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Psammous desmo-osteoblastoma

Ultrastructural and immunohistochemical evidence for an osteogenic histogenesis

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Summary. Fibro-osteo-cemental lesions of the jaw bones are a heterogeneous group of diseases which present problems in classification. Psammous desmo-osteoblastoma is one of four newly proposed entities (Makek 1983) and has until now been characterized by its light microscopic, clinical and radiological features. On electron microscopy this tumour exhibits fibroblastic (preosteoblastic), osteoblastic and osteocytic cells and a globular mineralization unlike the mineralization of the psammoma bodies. Immunohistological investigations with anti-osteonectin, a bone specific protein linking mineral to collagen, showed positive intracellular staining in all tumour cells and extracellular staining in the osteoid. The psammoma bodies were, however, not stained. These results confirm the view of the osteogenic histogenesis of psammous desmo-osteoblastoma, with an osteogenic differentiation of the tumour cells, bone formation and association of psammoma bodies which are not of bone origin. This combination of findings supports the view that psammous desmo-osteoblastoma represents a new and distinct entity occuring in desmal preformed cranio-facial bones which should be incorporated in a revised WHO-classification.

Key words: Psammous desmo-osteoblastoma – Ultrastructure – Osteonectin – Bone tumours – Fibro-osseous lesion

Introduction

Fibro-osteo-cemental lesions in the bones of the jaw and skull represent a very heterogeneous group of diseases (Waldron and Giansanti 1973; Hoppe 1986). The variety of the clinical pictures and morphological features, especially the histological similarity between various lesions, sometimes leads to uncertainties and confusion (Makek 1986; Donath 1986a). These difficulties cannot be prevented by the WHO-Classification of Pindborg and Kramer (1971). The identification and separation of these fibro-osseous lesions into distinct pathological entities is fully justifiable on their differences in biological behavior. Some are innocuous and can be treated conservatively with excellent results, whereas others are aggressive and require initial, early radical therapy to effect a cure (Sailer and Makek 1986). For this reason a reclassification has been suggested by several authors (Makek 1983; Burkhardt 1985; Waldron 1985). Based on morphological, clinical and radiological findings, psammous desmo-osteoblastoma (PDOB) was proposed and isolated as one of four new entities by Makek (1983). The main characteristics of PDOB consist clinically of aggressive growth and radiologically of a well defined area of dissolved bone. Viewed microscopically PDOB exhibits whirled fibrous tissue containing spindle cells, woven bone and psammoma bodies, which formerly led to the diagnosis ossifying fibroma, cementifying fibroma, aggressive cemento – ossifying fibroma, psammo osteoid fibroma etc.. As the morphological findings of psammous desmo-osteoblastoma are still based on light microscopic investigations we would like to add some ultrastructural and immunohistological findings to elucidate histogenesis.

Material and methods

A large osteolytic lesion was detected incidentally in the ascending ramus and condyle of a 17 year old female patient, resulting in a painless swelling without any change in sensation. The radiological appearance exhibited an osteolytic area of 3 cm

