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Case Report

Spheroidal Filamentous Inclusion Body Cells in von Recklinghausen's Disease

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Summary. Cells with spheroidal filamentous cytoplasmic bodies, distinctive by both light and electron microscopy, were found in a neoplasm arising from the sciatic nerve of a patient with von Recklinghausen's disease. Tissue fixed with formalin and embedded in paraffin for three years was deparaffinized, reprocessed, and examined with the electron microscope. The morphology of the spheroidal body cells, the close resemblance to erythrophagocytosis, and the possible significance of the changes are discussed.

Key words: Neurofibromatosis – Cellular inclusions – Myofibrils – Neurilemmona – Rhabdomyosarcoma.

Introduction

Cells with spheroidal filamentous cytoplasmic bodies, distinctive by both the light and electron microscope, were found in a tumor arising from the sciatic nerve of a patient with von Recklinghausen's disease. Tissue fixed with formalin and embedded in paraffin for three years was deparaffinized, reprocessed, and examined with the electron microscope. This report describes the morphology of the spheroidal body cells, with discussion of the close resemblance to erythrophagocytosis, and their possible significance. As far as can be ascertained, these cells have not been recognized previously.

Case Report

A 45-year-old male with multiple palpable subcutaneous tumors and several café-au-lait spots of the trunk and lower extremities developed a painful mass in the left posterior thigh. There was no available family history of von Recklinghausen's disease. He had had operations in the past, including removal of a subcutaneous left anterior costal margin neurofibroma (age 18), cervical laminectomy with removal of left extradural (C1, C2) and left intradural (C7–T1) tumors (age 42), cervical laminectomy with removal of extradural (C6–7 left) and intradural (C4–5) tumors. The left sciatic nerve from about 10 cm below the sciatic notch was thickened by an infiltrating tumor extending 15 cm. At its midpoint there was a larger tumor nodule measuring 10×8 cm, grossly intermingled with fibers of the sciatic nerve. The large tumor nodule was removed and

