

Expression of Proteases in Giant Cell Lesions of the Jaws, Tendon Sheath and Salivary Glands

REINHARD E. FRIEDRICH¹, JULIKA EISENMANN², KERSTIN RÖSER²,
HANNA A. SCHEUER³ and THOMAS LÖNING^{2,4}

¹Oral and Maxillofacial Surgery, ²Department of Oral Pathology, and
³Orthodontics, Eppendorf University Hospital, Hamburg, Germany;
⁴Institute of Pathology, Albertinen-Krankenhaus, Hamburg, Germany

Abstract. *Introduction: Peripheral giant cell granuloma (GC) of the jaw is a tumour-like lesion, situated on the gingiva. The aim of this study was to: (a) better define the cellular compartments of the lesion and (b) compare the protease expression-profile in GC lesions of the jaws to GC lesions of other sites. Materials and Methods: This study comprised 54 GC lesions (jaws: 30, tendon sheaths: 22, salivary glands: 2). A microarray technique was applied to the study of osteoclast-specific or osteoclast-like features of different sites (CD68, CD51, RANK, M-CSF). Proteases were immunohistochemically identified [cathepsin K, L, S and matrix metalloproteinase 9 (MMP9)]. Results: The GC of all lesions were immunoreactive for CD68 and CD51. Factors indicating the differentiation and activation of osteoclasts were detected in all lesions (RANK, M-CSF, cathepsin K, MMP9). The expression profile of M-CSF in GC and stroma cells was of a medium grade in cases with no apparent destruction of bone, whereas RANK was expressed only weakly in mono- or multinuclear CD68-positive cells. Conclusion: The results of this study reveal an identical cellular composition for all lesions irrespective of site. GC of lesions at all sites contain the same osteolytic proteases and express cytokines that are effective in bone metabolism. The reason for the absence of osteolysis in some 'epulis' cases may be due to the topography of the lesion. Furthermore, the reduced number of binding sites, revealed by the low expression profile of RANK, may possibly be responsible for an absence of or only superficial osteolysis in these cases, despite evidence of M-CSF.*

Correspondence to: Professor Dr. R.E. Friedrich, Oral and Maxillofacial Surgery, Eppendorf University Hospital, University of Hamburg, Martinist. 52, D-20246 Hamburg, Germany. Tel: +49 40428033259, Fax: +49 428038120, e-mail: rfriedrich@uke.uni-hamburg.de

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The term 'epulis' refers to benign neoplasms that arise from the gingiva and expand in a hemispheric or mushroom-like pattern. They are judged not to be a true neoplasm in terms of an autonomous growing tumour but rather of a proliferating tissue type (1-5). Inflammation may be the stimulus of an epulis but this is not evident in many cases. Epulis is found predominantly in women and the patients are frequently 20 to 40 years of age at the time of diagnosis (1-5). Three types of epulis can be distinguished microscopically: epulis granulomatosa, consisting of granulation tissue; epulis fibrosa, characterized by a collagen-rich connective tissue; and epulis gigantocellularis, formed by a cell-rich granuloma, numerous multi-nuclear giant cells with bone resorptive capacity and a dense vascularisation. Both epulis fibrosa and granulomatosa are proliferating tissues with well-defined margins. In contrast, epulis gigantocellularis shows ill-defined margins, with a high rate of local recurrence. Haemorrhage occurs frequently in epulis gigantocellularis and sections show haemosiderin deposits inside the lesion. The multinucleated giant cells resemble osteoclasts (1-7). Large osteolytic lesions sometimes occur adjacent to the giant lesions. However, the pathomechanism of osteolysis (cell types, proteases) in the lesions is unclear. Recent studies on bone resorption revealed the proteases cathepsin K, L and matrix metalloproteinase 9 (MMP9) are abundantly expressed in osteolytic regions (8-14). However, an elevated enzyme activity has been proven only for cathepsin K. Cathepsin K is detected exclusively in osteoclast-like giant cells. These findings support the hypothesis that cathepsin K is a predominant actor in osteolysis (15). Given these observations, it is likely that the soft tissue tumour epulis gigantocellularis shows a correlation between the osteolysis and increased cathepsin K expression in osteoclast-like giant cells.

