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In vitro bone resorption by isolated multinucleated giant cells from giant cell tumour of bone: light and electron microscopic study

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Summary. The behaviour of multinucleated giant cells (GCs), obtained from a giant cell tumour of the tibia and cultured on glass coverslips or on devitalized bone slices, was studied using light and electron microscopy. Monitoring the GCs on bone slices by phase-contrast microscopy revealed that they had removed calcified bone matrix resulting in excavation of lacunae, with subsequent lateral extension and perforation of the bone slices. Electron microscopy demonstrated for the first time that the GCs responsible for exavating lacunae had two specific membrane modifications, ruffled border and clear zone, and showed basically similar cytoplasmic fine structures to those of osteoclasts. Fluorescence images of the GCs on glass and on bone after rhodamine-conjugated phalloidin staining revealed that most of the GCs had an intensely fluorescent peripheral band composed of a number of F-actin dots called podosomes. Some GCs showed unusual arrangements of podosomes suggesting abortive attempts at GC formation. We have demonstrated that the band structure of the GCs cultured on bone is intimately involved in bone resorption. Two stromal cell types could be recognized. The predominant type, which seemed to be the only neoplastic element because of its proliferative capability, showed quite different fine structural and cytoskeletal features from the GCs. The other type, which was much less frequent and seemed not to proliferate, had morphological similarities to the GCs, and seemed to be their precursor. Importantly GCs cultured on bone and the osteoclasts share common structures for adhesion to and resorption of bone, strongly supporting the view that the GCs of the giant cell tumour of bone are potentially active bone resorbers and can be regarded as osteoclasts.

Key words: Giant cell tumour of bone – Bone resorption – Osteoclast – F-Actin – Podosomes

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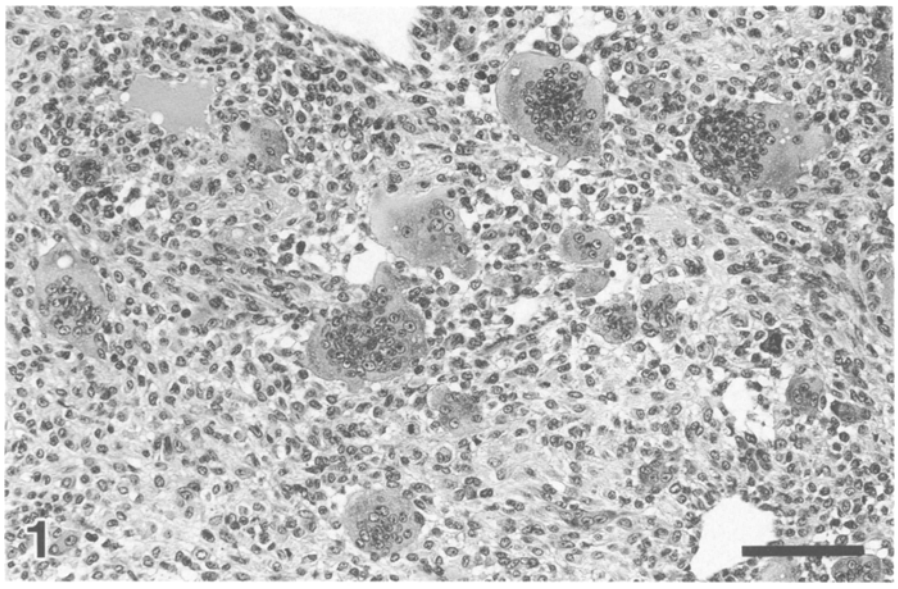
Introduction

Bona fide giant cell tumour of bone is a distinctive neoplasm that produces an expansile osteolytic lesion. The histopathological appearance of the tumour is characterized by the presence of abundant multinucleated giant cells embedded in stromal cells. The significance and histogenesis of the giant cells in the tumour and their relationship to the behaviour of the tumour have been under discussion for many years. Some authors have assumed that the giant cell participates in bone resorptive processes of the tumour, since they have observed that there is a certain resemblance in histochemical and electron microscopic features between giant cells and osteoclasts (Göthlin and Ericsson 1976; Schajowicz 1961). However, some morphological differences in the two cell types have led others to conclude that the cells are not identical (Aparisi 1978; Aparisi et al. 1977a).

Recently a growing body of evidence has strongly supported the view that the giant cells of the tumour are osteoclastic. The evidence includes their possession of calcitonin receptors (Goldring et al. 1987; Nicholson et al. 1987), specific reactivity of monoclonal antibodies against the giant cell tumour with mammalian osteoclasts (Horton et al. 1985) and their capability to excavate resorption lacunae on devitalized bone slices in vitro, one of the most distinctive features of osteoclasts (Chambers et al. 1985). However, development of two membrane modifications, a clear zone and a ruffled border, which are believed to be the most important and convincing morphological criteria for functioning osteoclasts, has not been demonstrated (Akisaka et al. 1988; Bonucci 1981; Göthlin and Ericsson 1976; Holtrop and King 1977; Holtrop et al. 1974, 1979; Miller 1977, 1978, 1981). Furthermore, although a band of "podosomes" (Tarone et al. 1985), which are peculiar F-actin-containing dot-like adhesion structures previously described in Rous sarcoma virus-transformed



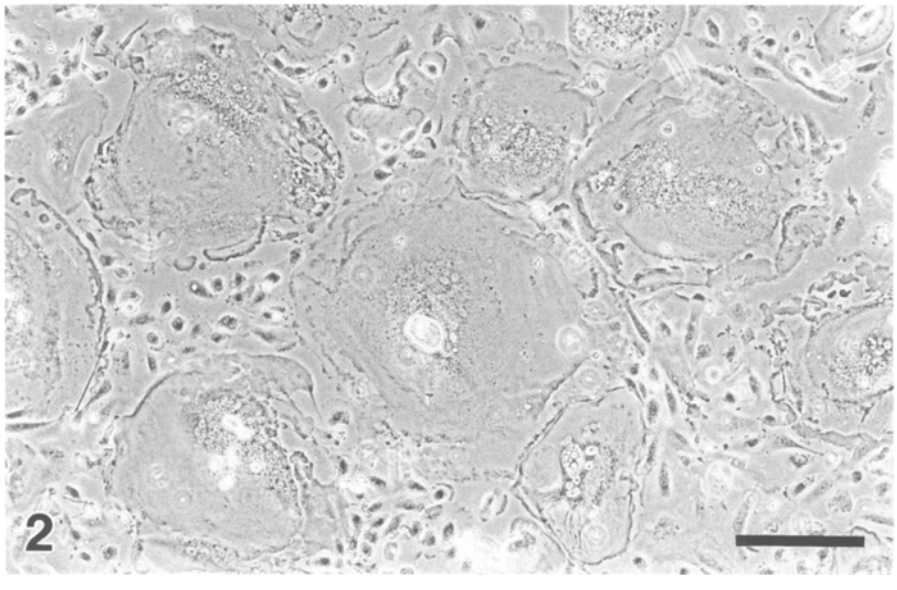


Fig. 1. Curetted tumour tissue showing variously sized multinucleated giant cells in a background of mononuclear stromal cells. *Bar* = 100 μm. H&E, ×160

Fig. 2. Well-spread giant cells cultured on a glass coverslip. 1 day of culture. The cells are surrounded by numerous mononuclear stromal cells. *Bar* = 100 μm. Phase-contrast, ×175

cells (Rohrschneider 1980) and then found in osteoclasts (Marchisio et al. 1984, 1987; Turksen et al. 1988), has been reported to be expressed in giant cells cultured on glass coverslips (Zambonin-Zallone et al. 1989), it has not been determined whether the structures are actually engaged in the bone resorption processes.