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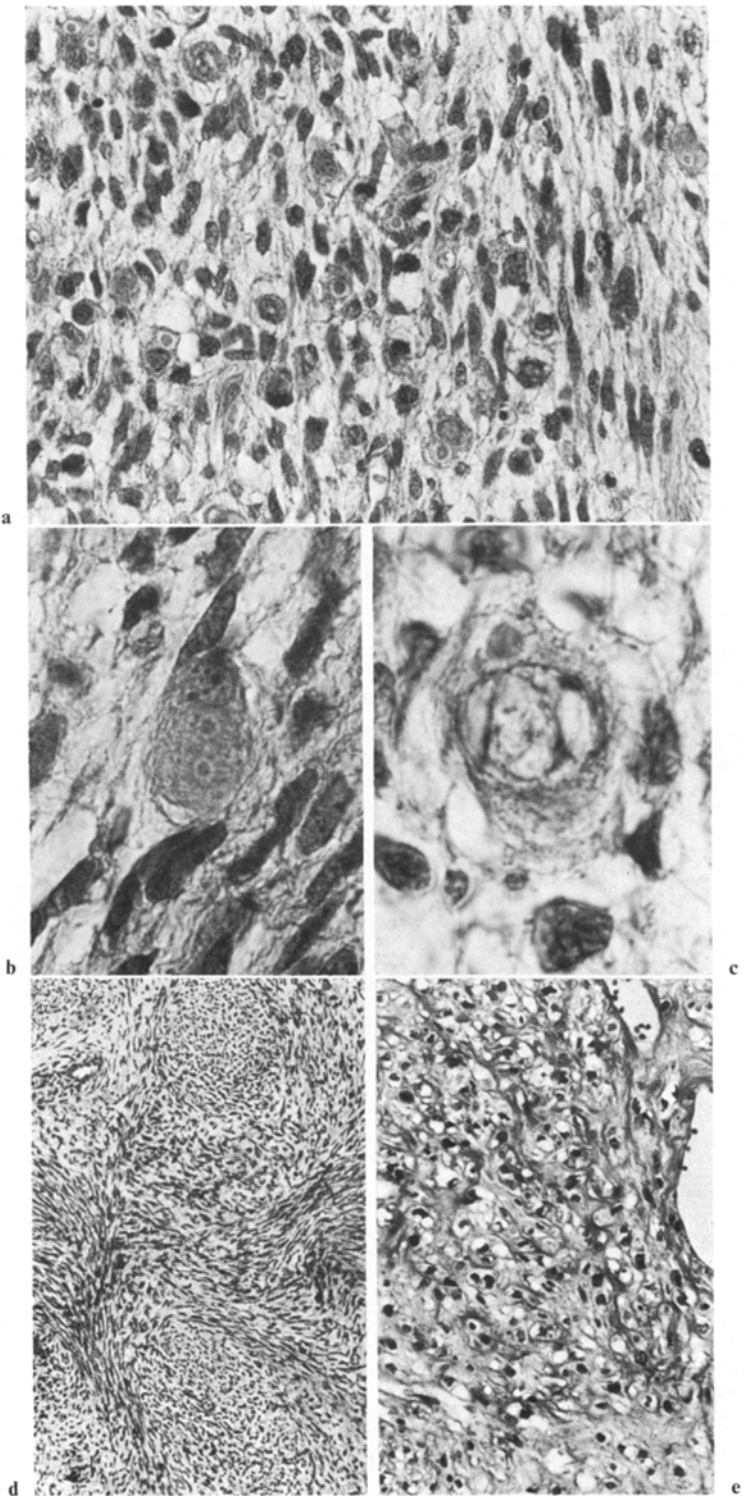
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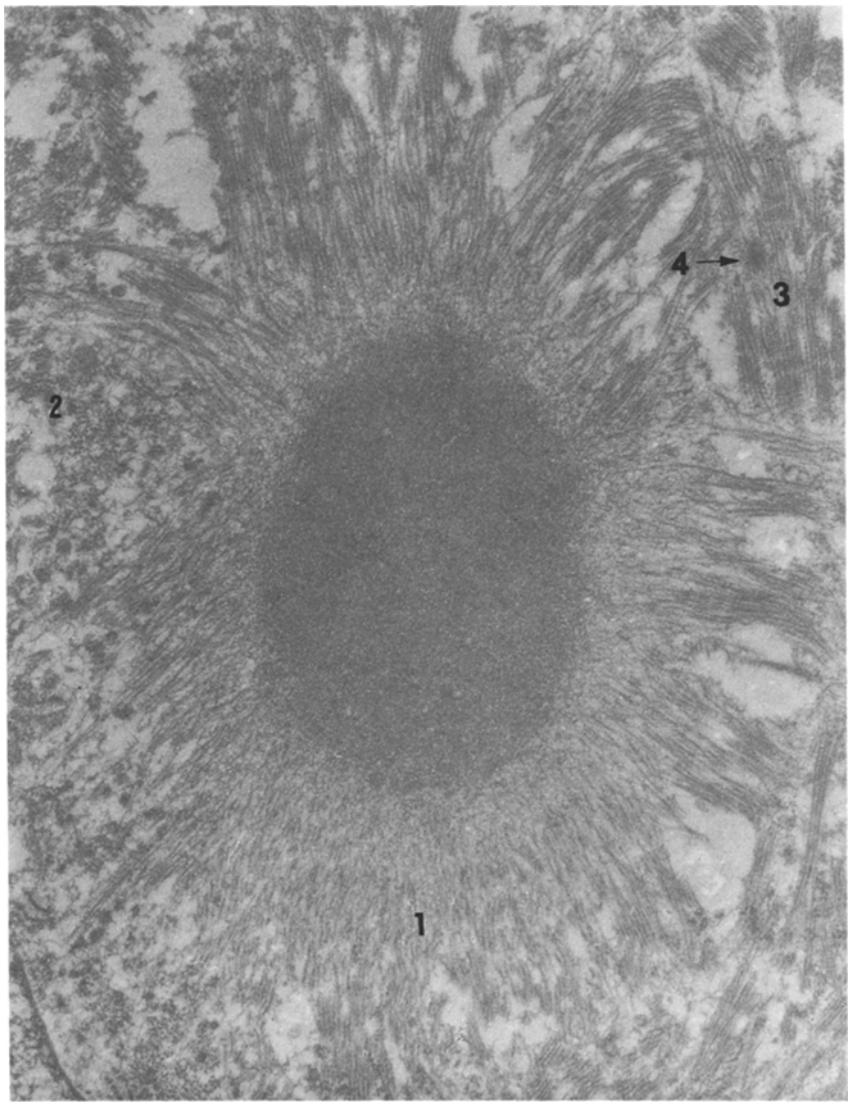

