sucrose at 4°C for 12 hr. The blocks were embedded in paraffin at 50°C for one and a half hr and sectioned at 3–5 µm thickness. Sections were incubated in Gomori medium (pH 5.0) using β-glycerophosphate as a substrate for 30 min at 37°C (5, 6). Then they were treated with dilute ammonium sulfide to develop a visible reaction product, and mounted with Entellan. For electron microscopic histochemistry, the samples were fixed for one hr in the solution, minced by hand or sectioned at about 50 µm with a tissue sectioner. The sections were rinsed in cacodylate buffer (0.1 M, pH 7.4) containing 0.1 M sucrose and kept 12 hr at 4°C. They were incubated in Gomori medium (pH 5.0) at room temperature for 30 min using β-glycerophosphate as a substrate for the demonstration of acid phosphatase using lead as a capture ion.

Media containing 10 mM sodium fluoride or 0.5 M L-(+)-tartaric acid or
0.5 M D-(−)-tartaric acid were used for inhibition studies at the same condition of histochemical studies. Control experiments was performed by incubating the section in the medium lacking substrate. Following incubation, the sections were rinsed several times in cacodylate buffer solution with 0.1 M sucrose (0.1 M, pH 7.4) for 1–2 hr. After dehydration, the materials were embedded in Epon 812. Observation was performed as mentioned previously.

RESULTS

A: Light microscopy

Paraffin-embedded sections were stained with hematoxylin and eosin. All cases except case 3 were diagnosed as giant cell tumors of bone of grade 2. Case 3 was diagnosed as giant cell tumor of bone of grade 3. The sections of each four tumor tissues had various morphological appearances. In general, two main cell types could be found. They were mononuclear stromal cells and multinucleated giant cells. In many areas, a large number of multinucleated giant cells were surrounded by mononuclear cells (Fig. 1A). In other parts, the sections contained spindle-shaped fibroblasts, collagen fibers, and relatively few giant cells. The giant cells appeared in close relation with capillaries. In areas where a large number of giant cells and mononuclear stromal cells clustered, vascularity was most prominent. But vascularity was sparse in areas with fibroblasts and collagen fibers. The giant cells were usually irregularly shaped, varied in size, and contained various numbers of nuclei. Most giant cells contained 10 to 15 nuclei.

B: Electron microscopy;

The mononuclear stromal cells were classified into several morphological types. The most frequently observed stromal cell was of the fibroblastic type in appearance. Characteristic features of these cells were the abundance of rough surfaced endoplasmic reticulum and the well developed Golgi apparatus in the cytoplasm. Cellular and nuclear outlines were irregular. A second type of stromal cells showed macrophage-like appearance with many electron dense mitochondria and lysosome-like bodies, but with a poorly developed endoplasmic reticulum. A third type of stromal cells was fewer in number compared to the former two types. Its characteristic feature was an electron dense cytoplasm with dilated endoplasmic reticulum. The giant cells were encountered frequently and had a highly variable number of nuclei. And one or two irregular nucleoli of varying size were found in most nuclei. The cytoplasm of the giant cells contained numerous electron dense small mitochondria with angulated cristae throughout the cytoplasm. Rough surfaced endoplasmic reticulum was usually located in the periphery of the cytoplasm. Golgi complexes were rather small and were often located in the vicinity of nuclei. Lysosome-like organelle could be observed in any position of the cell and often tended to be accumulated in the vicinity of the Golgi apparatus.