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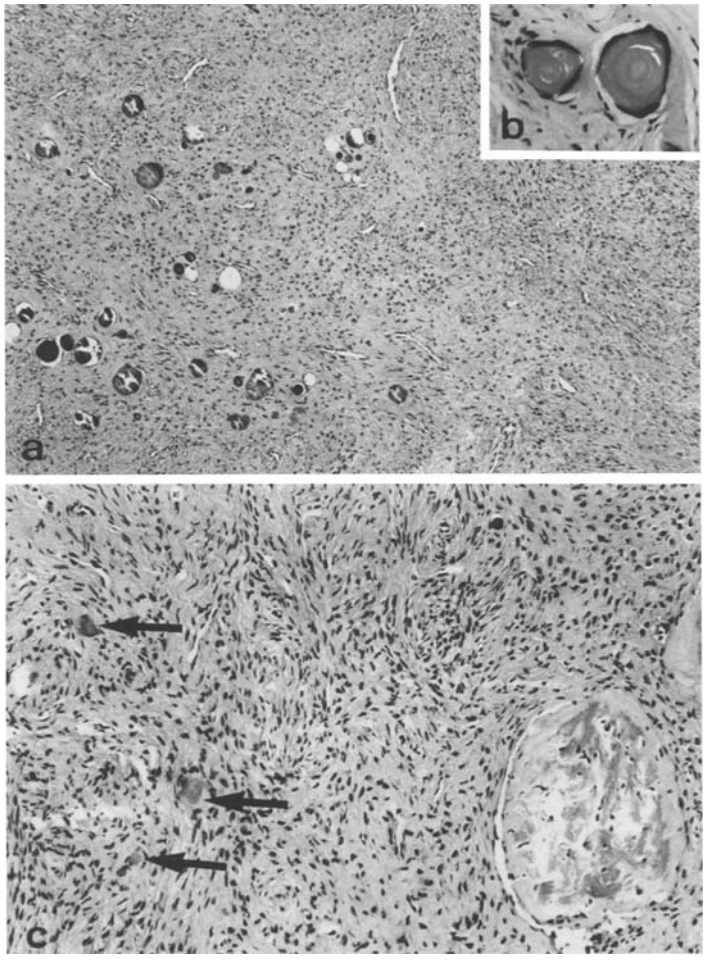

Fig. 1. a Fibrous parts of PDOB build up of fasciculi and whorls and interspersed psammoma bodies; HE; 64 × ; **b** Psammoma bodies with target like calcification; HE; 176 × ; **c** Fascicular tumour tissue with originating psammoma bodies (↑) close to a small osseous trabeculum; HE; 128 ×

diameter with perifocal osteosclerosis and some slight radiopaque foci in the central region of increased translucence. Surgical therapy consisted of resection including the buccal cortex layer without filling up the osseous defect. After 6 month there was complete bone healing with involution of the initial thickened condyle area. An 18 month long follow up period showed no evidence of relapse.

Tumour tissue was fixed in 8% buffered formaldehyde and decalcified when needed. Consecutive prepared paraffin sections (5 μ thick) were stained with hematoxylin-eosin, van Gieson and Giemsa.

Immunohistological staining was carried out on paraffin sections with an antihuman antibody against osteonectin, a bone specific protein (Termine et al. 1981). The biotin – avidin method was applied and peroxidase used as a marker enzyme (Polak and van Noorden 1983). Conventional positive and negative controls were done. For electron microscopy tissue blocks of 2 × 1 × 1 mm size were fixed in 4% glutaraldehyde and embedded in Epon. Semithin sections were stained with methylene blue. Ultrathin sections were contrasted with lead citrate and uranyl acetate and finally viewed with a Philipps electron microscope.

Results

On light microscopy most parts of the lesion consisted of fasciculi and whorls of varying cell density composed of a poorly vascularized fibrous tissue containing spindle cells of medium size (Fig. 1a, c). The nuclei were spherical or oval in shape with a low chromatin content. In these fasciculi bundles of collagen fibers and extensively basophilic calcified structures were found, frequently spheroidal or target like, the so called psammoma bodies (Fig. 1b). Sometimes they were concentrically surrounded by elongated spindle cells. In other areas a large amount of hard tissue formation was detected consisting of foci of osteoid and woven bone trabeculae (Fig. 2a). At the mineralization border rounded osteoblastic cells appeared but prominent osteoblastic rimming was absent (Fig. 2b). Completely incorporated cells resembled typical osteocytes while multinucleated osteoclasts were missing. Furthermore irregular basophilic cementlines and organisational tendencies such as lamellar bone formation and osteones were not found in the trabeculae. Regressive changes in the central parts of the tumour resulted in a loss of stroma cells accompanied by an increase of fibrosis and accumulation of bizarre thorn – shaped masses of

