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Culturing of cells from giant cell tumour of bone on natural and synthetic calcified substrata: the effect of leukaemia inhibitory factor and vitamin D3 on the resorbing activity of osteoclast-like cells

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Abstract Osteoclastic cells from giant cell tumour of bone (GCT) of bone provide a rich source for investigation of cellular mechanisms leading to formation of multinucleated cells, the resorption process and involvement of hormones and cytokines in these events. In the present study we investigated the effect of 1,25-dihydroxyvitamin D3 (VD3) and leukaemia inhibitory factor (LIF) on the resorbing potential of osteoclast of GCT origin using quantitative image-analysis of resorption lacunae in an in vitro dentine model. While VD3 unsignificantly increased the number of resorption pits and implicated surface after 7 days of GCT cell culturing, the stimulative effect of LIF was statistically significant. In cultures supplemented with LIF (5000 U/ml) the number of lacunae and resorption surface increased by 38% and 55%, respectively, when compared with control cultures. We suggest that both osteotropic agents increased osteoclastic activity, as the number of multinucleated cells was similar in control and experimental cultures. Seeding of GCT cells on biphasic calcium phosphate substratum revealed the relative inability of osteoclastic cells to resorb this synthetic material.

Key words Giant cell tumour of bone · In vitro resorption · Leukaemia inhibitory factor · Synthetic calcium phosphate · Electron microscopy

Introduction

Giant cell tumour of bone (GCT) is a relatively rare benign tumour that produces local lytic skeletal lesions by virtue of its osteoclastic component. As a benign tumour GCT has some uncommon features: a high recurrence rate for patients treated by means of conservative surgery [10, 39]; a capability to metastasize to the lung [46]; occasional malignant transformation [10, 13].

Histologically GCT is characterized by a large number of multinucleated giant cells (MNC), admixed with a minor subpopulation of mononuclear cells expressing T-cell-associated antigens [15] along with two major subpopulations of mononuclear cells: the first one possessing functional characteristics of the monocyte/macrophage lineage and the second corresponding to mesenchymal cells [4, 8, 15, 23, 36]. Currently, mesenchymal fibroblast-like cells are believed to represent the neoplastic tumour population which produces several factors presumably involved in the recruitment of osteoclasts and their monocyte/macrophage precursors [15, 22, 36]. MNC from GCT are osteoclasts, as they share with these latter common phenotypic and functional characteristics, such as possession of calcitonin receptors [15], expression of osteoclastic antigens [19] and the ability to excavate resorption lacunae on natural mineralized substrata [7, 21]. GCT osteoclasts and their mononuclear precursors thus provide a rich cellular source for the investigation of the cellular mechanisms leading to the formation of MNC, the differentiation pattern of their progenitors, the resorption process and the involvement of hormones and cytokines in these events.

The extensive usage of calcium-phosphate (Ca-P) bioceramics in human surgery requires new approaches to model the activity of cells involved in the remodelling process of implanted ceramics. The particular interest in investigating osteoclast-ceramic interactions is based on the fact that Ca-P ceramics have been used successfully for filling bone defects after conservative surgery of GCT [14, 43, 44]. Although there is distinct in vitro evidence of osteoclast resorbing activity against dentine and bone (reviewed by [3]) data on the ability of osteoclasts to resorb Ca-P ceramics are controversial [5; 16; 20; 40]. For our current study of interactions between ceramic and osteoclasts of GCT we chose biphasic Ca-P (BCP) ceramic. This material has been applied successfully in

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human surgery [9, 17, 32] and has certain advantages when compared with monophasic ceramics [31].

A wide spectrum of hormones and cytokines are involved in the regulation of bone resorption (for reviews see [2, 38]). The key role of 1,25-dihydroxyvitamin D3 (VD3) in the differentiation of osteoclast progenitors, the fusion of preosteoclasts and the resorbing activity of mature cells have been very well established [18, 24, 33, 42]. Among various cytokines involved in bone metabolism, the human interleukin for Da1 murine leukaemic cell line (HILDA) which is identical to leukaemia inhibitory factor (LIF) [30] was shown to have effects on bone remodelling both in vivo and in vitro [29, 35]. However, in vitro data on the action of HILDA/LIF on osteoclastic resorption are controversial, since both stimulation [25, 35] and inhibition [45] of this process have been reported.

In the present study we investigated the effects of VD3 and HILDA/LIF cytokine on the resorbing potential of osteoclasts of GCT origin using an in vitro dentine model and quantitative image-analysis of resorption lacunae.

Materials and methods

A GCT was obtained surgically from a 21-year-old man. The patient was firstly admitted to the hospital due to the buttock pain. Roentgenographs, computer tomography data and magnetic resonance image analysis showed a well-delineated area of bone destruction, 3 cm wide, located in the right ischial tuberosity. After open biopsy the diagnosis of GCT was made. According to the roentgenographic classification of Campanacci et al. [6] the tumour was attributed to stage 2 (active). A wide en-bloc resection was carried out.

At the time of biopsy and bone resection the concentration of HILDA/LIF cytokine was estimated in urine and serum of the patient according to De Groote et al. [11].

The following substrata were used for culturing of GCT cells: dentine, synthetic ceramic material and Thermanox coverslips (NUNC, Roskilde, Denmark).

Transverse sections of sperm whale dentine (about 100 µm thick) were cut with a water-cooled diamond saw (ISOMET, (Buehler) Illinois, USA). Dentine slices were stored in a 70° ethanol/water solution and then washed three times in phosphate buffer solution and culture medium before culturing.