The aim of this study was to analyse the cell types and the proteases found in epulis gigantocellularis that might be relevant for the bone lesions in the jaws. Therefore, different types of giant cell lesions were compared for their protease-expression profiles and the cellular distribution of these enzymes in cells and tissues.

Table I. Primary and secondary antibodies.

Primary antibody	Species	Dilution (2% BSA)	Supplier
Cathepsin K (N-20), No. SC-6507	Goat	1:750	Santa Cruz Biotech., Santa Cruz, U.S.A.
Cathepsin S (M-19), No. SC-6505	Goat	1:500	Santa Cruz Biotech., Santa Cruz, U.S.A.
Cathepsin L (S-20), No. SC-6500	Goat	1:750	Santa Cruz Biotech., Santa Cruz, U.S.A.
MMP9 Clone: 56-2 ⁴ , No. Ab-3	Mouse	1:1000	Calbiochem., EMD Bioscience, Inc. Merck, Darmstadt, Germany.
RANK, No. AF 683	Goat	1:20	R&D Systems Minneapolis, U.S.A.
M-CSF, No. SC-1324	Goat	1:1000	Santa Cruz Biotech., Santa Cruz, U.S.A.
CD68, Clone: 514H12, No. SM1718	Mouse	1:500	Serotec, Duesseldorf, Germany
CD1a, Clone: 1CA04, No. DM363	Mouse	Pure	Neomarkers/Lab Vision, Fremont, U.S.A.
CD 51, Clone: CJ00, No. NCL-CD51	Mouse	1:200	Novocastra Lab., Newcastle, U.K.
Secondary antibody		Dilution (1 x PBS)	Supplier
Anti-goat		1:200	Vector, Burlingame, U.S.A.
Anti-mouse		1:200	Vector, Burlingame, U.S.A.

PBS, phosphate buffered saline; BSA, bovine serum albumin.

Materials and Methods

Patients. Fifty-four giant cell lesions were identified in the archives of the Institute of Pathology, Hamburg University. The specimens had been archived between 1973 and 2005. The oral tissues were predominantly excised from patients of the University Clinic for oral and maxillofacial surgery, along with some further tissues from external practitioners. The oldest patient was 91 years of age and the youngest 7 (female:male=27:27). The pathological diagnoses summarised as 'giant cell lesion' included original descriptions such as epulis gigantocellularis, peripheral giant cell granuloma, giant cell tumour, giant cell tumour of the tendon sheath and central giant cell granuloma. Epulis gigantocellularis and peripheral giant cell granuloma were considered to be synonymous in this study.

Methods. 3-Amino-propyl-triethoxysilane)-coated slices of 1 µm-thick were fixed on slides (SuperFrost plus, Fa. Menzel, Braunschweig, Germany) and dewaxed in graded ethanol. Menthol and H₂O₂ were used to block the endogenous peroxidase. Microwave pre-treatment of tissues was accomplished by addition of adequate buffers. These slices were used for the immunohistochemical study of cathepsin K, L, S, and MMP9 (Table I). Punch biopsies of representative areas of the lesions were collected and prepared for a tissue microarray (TMA) to study the expression of CD1a, cluster of differentiation (CD) 68, macrophage-colony-stimulating factor (M-CSF), receptor activator of NF-kappa B (RANK) and CD51. ABC kits (Vector Laboratories, Burlingame, CA, USA) were applied to visualize the reaction products (hemalaun counterstain).

Evaluation of immunohistological evidence for CD1a, CD68, CD51, M-CSF and RANK was performed using microarrays. The tissues from 17 cases were incubated with antibodies raised against CD1a, CD68, CD51, M-CSF and RANK (peripheral giant cell granuloma: n=4, central giant cell granuloma: n=2, giant cell tumour: n=3, giant cell tumour of tendon sheath: n=8). The semiquantitative evaluation followed a standardized grading system (weakly positive: +, moderately positive: ++, strongly positive: +++, negative: -).

It was possible to evaluate orthopantomograms for associated bone pathologies in the region of the granulomas in 10 cases.

Results

The 54 giant cell lesions were: epulis gigantocellularis (or peripheral giant cell granuloma) (total 20 cases), central giant cell granuloma (7 cases), giant cell tumour (5 cases) and giant cell tumour of the tendon sheath (22 cases).

