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Splenic Involvement in Tuberous Sclerosis

Report of Three Cases

Bruce L. Bender[1], and Eduardo J. Yunis[2]

Departments of Pathology,
[1] Presbyterian-University Hospital,
[2] Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine,
Pittsburgh, Pennsylvania 15213, USA

Summary. Three cases of tuberous sclerosis in neonates were found to have focal, frequently perivascular, collections of large cells with abundant eosinophilic cytoplasm. These cells resembled those found in brain lesions of tuberous sclerosis but did not stain for acidic protein. Ultrastructurally, they were characterized by many membrane bound cytoplasmic bodies, 90 to 270 nm in diameter, with amorphous contents. Filaments were not demonstrated. Their appearance is considered most consistent with histiocytic origin.

Large cells with a histiocytic appearance and a superficial resemblance to those seen in the brain in tuberous sclerosis, but a different ultrastructure and reaction to GFAP staining, may be found in the spleen of neonates with this disease.

Key words: Tuberous sclerosis – Spleen – Ultrastructure – Glial fibrillary acidic protein (GFAP)

Introduction

The tuberous sclerosis complex includes central nervous system lesions, cardiac rhabdomyomas, renal cysts and adenoma sebaceum. Pulmonary, skeletal and retinal lesions are often described (Donegani et al. 1972). Five reports of splenic involvement are available (Darden et al. 1975; Morales 1961; Östör et al. 1978; Tsakraklides et al. 1974; Van Heerden et al. 1967). Two describe vascular hamartomas in adolescents (Darden et al. 1975; Van Heerden et al. 1967). The other three (Morales 1961; Östör et al. 1978; Tsakraklides et al. 1974) describe nodules of large cells with eosinophilic cytoplasm in the spleen of three neonates.

Upon reviewing the spleen in five autopsied cases of tuberous clerosis in neonates, three cases with collections of cells similar to those reported by Morales (1961) were found. In one case immunostaining for glial fibrillary acidic protein and electron microscopy were performed.

Offprint requests to: Dr. Bender, Presbyterian-University Hospital, Department of Pathology, DeSoto at O'Hara Streets, Pittsburgh, PA 15213, USA

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Table 1. Clinical and autopsy findings

Case	Age at diagnosis	Presentation	Age at death	Organ involvement
1	5 days	increasing head circumference	5 days	CNS, heart, skin, kidneys, spleen
2	2 days	congenital heart disease	2 days	CNS, heart, kidneys, eyes
3	4 days	congenital heart disease	5 days	CNS, heart, kidneys
4	5 weeks	seizures, 6 h	5 weeks	CNS, heart, focal pancreatic and hepatic dysplasia
5	2 days	congenital heart disease	2 days	CNS, heart, kidneys, ?skin, adrenal adenoma, thyroid dysplasia

Materials and Methods

The clinical information in the five neonates examined is summarized in Table 1. All but one of these children were five days old or less. The first three patients had splenic involvement. Autopsy material from two older children with tuberous sclerosis was also examined and splenic involvement was not demonstrated.

In all cases, portions of spleen were fixed in 10% formalin. Blocks were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Periodic Acid-Schiff (PAS) with and without diastase, Ziehl-Neelsen, and Naphthol-ASD stains were performed on selected blocks. Fluorescent and polarized light microscopy were also performed.

When lesions were found microscopically, the remaining pieces of fixed tissue were sectioned with alternate slices submitted for paraffin sections. If lesions were present in these slides (only Case 3) the corresponding areas in adjacent pieces of tissue were washed in Sorensen's buffer, and processed for electron microscopy. Paraffin embedded sections were cut and immunostained for GFAP by the Division of Neuropathology using the bridge technique and rabbit antibody to bovine GFAP, provided by Dr. Lawrence Eng (1978).

Results

The lesions were the same in all three patients. The spleens were grossly unremarkable, even upon close examination of tissue known to contain lesions. Most tissue was also microscopically unremarkable.

There were rare, usually perivascular, foci of abnormal cells scattered throughout the parenchyma. They ended abruptly but were not encapsulated and did not distort the splenic architecture (Fig. 1). The largest collections were no more than a millimeter in diameter.