The synthetic ceramic material (BCP: Triosite, Zimmer, France) consisted of hydroxyapatite and beta-tricalcium phosphate in 60:40 proportions as was determined by X-ray diffraction. BCP granules 40–80 µm in size were selected. Ten millimetre-diameter, 1 mm-thick disks were produces by compacting 200 mg of preselected granules at 130 mpa for 20 s (Specac, Kent, UK). Disks were sterilized by heating at 180 °C for 2 h. All ceramic samples were preincubated in 24-well plates (Greiner Labortechnik, GREINER, OSI, Paris, France) for 18 h in α (α-MEM, Alpha Minimum Essential Medium) with antibiotics at 37 °C before cell seeding.

Cultures on Thermanox coverslips were performed to investigate cell morphology, histochemical characteristics and number of nuclei in MNC.

The methods used for cell preparation were described elsewhere [21] with some modifications. Tumour specimens were stored for about 1 h in sterile α-MEM, supplemented with 1% antibiotic mixture (10,000 U/ml penicillin, 10,000 µg/ml streptomycin solution, GIBCO, BRL, Eragny, France) and heparin (50 U/ml). Necrotic and haemorrhagic areas were removed under sterile conditions and the tissue was dissected and mechanically disaggregated. Three subsequent trypsinization procedures were performed with a mixture of 0.05% trypsine and 0.02% ethylene diaminetetracetic acid in special salt solution (FLOW, Puteaux, France). The suspensions was agitated with a pipette, and the larger fragments were allowed to settle for 1–2 min. The resulting suspensions were diluted with a α-MEM supplemented with 15% of fetal calf serum (FCS; Dominique Dutscher, Brumath, France), and washed. After centrifugation cells were dissolved in a small volume of complete culture medium consisting of a α-MEM (Gibco), 15% of FCS, 2

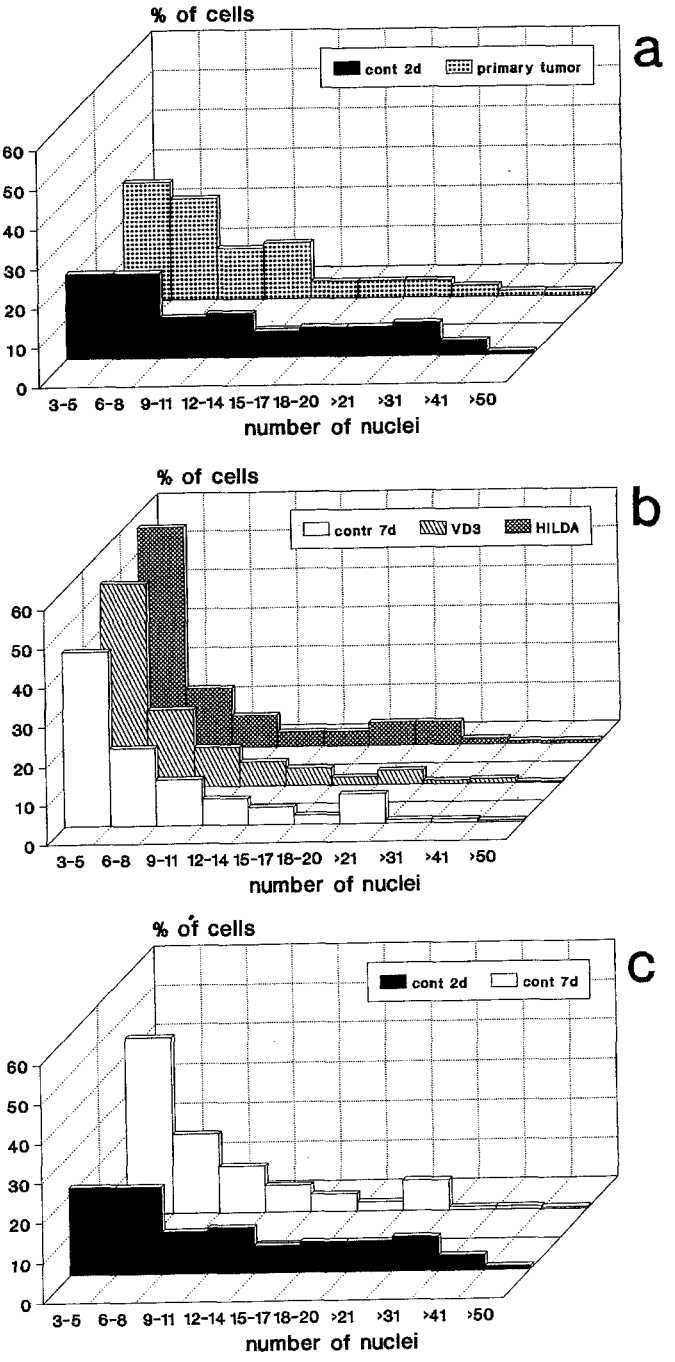

Fig. 1 Distribution pattern of nuclear number in multinucleated cells from giant cell tumour of bone (GCT) in (**a**) primary tumour and after 2 days of culturing, (**b**) 7-day control and experimental cultures and (**c**) 2- and 7-day control cultures. (*VD3* 1,25 dihydroxyvitamin D3, *HILDA* human interleukin for DA1 murine leukemia cell line)