In this study, we have investigated the behaviour of giant cells cultured on glass or bone substrate using light and electron microscopy, with special reference to cell-substrate relation. We wished to understand the nature and significance of the giant cells in this tumour further.

Materials and methods

A giant cell tumour of bone was obtained from a 26-year-old Japanese male patient. The case history of the patient was that he was first admitted to the hospital due to pain in his left knee. Roentgenographs revealed a well-delineated area of bone destruction located in the proximal end of the left tibia. After a histopathological diagnosis of giant cell tumour of bone had been made, the lesion was curetted out and the resulting cavity was packed with autologous bone and cement. Ten years later, the patient developed severely painful swelling with limitation of motion in the affected knee. No clinical or biochemical features of hyperparathyroidism were present and the values of serum calcium and alkaline phosphatase were within normal ranges. The diagnosis of recurrent giant cell tumour of bone was made based on clinical, roentgenographic and histopathological features, and another curettage was carried out.

The methods used for cell preparation were essentially those previously described for rodent and rabbit osteoclasts (Boyde et al. 1984; Chambers and Magnus 1982; Chambers et al. 1984). Briefly, the curetted tumour tissue was minced in Eagle's minimal essential medium (Gibco, Grand Island, N.Y.) supplemented with 15% fetal bovine serum (Flow Laboratories, Northryde, Australia) and antibiotics (50 IU/ml penicillin, 50 μg/ml streptomycin, and 2.5 μg/ml fungizone; Flow Laboratories) and then mechanically disaggregated into the medium using fine forceps and Pasteur pipette. After tissue debris had settled, aliquots of the resulting cell suspension were transferred to 24-well tissue culture plates. Each well contained a 12-mm round glass coverslip or a thin devitalized bone slice (approximately 8 mm × 8 mm in size and 15–20 μm or 200–250 μm in thickness) prepared from bovine femoral cortical bone as described elsewhere (Kanehisa and Heersche 1988) and 1 ml of the culture medium. After 1 h of incubation at 37° C, glass coverslips or bone slices were rinsed very gently to remove teased

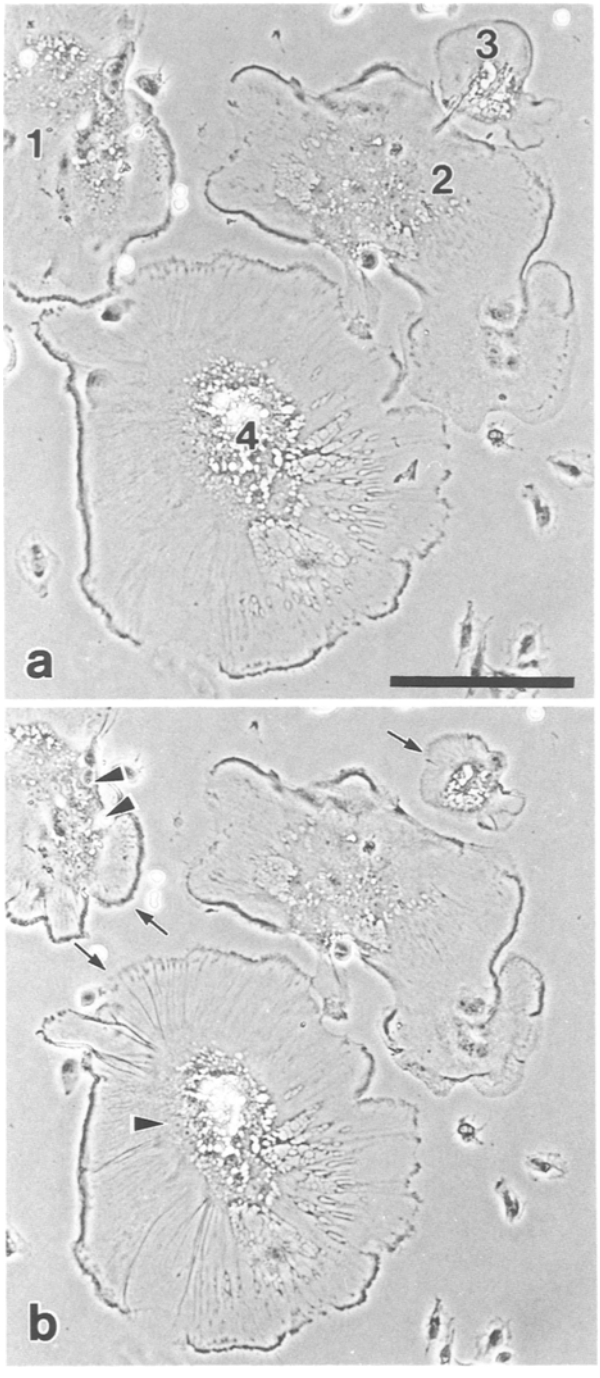


Fig. 3a, b. Four giant cells cultured on a glass coverslip, 1 day of culture. **a** Just before addition of calcitonin (50 mMRC units/ml). **b** Three minutes after calcitonin addition. Cytoplasmic motility of the giant cells ceased and the cells started contracting. Note marked retraction of the cell periphery (*arrows*) and compactness of the nuclear region (*arrowheads*). Reduced plan area of cytoplasmic spreading of the cells is expressed as a percentage of the value before calcitonin addition as follows: 1, 67.6%; 2, 90.3%; 3, 64.7%; 4, 84.6%. *Bar* = 100 μm. Phase contrast, × 240

