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Histochemistry of tartrate-resistant acid phosphatase and carbonic anhydrase isoenzyme II in osteoclast-like giant cells in bone tumours

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Summary. Using routinely processed, paraffin-embedded tissue specimens, osteoclast-like giant cells in giant cell tumour of bone (GCT), chondroblastoma, osteoblastoma and osteoblastic osteosarcoma were examined histochemically for osteoclast-specific enzymes tartrate-resistant acid phosphatase (TRAP) and carbonic anhydrase isoenzyme II (CA-II). Osteoclast-like giant cells and some mononuclear cells possessed TRAP activity. These were further classified with respect to CA-II immunoreactivity, i.e. cells with CA-II were seen in GCT and chondroblastoma, while those in osteoblastoma and osteoblastic osteosarcoma were negative for CA-II. All the cellular components in malignant fibrous histiocytoma and various extraosseous inflammatory lesions including malignant giant cells and macrophage polykaryons were negative for both TRAP and CA-II. These results indicate that osteoclast-like giant cells in GCT, chondroblastoma, osteoblastoma and osteoblastic osteosarcoma are all osteoclasts and generated by fusion of mononuclear cells with the same histochemical characteristics as osteoclast-like giant cells. The difference in CA-II immunoreactivity suggests the functional or maturational difference between osteoclast-like giant cells in GCT and chondroblastoma and those in osteoblastoma and osteosarcoma.

Key words: Osteoclast-like giant cells – Osteoclast – Bone tumours – Tartrate-resistant acid phosphatase – Carbonic anhydrase isoenzyme II

Introduction

Osteoclast-like giant cells are significant components of certain neoplastic lesion of bone, such as giant cell tumour of bone (GCT), non-ossifying fibroma, chondroblastoma, osteoblastoma, osteosarcoma and malignant fibrous histiocytoma (MFH) (Dahlin and Unni 1986; Mirra et al. 1989). The morphology and acid phosphatase histochemistry indicate that these giant cells are either osteoclasts or macrophage polykaryons; without further characterization of these giant cells, they have been referred to "osteoclast-like" giant cells because of their localization in bone lesions.

The osteoclast is now believed to originate from a progenitor cell similar to that of the monocyte-macrophage, and is considered to be a member of the mononuclear phagocyte system (Mundy and Roodman 1987; Vaes 1988). Therefore, the osteoclast and monocyte-macrophage bear some common cytochemical characteristics (Chambers 1978). However, there are several phenotypic characteristics which distinguish the osteoclast from the monocyte-macrophage (Mundy and Roodman 1987). Tartrate-resistant acid phosphatase (TRAP) and carbonic anhydrase isoenzyme II (CA-II) are examples of enzymes which are not found in the monocyte-macrophage or macrophage polykaryon and are considered as specific markers for distinguishing osteoclasts from other morphologically similar cells (Hammarström et al. 1971; Sundquist et al. 1987; Väänänen 1984; Van de Wijngaert and Burger 1986).

In an attempt to characterize the osteoclast-like giant cells in various bone tumours more precisely, we examined these cells in routinely processed, paraffin-embedded tissue specimens for TRAP enzymatic activity and CA-II immunoreactivity.

Materials and methods

The bone tumours examined in this study were GCT (7 cases), chondroblastoma (2 cases), osteoblastoma (2 cases), osteoblastic osteosarcoma (5 cases) and MFH (4 cases) (Table 1). In addition, 4 cases of extraosseous inflammatory lesions containing macrophage polykaryons (tuberculosis in cervical lymph nodes, sarcoidosis in cervical lymph nodes, rheumatoid nodules in the knee joint and non-specific inflammatory granulation tissue in gingiva) were also examined. Two GCT cases showed lung metastasis. In these

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Table 1. Materials used and histochemical results[a] of multinucleate giant cells