Histology

Epulis gigantocellularis. Epulis gigantocellularis (synonym: peripheral giant cell granuloma) is characterized by a fine mesh of collagen fibres, a dense network of vessels and numerous polynuclear giant cells. Bleeding from these lesions occurs frequently. Residues of former haemorrhage constitute haemosiderin deposits inside the lesion.

Central giant cell granuloma. The cellular and extracellular compositions of central and peripheral giant granulomas are identical under the microscope. The difference in terminology refers to the topography of the lesions, whereby the 'central' type granuloma is located inside the bone and the 'peripheral' type outside of the bone tissue. Clinical distinction refers to aggressive *vs.* non-aggressive types. The phenotype of non-aggressive giant cell granuloma is typically characterized by an asymptomatic circumscribed osseous lesion, and is found predominantly incidentally. In contrast, aggressive lesions appear by a rapid expansive growth with an often extensive osteolysis of cortical bone and resorption of adjacent dental roots (16).

Giant cell tumour of the tendon sheath. The giant cell tumour of the tendon sheath is a nodular tumour arising from soft tissue of the synovia or the tendon sheath. Fingers and toes are predominantly affected. The lesion is composed of a mixture of undifferentiated, spindle-like mesenchymal cells, osteoclast-type giant cells and foam cells. These tumours might show different grades of fibrosis and contain a certain amount of iron pigment. They are classified as granulation ulcers and arise from excessive growth as a result of micro traumata.

Giant cell tumour. The giant cell tumour of bone consists of fibro-histiocytic tissues that probably constitute the neoplastic part of the tumour. Other components of the tumour are polynuclear giant cells. The giant cells are supposed to be non-neoplastic in nature but to arise from monocytes as the consequence of cytokine activity of the tumour on these inflammatory cells.

Immunohistology.

The evaluation of immunostaining was performed on standard slices (cathepsin K, L, S and MMP9) and microarrays. Finally, the results were compared with radiographs of the jaws. The expression of cathepsins K, L, S and MMP9 was studied in all specimens. A subgroup of 17 cases was selected for tissue microarrays (TMA) and studied simultaneously for the expression of CD1a, CD68, CD51, M-CSF and RANK.

Cathepsin K. Cathepsin K was expressed in the giant cells in 53 cases. The expression was strong in 85% of peripheral giant cell granulomas. The cathepsin K expression in central giant cell granuloma was heterogeneous. The expression was strong in about half of the cases (57%), and showed moderate or weak staining in 28.6% and 14.3%, respectively. In giant cell tumours, strong immunoreaction predominated (60%) and the staining was moderate in a further 40% (no cases of weak staining). The expression pattern of cathepsin K in the giant cell tumours of the tendon sheath was similar to that of peripheral giant cell granuloma. In one case, no immunostaining was noted (Figure 1).

Cathepsin L. All 54 cases were immunoreactive for cathepsin L in giant cells. Peripheral giant cell granulomas showed a moderate (65%) or weak (35%) immunoreaction. The staining pattern of central giant cell granulomas was reversed compared to the peripheral type (weak expression: 85.7%, moderate expression: 14.3%). The intensity of cathepsin L expression in giant cell tumours was weak (60%) or moderate (40%). In giant cell tumours of the tendon sheath, the intensity of immunostaining was evenly distributed (moderate 40.9%, weak 54.5%). A strong cathepsin K expression in this entity was restricted to a single case. In addition to giant cells, mononuclear cells were also stained in all entities.