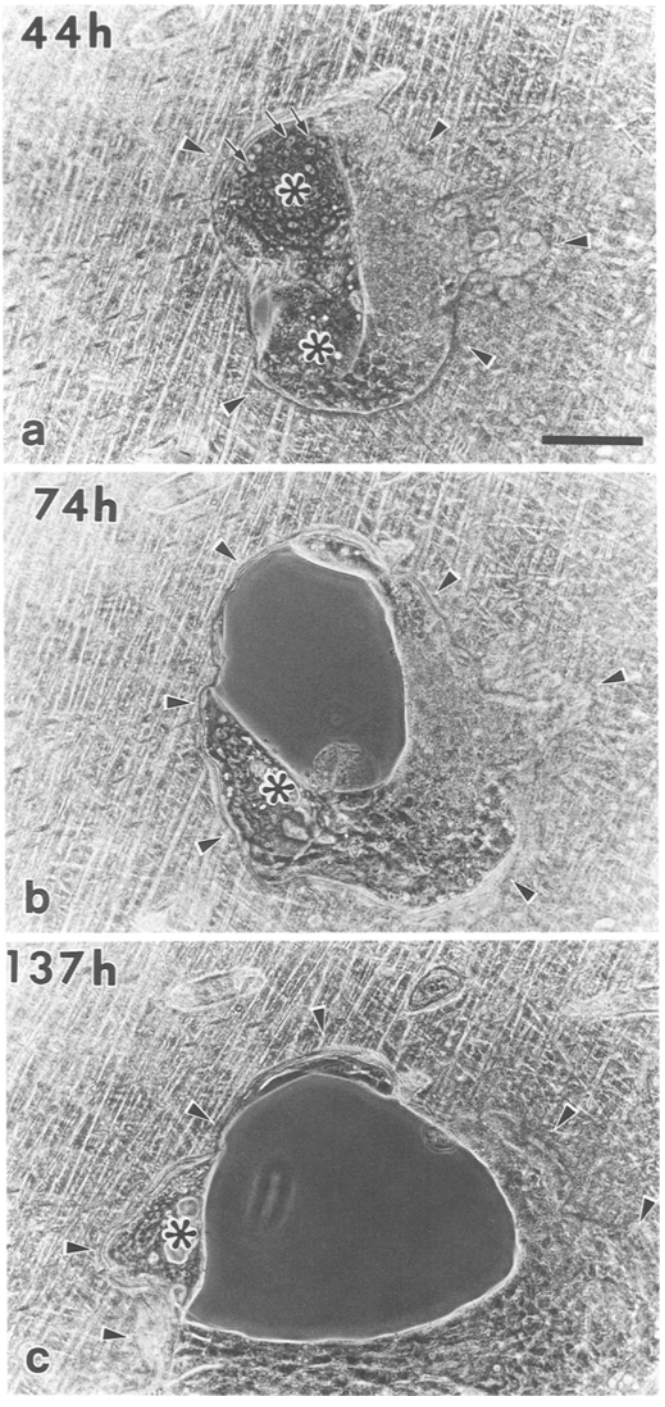


Fig. 4a–c. A resorption lacuna (*arrhowheads*) and the associated two giant cells (*asterisks*) on a bone slice. **a** Two giant cells within the lacuna. Multiple nuclei can be seen in the cells (*arrows*); 44 h of culture. **b** The lacuna had extended laterally and the bottom of the lacuna was partly perforated. The upper giant cell seemed degenerated and the lower one showed small vacuoles; 74 h of culture. **c** The lacuna had extened further. The upper giant cell disappeared and the vacuoles became larger in the lower one; 137 h of culture. A number of bright lines from the top of the bottom are scratches made during preparation of bone slices. *Bar* = 100 μm. Phase contrast, × 165

tissue debris and non-adherent cells, and each coverslip or bone slice was transferred to a 25-cm^2 tissue culture flask containing 5 ml culture medium. The cultures were incubated at 37° C in a humidified atmosphere (5% carbon dioxide – 95% air) until needed.

Light microscopic examinations were performed on several pieces of the curetted tumour tissue embedded in paraffin and stained with haematoxylin and eosin. A culture flask with a coverslip or a thin bone slice was sealed (air-tight) to maintain the appropriate pH and then placed in a 37° C incubation chamber mounted

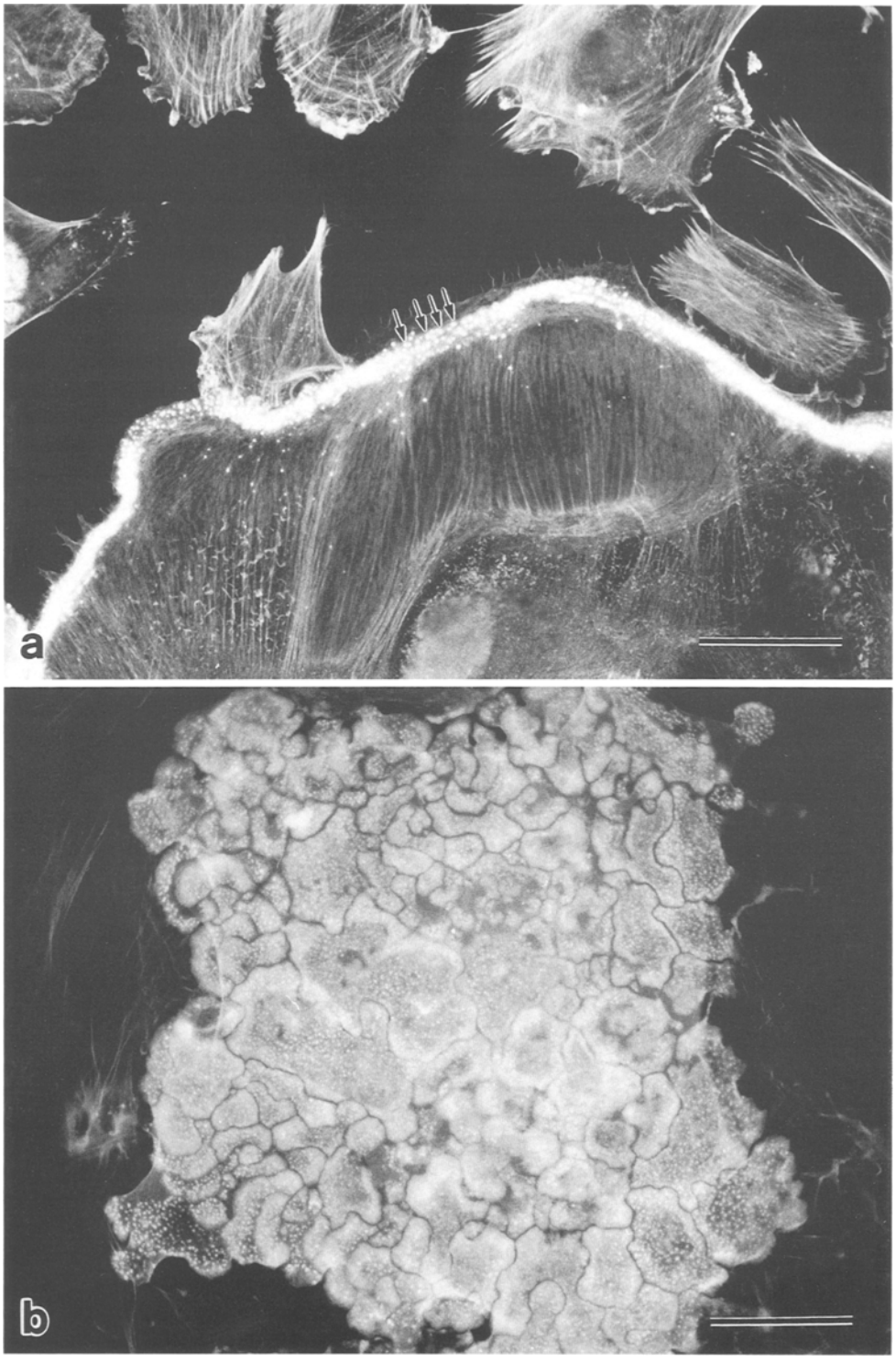


Fig. 5a–d. Fluorescence image of F-actin in giant cells cultured on glass as visualized by rhodamine-conjugated phalloidin staining. **a** Peripheral part of a giant cell and stromal cells. The bright fluorescent band consists of a large number of bright dots (*arrows*) called podosomes. F-actin is also present as a network of microfilaments throughout the cytoplasm of giant cell. The stromal cells show stress fibre type F-actin bundles and "arcs" of F-actin bundle. *Bar* = 50 μm. ×460. **b** A giant cell displaying a conglomerate of abundant discrete patches, each consisting of a number of podosomes. *Bar* = 50 μm. ×460. **c** A giant cell displaying diffuse distribution of a large number of podosomes throughout the whole cell without distinct organization. *Bar* = 50 μm, ×460. **d** A less frequent stromal cell type which shows a band of podosomes is surrounded by the other predominant stromal cell type with a different F-actin cytoskeleton. *Bar* = 50 μm. ×460

onto a Nikon Diaphoto TMD inverted microscope equipped with phase-contrast optics. The behaviour of cultured giant cells on coverslips was then monitored to see whether they could create resorption lacunae on thin bone slices, as described elsewhere (Kanehisa 1989; Kanehisa and Heersche 1988; Kanehisa et al. 1990a, b). Synthetic eel calcitonin (CT) (Toyojozo, Tokyo, Japan) was added to some cultures on coverslips at a final concentration of 50 mMRC units/ml and a plan area corresponding to cytoplasmic spreading of each giant cell before and after addition of CT was calculated in order to evaluate the effect of CT on the giant cells using a Magiscan 2A (Joyce Loebl, Eateshead, UK).

