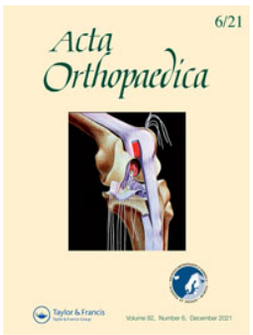

Acta Orthopaedica Scandinavica

ISSN: 0001-6470 (Print) (Online) Journal homepage: informahealthcare.com/journals/iort19

Giant Cell Tumor of Bone: Electron Microscopic and Histochemical Investigations

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To cite this article: Tomas Aparisi (1978) Giant Cell Tumor of Bone: Electron Microscopic and Histochemical Investigations, Acta Orthopaedica Scandinavica, 49:sup173, 1-37, DOI: 10.3109/ort.1978.49.suppl-173.01

To link to this article: https://doi.org/10.3109/ort.1978.49.suppl-173.01

ACTA ORTHOPAEDICA SCANDINAVICA
SUPPLEMENTUM NO. 173

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Giant cell tumor of bone

Electron microscopic and histochemical investigations

BY

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MUNKSGAARD · COPENHAGEN

ISBN 87-16-02984-4
ISSN 0300-8827

The present work is based on studies reported in the following papers:

I Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. Detailed fine structural analysis of different cell components. Virchows Archiv A. Path. Anat. and Histol. 376:273-298, 1977.

II Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. Fine structural localization of acid phosphatase. Virchows Archiv A. Path. Anat. and Histol. 376:299-308, 1977.

III Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Malignant giant cell tumor of bone. Fine structure and localization of acid phosphatase. Submitted for publication, 1978.

IV Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. Fine structural localization of alkaline phosphatase. Virchows Archiv A. Path. Anat. and Histol. (In press) 1978.

V Aparisi. T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. Variations in patterns of appearance of different cell types. Submitted for publication, 1978.

These papers will be referred to in the text by their Roman numerals.

CONTENTS

INTRODUCTION AND HISTORICAL SURVEY

Genuine giant cell tumor of bone represents a definite entity characterized by certain clinical and radiological findings, characteristic histopathological features, and usually favourable prognosis when adequate local therapy is instituted.

The nomenclature, diagnosis, and treatment of giant cell tumor of bone have, in different periods, been in dispute and only in more recent time some measure of agreement has emerged.

The genuine giant cell tumor of bone usually grows slowly, and tends to recur locally. In some instances, it may acquire malignant properties and metastasize.

Sir Astley Cooper (1818) first recognized this tumor, but it was Lebert (1845) who pointed out the benign nature of this lesion. The first classical contribution in the English literature was made by Paget (1853) in his Lectures of Surgical Pathology; he termed this lesion "myeloid sarcoma". The clinical and histopathological features of genuine giant cell tumors were first described, in a systematic manner, by Nelaton (1860), who considered this process benign or only locally malignant.

Virchow (1864-65) was of the opinion that the lesion might recur and even "degenerate into cancer". The difficulties to distinguish giant cell tumor of bone from malignant sarcoma was pointed out by Gross (1879) in a detailed analysis of some seventy cases.

Before the advent of roentgenographic diagnosis - which made more exact identification of this tumor much easier - the lesion was commonly referred to as "giant cell sarcoma", and according to this interpretation the treatment recommended consisted in amputation.

In the beginning of the 20th century this view concerning the malignancy of giant cell tumors of bone was strongly denied by Bloodgood (1923), who cautioned against needless amputation. This opinion was in part supported by Coley (1927); however, the latter investigator stated that "the great majority of cases are benign or at least only locally malignant, but the lesion should still be classed as a sarcoma since in certain cases it has all the clinical features of a malignant bone tumor causing death by metastases".

The true nature of the tumor remained obscure until Jaffe, Lichtenstein, and Portis (1940) made an effort to clarify the situation, separating a number of bone lesions containing giant cells from the genuine giant cell tumor of bone and pointing out the significance of the stromal cells in the histopathological diagnosis. From this detailed study and on the basis of the morphological differences between individual giant cell tumors and the variations among stromal cells, they subclassify giant cell tumors in three grades. This classification is particularly directed towards an evaluation of the aggressiveness of the tumor and is mainly based on the appearance and differentiation of the stromal cells.

Williams, Dahlin, and Ghormley (1954) analyzed 101 cases of giant cell tumor from the Mayo Clinic series of bone tumors and found that 10 per cent of them, although originally considered as benign, later underwent malignant transformation. This is in agreement with earlier studies (Willis, 1949; Russel, 1949) and has been confirmed by later observations (Coley et al., 1958; Jewell and Bush, 1959; Pan et al., 1964; Spjut et al., 1971; Dahlin et al., 1970, Larsson et al., 1975; Meary et al., 1975).

There is now almost general agreement that the histological grade is of no prognostic value.

The origin, behaviour, and significance of the different cells constituting the genuine giant cell tumor has also been a matter of discussion. The multinucleated giant cells have been suggested to originate from bone marrow, foreign body, or endothelial cells. Some consider the giant cells as osteoclasts, actively functioning as absorbers of bone, while others hold that they do not participate in the neoplastic process. The stromal cells have generally been considered as the "principal" or true neoplastic cells in this lesion.

The first electron microscopic description of giant cell tumor of bone was given by Miller and Monteleone (1957). Since then, the fine structure of the different cell components of benign giant cell tumor of bone has been presented in comparatively few studies, mainly from Japan (Fujimoto, 1960; Hirota, 1960; Horie, 1961; Tsuru, 1962; Ueno, 1964; Iwashita, 1965; Kuwana, 1967). In the occidental literature, occasional reports (Marchi and Santacroce, 1969; Steiner et al., 1970; Hanaoka et al., 1972; Boquist et al., 1976) have contributed to a better knowledge of this lesion.

AIMS OF THE PRESENT STUDY

Although - as mentioned above - there are several studies of the fine structure of the various cells constituting the genuine giant cell tumor of bone in the literature of the last two decades, general agreement concerning their morphological characteristics has not been reached; furthermore, the interrelationships, functional significance, and histogenesis of the tumor cells have not been unravelled.

The aim of this study has been a) to describe in detail and define the ultrastructural appearances of the cells; b) on the basis of these ultrastructural studies to attempt to explain interrelationships and possible functions of these cells as well as their histogenesis. In order to achieve these latter goals, the fine structural distribution and localization of acid and alkaline phosphatase in the tumor cells was studied.

MATERIALS AND METHODS

Light microscopy

Sections of tissues fixed in 4% paraformaldehyde (buffered to pH 7.2 with 0.15 M cacodylate buffer) and embedded in paraffin, and 1 μ thick sections prepared from Epon embedded tissues processed for electron microscopy were examined.

Electron microscopy

Small pieces of tumor tissue were trimmed to cubes with a side of ~1 mm under a drop of fixative, and were subsequently immersed in the same fixative. Fixation was carried out either in 2% osmium tetroxide (OsO_4) buffered with s-collidine (0.067 M) or in 3% purified glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and 0.1 M sucrose (Arborgh et al., 1976) for 4 to 24 hours. The glutaraldehyde-fixed tissues were postfixed in s-collidine buffered OsO_4. All the material was dehydrated in ethyl alcohol solutions of increasing strength. Uranyl acetate was included in the 100% alcohol to obtain en bloc staining. The material was embedded in Epon 812. Thin sections were prepared on an LKB Ultrotome, stained with lead citrate, and studied in a Jeol 100 C electron microscope.