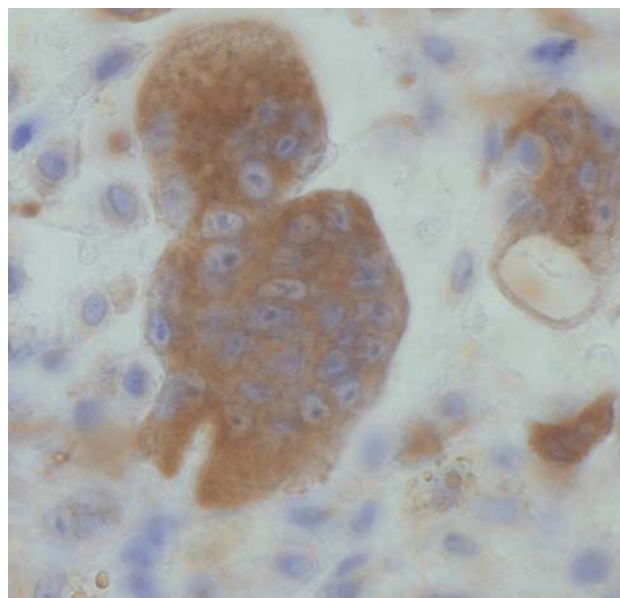


Figure 1. Immunolocalization of cathepsin K in the cytoplasm of multinuclear giant cell of epulis gigantocellularis (magnification $\times 40$).

Cathepsin S. Cathepsin S was not expressed in any of the 54 cases. However, in giant cell lesions of the tendon sheath, clusters of mononuclear cells were stained surrounding the non-stained giant cells.

MMP9. MMP9 was detected in the giant cells of 51 out of the 54 cases. Peripheral giant cell granulomas showed a predominantly strong expression (55%), but a weak or lack of staining was also observed. Central giant cell granuloma showed a heterogeneous MMP9 expression (strong: 42.9%, moderate or weak: 28.6% each). The MMP9 staining pattern in giant cell tumours was moderate (60%) or strong (40%). The giant cell tumours of the tendon sheath showed a predominantly strong expression (72.7%) and a moderate expression in a small subgroup (18.2%). One case each was weakly stained or negative.

CD1a. Fifteen out of 17 cases were CD1a positive (predominantly weak staining; 2 tissue samples could not be evaluated for technical reasons). Merely 2 cases of giant cell tumours showed a strong expression.

CD68. All 17 cases were strongly immunoreactive for CD68 in giant cells.

M-CSF. All tissues were immunoreactive for M-CSF (one specimen was excluded from evaluation for technical reasons). Moderate immunostaining predominated in all lesions.

Table II. Clinical data of patients with giant cell lesions.

Diagnosis*	Localisation*	Region	Gender	Age (years)	Osteolysis on X-ray	X-Ray: affected region	Cathepsin			MMP9
							K	L	S	
Central giant cell granuloma	Mandible	Region tooth No. 46	M	52	Yes	Region tooth No. 46 and mandibular Ramus	++	+	Negative	++
Central giant cell granuloma	Mandible	Region tooth No. 46	F	75	Yes	Region tooth No. 46 (apical, following apical resection)	+	+	Negative	+
Central giant cell granuloma	Mandible	Region tooth No. 46 to 47	F	33	Yes	Region tooth No. 48 to 45	+++	+	Negative	+
Giant cell tumour	Mandibular angle, extraorally located	Unknown	M	8	Yes	Mandibular angle (small, synclinal)	+++	+	Negative	+++
Peripheral giant cell tumour	Not specified	Unknown	M	9	No	Unknown	+++	+	Negative	+
Peripheral giant cell granuloma	Mandible	Region tooth No. 45	M	67	No	Unknown	+++	+	Negative	++
Peripheral giant cell granuloma	Maxilla	Region No. 11 to 12	M	60	No	Unknown	+++	++	Negative	+
Peripheral giant cell granuloma	Maxilla	Region tooth No. 21 to 22	M	43	No	Unknown	+++	++	Negative	+++
Peripheral giant cell granuloma	Mandible	Region tooth No. 35 to 37	F	69	No	Unknown	+++	+	Negative	+++
Central giant cell granuloma	Mandible	Region tooth No. 33 to 34	F	13	Yes	Same region with deviation of tooth roots)	++	+	Negative	++

F, Female; M, male; Region: region of the alveolar process of jaws determined by the appropriate position of the tooth. The positional code of a single tooth follows the recommendations of the Fédération Dentaire Internationale.

RANK. All lesions were immunostained for RANK expression in giant cells. The expression pattern was weak to moderate in all cases.

CD51. All cases were strongly immunoreactive for CD51, indicating the osteoclast-capacity of giant cells.

Evaluation of clinical parameters. Orthopantomograms of 10 cases were evaluated for 3 distinct findings: (a) Osteolytic process in the region of the jaws where the giant cell lesion was diagnosed, (b) extension of the osteolytic process on radiograms and (c) correlation of findings to the cathepsin expression. The orthopantomograms were from peripheral giant cell granulomas in 5 out of the 10 cases. These 5 cases showed no osteolysis in the region of interest (Table II).