After 5 days in culture, cells cultured on thin bone slices or coverslips were fixed with 3.7% (vol/vol) formaldehyde in phosphate buffered saline (PBS) for 5 min at room temperature and processed for visualization of F-actin (Opas and Kalnins 1983; Turksen et al. 1988). Following fixation, cultures were extracted for 5 min at room temperature with 1,4 piperazine diethanesulphonic acid (PIPES) buffer; 0.1 M PIPES, 1 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), 0.1% (vol/vol) Triton X-100 and 4% (wt/vol) polyethylene glycol (PEG) 8000, pH 6.9 (Opas et al. 1985). Cells were then rinsed with PBS, and incubated for 30 min at room temperature with 0.3 μM rhodamine-conjugated

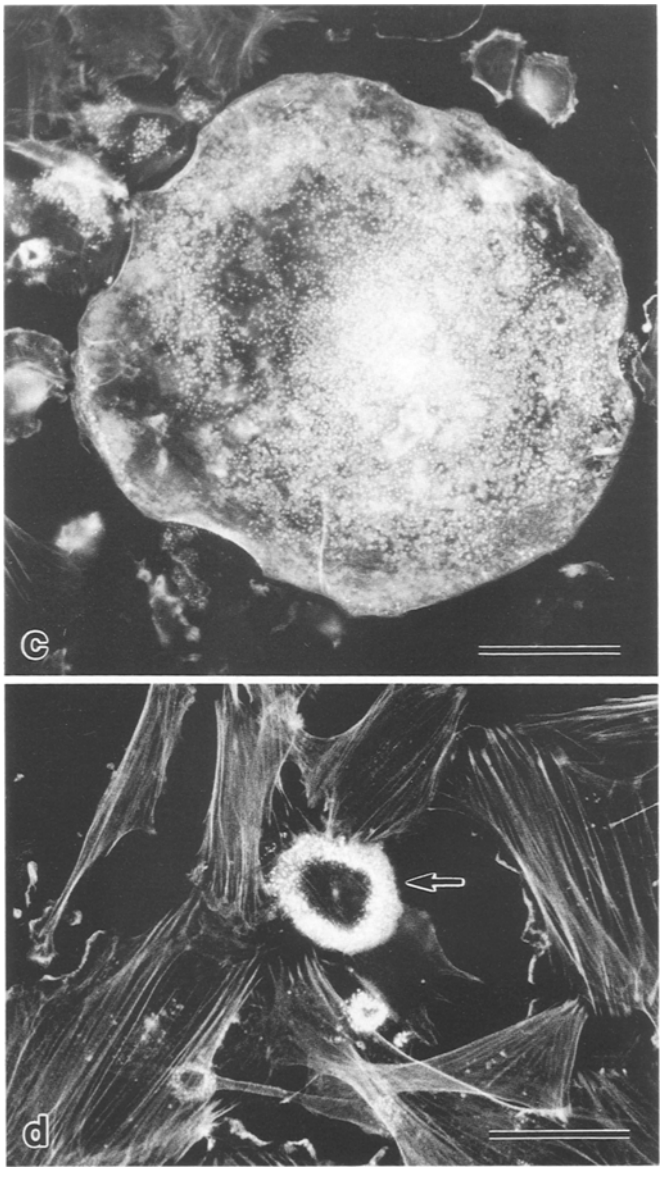


Fig. 5c, d

phalloidin (Molecular Probes, Junction City, Ore.) After rinsing, the cells were mounted with Geltol (Immunon, Detroit, Mich.). Labelled cells were observed with a Nikon Fluophoto equipped for epifluorescence.

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM) several bone slices with cells were processed after 3–5 days in culture. For SEM, bone slices, with or without pre-treatment with 0.25 M ammonium hydroxide for 10 min to remove the cells, were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 h at 37° C, rinsed in the same buffer, post-fixed in 2% osmium tetroxide in the same buffer for 1 h at 4° C and rinsed. The specimens were then dehydrated, critical-point dried, coated with gold by sputter-coating and observed using a JSM-35 SEM.

For TEM, the bone slices with cells were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for 1 h at 37° C and then for the following 5 h at 4° C. The fixed specimens were decalcified with 5% ethylene diamine tetraacetic acid (EDTA) containing 7% sucrose for 3–5 days at 4° C, rinsed in 0.1 M cacodylate buffer, post-fixed in 2% osmium tetroxide in 0.1 M cacodylate buffer and routinely dehydrated and embedded in Taab 812 Resin (Aldermaston, England). Ultra-thin sections were cut on an LKB Ultratome (Bromma, Sweden), stained with uranyl acetate and lead citrate, and examined in a JEM-1200 EX (Jeol, Tokyo, Japan).

Results

The histopathological appearance of paraffin-embedded and haematoxylin and eosin stained sections of the curetted tumour specimens revealed that the tumour was composed of mononuclear stromal cells and multinucleated giant cells, with a small amount of collagenous matrix (Fig. 1). The mononuclear cells showed a minor degree of cellular atypia and a small number of mitoses. The giant cells were quite numerous, varying considerably in shape, size and in the number of nuclei from several to some dozens. The nuclei were usually gathered and located centrally with some peripheral cytoplasm.

From the curetted specimens, numerous viable giant cells were disaggregated mechanically along with mononuclear stromal cells and adhered to glass coverslips and devitalized bovine bone slices. After 1 day of culture, most of the giant cells were well spread on the glass and showed active cytoplasmic activity consisting of continual development and regression of broad lamellipodia and lobopodia and ruffling activity at their periphery (Fig. 2). The number of nuclei in giant cells were generally greater than that for rabbit, rat or avian osteoclasts. When CT was added to the well-spread giant cells, the motility of the giant cells was suppressed and the cells contracted (Fig. 3) as Chambers et al. (1985) have described. There was heterogneity in responsiveness of the giant cells to CT in terms of cytoplasmic spreading as Kanehisa (1989) reported elsewhere with regard to isolated rabbit osteoclasts.

The multinucleated giant cells cultured on devitalized bovine bone slices had the capability of excavating resorption lacunae, which could be recognized as area sharply delineated by curvilinear lines under phase-contrast or ordinary bright-field light microscopy. The resorption lacunae generally extended laterally with time in culture, but the thin bone slices of 15–20 μm thickness were easily perforated by the giant cells (Fig. 4). In some cases the giant cells responsible for on-going bone resorption were relatively easily recognized, but in other cases the giant cells were difficult to observe on bone slices under phase-contrast microscopy. Under ordinary bright-field light microscopy usually it was very difficult to see the cells on bone slices or on coverslips. It was also noticed from observation of the cultures on coverslips that the number of giant cells decreased with time in culture, while the mononuclear stromal cells proliferated and took over the surface of coverslips.