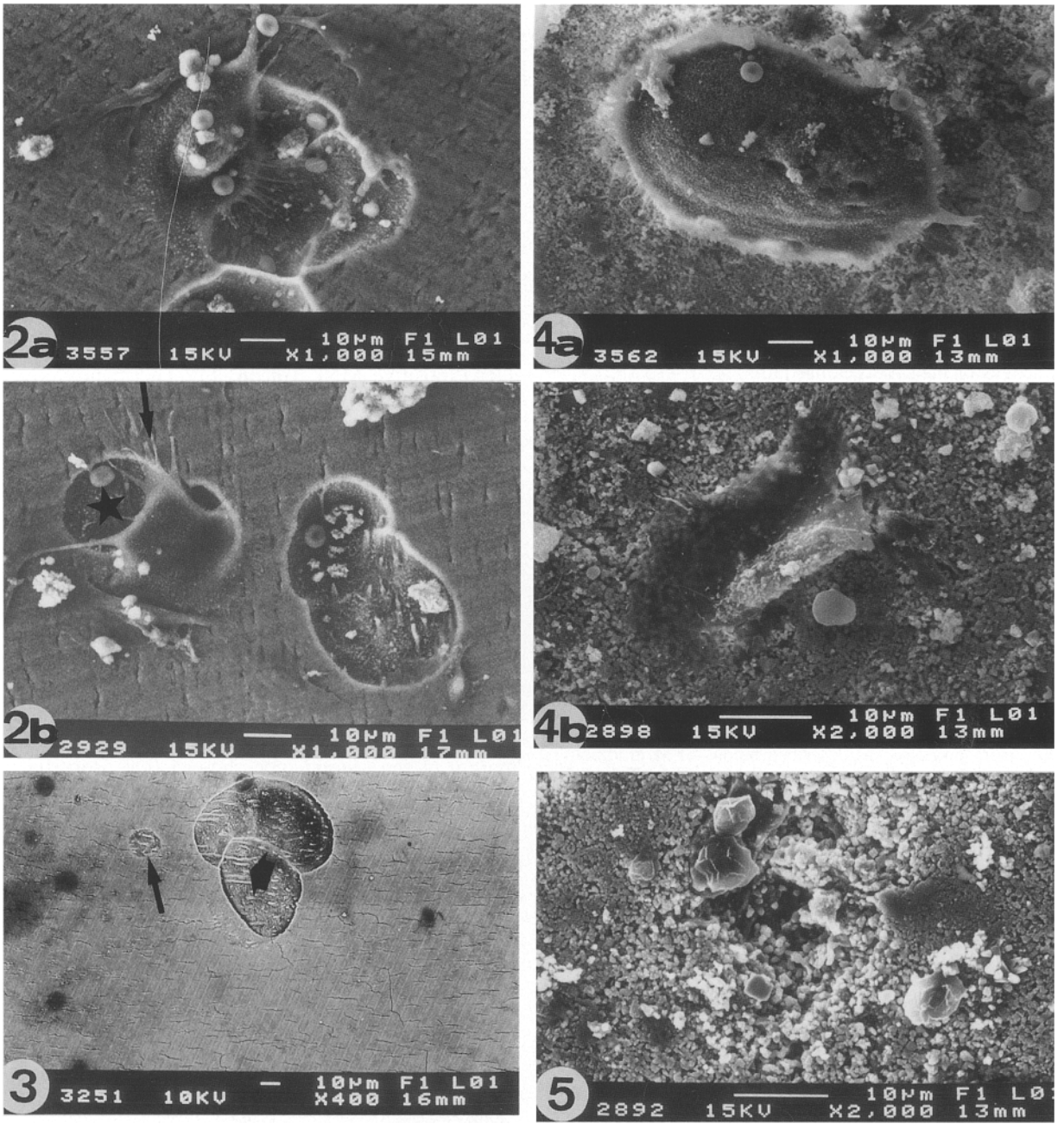

Fig. 2a, b Scanning electron micrographs (SEM) of dentine slices co-cultured with GCT cells for 2days in control conditions. **a** „Dome"-shaped with microvilli on surface. **b** Flattened cell with smooth appearance and filopodia *(arrow)* neighbouring resorption lacunae *(asterisks)*. Critical point preparation

Fig. 3 SEM of small simple *(arrow)* and complex resorption lacunae on the surface of dentine. Note the relative resistance of peritubular dentine *(arrow-head)* to resorption. Two-day culture. Ultrasonicated dentine sample

Fig. 4 SEM of "dome"-shaped (**a**) and flattened (**b**) giant cells on the surface of biphasic calcium phosphate (BCP) in a 2-day culture. Critical point preparation

Fig. 5 SEM of the surface of BCP disk after 2 days co-culturing with GCT cells. Small-sized (10–15 µm lacuna-like area). Hydrazine treatment

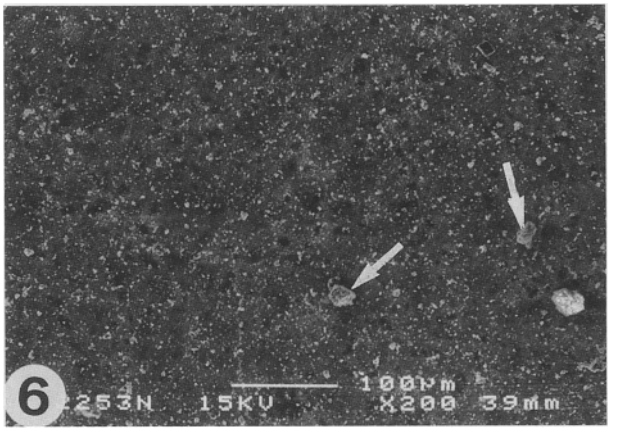

Fig. 6 SEM of the surface of BCP disk after 2 days incubation in culture medium without cells. Note the presence of small and great precipitated particles *(arrow)* and the absence of erosions. Hydrazine treatment