Histochemistry

Acid phosphatase. Fixation was carried out in the aforementioned glutaraldehyde solution for 4-24 hours. The tissues were washed and stored at $4^{o}C$ in 0.1 M cacodylate buffer containing 0.1 M sucrose. Approximately 12 hours prior to cutting, the material was immersed in a solution of 0.1 M cacodylate buffer and 0.1 M sucrose with 10% dimethylsulfoxide (Göthlin and Ericsson, 1973; Helminen and Ericsson, 1970). Sections cut at 50 μ were then prepared on a freezing microtome (Leitz) and incubated in a sodium β-glycerophosphate medium for the demonstration of acid phosphatase using lead as capture ion (Göthlin and Ericsson, 1973). Control incubations were performed in a medium containing sodium fluoride or sodium-L-tartrate as enzyme inhibitors or in a medium lacking the substrate. Incubation times varied between 10 and 60 minutes.

Following incubation, the tissue samples were rinsed several times in 0.1 M cacodylate buffer with 0.1 M sucrose and were postfixed in 2% OsO_4 buffered with s-collidine. The material was then treated for electron microscopy as described previously (see above).

Alkaline phosphatase. Small specimens of tumor tissue were fixed by immersion in the above-mentioned glutaraldehyde solution for periods varying from 75 min to 20 hours. Approximately 50 μ thick sections were subsequently prepared on a Leitz freezing microtome, and incubated in a Gomori type medium for the demonstration of alkaline phosphatase (Barka and Anderson, 1963; Göthlin and Ericsson, 1973). The specificity of the reactions was tested by using EDTA as enzyme inhibitor. Control sections were incubated in media lacking the substrate (β-glycerophosphate). After incubation, the sections were treated as described before.

Decalcification. When necessary, decalcification was performed in isotonic, neutral 4.13% solution of ethylenediaminetetraacetate (EDTA) (disodium salt) for 72 hours (Bélanger et al., 1967; Ericsson and Göthlin, 1971).

Treatment with dimethylsulfoxide. In the procedures for the demonstration of acid phosphatase at the ultrastructural level, DMSO (dimethylsulfoxide), (Helminen and Ericsson, 1970) was used in order to avoid tissue damage during preparation, and to facilitate the passage through the cell

membranes of the substrate and capturing ions. DMSO was added to the buffer prior to the preparation of the frozen sections in a concentration of 10%.

CASE HISTORIES

This study comprises 11 cases of benign and 1 malignant giant cell tumor of bone occurring over a period of 5 years and treated at the Section of Orthopedic Oncology, Department of Orthopedic Surgery, Karolinska Hospital, Stockholm (but for one case which was treated at the Sahlgren Hospital, Gothenburg, Sweden). Some of these patients were treated elsewhere before they were referred to the Karolinska Hospital because of recurrence.

The tumors occurred in 8 females and 4 males of widely different age (17-62 years). Ten of the tumors arose in sites typical of giant cell tumors of bone. One was located in the second metatarsal bone and one in the body of the 11th vertebra. A summary of sex, age, location, histologic type (Jaffe et al., 1940), treatment, and follow up is presented in Table 1.

RESULTS

I Benign tumors

A. Light microscopy: paraffin sections

There occurred some variability in the appearance of cellular components both among the different tumors and within the same tumor. Some areas were highly cellular, while others were rich in collagen and the number of cells fairly small. The stromal cells, each containing a single, large nucleus located in an indistinctly defined cytoplasm, often exhibited some degree of cellular atypia and occasional mitoses were observed. The giant cells were quite numerous, varying considerably in size and in the number of nuclei. The giant cell nuclei, which usually were placed centrally, were similar in appearance to those in the stromal cells. The cytoplasm of the giant cells was often granular and occasionally vacuolated. The giant cells occurred in most areas of the tumor, and were often closely related to the capillaries.

B. Light microscopy; Epon sections

More detailed information concerning the structural characteristics of

Table 1.

Case No	Pat	Age	Sex	Location	Initial treatment	Histologic type	Local recurrence	Treatment of recurrence	Follow up after last procedure
1	S G	62	F	Sacrum	Resection of sacrum	Jaffe 2			4 years
2	G B	34	M	Distal femur	Curettage	Jaffe 2	1st after 1 yr 2nd after 2 yrs	Knee endo-prosthesis	2 years
3	M A	17	F	2nd meta-tarsal bone	Curettage	Jaffe 1	After 9 months	Local resection	1 year
4	C J	28	F	Proximal tibia	Curettage	Jaffe 2	After 6 months	Curettage	11 months
5	E A	25	F	Distal ulna	Curettage	Jaffe 2			1 year
6	J H	31	M	Distal radius	Curettage	Jaffe 2	1st after 6 months 2nd " 3 "	Curettage	6 months
7	I A	41	F	Distal femur	Homotrans-plantation hemijoint	Jaffe 1			4 years
8	S T	33	F	Distal humerus	Curettage	Jaffe 1	After 6 months	Curettage	2 years
9	S A	25	M	Distal ulna	Curettage	Jaffe 2	After 4 years	Resection of ulna	4 years
10	L S	33	F	Th 11	Resection Th 11 body	Unclassified (benign)			3 years
11	T J	47	M	Proximal tibia	Curettage	Jaffe 2			6 months
12	A J	42	F	Distal fibula	Resection + ankle fu-sion	Malignant			19 months

the tumor cells could be obtained in toluidine-blue-stained sections of Epon-embedded tissues. The mononuclear stromal cells were irregular in shape, often ovoid, oblong or elongated, containing a single nucleus. The chromatin showed a tendency toward margination. One or two nucleoli were usually present. The giant cells had abundant cytoplasm with vacuoles and marked granularity. Some of the cells showed interconnecting bridges between the different portions of the cells. The latter cells were often located close to capillaries or lakes of red blood cells.

C. Electron microscopy

1. Stromal cells type 1 (I, V). These cells were of fibroblast-like appearance, in some areas tightly packed to form compact groups, in other portions more loosely arranged. Their shape and size varied considerably, often being ovoid with long, irregular extrusions. The cytoplasm contained a well developed endoplasmic reticulum (ER), mainly of the rough-surfaced variety. The cisternae of the ER were irregularly expanded (Fig. 1), with a finely granular content. Widening of the cisternae was observed more frequently in primarily osmium-fixed tissue, but could be observed also in the glutaraldehyde-fixed material. In some cells, the ER occupied almost the whole cytoplasm, creating a profusion of often intercommunicating, largely dilated cisternae.

Prominent, often perinuclearly located, Golgi regions were obvious in most cells (Fig. 1). Organelles presumed to belong to the lysosomal system were rather few. The cytoplasmic ground substance contained particulate glycogen, a well developed system of microfilaments and sparse free ribosomes. In some cases, an extreme folding and irregularity of the nuclear membrane could be observed, with occurrence of pseudoinclusions. Some of these inclusions were composed of different cytoplasmic organelles with appearances resembling those in the cytoplasm. Other inclusions contained elements, often lipid droplets, suggesting a degenerative change.

2. Stromal cells type 2 (I, V). These cells were less frequent than the type 1 cells in the tumors. Their shapes were irregular, and they formed abundant, thin pseudopods and peripheral projections. In general, the cytoplasm contained numerous mitochondria and a well developed lysosomal system

(Figs. 2 and 7). Both the Golgi regions and the ER were poorly developed. Glycogen particles were lacking in the cell sap which contained a moderate number of free ribosomes and microfilaments. The nuclei were rounded with slightly folded membranes and the chromatin focally condensed throughout the nucleoplasm (Fig. 2).