F-actin arrangement of the giant cells on coverslips was basically similar to that of mammalian and avian osteoclasts. Most giant cells displayed a characteristic brightly fluorescent peripheral band consisting of a large number of F-actin dots called podosomes (Fig. 5a). The band of the giant cells of this tumour appeared to be more conspicuous than that of osteoclasts. The microfilaments were also prominent, but no stress fibres were

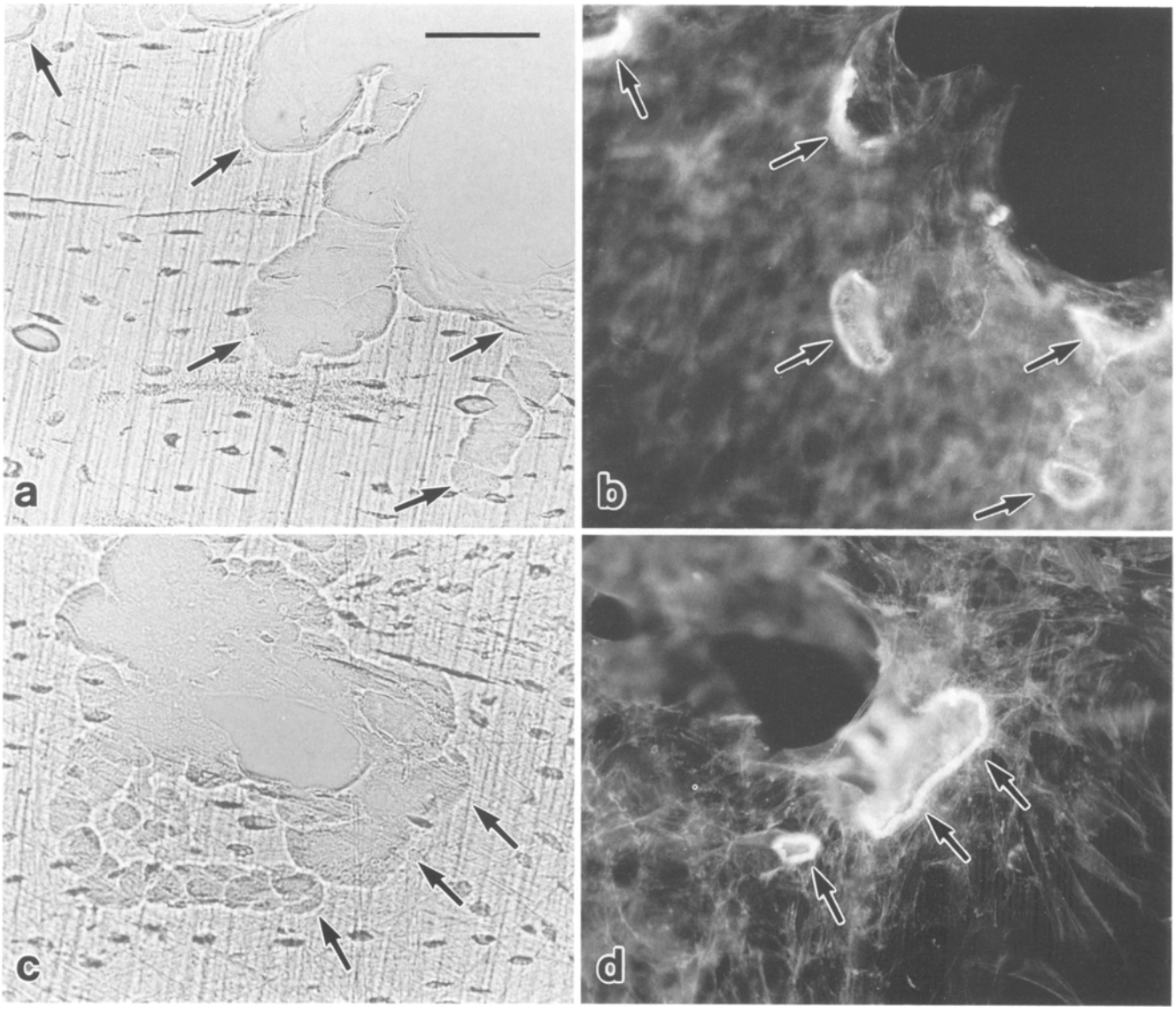


Fig. 6a–d. Bone slices with cultured cells after fixation, extraction and staining with rhodamine-conjugated phalloidin. **a**, **c** Bright-field image of resorption lacunae excavated by giant cells. The lateral boundary of the lacunae at the advancing resorption front is indicated by *arrows*. The responsible giant cells are not visible. **b**, **d** Fluorescence image of the same fields. The bone resorbing giant cells are visualized and show the bright bands (*arrows*) which exactly fit the advancing edge of the resorption front. *Bar* = 100 μm. ×170

found; they sometimes terminated at the peripheral band. A small percentage of the giant cells showed different patterns of podosome assembly from the typical peripheral band. Some giant cells had small discrete patches, rings or crescents consisting of podosomes, and in some cases the whole cell was furnished with compactly arranged discrete patches of podosomes displaying a jigsaw puzzle appearance (Fig. 5b); in other cases a number of podosomes were diffusely distributed through the whole cell without development of peculiar organization (Fig. 5c). However, two stromal cell types could be distinguished. The predominant cell type showed quite different F-actin arrangement which consisted of straight stress fibres-type F-actin bundles and arc-shaped F-actin bundles (Soranno and Bell 1982), which were perpendicular to the straight bundles (Fig. 5a). The other cell type was much less frequent than the previous one and had a small circular band of podosomes (Fig. 5d), which is similar to a single small discrete ring in the giant cell.

Since we previously found that the band of podosomes is intimately engaged in bone resorption by osteoclasts and is an essential part of the resorption apparatus of osteoclasts (Kanehisa et al. 1990b), we examined the relationship between resorption lacunae excavated on bone slices and the band of responsible giant cells. Combined use of the bright-field image of the resorption lacunae and fluorescence image of the giant cells after staining with rhodamine-conjugated phalloidin revealed that the bright band of F-actin containing podosomes in the giant cells exactly corresponded to the lateral boundary of the lacunae at the advancing resorption front (Fig. 6).