mM L-glutamine (Flow) and 1% antibiotics mixture. Cells in 20 µl aliquots were dropped on the surface of the substrata placed into 24-well plates (Nunc; 0.85×10^6/well). After 10 min incubation at room temperature, 1 ml of complete culture medium was gently added to each well to avoid reattachment of cells from the substrata, and plates were then incubated for an additional 30 min (37 °C, 5% carbon dioxide in atmosphere). After this short-term incubation, all the medium was replaced with fresh medium, and the cultures were incubated for an additional 2 and 7 days (37°, 5% carbon dioxide in atmosphere, 90% relative humidity).

In part of the cultures VD3 (kindly provided by Laroche Hoffman, Switzerland) was added at day 0 at a concentration of 10^{-7} M. In other cultures, HILDA/LIF cytokine was added at a concentration of 5,000 U/ml at day 1. Recombinant human HILDA/LIF was used as a conditioned medium from CHO, Chinese Hamster Ovary cells transfected with full-length cDNA coding for HILDA/LIF. Its biological activity was determined by its growth stimulating activity on the DA1a cell line [30].

Half of the culture medium in control and experimental GCT cultures (without or with additives, respectively) was replaced every 2 days.

The morphology of cells before culturing (cytocentrifuge preparations) and on Thermanox coverslips was investigated by routine light-microscopy methods (May-Grünwald-Giemsa staining). The number of MNC was scored on the whole Thermanox coverslip square and expressed as "number of MNC per culture". The number of nuclei per MNC was scored in at least 100 cells. Cells on cytocentrifuge preparations and on Thermanox coverslips were also studied for their tartrate-resistant acid phosphatase (TRAP) activity using a Sigma kit for leukocyte acid phosphatase (catalogue number 387-A).

The following scanning electron microscopy (SEM) observations were performed. After 2 days of culturing substrata were processed to investigate cell morphology. To study the surface modifications of dentine slices and BCP disks cultured for 2 and 7 days they were processed as described below. SEM morphology observations of 7-day cultures were carried out an the Thermanox coverslips.

For studies of cell morphology by means of SEM dentine slices, biomaterials and coverslips were fixed with 4% glutaraldehyde in phosphate-buffered saline (1 h, 4 °C) and postfixed with 1% osmium tetroxide (1 h, room temperature). Samples were dehydrated through a graded ethanol series, critical point dried and sputter-coated with gold-palladium.

For observations of the surface state of BCP disks, cells were eliminated from the surface. Disks were incubated for 15 min at 55 °C with hydroxyl-hydrazine, followed by absolute ethanol treatment (1 h, room temperature), drying and preparation for SEM observations by gold-palladium coating. This treatment resulted in minimal damage to the surface morphology of BCP disks (see Results).

To examine resorption lacunae features, dentine slices were placed in distilled water, ultrasonicated for 1–2 min, dried and prepared for SEM by gold-palladium coating.

SEM observations (JEOL 6300, Tokyo, Japan) were performed by means of secondary and backscattered electrons (BSE) under 15 kV. Quantitation of resorption pits made up by culturing osteoclasts on dentine was performed using an image analysis system (OXFORD Instruments (UK) Ltd. Bucks, England) linked to an SEM (in BSE). The areas of pit images were measured in at least 30 randomly selected areas per sample; the number of resorption lacunae were scored in the same areas visually. The results were expressed as mean percent of resorption surface per random field (about 50,000 μm^2) of duplicate cultures and mean number of lacunae per field ± standard error. All data were analysed using the Student's *t*-test to establish the statistical significance of observed differences.

For transmission EM (TEM) studies dentine slices were fixed as for SEM, dehydrated, embedded in Epon and polymerized at 60 °C. Ultrathin sections prepared on an LKB ultramicrotome were routinely contrasted with uranyl acetate and lead citrate and examined in a Jeol 200 CX TEM.

Results

MNC disaggregated from GCT by three subsequent processes of trypsinization amounted to about 1% of the tumour specimen. The distribution pattern of their nuclei number (determined on routine May-Grünwald-Giemsa preparations) is presented in Figure 1a. Most of the MNC were TRAP-positive.

Cells from GCT were seeded for 2 and 7 days in cultures containing the investigated substrata with or without addition of VD3 and HILDA/LIF cytokine (which effects were investigated only in 7-day cultures).

TRAP staining of Thermanox coverslips containing 2-day control cultured cells revealed the presence of TRAP-positive MNC (mean: 112/Thermanox coverslip) as well as numerous TRAP-positive mononucleated cells and TRAP-negative stromal cells. Practically no TRAP-negative MNC were observed. The distribution pattern of nuclei in cultured MNC did not differ significantly from those in primary tumour preparations (Fig. 1a).

SEM observations of dentine slices cultured for 2 days in control conditions with GCT cells, showed that fairly small cells as well as giant cells were implicated in the dentine resorption process. Morphologically, two types of resorbing cell were observed: a first type of roundish dome-shaped cell with microvilli, usually aggregated near the body of a cell bearing lamellipodia (Fig. 2a) and a second type of flattened cell with lobulated and folded pseudopodia in some areas of the cell periphery. The cell margin of the second type showed fine filopodia which were irregular in length and diameter. Cell polarization was sometimes characterized by broad pseudopodia at one pole and filopodia at the other (Fig. 2b).