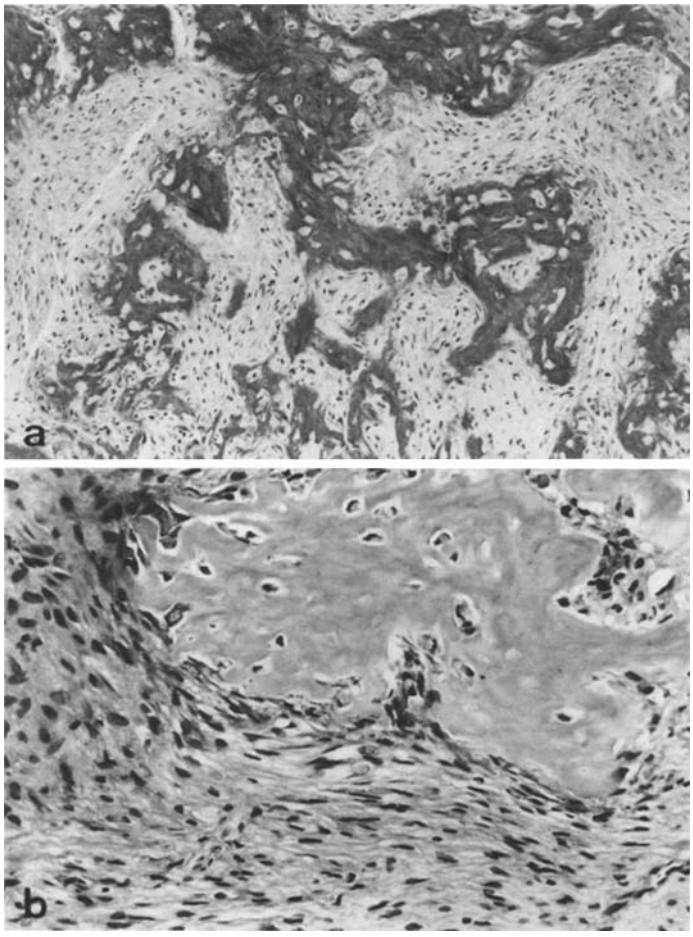

Fig. 2. a Ossifying parts of PDOB with desmal ossification forming irregular trabeculae embedded in a fusiform tumour stroma; HE; 112 × ; **b** Desmal ossification zone with growing osteoblastic cell differentiation towards the mineralization front; HE; 224 ×

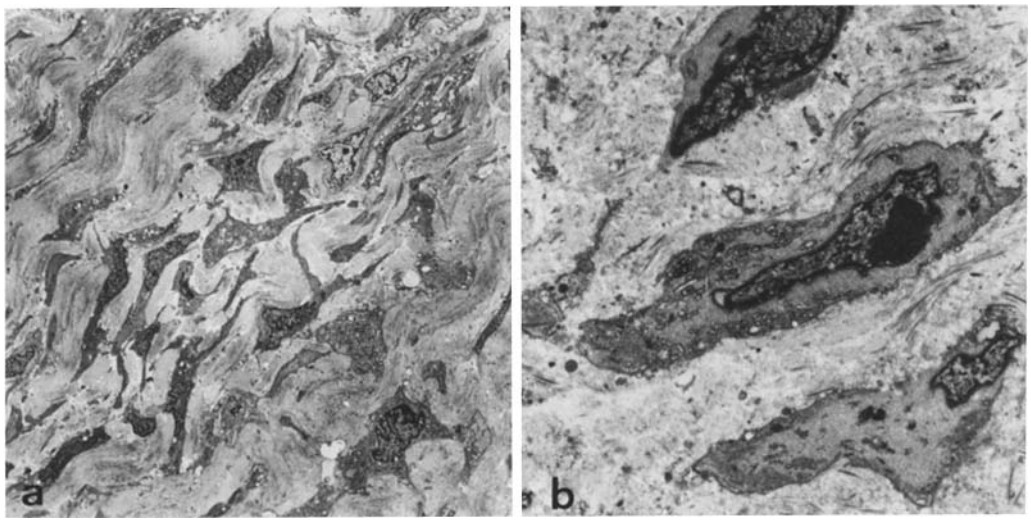

Fig. 3. a Fibrous parts of PDOB with elongated ramified spindle cells and large amounts od collagen fibers in the interstice; 976 × ; **b** Spindle cells with pitted nuclei and a big nucleolus as far as amounts of microfilaments and short profiles of endoplasmic reticulum in the cytoplasm; 4784 ×