The smallest of the cells were approximately 20 μ in diameter, with the largest approaching 75 μ. They had a large, round, central nucleus with unremarkable chromatin and no nucleolus. Occasional cells were binucleate. No mitoses were seen. The cytoplasm was abundant, homogeneous and acidophilic. There was granular, diastase resistant, PAS positivity, but no staining with

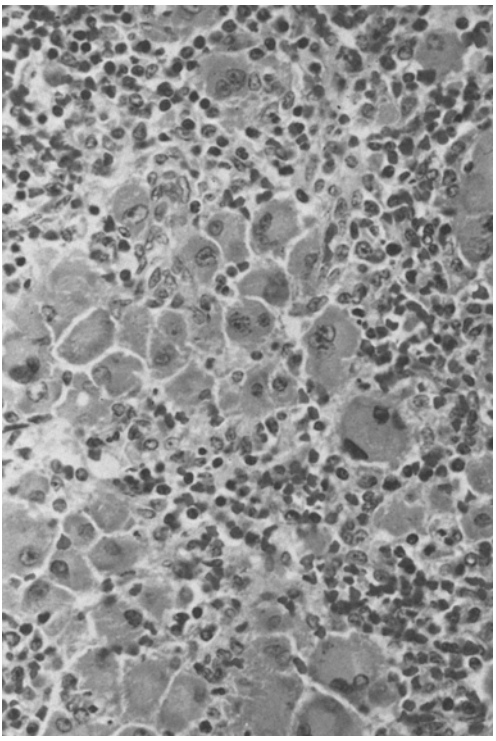

Fig. 1. A focus of large abnormal cells in a spleen. They have a histiocytic appearance by light microscopy, are not circumscribed and do not compress the surrounding tissue (H & E ×304)

Ziehl-Neelsen or Naphthol-ASD and no auto-fluorescence or birefringence. The cells did not form glands or demonstrate any organization. They appeared, instead, to lie loosely within the spleen.

Ultrastructurally, the nucleus of these cells was round or oval with chromatin partly dispersed and partly marginated. A single inconspicuous nucleolus was occasionally present (Fig. 2). There were many 90 to 270 nm membrane bound structures with an amorphous, moderately electron dense, interior (Fig. 2). Frequently round apparently membranous structures were present within these organelles, usually arranged as multiple small circles (Fig. 2, insert). Occasionally an oval area of increased, but not intense, electron density was present within an organelle. There was no halo separating the contents from the limiting membrane. The membrane was tri-laminar. The above structures tended to be in higher concentration around the nucleus. Unremarkable mitochondria were frequent, as were variably-sized cisternae of rough endoplasmic reticulum containing a small amount of lacy, moderately electron dense material. No fibrils were seen. The cell borders were poorly defined but appeared to abut upon each other without forming specialized junctions (Fig. 3). No basement membranes or processes were apparent. Interdigitated red blood cells were present (Fig. 3).