In many multinucleated giant cells, large lysosome-like organelle, secondary lysosome-like organelle and large digestive vacuoles were sometimes observed. Some digestive vacuoles contained free ribosomes and vesicular elements. Other digestive vacuoles showed membranous structures which appeared to be myelin-like material (Fig. 2). In the peripheral region of a multinucleated giant cell, the plasma membrane revealed a complex structure. In some regions it formed microvilli, while it was smooth in other regions. And sometimes peripheral digestive vacuoles
Fig. 1A. A large number of multinucleated giant cells and mononuclear stromal cells are found. Multinucleated giant cells are surrounded by mononuclear cells. Case 2: ×400

Fig. 1B. Dense reaction product is shown in giant cells. Media containing 0.5 M L-(+)-tartrate solution and 10 mM sodium fluoride solution as inhibitors, no reaction product is found. Stand.: incubated in a Gomori medium. L-(+)-Tar.: incubated in a Gomori medium containing 0.5M L-(+)-tartrate. D-(−)-Tar.: incubated in a Gomori medium containing 0.5M D-(−)-tartrate. NaF: incubated in a Gomori medium containing 10 mM sodium fluoride. Case 2: ×400
FIG. 4.

FIG. 5.
FIG. 2. A degenerating organelle and digestive vacuole (V) appear to be fusing. (arrows) Digestive vacuoles contain membranous structures appearing myelin-like materials. Case 1: ×30,000

FIG. 3. Myelin figure-like structures are excreted from a digestive vacuole (V). (arrow) rER: rough surfaced endoplasmic reticulum. Case 1: ×10,000

FIG. 4. Dark and degenerating giant cell is observed. GC: giant cell, S: stromal cell. Case 4: ×3,000

FIG. 5. In stromal cells, the reaction product is precipitated over lysosome-like organelles (arrows). GC: giant cell, S: stroma cell. Case 4: ×7,000

FIG. 6. The giant cell contains distinct reaction product located in numerous vesicular structures and in some lysosome-like bodies (L). M: mitochondria, G: Golgi apparatus. Case 2: ×24,000

FIG. 7. Detail of lysosome-like bodies (L) in a giant cell. Lysosome-like bodies are covered by reaction product. M: mitochondria. Case 2: ×42,000


FIG. 9. High magnification electron micrograph of a giant cell showing lysosome-like bodies (L) and secondary lysosome-like bodies (SL) with marked positive reaction product. Case 1: ×28,000

FIG. 10. High magnification electron micrograph of a giant cell composed of abundant lysosome-like organelles and digestive vacuoles (V) of autophagic or of a little heterophagic origin. Reaction product is confined to lysosome-like organelles of varying size and shape. Small amounts of precipitate are also deposited over parts of digestive vacuoles. Case 1: ×28,000

FIG. 11A. Inhibition study; incubated in a medium containing sodium fluoride. No final reaction product is located in lysosome-like bodies (L) and secondary lysosome-like bodies (SL). Case 1: ×28,000

FIG. 11B. Inhibition study; incubated in a medium containing L-(+)-tartrate. No final reaction product is located in lysosome-like bodies (L) and secondary lysosome-like bodies (SL). Case 1: ×24,000
appeared to excrete digested material to the extracellular spaces (Fig. 3). The degenerating giant cells were occasionally observed (Fig. 4).

**HISTOCHEMISTRY**

With a light microscope, giant cells showed dense reaction products indicating acid phosphatase activity, but stromal cells showed none or a small amount of precipitates of reaction products. No reaction product was observed in tissues incubated in a medium containing sodium fluoride and sodium L-(-)-tartrate (Fig. 1B). With the electron microscope, in the mononuclear stromal cell, reaction product was precipitated over the lysosome-like organelles of varying sizes and shapes (Fig. 5). In the multinuclear giant cell, the reaction product was precipitated over lysosome-like organelles distributed through the cytoplasm (Figs. 6, 7). Reaction products were observed in large secondary lysosome-like organelles (Figs. 8, 9). In most cells, precipitates were localized within digestive vacuoles. The amount of precipitates within the digestive vacuoles was less in the peripheral region of the cell than in the perinuclear region of the cytoplasm (Fig. 10). Precipitation was not seen over the Golgi complex, rough surfaced endoplasmic reticulum or plasma membrane. No deposit was observed in tissues incubated in a medium lacking the substrate or containing sodium fluoride (Fig. 11A) and sodium L-(-)-tartrate (Fig. 11B).