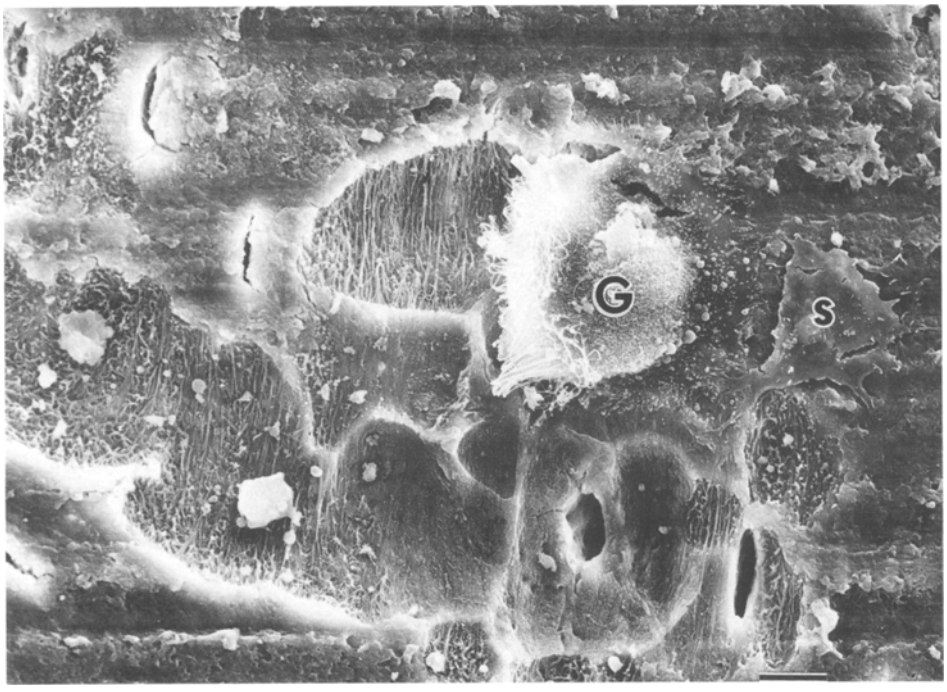


Fig. 7. Scanning electron micrographs of multilocular resorption lacunae excavated by a giant cell (*G*) which is dome-shaped in the centre and has cytoplasmic skirt and dense filopodial projections at the cell periphery. The upper surface of the cells has a lot of blebs. A flattened stromal cell (*S*) with smooth surface is also seen. Surface of the lacuna is usually coarsely fibrillar, but in some areas the surface is rather smooth. *Bar* = 10 μm. ×1000

Under SEM the giant cells on bone slices were observed as large cells having complex morphology with fine filopodial projections, microvilli and blebs over the upper cell surface and with a peripheral cytoplasmic skirt (Fig. 7). The stromal cells had flattened, polyhedral fibroblast-like morphology with a relatively smooth surface. Most of the giant cells were associated with multilocular resorption lacunae. A part of each lacuna, which was probably an advancing resorption front, was covered by a cell body of the giant cell. The surface of the resorbed area usually had a coarse fibrillar appearance caused by an exposed collagenous matrix (Fig. 7).

TEM confirmed that the giant cells were engaged in deep resorption concavities on bone slices (Fig. 8). They had the resorption apparatus possessed by authentic osteoclasts, that is two membrane modifications, the ruffled border and clear zone, both of which were apposed to the bone surface. The ruffled border eroded calcified bone matrix and the clear zone was tightly attached to non-resorbed bone surface with development of parallel dark bands perpendicular to the bone surface (Fig. 8b). The cells contained multiple nuclei and the cytoplasm was strikingly rich in organelles. In the deep portion of the ruffled border there were numerous relatively small membrane-bound vesicles, some of which communicated with channels between villus-like processes of the ruffled border (Fig. 8c). In the cytoplasm adjacent to the basolateral membrane and in the perinuclear region, there were abundant rough endoplasmic reticulum and mitochondria (Fig. 8d). The Golgi apparatus was relatively well developed and usually localized in the nuclear region. Numerous variously sized electron-dense granules were distributed only within the cytoplasm of the giant cell. The granules sometimes appeares to be associated with myelinoid bodies.

Under TEM there were two different mononuclear stromal cell types in the culture (Fig. 9). One was obviously predominant and appeared to be only the cell type that proliferated with time. This cell type was very similar to "stromal cell type 1" described by Aparisi et al. (1977a). These cells showed prominent nuclear polymorphism and the cytoplasm contained abundant well-developed rough endoplasmic reticulum, whose cisternae were irregularly expanded containing finely granular substance. The number of mitochondria varied and the size was relatively small. The Golgi apparatus was usually prominent and was located in the perinuclear area.

The other cell type was rare and hardly found in the culture (Fig. 9). The electron microscopic appearance was completely different from that of the stroma cell type 1, but very similar to that of the multinucleated giant cell. This stromal cell type and the giant cell shared an abundance of slender rough endoplasmic reticulum without dilatation, larger mitochondria than the previous type, and numerous electron-dense granules. However, it could not be confirmed whether this cell type corresponded to "stromal cell type 2" described by Aparisi et al. (1977a) because of some different morphological details.

Discussion

In accordance with Chambers et al. (1985) we were able to extract a number of viable multinucleated giant cells from the tumour tissue, and under SEM we observed that the cultured giant cells on devitalized bovine bone slices excavated resorption lacunae which were very similar to the Howship's lacunae found in vivo. In addition,