Case	Sex	Age (years)	Localization	TRAP	CA-II
Giant cell tumour of bone					
1	M	44	Femur	++	++
2	M	47	Femur	++	+
3	M	45	Femur	++	++
4	F	30	Cervical vertebra	+	++
5[b]	F	39	Thoracic vertebra	++	++
6[b]	M	36	Femur	++	++
7	F	30	Sacrum	++	++
Chondroblastoma					
1	M	17	Ilium	+	++
2	F	21	Ilium	++	+
Osteoblastoma					
1	M	14	Tibia	++	−
2	F	9	Pubis	+	−
Osteosarcoma					
1	F	14	Femur	+	−
2	M	11	Femur	+	−
3	F	11	Tarsus	+	−
4	M	12	Tibia	+	−
5	F	10	Femur	+	−
Malignant fibrous histiocytoma					
1	F	40	Humerus	−	−
2	M	29	Elbow	−	−
3	M	54	Scapula	−	−
4	M	79	Femur	−	−
Inflammatory lesion[c]					
1	M	48	Lymph node	−	−
2	F	40	Lymph node	−	−
3	F	55	Knee joint	−	−
4	M	63	Gingiva	−	−

CA-II, Carbonic anhydrase isoenzyme II; TRAP, tartrate-resistant acid phosphatase

[a] ++, Strongly positive; +, positive; −, negative

[b] Including metastatic lung lesion

[c] 1, tuberculosis; 2, sarcoidosis; 3, rheumatoid arthritis; 4, non-specific inflammation

cases both primary and metastatic lesions were examined. Tumour tissues were obtained at surgery and fixed in neutral buffered formalin. Paraffin sections (4 μm in thickness) were used for histochemical demonstration of TRAP and CA-II as well as for routine haematoxylin and eosin (H&E) staining.

Histochemical demonstration of TRAP activity was performed according to Van de Wijngaert and Burger (1986). Briefly, deparaffinized sections were pre-incubated with 0.2 M acetate buffer (pH 5.0) and then incubated with 0.05 M Veronal acetate buffer (pH 5.0) containing naphthol AS-BI phosphate (Sigma, St. Louis, Mo.) as substrate, hexazonium pararosaniline as coupler and 20 mM L(+)-tartaric acid (Wako, Osaka, Japan). These procedures were carried out at 37° C in a moist chamber for 2 h each. After incubation, sections were rinsed and counterstained with Carazzi's haematoxylin. For negative controls, the histochemical reaction was inhibited by the following procedures: (1) L(+)-tartaric acid was substituted with 10 mM sodium fluoride; (2) incubation was carried out without the substrate, naphthol AS-BI phosphate; and (3) deparaffinized sections were enzymatically inactivated by boiling in 0.2 M acetate buffer in an autoclave (120° C, 30 min) before incubation.

For immunohistochemical demonstration of CA-II, the avidin-biotin method was employed. Deparaffinized sections were incubated with 0.3% H_2O_2 in absolute methanol for 30 min to inactivate endogenous peroxidase, and then with 1:10 dilution of normal rabbit serum for 10 min to block non-specific binding. Thereafter, the sections were incubated with goat anti-human erythrocyte CA-II antiserum (1:300; Green Cross, Osaka, Japan) for 1 h followed by biotin-conjugated rabbit anti-goat IgG antiserum and peroxidase-conjugated streptavidin for 30 min each (Biotin-StreptAvidin Amplified system, Biogenex, Dublin, Calif.). They were then incubated with 0.05 M Tris buffer (pH 7.6) containing 0.005% 3,3′-diaminobenzidine tetrahydrochloride and 0.003% H_2O_2 for 10 min, and counterstained with methyl green. All incubations were carried out at room temperature and followed by five 10-min washes with 0.01 M phosphate-buffered saline (PBS; pH 7.2). Negative controls for immunostaining were performed by substituting the primary antibody with PBS or normal goat serum (1:300).

