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Case Reports

The Occurrence of Extracellular Matrix Vesicles in Pulmonary Alveolar Microlithiasis

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Summary. Mineralization in pulmonary alveolar microlithiasis was studied by transmission electron microscopy. The disease is characterized by psammoma like calcifications composed of hydroxyapatite crystals. The calcifications were surrounded by typical forming cells and matrix composed of collagen fibers arranged in a longitudinal pattern. Abundant calcifying extracellular matrix vesicles were found between the cells and the calcified fronts. The type of calcification found in the present lesion is similar to ectopic primary mineralization in other diseases, embryonal ossification and bone wound healing.

Key words: Matrix vesicles – Pulmonary alveolar microlithiasis – TEM

Introduction

It has been shown in recent years that mineralization in various pathological conditions is associated with formation of extracellular matrix vesicles (Kim 1976; Muhlrad et al. 1978; Sela and Bab 1979b). These trilaminar membrane invested organelles, 0.05–0.25 μm in diameter, were originally described in developing epiphyseal cartilage (Anderson 1969; Bonucci 1970). This was followed by the study of their role in bone and healing of osseous defects (Sela et al. 1978; Sela and Bab 1979a; Muhlrad et al. 1980). It is accepted by most authorities that the vesicles bud from the cell membrane into the extracellular matrix and serve as initial loci of calcification. Isolated matrix vesicles show high activity of alkaline phosphatase, pyrophosphatase and different ATPases (Ali et al. 1970; Bab et al. 1979). These enzymes are operative in increasing the concentration of inorganic phosphate both within the vesicles and in the milieu. In addition, the degradation of pyrophosphate and ATP involves the removal of their inhibi-

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tory effect on calcification (Bosky 1979). Vesicular membrane-associated phospholipids play a role in the increase of calcium concentration within the vesicle by virtue of a lipid-calcium interaction (Wuthier 1976).

It has been previously established that pulmonary alveolar microlithiasis shows globular psammoma like calcifications (Sosman et al. 1957). Data concerning the ultrastructural nature of this disease have not been obtained. The purpose of the present transmission electron microscope study was to characterize the morphological patterns of calcification in pulmonary alveolar microlithiasis.

Materials and Methods

An open lung biopsy was performed in a 24 year old woman with a pulmonary condition subsequently diagnosed by light microscopy as alveolar microlithiasis. Tissue was allocated for electron microscopy as a routine procedure and fixed with glutaraldehyde-formaldehyde fixative (Glauert 1975). Osmification was followed by *en block* staining with uranyl acetate and dehydration in graded series of alcohols and propylene oxide. The specimens were embedded in Epon and ultrathin sections were restained with uranyl acetate and lead citrate. The sections were examined with a Jeol 100 cx electron microscope at 40 or 60 KV.

Results

Examination of the specimens revealed areas of normal alevolar septa. The septa containing the calcified foci were thickened due to a marked increase in the amount of collagen fibers. Abundant mesenchymal forming cells with rich rough endoplasmic reticulum and long processes were present in the septa near the calcific deposits (Fig. 1). These cells were surrounded by collagenous matrix; the collagen fibers were organized mainly in a longitudinal pattern. In some areas, the arrangement of fibers showed a feather like appearance (Fig. 2). Calcification formed longitudinal and calcospheritic patterns (Figs. 2, 3). The calcospherites 0.3–0.6 μm in diameter, were constructed of hydroxyapatite crystals; occasional crystals protruded from the calcifying fronts (Fig. 4). The calcified material was directly applied to the processes of the forming cells or to the collagenous matrix.

The extracellular matrix was scattered with vesicles with diameters ranging between 0.05 and 0.25 μm. The vesicles were located mainly between the cells and the calcified fronts (Figs. 3, 4). Some vesicles contained electron dense material and in others hydroxyapatite crystals could be located. The crystals were frequently located extruding through the vesicular membrane (Fig. 4). These calcifying vesicles were scanty around cells and abundant at the mineralized fronts.

Discussion

Transmission electron microscope observations on pulmonary alveolar microlithiasis revealed the occurrence of forming cells, actively engaged in primary mineralization. The psammoma like calcifications found were adherent to connective tissue components of the alveolar septa, a finding which differs from