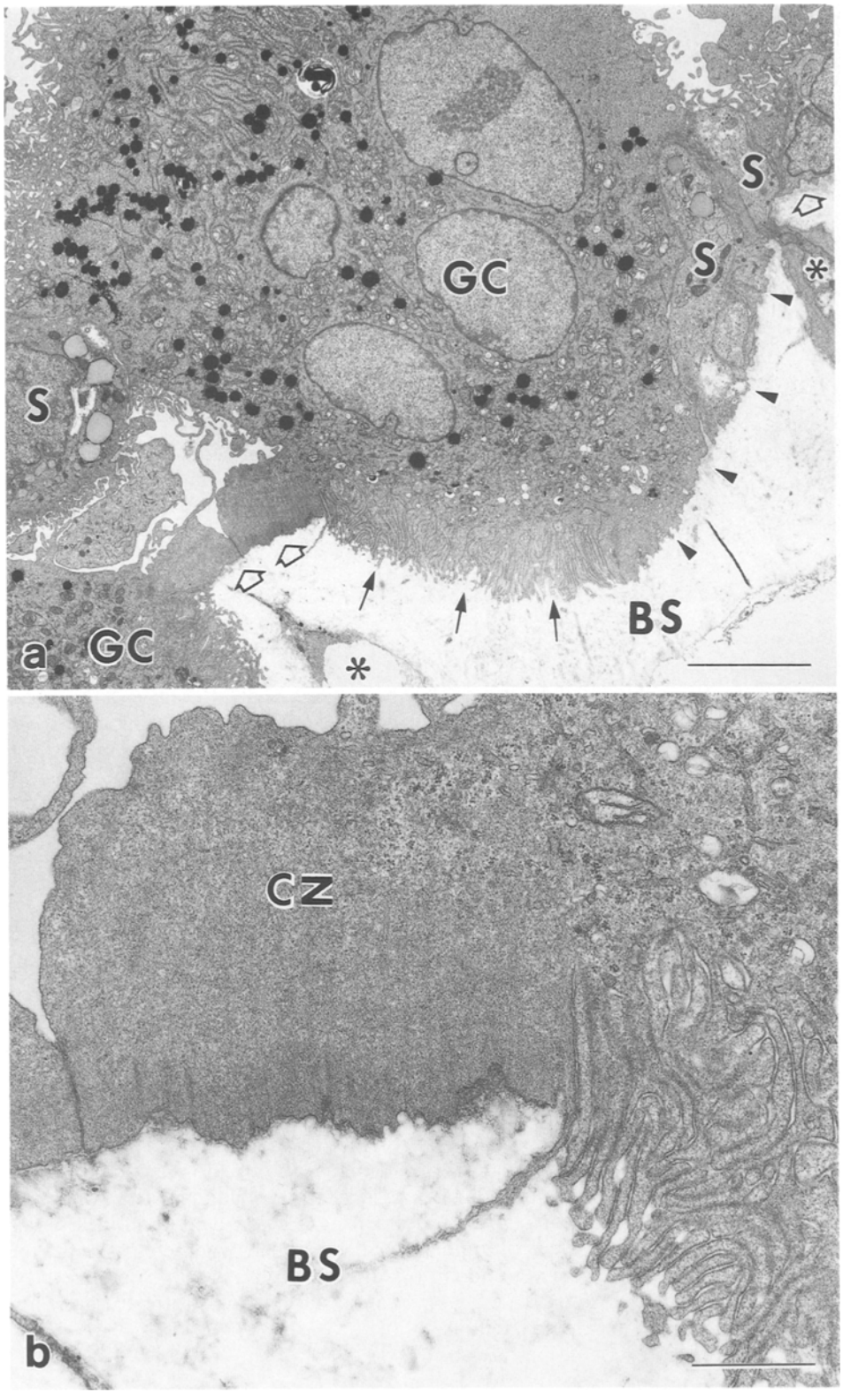


Fig. 8a–d. Transmission electron micrographs of well-polarized giant cells (*GC*) cultured on a bone slice (*BS*). **a** Low-power electron micrograph. The giant cells are located in resorption lacunae. Ruffled border is apposed to the bone surface undergoing resorption (*arrows*). Clear zone adheres to the unaffected bone surface indicated by *open arrows*. *Arrowheads* indicate the previously resorbed surface in the lacuna. Neighbouring stromal cells are marked with (*S*) and pre-existing osteocytic lacunae which contain dead osteocytes are marked with *asterisks*. *Bar* = 5 μm. × 4200. **b** The clear zone (*CZ*) with parallel dark bundles of filaments perpendicular to the bone surface, following the contour of bone surface. This region is clearly separated from the ruffled border. *Bar* = 1 μm. × 21 000. **c** Ruffled border consisting of complicated finger-like projections of the cytoplasm. The deep portion of the border contains relatively small vesicles which sometimes communicate with channels between the projections, and irregularly arranged cytoskeletal filaments and some myelinoid structures are also present. *Bar* = 1 μm. × 21 000. **d** The basolateral membrane shows numerous microvillous processes which are more slender than the ruffled border. The cytoplasm adjacent to this membrane is characterized by the abundance of rough endoplasmic reticulum and clusters of free ribosomes. This portion and the perinuclear region contain a number of mitochondria and electron-dense granules which sometimes appear closely associated with myelinoid bodies. *Bar* = 2 μm. × 10 500

we monitored for the first time the actual bone resorption sequences in which the giant cells removed bone substrate, resulting in development of optically identifiable resorbed area, progressive lateral extension of that area and then perforation of bone slice. This is convincing evidence that the giant cells do have an osteoclastic nature, because other cells of the mononuclear phagocyte system such as monocytes, macrophages and their polykaryon failed to create such lacunae in similar culture systems (Ali et al. 1984; Chambers and Horton 1984).

Earlier some authors have concluded that although there is a certain resemblance in ultrastructure between osteoclasts and the giant cells of this tumour, the cells

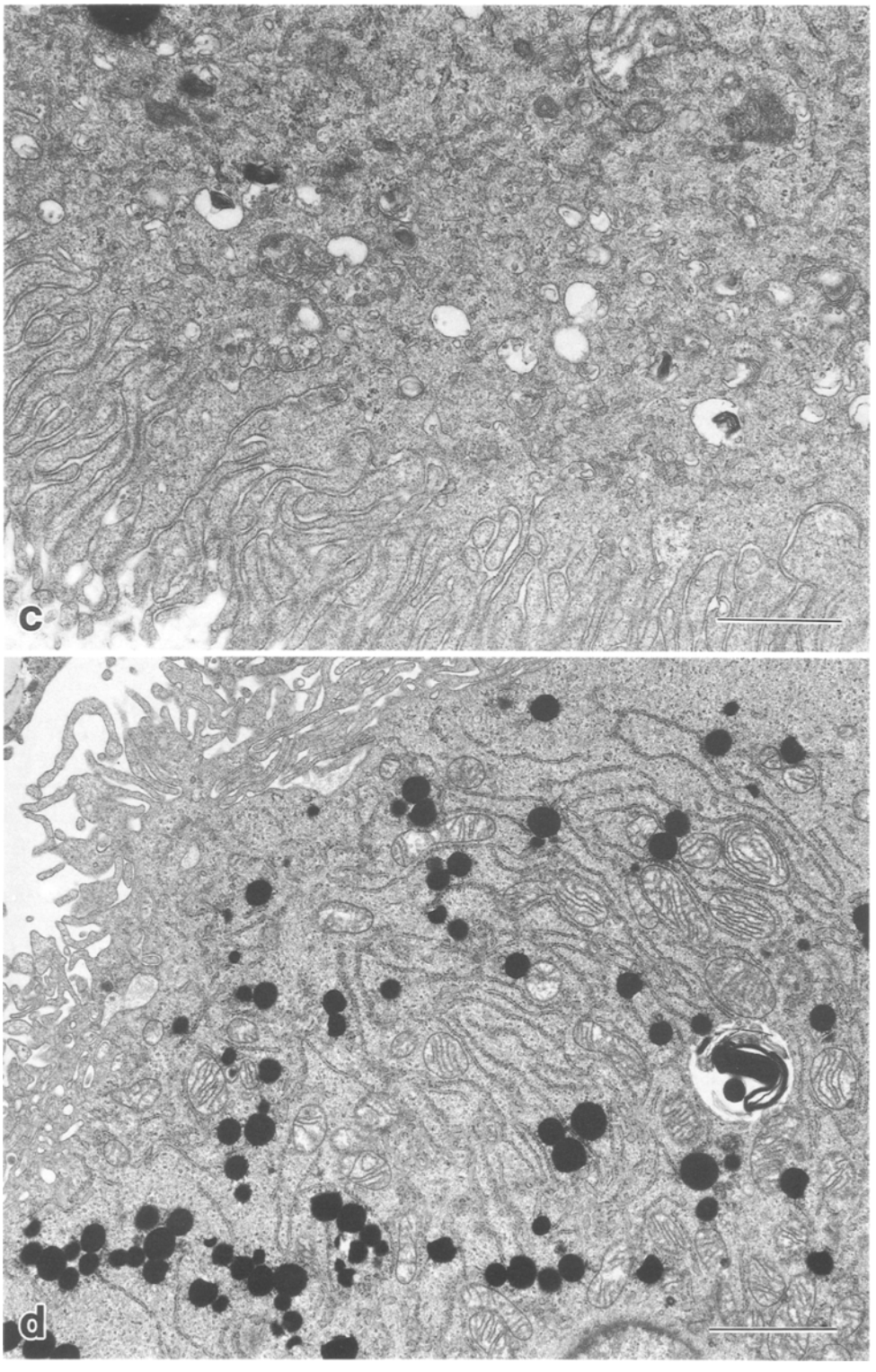


Fig. 8c, d

are not identical, mainly because the giant cells in the tumour tissue did not show a ruffled border or an "ectoplasmic layer"/clear zone, both of which have been reported for osteoclasts (Aparisi 1978; Aparisi et al. 1977a, Göthlin and Ericsson 1976). This view was supported by the studies on ultrastructural localization of acid and alkaline phosphatases in cell types (Aparisi et al. 1977b, 1978). However, in disagreement with the earlier studies mentioned above, our TEM study on the giant cells revealed for the first time that they were indeed engaged in excavating lacunae with the development of a ruffled border and a clear zone. The two membrane modifications of the giant cells were rather more conspicuous than those of authentic osteoclasts in vivo. This discrepancy between earlier studies and our work is probably due to lack of contact of the giant