Results

Except for MFH, all bone tumours contained osteoclast-like giant cells, which were most numerous in GCT (Fig. 1a–d). These giant cells were variable in shape and never showed mitotic figures. Nuclei were round to oval and uniform in size, and had one to two nucleoli. A relatively large amount of cytoplasm was frequently granular and vacuolated. Osteoclast-like giant cells in GCT were largest with the largest number of nuclei (Fig. 1a), whereas those in osteosarcoma were smallest with the smallest number of nuclei (Fig. 1d). Giant cells in MFH were clearly distinguishable for their malignant feature from the osteoclast-like giant cells of the rest of the examined tumours (Fig. 1e). Nuclei of these malignant giant cells were usually large, variable in shape and occasionally hyperchromatic. Benign giant cells (osteoclast-like giant cells) that have been reported to be relatively common in extraosseous MFH lesions (Enzinger and Weiss 1988) were not observed in this study of intraosseous MFH.

Results of histochemistry for TRAP and CA-II are shown in Table 1. In the periphery of the tumour mass of GCT, apparently normal osteoclasts were seen in shallow concavities in the surface of bone trabeculae (Howship's lacunae) and showed moderate to strong reactivity for both TRAP and CA-II (Fig. 2a, b). Osteoclast-like giant cells in GCT and chondroblastoma showed moderate to strong reactivity for TRAP and CA-II (Fig. 3a–d). In addition, some mononuclear cells with a round to oval nucleus in these tumours showed the same histochemical reaction as the osteoclast-like giant cells. Mononuclear cells with a spindle-shaped nucleus in GCT and those with an indented nucleus in chondroblastoma were negative for both enzymes. These findings were also seen in the metastatic lung lesions of GCT (cases 5, 6). In osteoblastoma and osteosarcoma, osteoclast-like giant cells and some mononuclear cells with a round to oval nucleus were positive for TRAP but not for CA-II (Fig. 3e–h). Neither TRAP nor CA-II reactivity was present in mononuclear cells rimming osteoid or bone trabeculae in osteoblastoma and anaplastic tumour cells lying within lace-like osteoid or bone in osteosarcoma. All the cellular components