3. Multinucleated giant cells (I, V). These cells were of frequent occurrence and had a highly variable number of nuclei. The nuclei of the giant cells were irregularly shaped with extrusions and deep infoldings of the nuclear membrane. They varied greatly in size. The chromatin was generally condensed toward the peripheral part of the nucleus. A moderately prominent or large nucleolus was often encountered. Nuclear pseudoinclusions were observed (Fig. 3) and resembled those already described in stromal cells type 1. Mitochondria usually occurred in abundance (Fig. 7), and exhibited variable size and shape in the sections. The matrix of the mitochondria was homogeneously dense in tissues fixed primarily in glutaraldehyde while in osmium-fixed tissues the mitochondria appeared slightly swollen with pale matrix.

The ER of giant cells was almost exclusively of the rough-surfaced variety. Generally, it was sparse in the central portions of the cells, but might be more prominent focally in peripheral areas - in some cells forming roughly parallel stacks of cisternae. Aberrations of the ER could be seen in other giant cells where the organelle formed abundant, interconnecting, widely dilated cisternae and tubules filled with moderately electron dense, finely granular material. In such giant cells, the areas with aberrant ER and sparse mitochondria could alternate with areas of more "common" appearance, rich in mitochondria. Furthermore, other cells contained wide portions of cytoplasm almost exclusively constituted of flattened, irregularly dispersed narrow cisternae or tubules of meandering rough-surfaced ER (Fig. 4). Occasionally, areas with rough-surfaced ER forming whorls or finger-print-like figures were encountered. Smooth-surfaced ER of conventional type was of rare occurrence. The Golgi regions were often large and frequently located in perinuclear portions of the cells. In the vicinity of conventionally structured Golgi areas, small vacuoles and expanded smooth-surfaced tubular, sausage-,

horse-shoe-, and ring-shaped elements appeared to form a specialized, peripheral part of the Golgi apparatus. Occasionally, coated vesicles, seemingly budding out from smooth-surfaced Golgi membranes, could be observed.

Occasional giant cells showed the occurrence of a highly characteristic intracytoplasmic tubular system composed of intercommunicating smooth-surfaced canals (Fig. 6).

Single membrane-limited, irregularly shaped bodies containing variable amounts of granular, membranous, or dense material - and probably representing lysosomes - were rather infrequent throughout the cytoplasm. Residual bodies and digestive vacuoles, containing rather well preserved cellular fragments, were observed in many of the cases, but only in some cells. Sometimes, clusters of residual bodies, lipofuscin-like structures and large lysosomes were present in cells with prominent digestive vacuoles.

The cytoplasm contained a fairly well developed system of microfilaments, while microtubules were sparse.

The plasma membrane of the giant cells was commonly smooth or slightly wavy, forming microvillous-like projections. These microvillous-like projections showed a tendency to form a true ruffled border in many cells (Figs. 4 and 5). In some areas - subjacent to the plasma membrane - vacuoles, vesicles, and other organelles were few or absent, and a tendency toward formation of an ectoplasmic layer was noted.

Some cells appeared to be very active in phagocytosis of various materials, such as fibrin and bits of cytoplasm. In the latter case, the cells formed deep invaginations sending large flaps around the material seemingly destined for phagocytosis. Some giant cells were closely surrounded by other cells (mainly stromal cells type 1, but also occasionally type 2 cells). Sometimes these cells were located in invaginations of the giant cells and might be in part surrounded by pseudopods and flaps ejecting from the surface of the giant cells.

4. Possible "intermediate" forms. Clearly identifiable transitional or intermediate forms have not been observed. Binucleated forms occurred occasionally in some tumors. Among the different cytoplasmic organelles in such cells, rough-surfaced ER and microfilaments were most conspicuous. Mitochondria were rather few and lysosomes not large or prominent. Morphologi-

cally, these cells shared more features with type 1 than type 2 cells. The characteristics of giant cells (ruffled border, intracytoplasmic tubular system, ectoplasmic layer, large number of mitochondria) were lacking.

5. Other cell types. Small, infrequent, mononuclear cells were seen in many - but not all - of the tumors examined. They had rounded or kidney-shaped nuclei, often lacking a clearly identifiable nucleolus. The cytoplasm was scanty and contained few organelles.

D. Histochemistry

1. Acid phosphatase (II, V)

Stromal cells type 1. Reaction product was deposited over conventional lysosomes of varying shape and size. Final product was also located over large inclusion bodies with dense matrix containing lipid-like, membranous and granular material.

Stromal cells type 2. Final product was precipitated over the numerous, irregularly shaped, and often large, lysosomes.

Multinucleated giant cells. The findings revealed presence of deposits, indicating activity of acid phosphatase, in single membrane-limited bodies of the conventional lysosome type. Some of the lysosomes showed tubular extrusions which also were covered by reaction product (Fig. 5). The Golgi cisternae and Golgi-associated vesicles and vacuoles often showed presence of distinct lead phosphate precipitate. Furthermore, deposition of final product was found over most of the tubule-, horse-shoe-, sausage-, and ring-shaped elements. some of which were located in areas very close to the Golgi regions. In some cells with prominent digestive vacuoles, fine structural evidence for the presence of acid phosphatase in these elements has been obtained. Finally, occurrence of reaction product was observed in the elaborate systems of intercommunicating canals located subjacent to the cell surface, and showing the appearance of labyrinths (Fig. 6).

Controls. No precipitate was observed in tissues incubated in a medium lacking the substrate or containing sodium fluoride or sodium-L-tartrate.

2. Alkaline phosphatase (IV)

Stromal cells type 1. Most of these cells showed deposits of reaction product located over the plasma membrane in a focal, spotty fashion, or all

the way around the circumference of the cells. In some areas, a fine sprinkling of deposits was noted in the extracellular matrix. Conspicuous precipitates of reaction product were observed in areas where cells were lying close together; these deposits were thinner on the "free" surfaces (Fig. 1). Deposits of lead phosphate were also observed over the membranes bordering vesicles and vacuoles subjacent to the plasma membrane. Occasionally, final product was also found to be associated with the membranes of infolded channels emanating from the cell surface, and with tail-like expansions of subplasmalemmal vacuoles. No reaction product was observed in other structures in the cells.

Stromal cells type 2. Deposition of reaction product was never observed within these cells or on their plasma membranes.

Multinucleated giant cells. Presence of faint deposits of reaction product could be observed over the plasma membrane in many of these cells in some of the tumors; in many other giant cells, there was a complete lack of precipitates. Occasionally, strong, focal deposits were noted on the plasma membranes, often in areas with cytoplasmic material resembling the "ectoplasmic layer". Other components of the multinucleated giant cells were devoid of precipitates.

Other cell types. Deposits of reaction product were noted on the membranes bordering erythrocytes and on the plasma membranes of endothelial cells in the small tumor vessels.

Controls. Tissues incubated in a medium lacking the substrate or containing EDTA as enzyme inhibitor lacked deposits signifying enzyme activity.

II Malignant tumor (III)

A. Light microscopy

Examination of tissue obtained both from the biopsy and the locally resected tumor confirmed the diagnosis of malignant giant cell tumor of bone. The tumor tissue contained areas rich in cells with fibroblast-like polymorphous appearances, showing numerous atypical mitoses and infiltrative-destructive growth. Giant cells were moderate in number and comparatively small. The number of nuclei varied considerably in these cells. Relatively

large regions showed presence of abundant, lipid-rich cells resembling foam cells or xanthoma cells.

B. Electron microscopy

1. Fibroblast-like cells. These cells were abundant in the tumor tissue, varied considerably in shape, and might be markedly irregular, with centrally or excentrally located nucleus, often containing 1-3 often irregular nucleoli. Like in the benign counterpart, the ER was the most conspicuous cytoplasmic organelle in the cells, presenting a great variability in the size and shape of individual cisternae. Mitochondria, with irregular dilation of the cristae, were of frequent occurrence. Phagocytic vacuoles were rare, as were also other types of vacuoles. Particulate glycogen and fat droplets have not been encountered.