Removing the cells from the surface of dentine allowed us to characterize the resorption pits morphologically and quantify their number and implicated surface

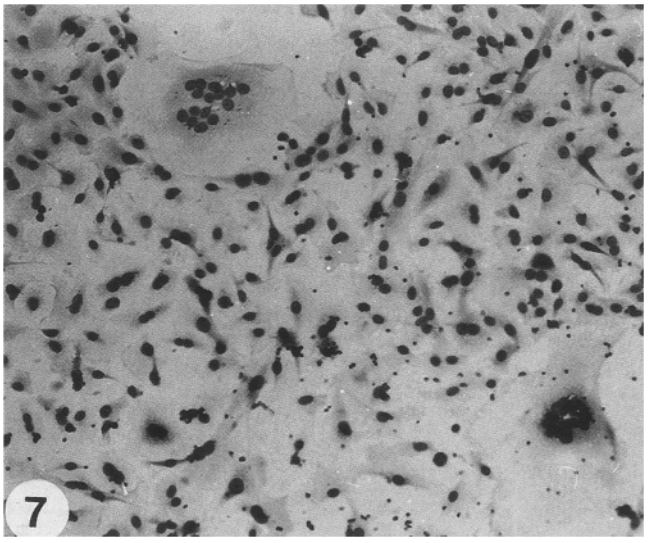

Fig. 7 Giant cell surrounded by numerous mononucleated stromal elements after 7 days of GCT culturing on Thermanox coverslip. May-Grünwald-Giemsa staining, ×200

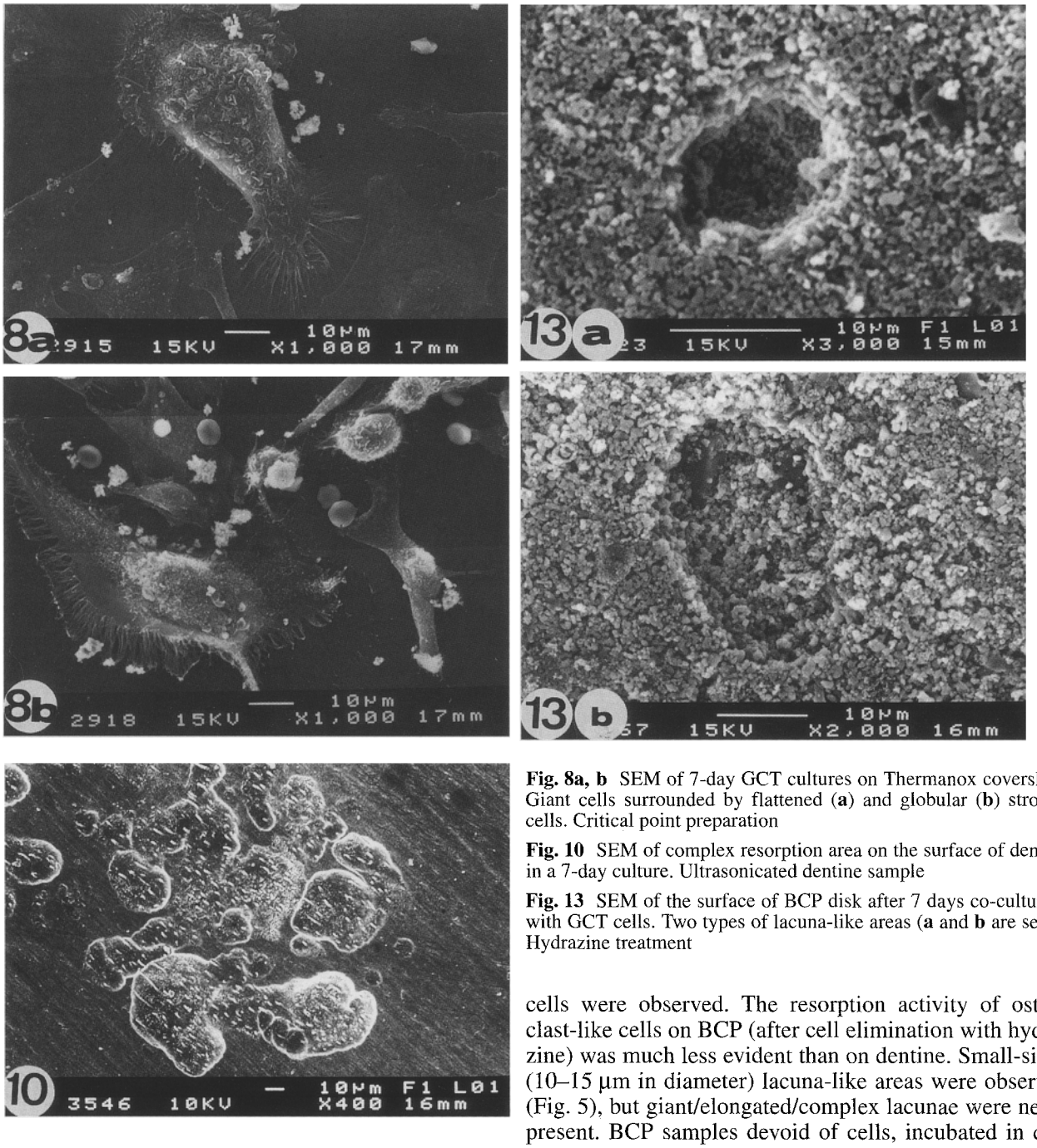

Fig. 8a, b SEM of 7-day GCT cultures on Thermanox coverslips. Giant cells surrounded by flattened (**a**) and globular (**b**) stromal cells. Critical point preparation