All 4 cases with central giant cell granuloma showed an osteolytic zone of different size. Intensity of cathepsin K expression differed accordingly. One case of giant cell tumour showed a small osteolysis that was strongly immunoreactive for cathepsin K.

Summary of results. In all entities, a strong cathepsin K expression was revealed, in particular in the peripheral giant cell granuloma group (85%). However, in this group no osteolysis of the jaws was detectable on orthopantomograms in 5 cases. Cathepsin L expression in giant cells was found in

all entities, at least at a weak level. The peripheral giant cell granuloma group differed in the expression pattern from the other entities in terms of stronger staining. Furthermore, in addition to giant cells, all mononuclear cells were immunostained in all entities for cathepsin L expression. Cathepsin S was not expressed in giant cells. However, some mononuclear cells were found to express cathepsin L. MMP9 was strongly expressed in the majority of cases, with the exception of giant cell tumour (moderate expression). CD1a expression in giant cells was predominantly moderate and merely weak in giant cell tumour. In contrast, a strong CD1a expression was observed only in giant cell tumour of the tendon sheath. CD68 expression in giant cells and macrophages was strong in all cases. M-CSF expression of giant cells was moderate in all cases with the exception of central giant cell granulomas (weak staining). RANK expression was predominantly weak. In contrast, the CD51 expression was strong irrespective of the entity.

Discussion

This study investigated the expression of proteases and associated enzymes and receptors in giant cell lesions of different sites.

In the course of epulis gigantocellularis, osteolysis of adjacent bone can be observed. This phenomenon is shared

by different tumour-like giant cell lesions (central giant cell granuloma, giant cell tumour). Following recent studies on giant cell tumours of bones, it is reasonable to assume that cathepsins are causative agents for osteolysis (5, 8, 9). In addition to this group of proteins, the cellular composition of the tumour-like lesion and the topography of the tumour in relation to the bone are clearly important for the degradation of bone.

The aim of this study was to determine the types of proteases and cell types relevant for the osteolysis of bone in cases with giant cell granulomas lesions and to determine whether osteolysis is specific for epulis gigantocellularis.

The characterization of macrophages by means of determining their specific membrane markers was performed to allow the assignment of proteases to certain cells. For example, CD1a identifies antigen-presenting cells (*e.g.* Langerhans cells and interdigitating reticulum cells). CD68 identifies macrophages/monocytes.

The immunophenotyping included seeking evidence for osteoclastic properties of giant cells by staining for specific receptors (vitronectin receptor, CD51) and cytokines, to allow inferences to be drawn on cellular function.

Cathepsins K, L, and S are relevant for the metabolism of intra- and extracellular proteins. These enzymes are lysosomal cysteine proteases involved in numerous pathological processes that are associated with the degradation of tissues. This study revealed that the expression of cathepsin K in the majority of cases was strictly restricted to the multinuclear giant cells. The expression of this enzyme was strong in the majority of cases. With the exception of one giant cell tumour of the tendon sheath (no staining) all giant cell lesions of this study showed the same staining pattern. The peripheral giant cell tumour showed a strong cathepsin K expression in 85% of cases. Despite this strong cathepsin K expression none of the 5 cases showed osteolysis adjacent to the lesion on x-rays of the jaws. Cathepsin L was expressed in all lesions both in mononuclear cells and giant cells. The expression for cathepsin L was moderate in peripheral giant cell granulomas and weak in other lesions of this type. Cathepsin S expression was restricted to mononuclear cells in this study. MMP9 expression was strong in peripheral giant cell granulomas and the giant cell tumour of the tendon sheath and moderate in other lesions.

Several studies that used different techniques have shown that cathepsin K expression in osteoclasts is related to bone resorption, *e.g.* by competitive RT-PCR (17) and *in situ* hybridization (5). Both these studies revealed a strong cathepsin K mRNA expression selectively in osteoclasts. These results are supported by immunohistological and *in situ* hybridization studies (18). However, the cathepsin K expression was also found in mononuclear cells, presumably the osteoclastic progenitor cells. Cathepsin K expression is

restricted to multinuclear giant cells and epithelioid cells, independent from pathological alterations and the topography of the lesion, and is not found in normal macrophages (4). The specific expression of cathepsin K in these lesions is supported by the immunohistochemical findings of Lindemann *et al.* (15) who identified an increased cathepsin K expression in particular in giant cell tumours of bones and only in 'osteoclast-like' giant cells. They concluded that cathepsin K might be a deciding factor for osteolysis in giant cell tumours.