2. Cells with abundant lipid inclusions and mitochondria. These cells are extremely irregular in shape, forming abundant, blunt extrusions and pseudopods and having deeply infolded areas of their plasma membranes. The nuclei with irregularly folded and protruding nuclear membranes had a tendency to imitate the cellular outlines. Sparse nucleoli occurred and tended to be highly irregular and large. The chromatin was in general more compact and dense than in the nuclei of fibroblast-like cells. The cells were easily distinguished by the presence of numerous large lipid "droplets" in the cytoplasmic matrix and in single membrane bordered lysosome-like organelles, abundance of mitochondria, occurrence of large and numerous organelles resembling lysosomes, and presence of large phagocytic vacuoles subjacent to the plasma membrane. In many cells, the bulk of the cytoplasm was almost completely occupied by the lipid droplets and droplet-filled lysosome-like elements. The mitochondria varied in size and shape; no dilatation of cristae, or other special features, were noted. Vacuoles of endocytic (phagocytic) type were frequently encountered subjacent to the plasma membrane. These vacuoles became more abundant in areas lying in the vicinity of necrotic or severely damaged cells. No dilatation or expansion of the moderately well developed ER was observed. Golgi regions were inconspicuous. Presence of particulate glycogen in the cell sap was not observed.

3. Multinucleated giant cells. In comparison with the giant cells in benign giant cell tumors, the corresponding cells in the malignant counterpart contained more abundant vacuoles, and also more numerous smooth-surfaced, ring-shaped organelles, and Golgi-associated tubular elements. Although microvillous-like protrusions of the plasmalemma were encountered, genuine ruffled borders were never observed. In some cells structures resembling the ectoplasmic layer in osteoclasts were found.

C. Histochemistry

Acid phosphatase

Fibroblast-like cells. The deposits of reaction product were limited to conventional lysosomes.

Cells with abundant lipid-inclusions and mitochondria. Lead phosphate precipitates were confined to the different lysosome-like bodies described above.

Giant cells. Reaction product occurred in conventional lysosomes and Golgi elements. Small vacuoles and vesicles located near the Golgi apparatus also showed presence of final product. Furthermore, precipitate was observed over smooth-surfaced tubular elements with ring-shaped (tire-like) appearances.

Controls. No precipitates were observed in tissues incubated in media containing sodium fluoride or L-tartrate, or lacking the substrate.

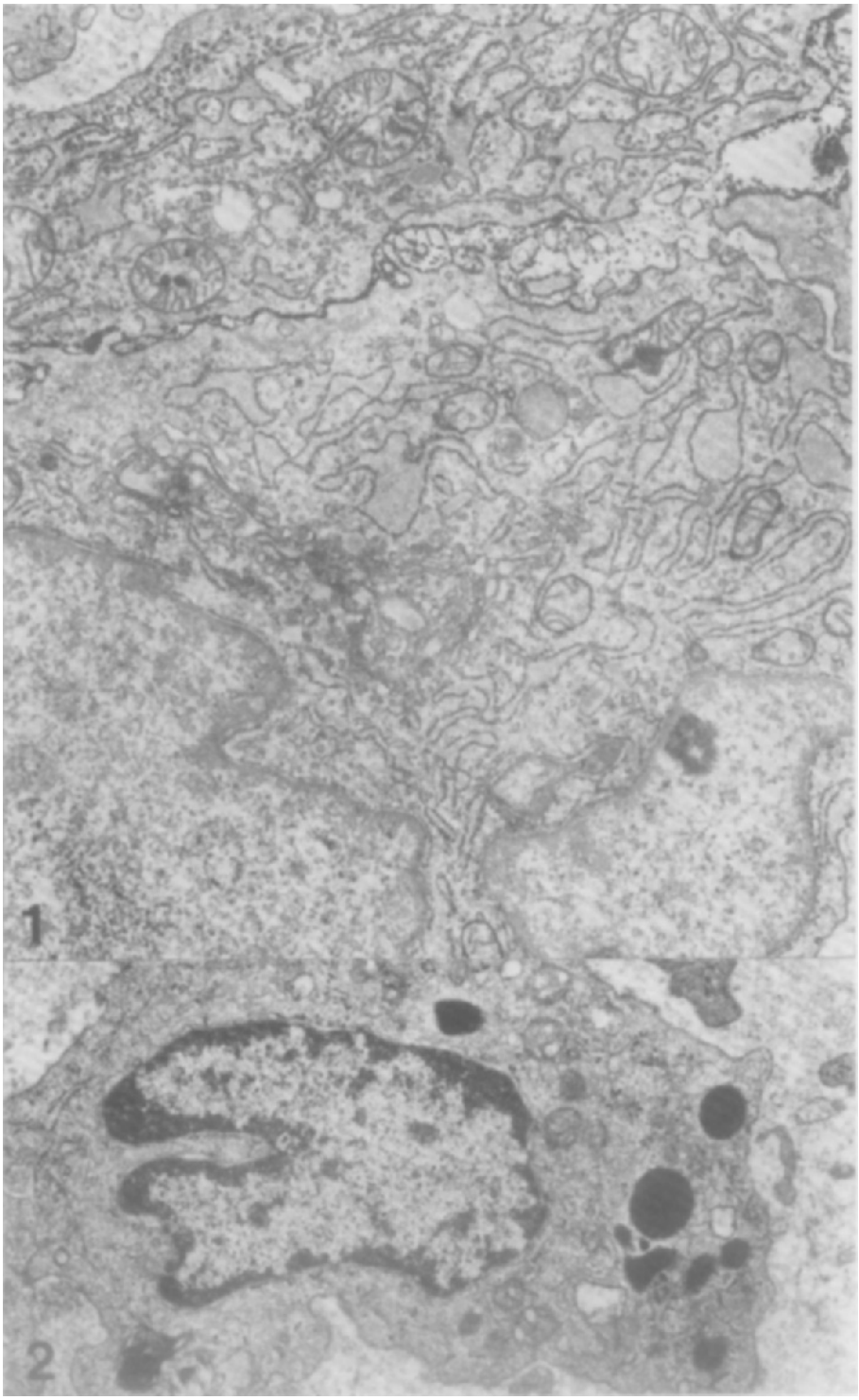
DISCUSSION

Ultrastructure of the different cells

Giant cell tumors of bone are characterized histologically by the presence of multinucleated giant cells, interspersed with areas of mononuclear stromal cells. Earlier, light microscopic studies have failed to distinguish between the different types of stromal cells (Hutter et al., 1962; Mnaymneh and Ghandur-Mnaymneh, 1967; Oberling et al., 1967; Oliva et al., 1974). On the basis of electron microscopic investigations, however, distinct subgroups of cells could be differentiated (Horie, 1961; Ueno, 1964; Iwashita, 1965; Hanaoka et al., 1970; Steiner et al., 1972).

Fig. 1. From tissue incubated for the demonstration of alkaline phosphatase. Electron micrograph showing portions of two stromal cells type 1 which are partially tightly applied. Reaction product indicating enzyme activity is deposited on the plasma membrane of both cells. Note irregular dilatations of the rather abundant rough-surfaced endoplasmic reticulum and the large Golgi apparatus in the binucleated cell in the lower part of the picture. Incubation time 90 minutes. x 14,000.