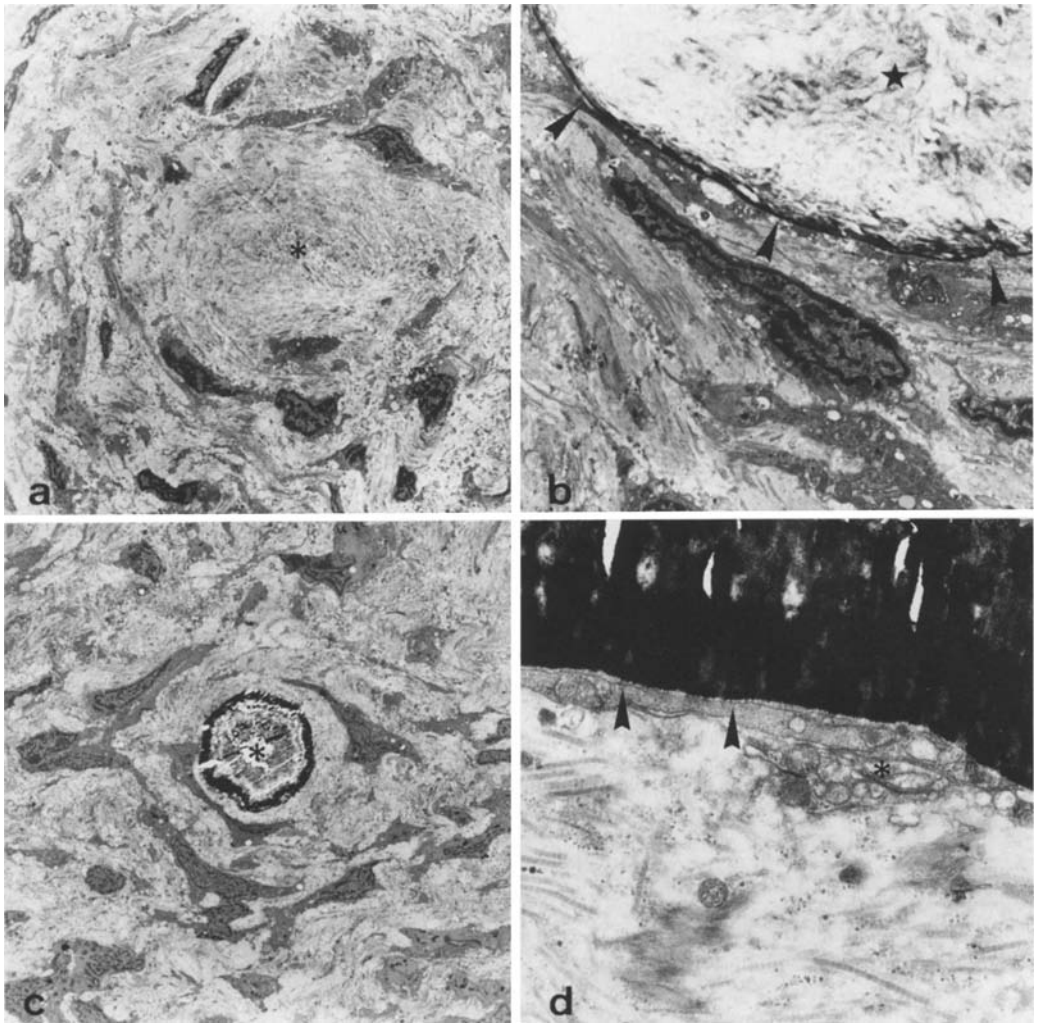

Fig. 4. a Fibrous parts of PDOB with a condensing collageneous whorl (*), the starting point of psammoma body formation; 1328 × ; **b** Early calcification of a psammoma body (*) with a marginal calcium impregnation of the collageneous whorl (▾); 3848 × ; **c** Target like layered psammoma body (*) with concentrically surrounding fibroblastic spindle cells; 1456 × ; **d** Border zone of an intensively calcified psammoma body with a sharply lined mineralization surface (▾) covered by a small cytoplasmic process (*); 15488 ×

acellular calcified matter. Occasionally the stroma was myxoid in appearance and interspersed with small pseudocysts filled with erythrocytes and sometimes bordered by haemosiderin deposits simulating aneurysmal bone cyst formation. Examination of the peripheral areas showed no clear evidence of encapsulation, but rather, the new bone formation appeared to be simply reactive and restricted to the production of slender trabeculae of woven bone.

Electronmicroscopically, two major tissue compartments could be distinguished, the first a cell rich zone with formation of collagen fibers and interspersed psammoma bodies (Figs. 3, 4) and the second a zone of osteoid and woven bone formation (Fig. 5).

Looking at the first tissue compartment we found spindle cells with elongated and pitted nuclei of loose chromatin content and with a prominent nucleolus (Fig. 3a). The cytoplasm exhibited ramified processes, many microfilaments, short profiles of endoplasmic reticulum and some mitochondria (Fig. 3b). Variable and densely packed fasciculi of parallel arranged collagen fibers with typical transverse striation (diameter 400–600 A) appeared in the interstices. Sometimes irregular whorls of collagen fibers were built up, concentrically surrounded by fibroblastic cells as previously described (Fig. 4a). These whorls represented the fibrous matrix of the acellular psammoma bodies. Their calcification resulted in a very homogeneous impregnation with emphasis on the marginal collagen fibers