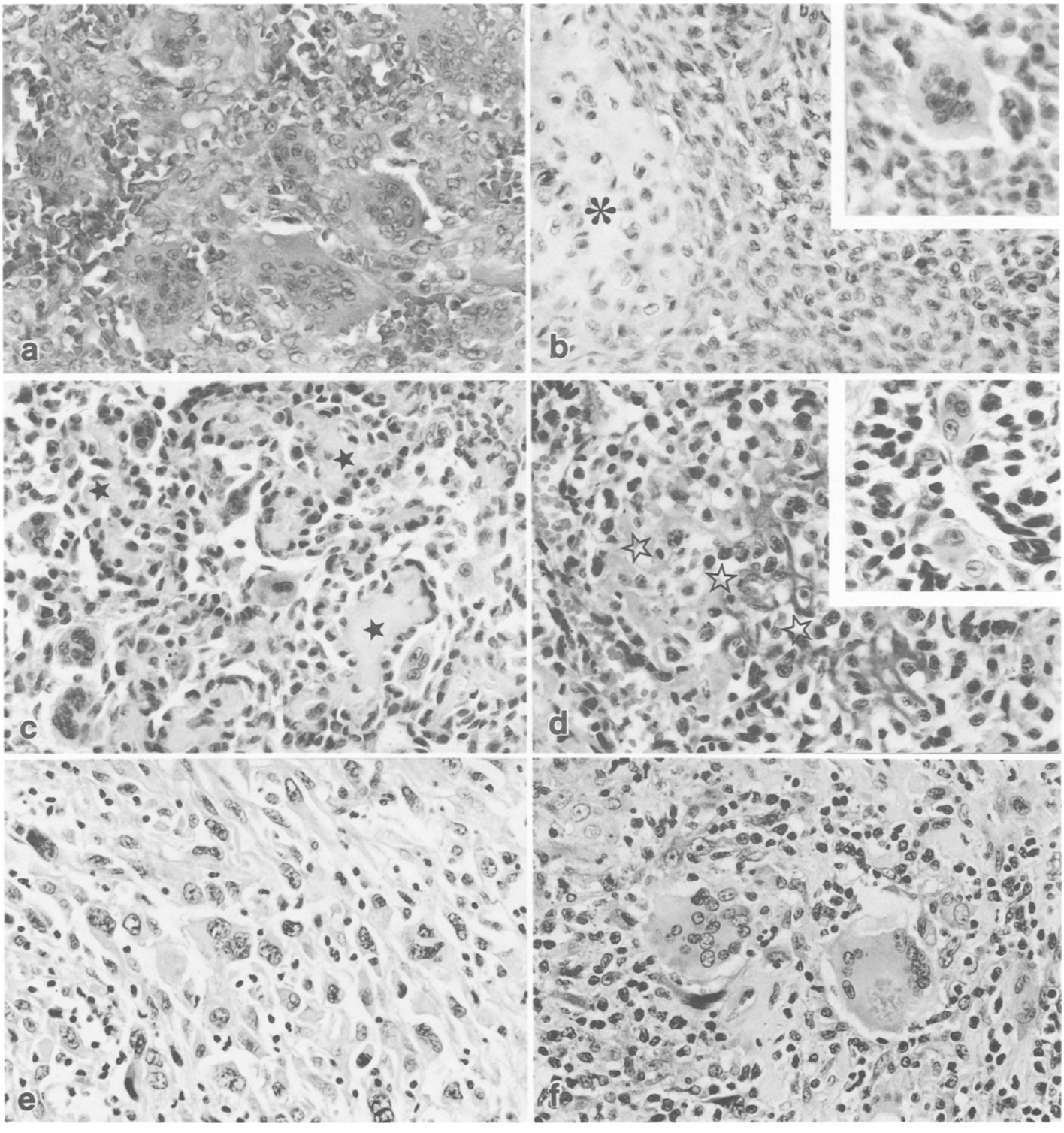


Fig. 1. Giant cells in bone tumours [**a** giant cell tumour (GCT); **b** chondroblastoma; **c** osteoblastoma; **d** osteoblastic osteosarcoma; **e** malignant fibrous histiocytoma (MFH); **f** non-specific inflammatory granulation tissue in gingiva]. Giant cells in GCT (**a**) are largest with the largest number of nuclei, whereas those in osteoblastic osteosarcoma (*inset* of **d**) are smallest with the smallest number of nuclei. Giant cells in chondroblastoma (*inset* of **b**) appear to be larger than those in osteoblastoma (**c**). Giant cells in MFH (**e**) show large and atypical nuclei. ❋, cartilage with neoplastic chondroblasts; ✶, osteoid rimmed by neoplastic osteoblasts; ✩, lace-like osteoid and bone with anaplastic tumour cells. H&E, ×300