Fig. 2. Picture illustrating the appearance of a stromal cell type 2. The cytoplasm contains several lysosome-like bodies with dense matrix. x 14,500.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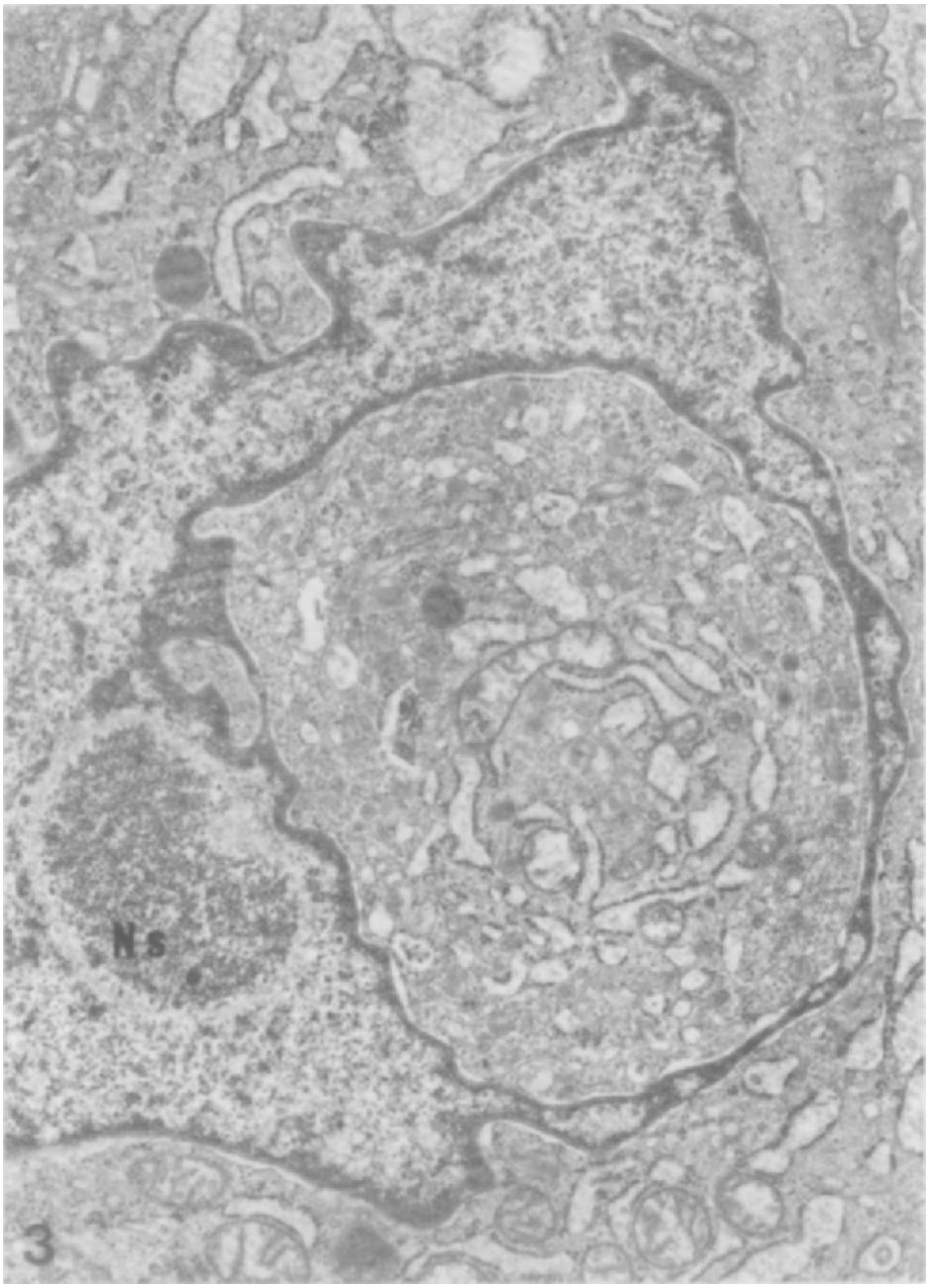

Fig. 3. Portion of giant cell with a large nuclear pseudoinclusion containing cytoplasmic organelles such as rough-surfaced endoplasmic reticulum, mitochondria and lysosomes. Ns, nucleolus. x 18,500.

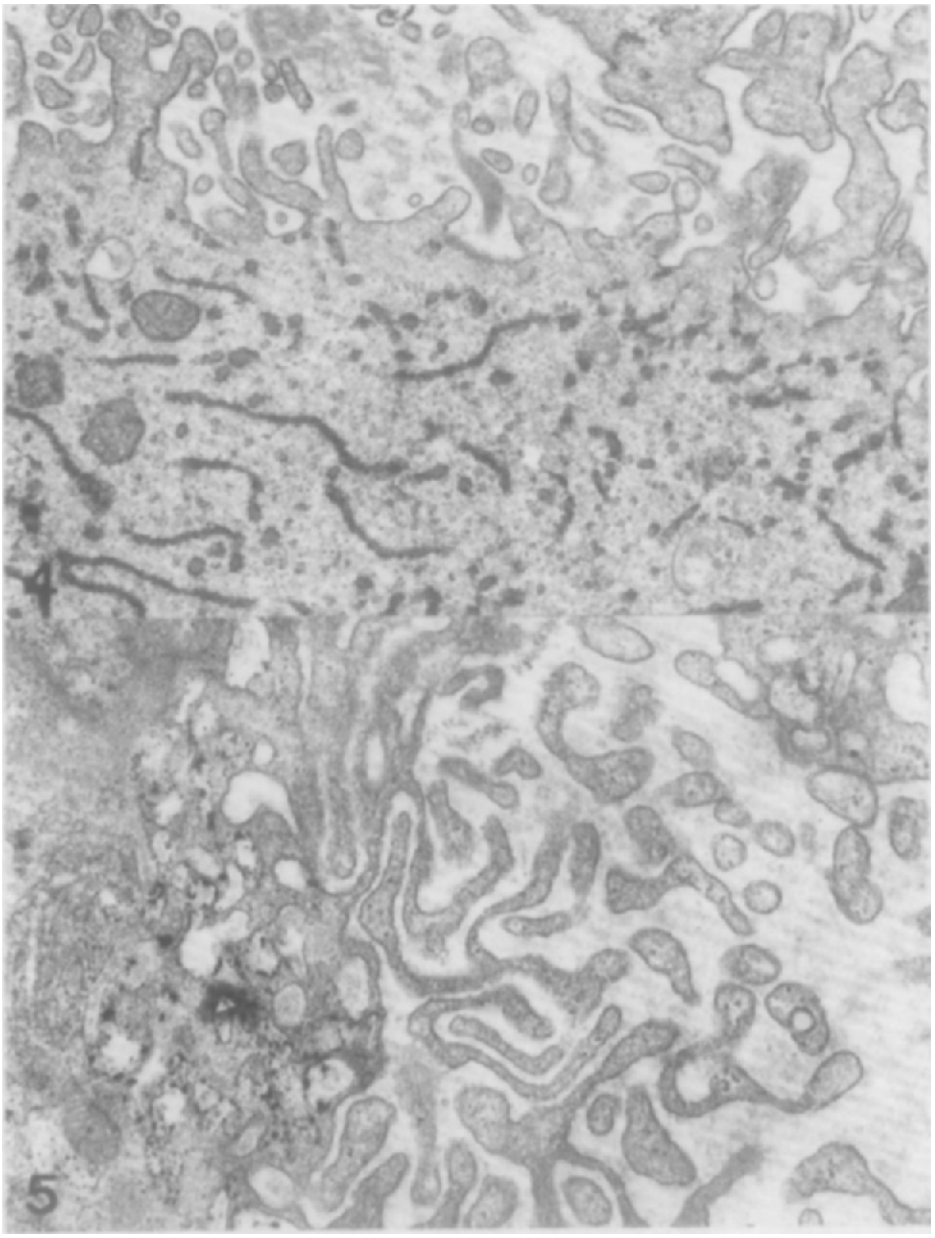

Fig. 4. Superficial area of a giant cell with irregular microvillous-like protrusions. Collapsed tubule-shaped elements of the rough-surfaced endoplasmic reticulum are haphazardly distributed in the subjacent cytoplasm. x 18,500.