Fig. 10 SEM of complex resorption area on the surface of dentine in a 7-day culture. Ultrasonicated dentine sample

Fig. 13 SEM of the surface of BCP disk after 7 days co-culturing with GCT cells. Two types of lacuna-like areas (**a** and **b** are seen). Hydrazine treatment

of resorption. The size of the resorption lacunae produced by GCT osteoclasts was very variable and fluctuated from 15 µm to 100 µm. Three main morphological types of pit could be observed: circular and relatively deep lacunae of small diameter, larger and shallower elongated excavations and complex, generally shallow, pits with irregular contours (Figs. 2, 3). The relative resistance of highly mineralized peritubular dentine to resorption (Fig. 3) was noteworthy when compared with less mineralized zones.

SEM studies of 2-days GCT cultures on BCP showed a giant cell morphology similar to that on dentine (Fig. 4a, b) but no evident resorption lacunae related to these cells were observed. The resorption activity of osteoclast-like cells on BCP (after cell elimination with hydrazine) was much less evident than on dentine. Small-sized (10–15 µm in diameter) lacuna-like areas were observed (Fig. 5), but giant/elongated/complex lacunae were never present. BCP samples devoid of cells, incubated in culture medium and treated with hydrazine failed to reveal the surface modifications observed in cell-containing samples (Fig. 6).

After 7 days of culture, morphological observations were made for experimental and control cultures on Thermanox coverslips at light microscopic (fig. 7) and SEM levels. Different morphological types of cells were observed in all cultures: giant cells reaching 70 µm with microvilli on their superior surface and lamellipodia at one or two poles of the cell (Fig. 8a, b), flattened giant cells (data not shown), elongated fibroblast-like cells and globular small cells (Fig. 8a, b). Histochemical analysis showed that practically all multinucleated cells were TRAP-positive. Their number in control and experimen-

tal cultures supplemented with VD3 or HILDA/LIF cytokine were similar (Fig. 9). The pattern of nuclear distribution did not differ significantly in all 7-day cultures (Fig. 1b), but differed notably from 2-day control cultures by the switch to remarkable predominance of 3–5-nuclei-containing cells (Fig. 1c).

The process of dentine resorption continued till day 7 of culture. Resorption lacunae began to be more pleomorphic in their size and structure: they ranged from single small-medium size pits (10–20 μm in diameter) to huge complex areas of resorption reaching 200–300 μm (Fig. 10). Their number was significantly increased by about 5.5-fold from 2-days to 7 days in control cultures (Fig. 11a). Image-analysis data of the pit surface (Fig. 11b) revealed a significant increase in the surface involved (about 4.4-fold). Fort both variables (number and surface of lacunae), all the 7-day cultures (control and experimental), differed significantly from 2-day cultures. In 7-day cultures supplemented with VD3 both the number of lacunae and their surface increased by 26% and 41%, respectively when compared with control cultures (Fig. 11a, b). Statistically significant increase of the number of lacunae and their resorption surface at day 7 (by 38% and 55%, respectively) was induced by HILDA/LIF (Fig. 11a, b).

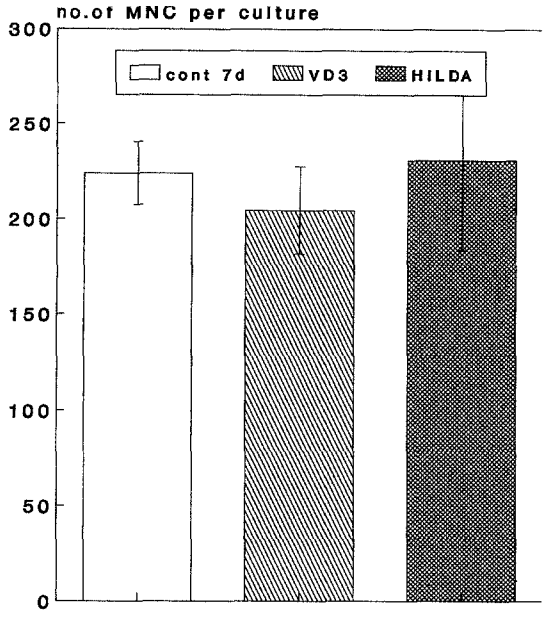

Fig. 9 Number of multinucleated cells in 7-day cultures of GCT with or without addition of 1,25-dihydroxyvitamin D3 (VD3) or human interleukin for DA1 muvine leukaemia all line/leukaemia inhibitory factor (HILDA/LIF). Results represent means± standard error. (Three replicate Themanox coverslip cultures were used for each group)

Despite the size and morphological composition of lacunae produced by osteoclasts in 7-day cultures, a relative resistance of peritubular dentine was observed. This feature was supported by TEM data. Ultrathin sections of the giant cell inside lacuna of resorption (Fig. 12a) showed the presence of finger-like structures (Fig. 12b) comparatively resistant to dissolution and probably corresponding to peritubular dentine. On the section examined, despite the evident resorption features, neither a ruffled border nor a clear zone was observed.