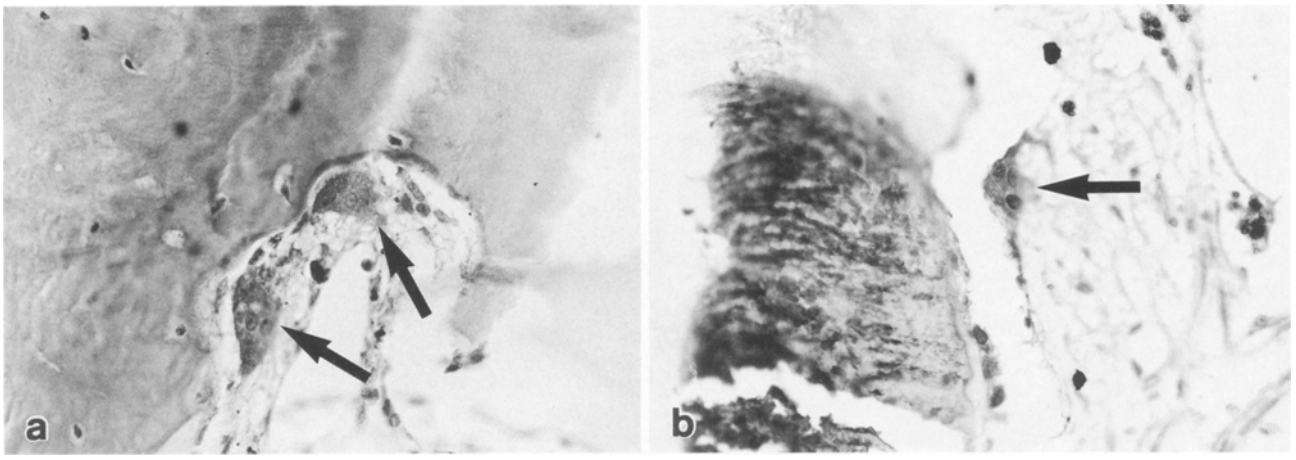


Fig. 2a, b. Histochemical staining of osteoclasts. Osteoclasts in the Howship's lacunae (*arrows*) show strong reactivity for both tartrate-resistant acid phosphatase (TRAP) (**a**) and CA-II (**b**). **a**, TRAP activity; **b** CA-II immunoreactivity. ×300

in MFH and various inflammatory lesions including malignant giant cells and macrophage polykaryons were negative for both TRAP and CA-II.

The specific histochemical reactions were not seen in the negative control sections.

Discussion

Osteoclast-like giant cells in bone tumours are so called because of their similarity to osteoclasts in morphology and acid phosphatase activity (Chambers 1978; Hanaoka et al. 1970; Komiya 1982; Mirra et al. 1989; Yoshida et al. 1982). These characteristics, however, are also shared by macrophages or macrophage polykaryons. To our knowledge, only osteoclast-like giant cells in GCT and MFH have proven to be osteoclasts.

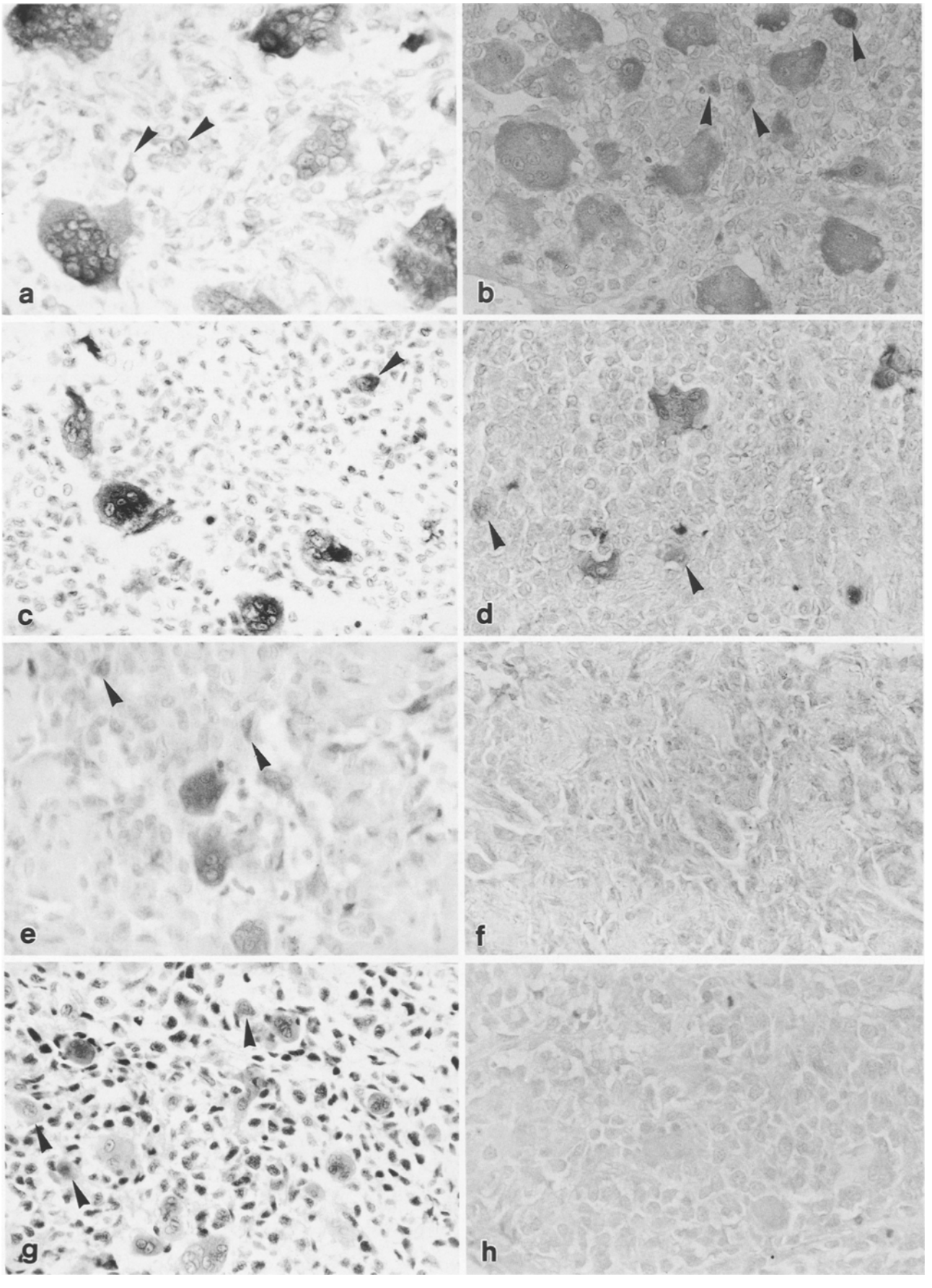
Multinucleate giant cells isolated from GCT have been shown to be able to resorb slices of devitalized bone, while macrophage polykaryons are unable to induce morphologically evident bone resorption under similar conditions in culture (Chambers and Horton 1984; Chambers et al. 1985). The GCT giant cells possess receptors for calcitonin (Goldring et al. 1986); calcitonin induces a quiescent state and causes a dramatic reduction in the volume of bone resorbed by the cells (Chambers et al. 1985). Resorption of bone slices by osteoclast-like giant cells of MFH has also been shown (Flanagan and Chambers 1989). Moreover, these MFH giant cells react with osteoclast-specific monoclonal antibodies. It should be noted, however, that the presently examined MFH giant cells were not benign or osteoclast-like giant cells capable of in vitro bone resorption. We suspect that the malignant MFH giant cells in this study may not have ability of bone resorption in vitro experiments like those described above for they lack both TRAP and CA-II (see below).