Fig. 2. Electron micrograph of a spheroidal filamentous body, showing dense central core region, annular peripheral region with microfilaments (*1*), merging with microfilaments and microfibrils in the rest of the cell. Longitudinal and cross-sectional (*2*) profiles of filaments are seen, as well as regions with primitive banding (*3*) and small filamentous clusters (*4*). Tissue fixed with formalin, embedded in paraffin for three years, deparaffinized, reprocessed and stained with uranyl acetate and lead citrate, ×16,000

Fig. 1. d Tumor, fasciculated spindle shaped cells in longitudinal and cross-sectional profiles,, Bodian ×100. **e** An area of chondroid differentiation within the tumor, phosphotungstic acid-hematoxylin, ×270. **a** A field of cells containing round cytoplasmic inclusions, most of which are similar in size to nearby erythrocytes, and which are fairly uniform in size, although smaller forms are seen also, H. and E., ×400. **b** Higher power of cell containing two spheroidal bodies showing dense central core surrounded by pale region with discernible radial projections, H. and E., ×1000. **c** Spheroidal body inclusion cell with cytoplasmic body, and nuclear pseudoinclusion, H. and E., ×1000

a nerve stimulator was placed around the sciatic nerve. Post-operatively there was pain relief with slight residual weakness of foot dorsiflexion, and three years post-operatively there has been no recurrence.

Material and Methods

Tissue obtained at the time of the operation was fixed in 10% formalin and embedded in paraffin. Three years after light microscopic diagnosis, selected tissue was removed from the paraffin block, deparaffinized, rehydrated, fixed in 3% glutaraldehyde, followed by 0.1 M sodium cacodylate, 1% osmium tetroxide, graded alcohols and propylene oxide and embedded in Spurr. Semi-thin sections were stained with toluidine blue and examined with the light microscope; ultrathin sections were double-stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Light Microscopy. Most of the tumor was necrotic and there were patches of hemorrhage. In preserved areas there were spindle-shaped cells in a palisading arrangement (Fig. 1 d). Laidlaw stain revealed a moderate number of intercellular fibres. The nuclei were large, hyperchromatic, and mitotic figures were frequent (up to 4 per high power field). Many nuclei had cytoplasmic indentations (pseudo-inclusions) (Fig. 1 c). In several areas large ovoid cells with similar nuclei but increased cytoplasm were seen. These were intimately intermingled with the rest of the tumor cells and stroma. Many had discrete cytoplasmic inclusions (Fig. 1 a–c), similar in size to adjacent red blood cells. The bodies had strongly eosinophilic central round cores, surrounded by annular pale rings and fine radial projections, and were similar in size, although a few smaller exceptions were also found. No instances of inclusions at the cell membrane or within the nuclei were found. The bodies also stained positively with phosphotungstic acid hematoxylin and Sudan black, mildly positive with Luxol fast blue and negative with periodic acid Schiff stains. In other parts of the tumor there was evidence of chondroid (Fig. 1 e) and pleomorphic rhabdomyosarcomatous differentiation. Large tapering cells with recognizable cross-striations, as well as cobweb, globoid, and granular forms were seen. On histological grounds the tumor was considered malignant.