The investigation of resorption activity of osteoclast against BCP after 7 days of co-culture showed the presence of some lacunae-like structures, the size and number of which were lower than those on dentine. The small pits (about 10 μm in diameter; Fig. 13) exhibited well-delimitated outlines; in larger lacunae (up to 20 μm) scalloped margins were observed (Fig. 13b). The pits varied in depth and surface and complex lacunae were never observed. By SEM neither secondary electrons nor BSE allowed us to apply image analysis to quantify the number of pits produced by osteoclast-like cells on BCP.

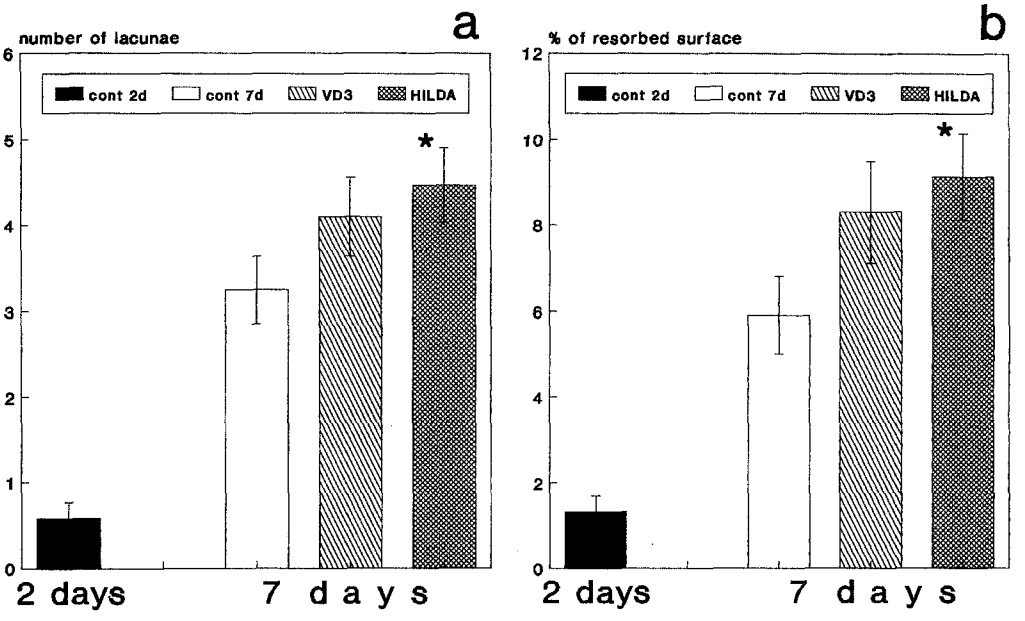

Fig. 11 Number of resorption lacunae (**a**) and percentage of resorbed surface (**b**) in 2- and 7-day cultures of GCT cells on dentine in presence or absence of VD3 and HILDA/LIF. The results are expressed as mean number of lacunae (**a**) and mean percent of resorption surface (**b**) per field (about 50,000 μm^2) of duplicate cultures ± standard error. At least 30 fields were for each sample analysed. *$P<0.05$ for group HILDA/LIF comparing with appropriate (7-day) control. All data for 7-day cultures differed significantly from those for 2-day cultures

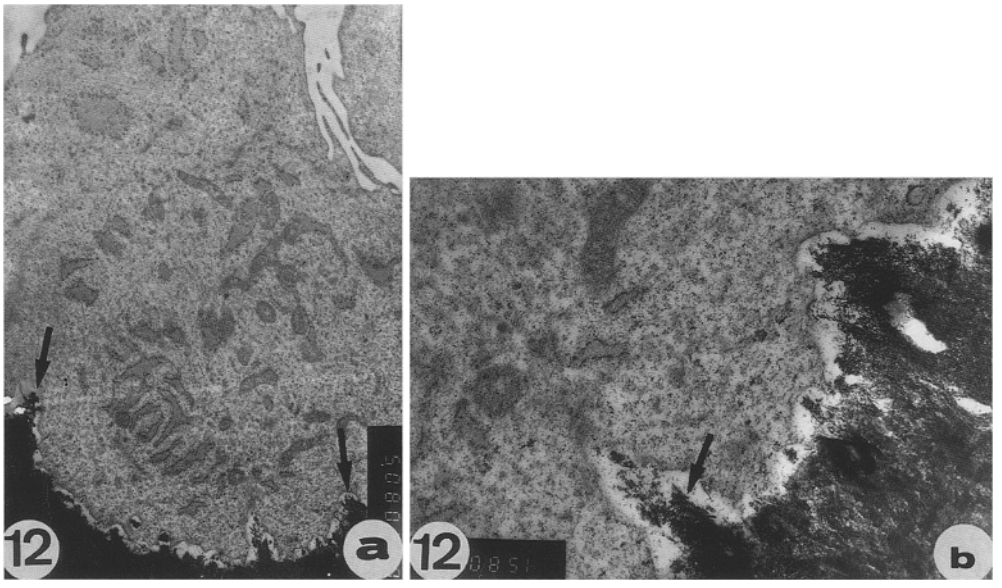

Fig. 12 Transmission EM of ultrathin sections of the giant cell inside lacuna of resorption (**a**). The edges of the lacuna are marked with *arrows*. Note the presence (**b**) of finger-like structure comparatively resistant to dissolution *(arrow)*. Seven day culture

Discussion

In agreement with others reporting on osteoclasts from GCT [7, 21] we were able to obtain significant number of viable MNC from a tumour specimen by means of mechanical tissue disaggregation and two cycles of trypsinization. Most of the MNC (along with some mononuclear cells) possessed strong TRAP activity and varied in size and nuclear number (from 3 to more than 50). Their osteoclastic nature was proven by their ability to excavate resorption lacunae after seeding on natural mineralized substratum. Image analysis of SEM observations of dentine pieces co-cultured with tumour cells allowed us to quantify the number and surface of resorption pits produced by osteoclasts in control cultures in 2- and 7-day intervals and to estimate the effect of vitamin D3 and of HILDA/LIF, a cytokine presumably involved in the bone remodelling process [29; 35].