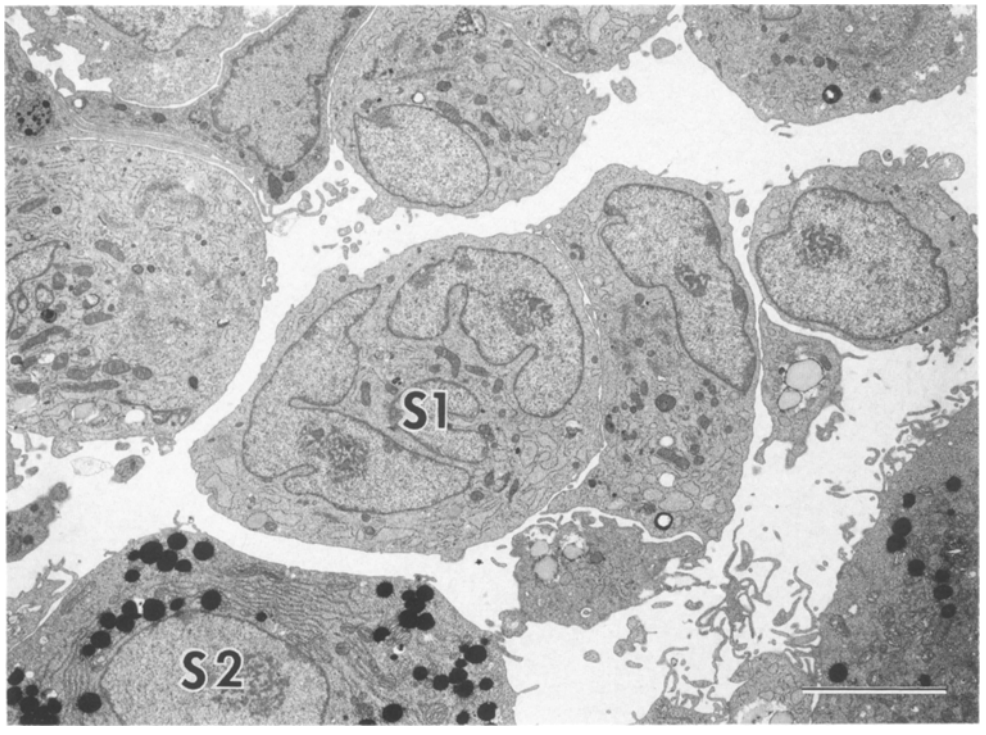


Fig. 9. Predominant stromal cell type (*S1*) is characterized by an abundant well-developed rough endoplasmic reticulum with expanded cisternae, nuclear pleomorphism of various degrees and a moderate number of relatively small mitochondria. The other type (*S2*), which is much less frequent, shows basically similar ultrastructural features to those of the giant cells. Numerous electron-dense granules are present only in this type and in the giant cells. *Bar* = 5 μm. ×4200

cells with bone surface. The observations made in this study led us to conclude that the giant cells of this tumour are potentially active bone resorbers and if they get an opportunity to attach to the bone surface, they can start resorbing bone like authentic osteoclasts. In fact the giant cells are formed and accumulated in the centre of the tumour where the pre-existing bone has disappeared and bone resorptive activity is no longer needed. Therefore, most of the giant cells are prevented from reaching their favourite substrate. Considering that the giant cell tumour produces an expansile osteolytic lesion, it appears plausible that the more the tumour grows, causing expansion, the more opportunities the interior giant cells can have to gain access to surrounding bone tissue.

Treatment of the giant cells with rhodaine-conjugated phalloidin allowed us to observe a band of F-actin containing podosomes in parallel with the observation made by Zambonin-Zallone et al. (1989). This unique structure has been found in osteoclasts (Marchisio et al. 1984, 1987) and has been suggested to be an essential part in the resorption apparatus of osteoclasts. This is based on observations that the band-like arrangement of podosomes of osteoclasts determines the specific shape of the resorption lacunae and is an adhesion structure providing a seal to allow creation of an acidic microcompartment for demineralizing and degrading bone matrix, and, in addition, podosomes disappear in osteoclasts treated with CT, resulting in the disruption of the band (Kanehisa et al. 1990b; Lakkakorpi et al. 1989; Turksen et al. 1988). In this study we observed that the band of podosomes corresponded to the lateral boundary of the resorption lacunae at the advancing resorption front, in accordance with our previous observations on the osteoclast (Kanehisa et al. 1990b). This evidence also supports that the authentic osteoclasts and the giant cells share a common apparatus for substrate adhesion and bone resorption. We have noticed, however, that a small percentage of giant cells displayed an unusual arrangement of podosomes which we have not observed in osteoclasts. The presence of these abortive giant cells might suggest that an unusual mode of giant cell generation occurs under the pathological environment of this tumour.

With respect to the cytogenetic relationship between giant cells and stromal cells, we have not obtained conclusive results in the present study. However, examination of F-actin cytoskeleton revealed that the F-actin pattern of the predominant stromal cell type was completely different from that of the giant cells. This type probably corresponds to "stromal cell type 1" described by Aparisi et al. (1977a), which had different ultrastructural features from the giant cell and the other stromal cell type judging from our TEM results. On the basis of these and the observation that the giant cells disappeared with time in culture with proliferation of the stromal cells, it seems that the stromal cell type 1 which may be a neoplastic element of this tumour is not a precursor of the giant cell. The other much less frequent cell type may be a precursor which seems not to proliferate in the cultures. This view agrees with the results of Roessner et al. (1984, 1987). They identified two stromal cell types: fibroblast-like cells which were capable of proliferating and cells of the mononuclear phagocyte system which showed alpha-naphthyl acetate esterase activity similar to that of the giant cells. They also found that part of the mononuclear cells and the giant cells stained positive with antibodies against the mononuclear

phagocyte system, whereas fibroblast-like stromal cells showed negative reactions to the antibodies. Thus, they have concluded that the giant cell tumour of bone is a neoplasm of fibroblastic cells with strong reactive infiltration of cells from the mononuclear phagocyte system and that the giant cells are derived from this system. In addition, Goldring et al. (1987) characterized cells cultured from giant cell tumour of bone and identified two cell types which were very similar to those described by Roessner et al. (1984, 1987). They have also suggested that cells of monocyte-macrophage lineage are the precursor of the tumour-associated giant cells. However, Aqel et al. (1988) analysed giant cell tumour of bone using various monoclonal antibodies specific for myeloid cells and HLA-DR, and since the giant cells failed to react with the majority of them, concluded that the giant cells are not of macrophage origin. Their conclusion is supported by the results of Wood et al. (1978), who demonstrated that macrophages in the tumour expressed Fc and complement receptors and were actively phagocytic, whereas the giant cells lacked these characteristics. The question as to whether the macrophage-like stromal cells are the precursor of the giant cells still remains to be answered. However, considering the fact that abundant giant cells continue to develop within the tumour where there are two major mononuclear stromal cell types and the fact that the neoplastic fibroblast-like stromal cells have very different phenotypic and functional features from those of the giant cells which, in turn, share many characteristics with macrophage-like stromal cells, it seems likely that the latter are the more probable precursor for the giant cells. This issue is highly relevant to many unanswered questions about the ontogeny and differentiation of osteoclasts, because of the similarity of the giant cells to osteoclasts both in morphology and behaviour.

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