The lysosomal cysteine protease cathepsin K plays a key role in the regulation of bone resorption. Bone resorption is correlated to the expression of cathepsin K (13, 18, 19, 20). It has also been shown that bone resorption is impaired in the absence of cathepsin K (11, 21).

Osteonectin and collagens are substrates for activated cathepsin K (3). Cathepsin K and MMP9 are able to cleave collagen type I and II (22). However, the degradation of human cortical bone is only dependent on the activation of cathepsin K and no further enzymes are needed for osteolysis (7).

The role of cathepsin L and S for bone resorption is presently unknown. Inhibition of cathepsin L has no impact on osteoclast-induced bone resorption (12). Cathepsin L is likely to play a minor role, compared to cathepsin K, in the degradation of bone (17).

Matrix metalloproteinases, in particular MMP9 (14), are suspected of being responsible for the locally aggressive behaviour of giant cell tumours. However, it is presently unknown whether this protease is directly involved in osteolysis.

In accordance with the present findings, Lindeman *et al.* (15) showed that both MMP9, cathepsin K and cathepsin L are expressed in giant cell tumours of bones.

In addition to the expression of proteases in tissues, it is important to know the activity of these enzymes at the place of expression.

Specific assays for protease activity that were performed with tissues from giant cell lesions allowed the conclusion that only cathepsin K had a high activity in tissue. In contrast, MMP9 was present as an inactive proenzyme in 98% of the cases. Therefore, this enzyme is of less importance in osteolysis. The proteolytic activity of cathepsin K is pH dependent and higher in an acidic milieu (23-26). This acidic milieu is established by ionic and proton pumps in the region of the 'ruffle borders' of osteoclasts after adhesion of these cells to the bone. In the case of insufficient adhesion of osteoclasts to the bone, the osteolytic proteases are not able to degrade bone (5). The inactivity of cathepsin K prevents the activation of MMP9. This may be the explanation for the present findings that despite strong expression of both cathepsin K and MMP9 in all lesions, osteolysis was not correlated to the immunolocalisation of osteolytic enzymes. *In vitro* studies with cell lines from giant

cell tumours on the osteolytic properties of mononuclear cells revealed that cathepsin K-positive mononuclear cells had no osteolytic potential and therefore were clearly not involved in the osteolytic process (15).

The results of this study indicate that a strong cathepsin K expression in osteoclast like giant cells does not necessarily lead to osteolysis. However, there is strong evidence for the key role of cathepsin K for the osteolysis of giant cell tumours of bone and the restriction of the protease to osteoclast-like cells (15). An explanation for these contradictory results may be that cathepsin K is not expressed as a mature enzyme but as procathepsin K.

A further aspect of cathepsin activity might be the topography of the giant cells in relation to the adjacent bone. Cathepsin K was cytochemically quantified in osteoclasts of giant cell tumours of bone and in non-affected bone. These studies revealed that osteoclasts more distantly located to the bone had high amounts of cathepsin K mRNA and protein but the enzyme was predominantly in an inactive form, the zymogen form (5). In contrast, osteoclasts close to the bone contain mature and active cathepsin K. Dodds *et al.* (5) further showed that the activation of cathepsin K *in vivo* takes place intracellularly prior to bone resorption. After this activation, cathepsin K is secreted *via* resorption lacunes.

Therefore, it is reasonable to assume that the topography of the peripheral giant cell granuloma to the bone is crucial for the capacity of the cathepsin K produced in the giant cells of this lesion to degrade bone. Possibly a peripheral localisation of the epulis with small regions adhering to the bone is associated with a high amount of cathepsin K as a proenzyme in osteoclast-like cells. In this case, the resorption of bone might occur very slowly or even not at all.