After 2 days of culture, osteoclasts presented in reference cultures on Thermanox coverslips were abundant, mostly TRAP-positive and were admixed with developing stromal fibroblast-like cells and TRAP-positive small mononuclear cells, assumed to be late osteoclastic precursors [42]. SEM observations of dentine slices co-cultured with tumour cells revealed that two types of osteoclastic cells were generally involved in the resorption process. Thus, rather small dome-shaped and flattened cells varying in size and bearing lobulated and folded pseudopodia were found to be associated with resorption lacunae. The morphology of these lacunae varied from simple small round pits to complex areas reaching 100 μm. Their size and number increased significantly during the following 5 days of culturing (by 5.5- and 4.4-fold, respectively), in agreement with the qualitative observations made by Kanehisa et al. [21].

In 7-day cultures, MNC were still abundant and possessed usual morphological features. The distribution pattern of nuclei in 7-day cultures differed from that in 2-day cultures by an increase in the proportion of multiple (three-five) nuclei-containing cells. This observation was attributed both to the formation of new cells from mononuclear precursors still existing in 7-day cultures and/or to better survival of cells with low numbers of nuclei.

Quantitative analysis of lacunar number and pit surface on dentine slices co-cultured with osteoclasts in the presence of VD3 and HILDA/LIF showed that both agents had moderate stimulating effect on resorption, although this influence was more pronounced for the cytokine. Since the number of multinucleated cells was similar in control and experimental 7-day cultures, the effects of both substances were very likely to increase the intrinsic cellular resorption activity. We cannot however, exclude the possibility that VD3 and HILDA/LIF increased the number of mononuclear TRAP-positive cells which possess osteoclastic activity [12, 34]. The scoring of these cells in 7-day cultures was very difficult.

The stimulation effect of VD3 on resorption activity in GCT cultures was not statistically significant. In this respect, a moderate action of VD3 on mature osteoclasts has been shown previously by McSheehy and Chambers [27] and was mentioned in the review of Blair et al. [2].

Data about in vitro action of HILDA/LIF on osteoclastic cells ranged from the inhibition of resorption in fetal mouse bone explants [45] to the absence of effect on the generation of osteoclast-like cells from adult mouse bone marrow [41], and to stimulation of bone resorption in cultures of neonatal mouse calvaria [25, 35]. Interestingly, Van Beek et al. [45] noted that with the increasing maturation of fetal bone explants the antiresorptive potency of LIF decreased. The differences in data thus could be explained by diverse effects of HILDA/LIF on stromal (osteoblast or fibroblast-like) cells which mediate the action of the cytokine on resorption activity or osteoclast formation. Indeed, LIF receptors have been identified on osteoblast-like and preosteoblastic cells but

not on osteoclasts [28, 37] and LIF was shown to stimulate the proliferation in isolated osteoblasts [26]. The resorbing stimulative effect of HILDA/LIF in the cultures of GCT cells so could be mediated by its action on the stromal cells of the tumour, the neoplastic component of which is thought to be osteoblastic [36].

Whether HILDA/LIF is involved in the pathogenesis of GCT is unclear. The level of this cytokine in the serum and urine of the patient before tumour curettage was not elevated when compared with control samples (data not shown), but local involvement of HILDA/LIF in the development of tumour is not excluded by these data. In this respect, activated monocytes have been shown to produce HILDA/LIF [1] and mononuclear cells of haemopoietic origin appeared to be the essential component of all GCT.

Lacunae of resorption produced by GCT osteoclasts on natural mineralized substratum revealed a characteristic feature: the relative resistance of peri-tubular dentine was observed by means of SEM and TEM. Similar findings by Jones and co-workers [20] showed that authentic mammalian and avian osteoclasts were unable to resorb highly mineralized areas of peritubular dentine. The limited capacity of GCT osteoclasts to resorb BCP observed in the present study could thus be explained by the high degree of mineralization of synthetic substratum. Other factors which could play a role in the restricted ability of osteoclasts to produce lacunae on BCP may be linked both to proper cell-material interactions and in vitro conditions. Extracellular matrix components are known to improve osteoclastic attachment (for review see [2]) and the resorption activity of Ca-P has been potentiated in cultures with mineralized extracellular matrix previously produced by osteoblastic cells [5]. The physico-chemical composition of synthetic substrata, that is the crystal structure, the sintering time and temperature, the grain and inter-grain size, all govern osteoclastic resorption [5, 16]. Another factor which can modulate the activity of osteoclasts is the concentration of Ca and P ions; increasing concentrations of these ions reduce osteoclast formation and decrease the activity of mature cells [47]. Due to the high content of Ca in BCP ceramics and its dissolution, the action of osteoclasts could be altered soon after the beginning of the resorption process.

In conclusion, the culturing of GCT cells on natural and synthetic mineralized substrata allows new approaches to the understanding of the mechanisms of osteoclastic cells actions, of their physiology and of the regulatory factors involved in their interactions with stromal cells and substrata. Such in vitro models will help to clarify the aetiology and pathogenesis of GCT.

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