Fig. 5. From tissue incubated for the demonstration of acid phosphatase. Picture showing ruffled border-like differentiation of the plasma membrane and the presence of tubular and vesicular enzyme-containing elements in the superficial portions of the cytoplasm. Incubation time 20 minutes. x 30,000.

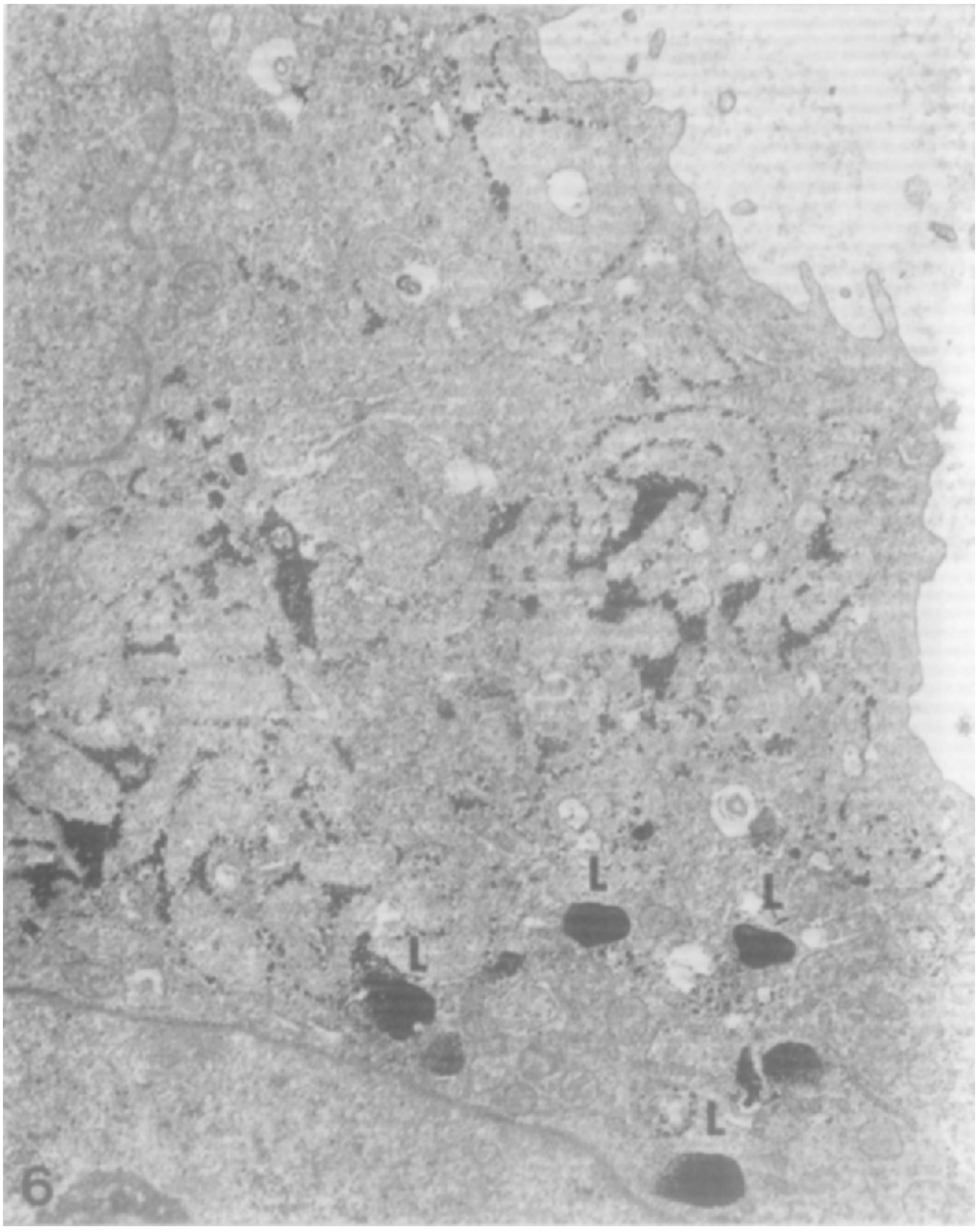

Fig. 6. From tissue incubated for the demonstration of acid phosphatase. Portion of a giant cell with abundant reaction product deposited over lysosomes (L) and a system of partly interconnected tubular and cisternal smooth-surfaced structures ("labyrinth"). Incubation time 15 minutes. x 15,000.

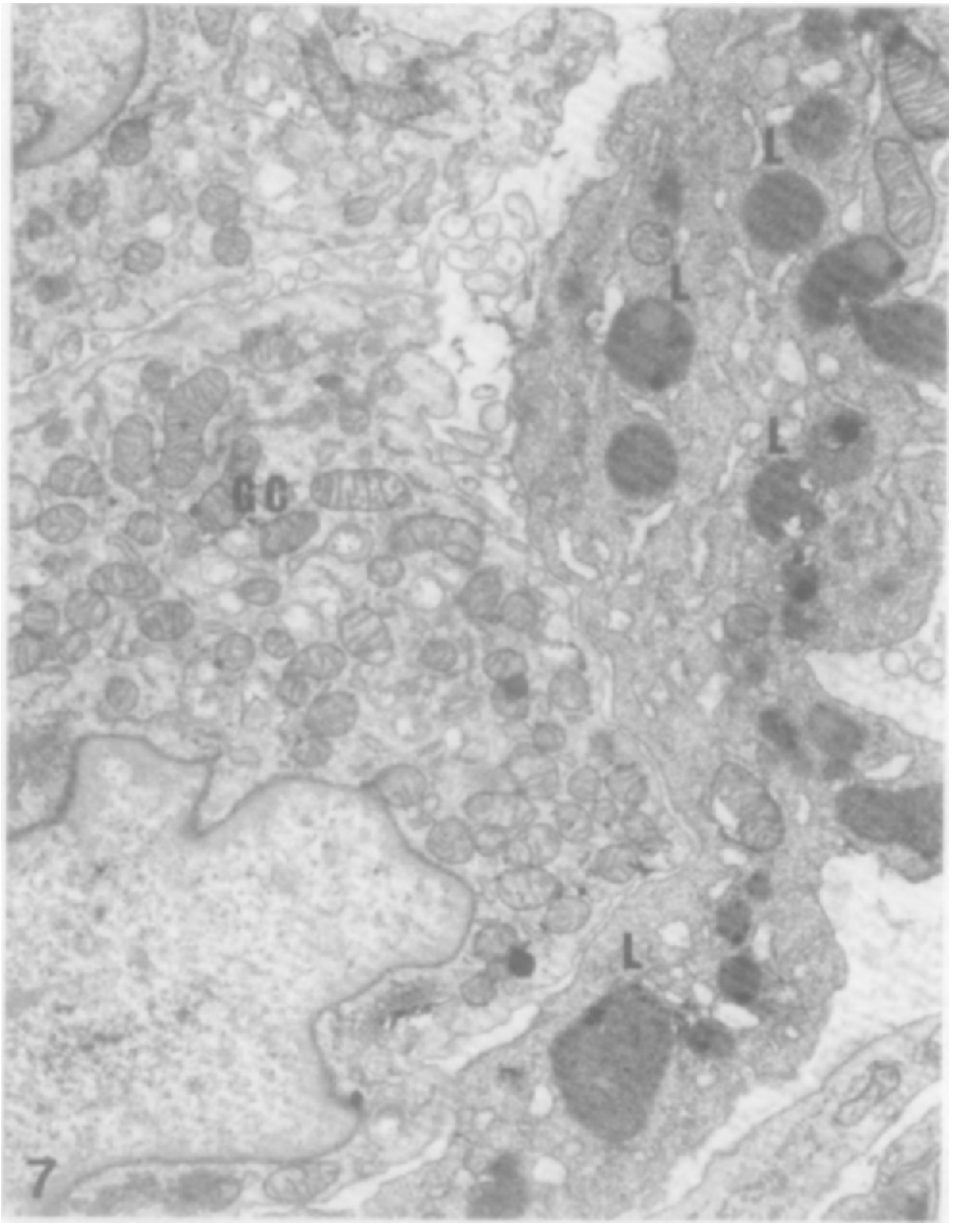

Fig. 7. Picture illustrating a portion of a giant cell (GC) with closely applied stromal cells type 2 containing numerous lysosomes (L) in its cytoplasm. x 15,000.