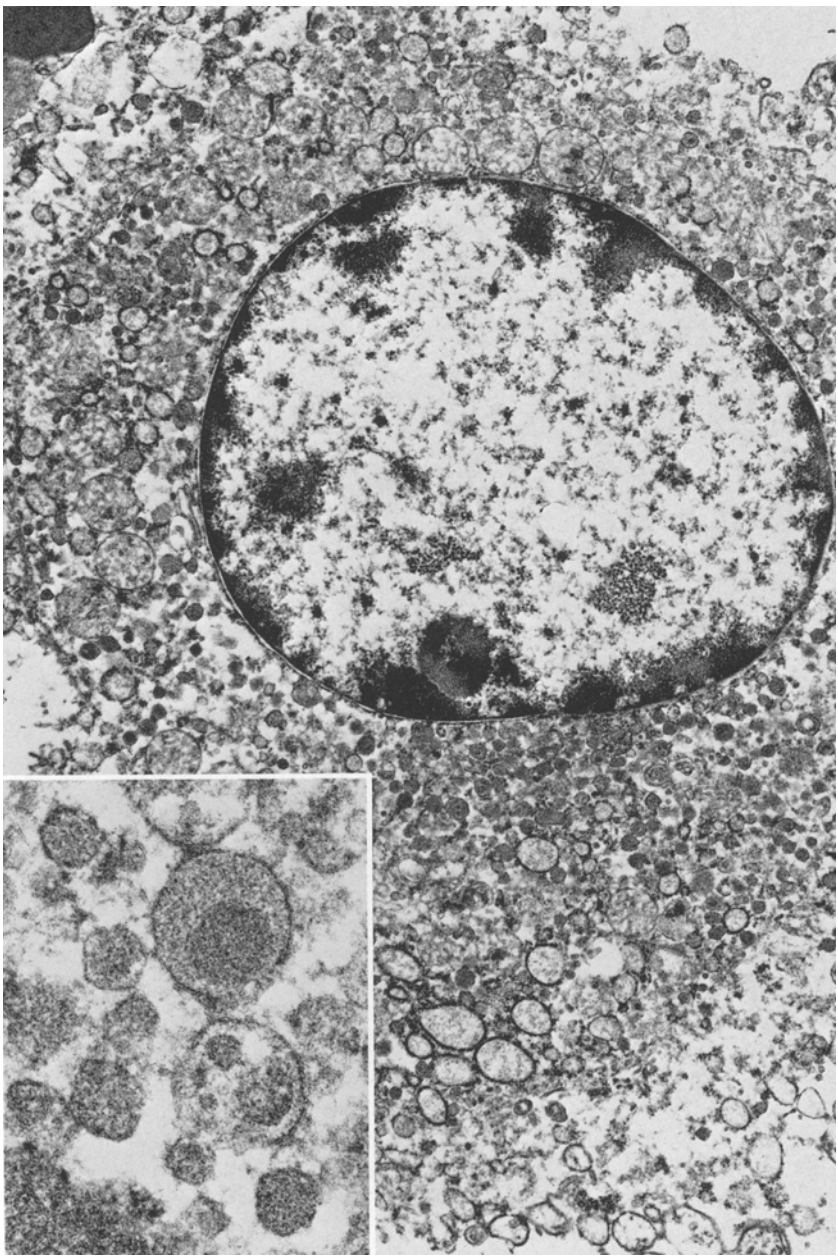

Fig. 2. An electron micrograph illustrating perinuclear membrane bound organelles mingling peripherally with dilated sacs of rough endoplasmic reticulum and mitochondria. No fibrils are seen. A small nucleolus is apparent (× 13,300). Insert: higher magnification illustrating membranous structures and variable density within organelles (64,400)

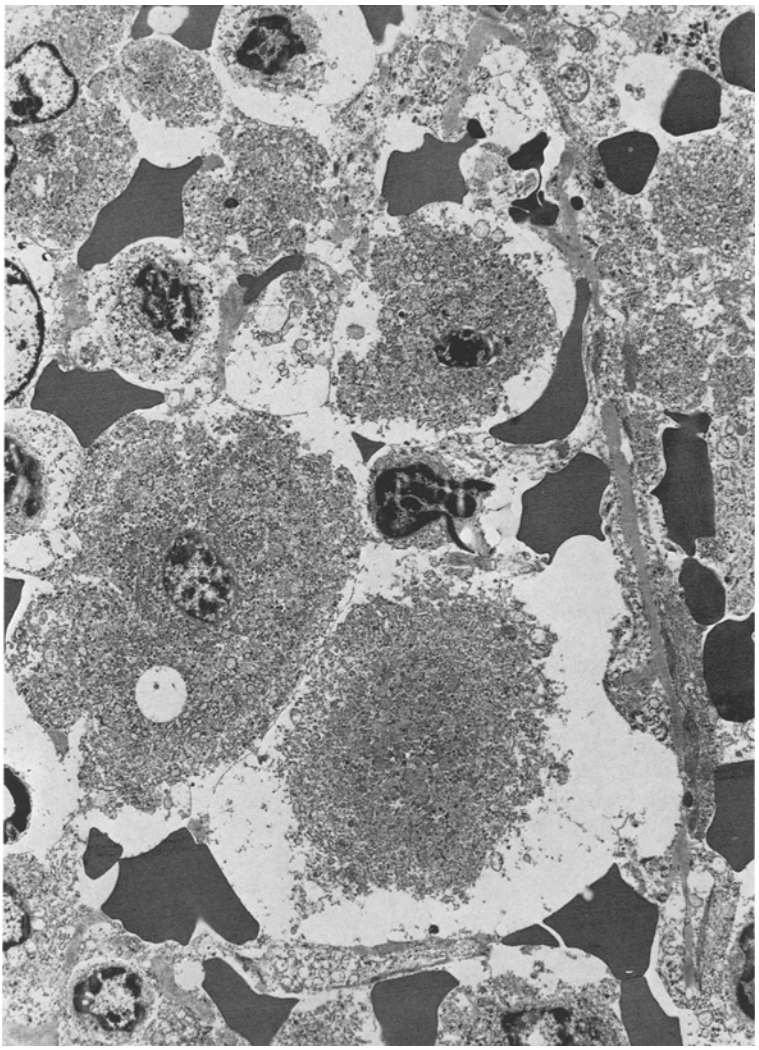

Fig. 3. A low power electron micrograph illustrating several abnormal cells with interdigitated red blood cells in a sinusoid. The cells do not have processes or specialized junctions (× 3,000)