Electron Microscopy. Despite the drawbacks of the fixation procedure, reasonably well preserved cells with the spheroidal bodies were found. These cells all contained recognizable, haphazardly arranged cytoplasmic filaments; longitudinal and cross-sectional profiles and regions with primitive banding were recognized (Fig. 2). The subunits measured 60–90 Å (thick) and 110–150 Å (thin) in diameter when discernible. Each body studied was quite similar in shape and configuration, having an electron dense central region of variable size (1.5–4.5 μ in maximum axis, but usually near to 4 μ), from which microfilaments in the range of 80–120 Å thickness were seen to emanate. After an annular region of decreased density measuring 0.2–1.0 μ in radial direction beyond the denser central core, these microfilaments merged with the microfilament and microfibril network of the rest of the cell, where primitive banding was recognized. With higher magnification the individual filaments within the dense central core of the bodies measured approximately 100 Å in diameter. The tumor cell nuclei were generally large, irregularly notched, and often contained regions of cytoplasm (pseudo-inclusions) with recognizable cytoplasmic organelles. The chromatin was usually clumped beneath the nuclear envelope. Free ribosomes were present throughout the cytoplasm in no specific pattern. In some cells a peculiar conformation of the rough endoplasmic reticulum in cross-section with concentric electron dense rings around a central collection of ribosomes was found (described by Warner and Kelly, 1974). Other organelles were sparse, except for the occasional cell which contained abundant mitochondria, which were poorly preserved. No basement membrane was noted surrounding any of the cells studied, and axons were encountered only rarely.

Discussion

The intracytoplasmic bodies described in this report have staining characteristics of red blood cells, moreover adjacent intra- and extravascular erythrocytes in

the same paraffin sections stain in the same way as the bodies. While rare examples are smaller, most of the structures are in the size and shape range of spherocytes, and except for the annular peripheral pallor and radial projections, the light microscopic appearance is in accord with that seen in normal spleen, malignant histiocytosis and certain rare tumors (Marin-Padilla, 1977). In experimental conditions erythrophagocytosis with optimal fixation shows stages of contact, adherence, engulfment by pseudopod, retraction of phagosome and degeneration (Essner, 1960; Edwards and Simon, 1970; Simon and Burke, 1970; Wakefield and Hicks, 1974; Zeligs and Wollman, 1977). Typical features of intracellular red cell destruction are a) an identifiable surrounding membrane, b) fragmentation or fission and breakdown of the cell, formation of "myelin-like" figures and dense residuum, and frequently c) ferritin particles in the vicinity. The spheroidal bodies thus do not represent erythrophagocytosis: this suggests that other instances referred to by light microscopy as erythrophagocytosis actually may not be so.

The histogenesis of tumors arising in association with the peripheral nerve sheath is problematical: the functional characteristics, criteria for identification, and embryogenesis of Schwann cell, fibroblast, and perineurial cell are unsettled. In these tumors the relation to axons, nature and content of collagen and elastin fibers, and presence or absence of palisading, foam cells, hemosiderin, mast cells and basement membrane are important features (Raimondi and Beckman, 1967; Cravioto, 1968; Harkin and Reed, 1968; Feigin, 1971; Russell and Rubinstein, 1971). There have been descriptions of formation of collagen, cartilage, and osteoid (White, 1971), elastin (Lassmann et al., 1975), bone (Wilson, 1941), presence of nevus cells, melanoma, angiosarcoma, rhabdomyosarcoma ("Triton" tumor, Masson, 1932; Woodruff et al., 1973), liposarcoma (D'Agostino et al., 1963), and glandular elements (Garre, 1892; Foraker, 1948; Woodruff, 1976). Malignant variants are usually referred to as sarcomata, but many terms (malignant schwannoma, neurosarcoma, etc.) are used; focal histological malignancy is said not to reflect biological potential (White, 1971). Of the ten reported cases with rhabdomyosarcomatous differentiation (five with demonstrated cross-striations, none with ultrastructural data), only one patient had survival longer than two years (Woodruff, 1973). The cells described in this report, as well as displaying unique structural features, thus may indeed signify a less lethal variant of this group of tumors.

Particular arrangements of sets of filaments are seen in many abnormal cells (Schochet and McCormick, 1972; Ogata et al., 1972; Gessaga and Anzil, 1975), which suggests that they may be non specific. However, the distinctive and consistent structure of the spheroidal filamentous body cells implies some order in the disorder of misaligned filaments: in tissue with the characteristics described above, a primitive aggregate of myofilaments suggests possibilities such as 1) a giant spheroidal Z-like region or 2) a filament "germinal center". Recognition of further examples may shed more light on these aggregating characteristics, in addition to the biology of this structurally distinct neoplasm.

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