The results of the present study revealed the existence of three distinct cell types which were always present in these tumors: stroma cells type 1 (fibroblast-like); stromal cells type 2 (macrophage-like); and multinucleated giant cells. Other, small, mononuclear cells of enigmatic origin, possibly emanating from the blood or the reticuloendothelial system, may also occur, but they can not be considered of importance in the neoplastic process.

The predominant type 1 stromal cell resembles the fibroblast both in shape and cytoplasmic content. The nucleus is irregular, however, with deep infoldings and pronounced lobulation. Many stromal cells type 1 showed nuclear pseudoinclusions. They were evidently created by the pronounced tendency of the nuclear membrane to fold and wrinkle. These nuclear pseudoinclusions have not been described previously in genuine giant cell tumors. The significance of these nuclear alterations is not clear, but similar nuclear aberrations occur in different types of tumor cells. The findings in the present study thus agree with the notion that stromal cells type 1 are true tumor cells. The prominent ER, indicating a high degree of protein synthesis, is frequently observed in tumor cells but can also be encountered in rapidly growing non-neoplastic, fibroblastic tissues, and similar appearances have been reported in cells that clearly were non-neoplastic (Brown et al., 1974; Cooper et al., 1973). Aberrations of the ER in the form of dilatation, irregularity, and increased overall size were noted in stromal cells type 1. In terms of interpreting the appearance of the ER, all that can be said at present is that the findings support the idea that stromal cells type 1 actively participate in the synthesis of proteins, probably -- among other compounds - the collagen which in some areas of the tumors is quite abundant; further that the pleomorphism of this organelle is compatible with the view, that the cells are neoplastic in nature.

The type 2 stromal cells, much less frequent than type 1 cells and with the appearance of macrophages, were a more homogeneous group of cells. The variability of their nuclei and the components of the cytoplasm was limited. This suggests that they are not active in the proliferative processes in the tumor tissue. These macrophage-like cells may instead play a role in the immunological balance in the giant cell tumor; perhaps their presence signifies

a host response against the tumor cells.

The multinucleated giant cells, by definition, are always present in genuine giant cell tumors of bone. The role of these cells in the biological properties of the tumor has been discussed on the basis of light and electron microscopic observations (Johnson, 1930; Jaffe et al., 1940; Schajowicz, 1961; Hanaoka et al., 1970; Steiner et al., 1972). The resemblance in structure between giant cells and osteoclasts has led some authors to conclude that the giant cells are active in resorptive processes in the bone and that they are responsible for the bone destruction resulting from the growth of this tumor (Schajowicz, 1961). The findings in the present study serve to illustrate the fact that there is a certain similarity between - at least some - tumor giant cells and osteoclasts; however, they are not identical. Thus, the organization of the cell surface structure differs in that osteoclasts mostly show a ruffled border, while the giant cells only rarely exhibited such a structural specialization. Also, the "ectoplasmic layer", often present in osteoclasts, is found more infrequently in the tumor giant cells. These differences in appearance do not exclude the possibility that the cells have developed from the same "stem" cells; they may reflect functional changes of the cells conditioned by the neoplastic environment. Alternatively, true osteoclasts and neoplastic giant cells may be present concomitantly in the same tumor.

Variations in the appearance of the ER were often noted in the giant cells. These aberrations were more pronounced than in the fibroblastic stromal cells. In addition, the giant cells might show presence of finger-print-like formations of the rough ER and thin, flattened widely dispersed cisternae without connection with one another. This variability of the rough-surfaced ER is presumed to express a considerable plasticity of this organelle in the giant cells, probably reflecting a propensity for accomodation to different functional states.

Phagocytic activity

Many digestive vacuoles, large inclusion bodies, and residual bodies were observed in the cytoplasm of the giant cells, supporting the idea that these cells actively participate in the digestive phase of the phagocytic process. This activity was exercised within the lysosomal system, since the

bodies showed ultrahistochemical evidence of presence of acid phosphatase, and accordingly are to be classified as lysosomes. The phagocytic vacuoles carrying acid phosphatase might either be heterophagic or autophagic.

The nature and origin of the vacuolar structures with tail-expansions, sausage-shaped, tubular elements and ring- and horse-shoe-shaped structures distributed haphazardly throughout the cytoplasm is somewhat uncertain. The result of the ultrahistochemical studies indicate that they are part of the lysosomal system, despite their morphologic appearance. One possibility is that all, or part, of this system may represent areas of GERL, since especially the sausage-shaped tubular elements appear to be closely related to the Golgi regions. Some ring-shaped elements may correspond to newly formed autophagic vacuoles. The evidence obtained seems to indicate that the phagocytic and lytic activity of giant cells becomes accentuated following malignant transformation.

Localization of alkaline phosphatase

Previous studies, at the light microscopic level, on the localization of non-specific alkaline phosphatase in giant cell tumors of bone have either demonstrated faint deposition of reaction product in the giant cells (Kraievski et al., 1970; Thiery et al., 1975), or failed to reveal the presence of this enzyme activity in both stromal and giant cells (Schajowicz, 1961; Mnaymneh and Ghandur-Mnaymneh, 1967; Gilmer, 1954; Jeffree and Price, 1965). Ores et al. (1969), in an electron microscopic study of one case of giant cell tumor of bone, were unable to provide evidence of the occurrence of alkaline phosphatase activity in either stromal or giant cells.

The findings in the present investigation have proven the existence of an enzyme causing the hydrolysis of β-glycerophosphate at an alkaline pH in or on the plasma membrane of giant cells and stromal cells type 1. This enzyme appears to be the same as was studied in previous reports by others and represents the non-specific alkaline phosphatase (E.C.3.1.3.1.) (Aparisi et al., 1978 a, b). The relatively weak deposits of reaction product may explain why earlier light microscopic studies have not revealed the enzyme activity. Strong inhibition of enzyme activity by the fixative has perhaps contributed to prevent identification of the enzyme in some of the previous

studies. The activity of alkaline phosphatase is comparatively strong in bone forming cells (Göthlin and Ericsson, 1973), and likewise in bone forming tumors (osteoblastic osteogenic sarcomas) (Jeffree and Price, 1965). Presence of alkaline phosphatase activity in stromal cells type 1 support the idea that these cells have a kinship with bone forming cells.

Osteoclasts have never been demonstrated to carry alkaline phosphatase. However, few investigations have been carried out at the ultrastructural level, and the possibility must be left open that in the studies of osteoclasts, complete inhibition of the enzyme activity by the fixative may have occurred. Since the localization of acid phosphatase in osteoclasts differs somewhat from that in giant cells, current knowledge may be taken to suggest that the two cells are unrelated to one another, the tumor giant cells seeming to be histogenetically more related to the alkaline phosphatase-carrying stromal cells type 1, and the osteoclasts to macrophage-like cells.