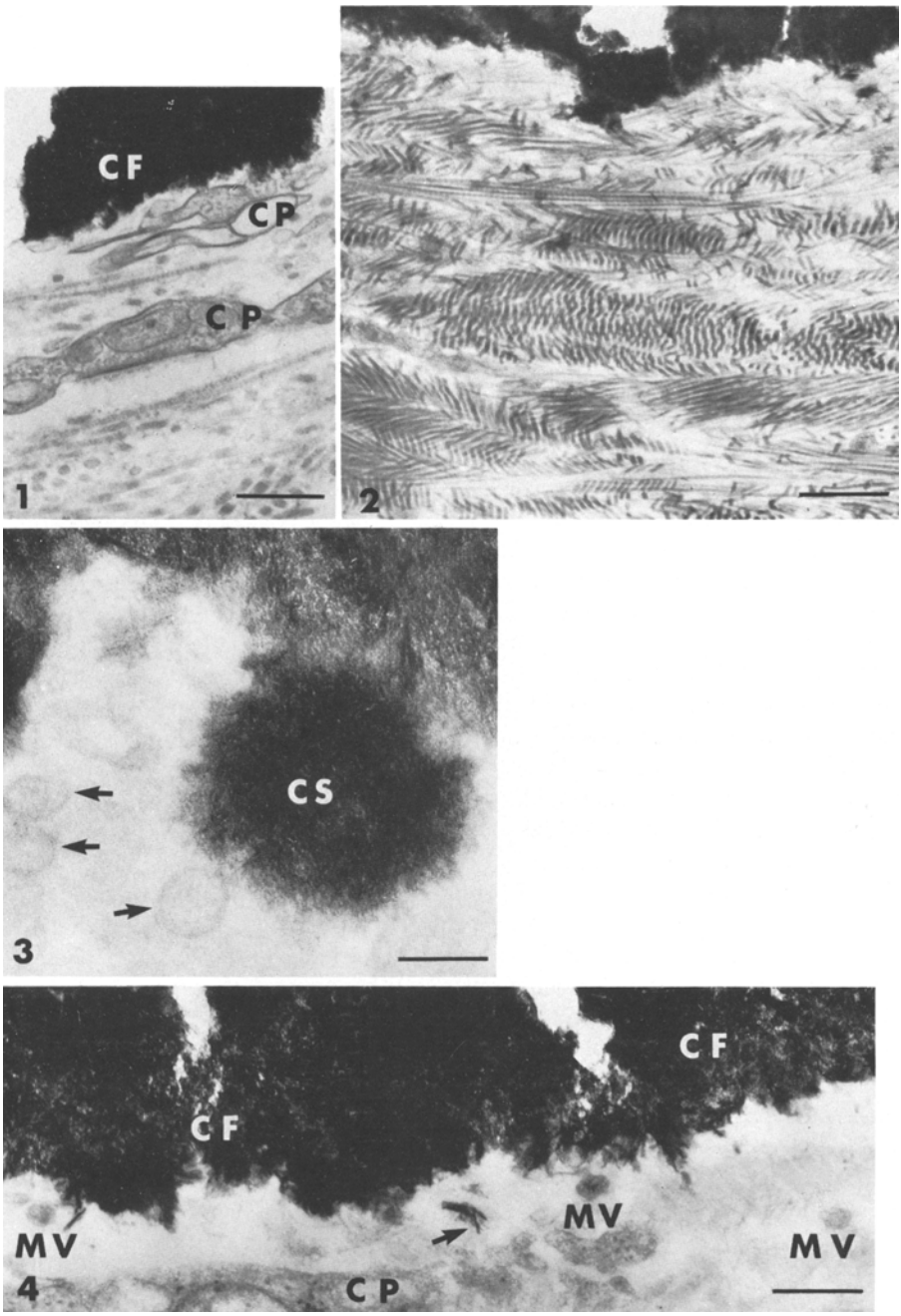

Fig. 1. Cell processes (*CP*) surrounded by collagenous matrix alongside calcified front (*CF*) (Bar = 0.43 μm; × 35,000)

Fig. 2. Collagen fibers organized in longitudinal feather like pattern. (Bar = 1.14 μm; × 13,200).

Fig. 3. Matrix vesicles (*arrows*) and calcospheritic structure (*CS*) attached to the calcified front (Bar = 0.18 μm; × 82,500)

Fig. 4. Matrix vesicles with electron dense material (*MV*) scattered between process of forming cell (*CP*) and the calcified front (*CF*). Note apatite crystals extruding from vesicle (*arrow*) and protruding from the calcified front (Bar = 0.38 μm; × 39,000)

light microscopic studies in which the calcifications were observed lying free in the alveolar lumen (Sosman et al. 1957). The latter phenomenon is probably an artifact resulting from differential shrinkage of hard and soft tissue during histological processing. The occurrence of matrix vesicles in large quantities is characteristic of this pattern of calcification. The present observations coroborate with previous descriptions on the nature of ectopic calcification in different tissues. The involvement of matrix vesicles in the process of mineralization was reported in the aorta (Kim 1976), meningiomal psammoma-bodies (Lipper et al. 1979) and in experimental cutaneous calcinosis (Boivin 1975). In addition matrix vesicle calcification was described in osteogenic neoplasms and in instances of new bone formation during the healing of osseous defects (Muhlrad et al. 1978; Sela and Bab 1979a; Sela and Bab 1979b; Muhlrad et al. 1980). In both normal and pathological calcifications, three morphological stages of matrix vesicles could be distinguished: "empty" vesicles, vesicles with electron dense material and vesicles containing apatite crystals. Another common denominator described in these instances involved the formation of calcospherites by conglomeration of apatite crystals around the initial intravesicular crystal. However, the organization of collagen in the matrix showed differing patterns. A longitudinal arrangement of fibers was observed in bone wound healing (Sela et al. 1978; Sela and Bab 1979a). The collagen fibers in neoplastic calcifying lesions are scattered hapharzardly (Muhlrad et al. 1978; Sela and Bab 1979b). The matrix in pulmonary alveolar microlithiasis presented with organized collagen fibers, closely similar to those found in bone wound healing. In addition, transverse fibers with a feather like appearance could be defined in the lesion described here. The morphological similarities found in lung microlithiasis, bone wound healing and embryonal bone formation must be considered in view of the differences found in matrix organization between non neoplastic and neoplastic ossification. Tumoral mineralizing fronts which form following the calcospheritic stage are highly disorganized, their morphology being dependent on the architecture of the collagen network. It is concluded that the morphological patterns of calcification in the lungs have been found to be highly similar to the non neoplastic type of primary ossification.

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