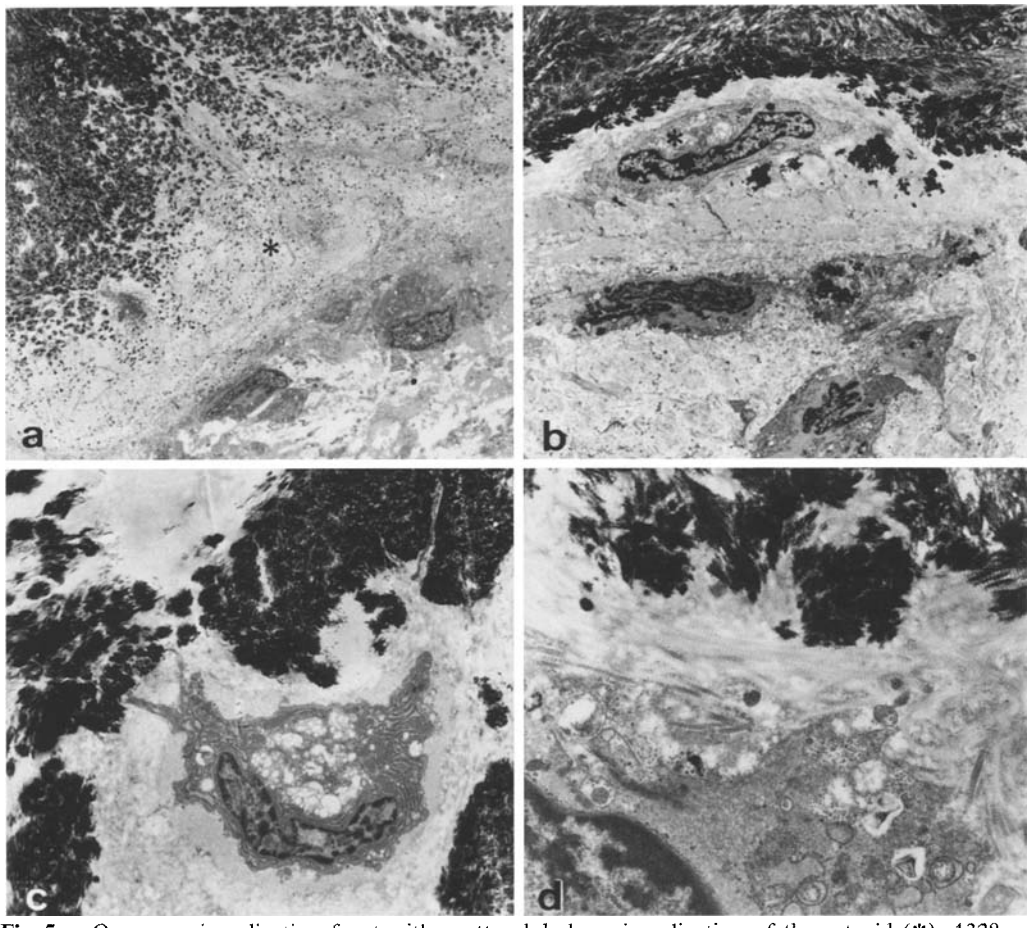

Fig. 5. a Osseous mineralization front with spotty globular mineralization of the osteoid (*); 1328 × ; **b** Increasing osteoblastic cell differentiation towards the mineralization front with polar arrangement of cytoplasmic organelles (*); 2252 × ; **c** Osteoblastic cell with a large golgi zone and densely packed parallel arranged lamellae of rough endoplasmic reticulum; 2992 × ; **d** Globular mineralization of calcium hydroxyapatite cristals on collagen fibers; 8960 ×

which led to a sharply lined outer surface (Fig. 4b). Appositional growth induced a target like arrangement of varying calcareous density (Fig. 4c). Only occasionally were cells and cytoplasmic processes found in close contact to the psammoma body surface (Fig. 4c). These cells showed an increased and sometimes vesicular rough endoplasmic reticulum, but neither maturation forms to osteoblasts and/or cementoblasts nor a typical globular mineralization at the mineralization front were detected.

The osteoid and woven bone formation (typical for the second tissue compartment) arose out of the basic fibrous stroma of the lesion (Fig. 5a, b). Characteristically the cells became more ovoid in shape and polarization of cytoplasmic structures appeared. The nuclei were located at the apical cell pole in opposite to the mineralization front and their chromatin was condensed toward the periphery. A large Golgi apparatus developed in the juxtanuclear region while the cytoplasm was filled with densely packed parallel arranged lamellae of rough endoplasmic reticulum (Fig. 5c). Only a few microfilaments remained. Between these osteoblastic cells, bundles of collagen fibers radiated from different directions into the mineralization front forming osteoid and woven bone trabeculae. Mineralization was caused by segmentation of matrix vesicles inducing a globular mineralization. This originated by confluence of growing needles and fascicular mineral nucleations of calcium hydroxyapatite cristals at the irregularly arranged mineralization front (Fig. 5d). Finally, complete mineralization was observed leaving small lacunae filled with osteocytic cells with less prominent rough endoplasmic reticulum and Golgi apparatus than seen in the osteoblastic cells.

Immunohistological investigations with antihuman osteonectin antibody proved positive intracellular and extracellular staining only in the tumour lesion and surrounding jawbone, while adherent portions of soft tissue i.e. periosteum, connective tissue and striated muscles were never stained.