Osteolytic activity

Presence of acid phosphatase in several cell components of the giant cell tumor of bone is in accordance with the notion that these organelles play a role in the osteolysis causing bone destruction. The activity seems to be specially strong in the giant cells and the stromal cells type 2. However, activity of acid phosphatase has so far only been demonstrated intracellularly. The cytoplasmic labyrinths present in some of the giant cells show a clear resemblance to subplasmalemmal tubular systems demonstrated in osteoclasts and are presumed to connect with the cellular surface (Göthlin and Ericsson, 1976). These structures in osteoclasts probably contribute to the secretion of lysosomal enzymes to the extracellular space (Göthlin and Ericsson, 1976; Lucht, 1971). If they have a direct connection with the surface in the case of giant cells is so far unknown. Convincing evidence that bone material is taken up by the giant cells has not been obtained.

Origin of giant cells

The mode of formation and origin of giant cells has been a matter of discussion. The possibility of amitotic division advocated by Schajowicz (1961) and Gallardo et al. (1970) in studies of tissue culture has been con-

sidered. In some fibroblast-like type 1 cells, the pronounced "lobulation" of nuclei might suggest a formative stage in amitotic division of nuclear segmentation. The close spatial association between stromal cells type 1 could, on the other hand, support the "fusion" theory. However, clear evidence for this mechanism is lacking in the present study.

The histochemical observations indicate that, with respect to the fine structural localization of acid phosphatase, there are clear differences between both types of stromal cells and giant cells. However, the findings can not be taken to exclude the possibility that any of these cells represent precursors of giant cells. Concerning the localization of alkaline phosphatase, the present study supports the view that stromal cells type 1 and multinucleated giant cells are histogenetically related. If, indeed, the fusion theory is correct, the observations support the concept that the mononuclear stromal cells type 1 represent the precursors of giant cells.

SUMMARY

The fine structure of the various cells constituting giant cell tumors of bone was studied in 12 cases, 11 of which were benign and 1 malignant. Besides pure morphological studies, ultrahistochemical investigations on the localization and distribution of nonspecific acid and alkaline phosphatase in the different cells present within these tumors were performed.

Three distinct cell types were found to always be present in these tumors: stromal cells type 1 (i.e. fibroblast-like); stromal cells type 2 (i.e. macrophage-like); and multinucleated giant cells.

Stromal cells type 1 of fibroblast-like appearance had an irregular outline, and, characteristically, the cytoplasm showed presence of abundant, often polymorphous, rough-surfaced endoplasmic reticulum and prominent Golgi regions; particulate glycogen was commonly observed in the cytoplasm in cells of the benign variant, but was lacking in the malignant counterpart. In the latter, the type 1 cells were pleomorphic and showed numerous atypical mitoses.

Stromal cells type 2 with the macrophage-like appearance were less frequent than the type 1 cells, and contained many mitochondria and lysosomes in their cytoplasm. while the endoplasmic reticulum was inconspicuous. No glycogen was noted in the cytoplasmic ground substance. In the malignant tumor, these cells showed an extremely irregular shape, forming numerous extrusions; the cytoplasm usually contained abundant, large, lipid-like droplets, and numerous mitochondria.

Multinucleated giant cells in the benign tumors were large, with variable numbers of nuclei. Mitochondria usually occurred in abundance. Generally, rough-surfaced endoplasmic reticulum was sparse in central portions of the cells but tended to be more abundant in the periphery. Golgi regions, often perinuclearly located, were large and prominent. Smooth-surfaced, tubular, sausage-, horse-shoe-, and ring-shaped structures were commonly observed in the vicinity of the Golgi regions. A fairly well developed system of microfilaments in the cytoplasmic ground substance was frequently encountered. Certain quantative differences were noted between the appearance and distribution of various organelles in the benign tumors and the malignant one.

The range of morphologic variability of the different cell types has been established. Variations in fine structural appearance include presence of nuclear pseudoinclusions in giant cells and type 1 stromal cells; aberrations in the configuration of the rough-surfaced endoplasmic reticulum in the same cell types; and presence of ruffled borders, ectoplasmic layers and cytoplasmic labyrinths in the giant cells.

The study of the localization of nonspecific acid phosphatase showed presence of reaction product in stromal cells type 1 and 2 solely in conventional lysosomes. In the multinucleated giant cells, lead phosphate precipitate was deposited over lysosome-like organelles, and Golgi cisternae, vesicles and vacuoles. Smooth-surfaced, tubular, sausage-, horse-shoe-, and ring-shaped structures also showed presence of final product. In addition, evidence for acid phosphatase activity was obtained in digestive vacuoles, residual bodies, and labyrinthal formations in the cytoplasm of the giant cells.

Deposition of final product indicating presence of alkaline phosphatase was obtained on the plasma membrane and the membranes bordering subplasmalemmal vesicles and vacuoles in the giant cells and type 1 stromal cells.

Occurrence of large acid phosphatase-containing digestive vacuoles, residual bodies and lysosomes in giant cells suggested that these cells participate very actively in phagocytic and lytic activities.

The morphologic evidence gathered favoured the notion that stromal cells type 1 are neoplastic; on the other hand, convincing neoplastic properties in stromal cells type 2 are lacking.

The nature, function, origin and possible participation in the neoplastic process of the multinucleated giant cells was discussed against the background of the fine structural and histochemical observations.

The results of the enzyme histochemical analysis of acid and alkaline phosphatase supported the view that stromal cells type 1 and multinucleated giant cells are histogenetically related. The possible role of stromal cells type 2 in the immunological balance in the giant cell tumor is mentioned. The fine structure of these cells suggest that one of their main functions is endocytosis, presumably of a predominantly phagocytic nature.

ACKNOWLEDGEMENTS

My thanks are due to Professor Sven Olerud, Head of the Department of Orthopedic Surgery at Karolinska Hospital, for his support and active interest throughout this investigation.

I also wish to express my deeply felt gratitude to Professor Bengt Engfeldt, Head of the Department of Pathology at Huddinge Hospital, for placing the facilities of his Department at my disposal.

This investigation was carried out under the guidance of Professor Jan L.E. Ericsson, Laboratory of Clinical Pathology, University of Uppsala. He gave me the benefit of his extensive experience and deep insight in electron microscopy and histochemistry, and I am greatly indebted to him both for this and for his unfailing encouragement and enthusiasm.

Thanks are also due to Associate Professor Ulf Nilsonne, Department of Orthopedic Surgery at Karolinska Hospital, for his generous support, his friendly encouragement and fruitful collaboration.

I am also indebted to Docent Bengt Arborgh, who introduced me to the field of electron microscopy and has taken an active part in the present investigation.

My old friend and colleague Docent Gustav Göthlin originally suggested the topic of this study and I wish to thank him for his continuous interest and encouragement.

My grateful thoughts also go to my former teacher and personal friend, the late Docent Hilding Wetzenstein, who taught me the valuable principle of combining a critical approach to basic research with undiminished interest in the patient and in clinical practice.

I gratefully acknowledge the skilful technical assistance of Miss Silwa Mengarelli and the photographic work performed by Mr. Magnus Norman.

My thanks are also due to Mrs. Brigitte Giertz and Mrs. Ingrid Lettström for their help in typing the manuscript.

I am indebted to my colleagues at the Department of Orthopedic Surgery Karolinska Hospital, for their loyal support and readiness to facilitate my work during this investigation.

This study was supported by grants from the Swedish Cancer Society.

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