Liu *et al.* (1) immunohistologically investigated different giant cell lesions of the jaws for their cathepsin K expression. They found that polynuclear giant cells of peripheral and central giant cell granulomas all shared the same phenotype of osteoclasts. Hansen *et al.* (9) revealed an increased cathepsin K mRNA and protein expression in giant cells of cholesteatomas, a lesion characterized by progressive erosion of bone. They found an increased cathepsin K expression in the giant cells of a giant cell tumour of the tendon sheath and concluded that there is a close relationship between these osteoclasts (8). However, numerous other diseases with invasive properties are able to express cathepsin K in osteoclast-like cells, *e.g.* pancreatic carcinoma with giant cells (10). In a case of anaplastic thyroid carcinoma, the increased cathepsin K expression in osteoclast-like giant cells was associated with the invasiveness of the tumour and its property to degrade the chondreal trachea (27).

The results presented here are supported by the findings of an *in situ* hybridization study where osteoclasts showed no or only weak cathepsin S and L mRNA expression (7). In contrast, Lindeman *et al.* (15) demonstrated mRNA profiles

that showed cathepsins K and L and MMP9 to be the predominantly expressed collagenases in giant cell tumours of bones, leaving cathepsin S as the enzyme of mononuclear cells

This study revealed cathepsin S expression to be restricted to mononuclear cells, independently of the type of lesion. Cathepsin K expression was found exclusively in giant cells, whereas cathepsin L was expressed both in giant cells and mononuclear cells.

Mature osteoclasts are characterized by the expression of the vitronectin receptor. This receptor mediates the cell-to-substrate interaction between osteoclasts and bone (28). This immunohistochemical study confirms the osteoclast-like character of the giant cells by revealing their strong CD51 expression.

The function of giant cells is not only dependent on the enzyme spectrum but also from the activity state of these cells. This activity state is regulated by osteoclast-inducing factors such as RANKL (receptor activator of NF-kappa B ligand) and M-CSF and their receptors RANK and M-CSF. RANKL and M-CSF are cytokines involved in the ontogenesis of osteoclasts (2, 29-31). The immunohistochemical investigation for RANK and M-CSF expression in these cells aimed to detect the activation of osteoclast-specific properties of the giant cells. RANK and M-CSF were universally revealed in the giant cells of all lesions. However, the expression of RANK was low in general, but moderate for M-CSF.

RANKL was assessed as a mediator of bone resorption. This cytokine acts as an intercellular signal transducer from osteoblasts to osteoclasts. RANK is the receptor of osteoclasts. In the presence of M-CSF, RANKL stimulates the differentiation of monocytes/macrophages *via* precursor cells of osteoclasts to mature osteoclasts (32).

M-CSF is a growth factor involved in inflammation and osteoclast differentiation. Loss of osteoclasts in op/op osteopetrosis mice is the consequence of insufficient or lack of M-CSF synthesis (33).

Evidence for RANK and M-CSF in all lesions allows the assumption that RANK, RANKL and M-CSF are involved in osteolysis. The weak expression of RANK in the majority of lesions may be due to the lack of osteolysis in these cases. Yoshida *et al.* (33) demonstrated the expression of RANKL- and M-CSF both in giant cells and mononuclear cells in giant cell tumours of the tendon sheath. They assumed that giant cells contribute to the osteoclast differentiation by exocytosis of these factors.

The present study confirmed the expression of M-CSF in giant cells. Morgan *et al.* (34) found a stronger mRNA and protein expression of RANKL in osteoclasts of giant cell tumours compared to the adjacent stroma. In the beginning of these studies it was suggested that neoplastic stromal cells express RANKL and transform normal monocytes to osteoclasts, and were thereby the causative cell type for bone destruction (2). On the contrary,

Tsurukai *et al.* (35) postulated a key role of RANKL expression in osteoblasts and assumed that progenitor cells of osteoclasts are already present in the osseous milieu. They argued for osteotropic factors triggering the velocity of osteoclast differentiation.

This study reveals that all giant cell lesions are able to activate osteoclast-mediated bone resorption at a cellular level. Giant cells express osteolytic proteases and osteoclast-activating cytokines that play a role in bone metabolism. Further studies are needed on peripheral giant cell granulomas with radiological evidence for osteolysis to verify the topography-dependent expression and activity of proteolytic enzymes and osteoclasts.

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