**DISCUSSION**

In previous studies on the fine structural localization of acid phosphatase in giant cell tumors of bone, some investigators (16) concluded that enzyme activity was present only in the giant cells and appeared to be absent in the stromal cells. Other investigators (1) reported distinct localization of reaction products in the stromal cells indicating the presence of acid phosphatase in the organelles which showed the characteristic morphology of lysosomes. They also observed that acid phosphatase was present in conventional lysosome, lysosome-like organelles, vesicles, vacuoles, the Golgi apparatus, and subplasmalemmal vacuolar and tubular structures associated with special function of the plasma membranes in the giant cells. In this study, the author observed that localization of reaction products appeared to be present in conventional lysosomes in stromal cells and with regard to the giant cells to be present in lysosome-like organelles, secondary lysosome-like organelles and digestive vacuoles of autophagic origin. But acid phosphatase activity appeared to be absent in the Golgi apparatus and plasma membranes.

In osteogenic sarcoma, acid phosphatase activity was present in lysosome-like structures and associated vesicles of almost all the cell-types (3). In osteoblastoma which contains three distinct types of cells: osteoblast-like, macrophage-like, and multinucleated giant cells, acid phosphatase activity was present in the lysosomes, most Golgi cisternae and vesicles in the osteoblast-like cells. This enzyme activity in the macrophage-like cells was present in the large and abundant lysosomes. In the multinucleated giant cells this enzyme activity was present in conventional lysosomes, Golgi regions and special organelles probably corresponding to GERL (2).

In osteoclasts acid phosphatase activity was found in tubular structures, vesicles and vacuoles located subjacent to brush border, some Golgi vesicles and Golgi cisternae, extracellular channels and channel expansions in the ruffled border, and such
lysosomal enzyme was released to the extracellular medium and found in cell-bone interspace (7, 8, 14). But giant cells in the giant cell tumor of bone did not show a well developed brush border, and were not in direct contact with bone and there was no evidence that they were involved in bone resorption. McCarthy et al. (15) suggested that secretory acid phosphatase activity was found in the vesicles of the giant cell cytoplasm. Certainly, in patients with giant cell tumor of bone, serum acid phosphatase activity is slightly elevated over normal range (21). The serum level of acid phosphatase activity was 10.7 K·Au in case 1, 6.0 K·Au in case 2, 2.8 K·Au in case 3 and 6.1 K·Au in case 4. The serum level was normal only in case 3. After resection of tumor elevated serum levels returned to normal (1.0–4.0 K·Au).

In this study, it was observed that secondary lysosome-like organelles and digestive vacuoles in perinuclear region of the cytoplasm contained higher acid phosphatase activity than those in the peripheral region of the cytoplasm. Occasionally the digested material appeared to be excreted from peripheral digestive vacuoles. Therefore, the author speculated that acid phosphatase would be released from the vacuoles to the extracellular spaces at the excretion of the digested material. Therefore, serum acid phosphatase activity in patients with giant cell tumor of bone is slightly higher, but it is not as high as in those with bone metastasis in prostatic cancer (21). Acid phosphatase activity would be low when acid phosphatase was excreted from digestive vacuoles, since it was exhausted by digesting the materials. The degenerating giant cells were sometimes seen, and the digestive vacuoles often contained degenerated mitochondria. In a study of cells cultured from giant cell tumors, the giant cells at first showed active movements by extending pseudopodia which eventually degenerated while the mononuclear cells continued to divide (4). These fact that giant cells have no ability of division and are often observed in degenerated form led us to believe that they are destined to death or autolysis. In conclusion, giant cells would be degenerated by autolysis and acid phosphatase would be excreted from cytoplasm at the time when the digested material is excreted. Therefore, the measurement of serum acid phosphatase could be a useful diagnostic tool for detecting occurrence, recurrence and metastasis.

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REFERENCES