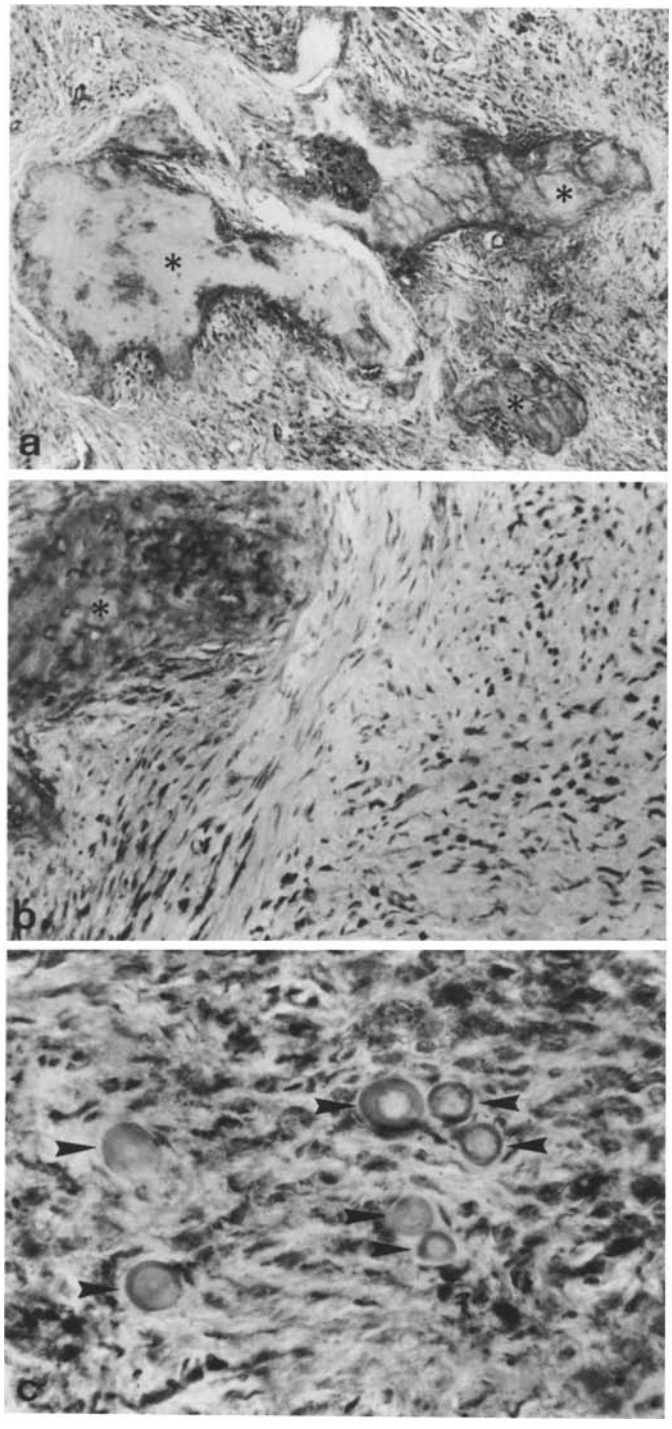

Fig. 6. a Immunohistological marking of osteonectin with a strong extracellular reaction at the mineralization zone of osseous trabeculae (*) peroxidase reaction; 112 × ; **b** Immunohistological marking of osteonectin in the osteoid of a newly formed osseous trabeculum (*) and intracellular in the surrounding spindle cells; Peroxidase reaction; 144 × ; **c** Immunohistological marking of osteonectin in the cytoplasm of the spindle (preosteoblastic) cells. Unstained psammoma bodies (▾); Peroxidase reaction; 224 ×

Maximum cytoplasmic staining reaction was observed in the osteoblastic cells of the mineralization front and to a much lesser extent in the osteocytes of the woven bone trabeculae (Fig. 6a, b). The spindle cells were stained faintly but became more pronouncedly stained towards the mineralization front, resulting in a gradient of increased staining intensity parallel to the progressive differentiation of osteoblastic/osteocytic cell forms. Extracellular staining was evident in the osteoid of the newly formed bone trabeculae (Fig. 6a, b), while the psammoma bodies did not stain with antiosteonectin antibody even after pronase predigestion of the calcified and decalcified tissue sections (Fig. 6c).