Hammarström et al. (1971) have shown that osteoclastic acid phosphatase activity can be distinguished from that in other cells of hard and soft tissues by its resistance to tartrate inhibition. Indeed, TRAP activity serves as a marker for osteoclasts as well as mononuclear osteoclast precursors, and is not found in monocyte-macrophages or macrophage polykaryons (Baron et al. 1986; Van de Wijngeart and Burger 1986). This TRAP activity has also been shown to be markedly sensitive to inhibition by fluoride and copper, and highly stable in routinely processed paraffin or glycol-methacrylate embedded tissue specimens (Eggert 1980; Eggert and Germain 1980; Van de Wijngeart and Burger 1986).

The present study demonstrated fluoride-sensitive TRAP activity in osteoclast-like giant cells in GCT, chondroblastoma, osteoblastoma and osteosarcoma as well as ordinary osteoclasts. In contrast, macrophage polykaryons in various inflammatory lesions and malignant giant cells in MFH never showed such enzyme activity. These results support the notion that, like those in GCT, the osteoclast-like giant cells in chondroblastoma, osteoblastoma and osteosarcoma are all osteoclasts.

Cumulative evidence indicates that an acidic environment under the ruffled border of osteoclast is important not only for causing mineral release but also providing an optimal environment for acid hydrolases to exert their effect during bone resorption (Vaes 1988). The reversible hydration of carbon dioxide catalysed by CA-II

Fig. 3. Histochemical staining of giant cells in bone tumours. **a**, **b**, GCT; **c**, **d**, chondroblastoma; **e**, **f**, osteoblastoma; **g**, **h**, osteoblastic osteosarcoma. Though giant cells in each bone tumour show TRAP activity, the enzyme activity of giant cells is stronger in GCT (**a**) and chondroblastoma (**c**) than those in osteoblastoma (**e**) and osteoblastic osteosarcoma (**g**). Some mononuclear cells which have the same nuclear morphology as giant cells are also positive for TRAP (*arrowheads* in **a**, **c**, **e** and **g**). As for CA-II, giant cells in GCT (**b**) and chondroblastoma (**d**) are positive for CA-II, whereas those in osteoblastoma (**f**) and osteoblastic osteosarcoma (**h**) are negative. Some mononuclear cells with the same nuclear profile as giant cells are positive for CA-II in GCT and chondroblastoma (*arrowheads* in **b** and **d**). **a**, **c**, **e** and **g**, TRAP activity; **b**, **d**, **f** and **h**, CA-II immunoreactivity. ×300


a
b
c
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e
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h

is thought to be a source of hydrogen ion supply that is necessary for generation of such an acidic environment, because acetazolamide, a specific inhibitor of CA, inhibits bone resorption both in vivo (Waite et al. 1971) and in vitro (Minkin and Jennings 1972). In addition osteoclast possesses CA-II (Väänänen 1984), and deficiency of CA-II accompanies an autosomal recessive syndrome of osteopetrosis, a disease characterized by a failure of bone resorption (Sly et al. 1983). Sundquist et al. (1987) have shown that CA-II is never detected in foreign body giant cells, peritoneal macrophages, lung macrophages and cultured peripheral monocytes. Therefore, it is fair to consider that CA-II is specific for functioning or mature osteoclasts.

The difference in CA-II immunoreactivity among osteoclast-like giant cells in this study may be related to their difference in maturation or resorptive activity. CA-II-positive osteoclast-like giant cells in GCT and chondroblastoma were larger and had more nuclei than CA-II-negative cells in osteoblastoma and osteosarcoma. Moreover, the former showed stronger TRAP activity than the latter. Minkin (1982) has shown that, when bone resorption is stimulated in cultured newborn mouse calvaria with either parathyroid hormone or $1,25(OH)_2$ vitamin D_3, there is a significant increase in TRAP activity in the medium, and proposed that there is a positive correlation between the TRAP and resorptive activities.

The reason for functional or maturational difference between osteoclast-like cells in GCT and chondroblastoma and those in osteoblastoma and osteosarcoma may be explained by the cellular composition of these tumours. Unlike GCT and chondroblastoma, osteoblastoma and osteosarcoma are bone-forming tumours and composed of neoplastic osteoblasts. Macrophage colony stimulating factor (M-CSF), which osteoblastic cells are able to produce, inhibits bone resorption by osteoclasts (Elford et al. 1987; Hattersley et al. 1988). Moreover, it has been suggested that M-CSF inhibits osteoclast differentiation (Van de Wijngaert et al. 1987).

Some mononuclear cells with round to oval nucleus exhibited the same histochemical reactivity as osteoclast-like giant cells. These mononuclear cells may represent precursors of osteoclast-like giant cells. It is generally accepted that the multinucleate osteoclasts arise locally by coalescence of mononuclear cell precursors. Though it is not known whether CA-II is present in osteoclast precursors, TRAP activity has been demonstrated in them (Baron et al. 1986). Generation of osteoclast-like giant cells by fusion of TRAP-positive mononuclear cells is further supported by the fact that osteoclast-like giant cells lack mitotic nuclear division and are similar in nuclear configuration to putative mononuclear precursors.

In summary, this study demonstrated the presence of TRAP activity, an osteoclast-specific enzyme activity, in osteoclast-like giant cells in GCT, chondroblastoma, osteoblastoma and osteosarcoma. Furthermore, these osteoclast-like giant cells can be divided into two groups, that is, cells with CA-II, which are seen in GCT and chondroblastoma, and those without CA-II, which are seen in osteoblastoma and osteosarcoma. This diversity among osteoclast-like giant cells may serve as a useful tool in the clinical diagnosis of a group of bone tumours containing these cells.

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