Discussion

Although the normal cells present in the current material have only been described in three previous patients, their presence in three of five spleens of neonates with tuberous sclerosis indicates that they are not rare in such cases. All of the current cases, and the previously reported one, occurred in neonates, although spleens from older children with tuberous sclerosis were also examined. It is likely, therefore, that the presence of such cells in the spleen of patients

with tuberous sclerosis is either transient, or restricted to very severely afflicted patients, most of whom do not survive infancy.

The abnormal cells described in this report have a light microscopic appearance very similar to that of affected brain cells in tuberous sclerosis. They differ with respect to immunostaining for GFAP and ultrastructure, however.

Approximately one third of tuberous sclerosis cells in the brain have GFAP positivity (Bender and Yunis 1980; Velasco et al. 1980). Although only small numbers of cells have been examined, the lack of such staining suggests different cytoplasmic composition of the cells in the spleen.

This difference is confirmed by ultrastructural examination. The abnormal brain cells in tuberous sclerosis are characterized by large numbers of 9–12 nm thick fibrils, the presumptive correlate of GFAP positivity, and frequent dense bodies with a crystalline appearance (Arseni et al. 1972; de Chadarevian and Hollenberg 1979; Ribadeau Dumas et al. 1973). They may have stacked rough endoplasmic reticulum with interdigitated polyribosomes. The cells in the spleen do not have any of these features, being characterized instead by membrane bound structures resembling secretory vesicles or lysosomes and dilated sacs of rough endoplasmic reticulum.

All of the current cases also had central nervous system lesions of tuberous sclerosis which were examined by GFAP immunostaining and electron microscopy and had the classical findings. It seems, therefore, that abnormal cells in these two sites are affected differently, or that the same injury leads to different morphologic and chemical changes in different cells. However, the less than ideal fixation of the splenic cells may have precluded visualization of fibrils.

Ultrastructural studies of lesions of tuberous sclerosis in the kidney (Mori et al. 1971) and the skin (Bhawan and Edelstein 1977) have revealed extracellular lumina, microvilli, numerous cytoplasmic organelles, lipid vacuoles and an absence of fibrils in the former, abnormal endothelial cells with prominent fibrils and occasional multilayered basement membrane couched in long spaced collagen, mast cells and fibroblasts in the latter. These disparate findings in various locations make it increasingly difficult to accept Inglis' (1954) theory that all the lesions of tuberous sclerosis arise from a single cell line which originates in the neural crest. It is nonetheless attractive to continue the search for an acceptable unifying concept of the pathogenesis of tuberous sclerosis. The idea that a single metabolic defect leads to disordered function in multiple cell lines fits the available evidence, although no direct evidence of such a defect exists at this time.

The nature of the splenic cells described in this report is unclear. By light microscopy they resembled histiocytes. The Naphthol-ASD stain was negative, excluding immature myelocytes.

Ultrastructurally, their identity is unclear. The cells do not have the characteristics of Schwann cells or fibroblasts, although these possibilities have been suggested in other lesions of tuberous sclerosis (Bhawan and Edelstein 1977; Mori et al. 1971). The presence of large numbers of moderately electron dense membrane bound bodies, and of dilated rough endoplasmic reticulum, suggests a secretory capacity. There are no specialized junctions or glandular structures to strengthen this hypothesis. The membrane bound bodies may represent lyso-

somes, although this appearance would be unusual. If they are lysosomes, it is very likely that the cells are histiocytes. At present we prefer to call these cells "histiocytoid" as they resemble histiocytes more than anything else, but refrain from adhering firmly to any single histogenetic theory until further evidence is accumulated.

In summary, clusters of abnormal cells with a histiocytic light microscopic appearance and abundant moderately electron dense cytoplasmic bodies, dilated rough endoplasmic reticulum and mitochondria ultrastructurally, were found in random sections in three of five cases of tuberous sclerosis in neonates. These cells have only been described in neonates. Their identity is unclear but they do not appear to be of the same origin as the abnormal cells in the brain of these or similar patients. They do appear to be a genuine part of the tuberous sclerosis complex.

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