Discussion

Since 1971 the diagnosis of osteogenic and odontogenic lesions of the jaw bones has been based on Pindborg and Kramer's WHO-classification, which was the first comprehensive description and recommended terminology of these lesions. Recent publications have increasingly called for reclassification as a growing number of difficulties, unclassifiable lesions and proposed entities are published (Makek 1983; Burkhardt 1986; Donath 1986a; Makek 1987). In this regard the fibro – osteo – cemental lesions of the jaw represent a problematic group (Smith et al. 1981; Bhaskar 1981; Robinson and Miller 1983; Hoppe 1986). One of the main reasons is their complex histogenesis (Burkhardt 1986). Knowledge of which is the basis of each morphological classification of tumours. Since systematic electron microscopic investigations, such as Schulz's study on bone tumours (1980), and additional immunohistological and histochemical investigations, such as Roesner's study on bone tumours (1984), have not been reported in this field we used these methods to study the morphology of psammous desmo-osteoblastoma, one of four new entities proposed by Makek (1983).

At the light microscopic level this lesion is primarily characterized by a fibrous fusocellular tissue with additional production of hard material i.e. psammoma bodies and woven bone trabecule. Ultrastructurally we were able to indentify non-bone forming cells and bone forming cells in PDOB. The non-bone forming cells resemble those fibroblastic cells described by Hirohata and Morimoto (1971) and Schulz (1980) in fibrous dysplasia while the bone forming cells fullfill all criteria of osteoblasts and osteocytes as described by Steiner (1977), Knese (1979) and Schulz (1980). Yet at the mineralization front the osteoblastic cells seem to develop out of the fibroblastic cell pool and initiate typical desmal ossificiation with globular calcification on mature collagen fibers. Thus mineralization and bone production in PDOB exhibits differences to cementogenesis which is characterized by appositional mineralization (Schroeder 1982; Burkhardt 1986) without primary involvement of the collagen fibers (Donath 1986b). Because of their collageneous mineralization core psammoma bodies in PDOB should not be interpreted as being of cementum origin although they show concentric appositional growth. However, the mineralization of psammoma bodies clearly differs from that of normal bone and resembles the non-osteoblastic mineralization seen in psammoma bodies of meningiomas (Kepes 1982; Moss 1986). This observation is confirmed by Aoba et al. (1978) and Störkel et al. (1986) who found a different calcium content in the psammoma bodies of jaw tumours with EDEX-analysis when compared with the similar calcium content of cementum and bone. Based on our ultrastructural analysis of PDOB we favour a tumour of fibro/osteoblastic origin with production of woven bone and development of non osseous and non cemental psammoma bodies.

In 1981 Termine et al. described a non collageneous bone specific protein called osteonectin linking hydroxyapatite and collagen type I and according to Romberg et al. (1985) playing a role in bone hemostasis. In PDOB, our investigations with anti-osteonectin-antibody exhibited a positive staining reaction, extracellular in the osteoid and intracellular in the osteoblastic/osteocytic cells which is in agreement with the findings of Schulz et al. (1985) and Jundt et al. (1986) in bone tumours of the skeletal system. The positive staining reaction in the cytoplasm of the fibroblastic appearing spindle cells confirms the opinion of the pre-osteoblastic or functional osteoblastic nature of these tumour cells. Similar observations were published by Schulz (1980) who found a strong alkaline phosphatase reaction characteristic for osteoblastic cells in the fusiform fibroblastic cells of fibrous dysplasia and concluded they were of osteoblastic origin. Psammoma bodies in PDOB were never stained, which indicates that their mineralization is not of the osseous type and supports the opinion based on the ultrastructural findings that they are not of bone or cementum origin.

Conclusions

In summary, PDOB, a lesion first described on the basis of lightmicroscopical and clinical investigations exhibits ultrastructural and immunohistological evidence of an osteogenic histogenesis. When compared with other fibro-osseous lesions of the cranio-facial bones, the available clinical, radiological and morphological characteristics support the view that PDOB is a distinct entity which should be incorporated in a revised WHO-classification.

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