

An autopsy case of tuberous sclerosis. Histological and immunohistochemical study

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Summary. We report an autopsy case of tuberous sclerosis. A 19-year-old Japanese man had shown facial adenoma sebaceum, intractable convulsive seizures and severe mental retardation. Gross inspection of the brain showed a cortical tuber from the orbital frontal lobe to the rhinencephalon of the left side and a few subependymal nodules. Histological examination revealed many cortical tubers in the cerebral hemispheres, a few subependymal nodules with calcification and multifocal clusters of heterotopic cells in the white matter (white matter nodules). In these lesions, massive giant cells with abundant eosinophilic cytoplasm and without Nissl substances were found. Although the size and shape of the giant cells were variable, the majority of them were gemistocytic, ovoid or polygonal. Immunohistochemistry was employed in these lesions using antibodies against neurofilament protein (NFP), glial fibrillary acidic protein (GFAP), vimentin (VM) and myelin basic protein (MBP). In the cortical tuber, the majority of the giant cells were positive for both NFP and VM, but a few were positive for GFAP. All of them were negative for MBP. In the subependymal nodule and white matter nodule, the majority of the giant cells were positive for NFP, but a few were positive for VM, and none were positive for either GFAP and MBP. These findings suggest that the majority of the giant cells may be immature cells toward neuronal series and a few may be those toward astroglial series. These findings also indicate that the giant cells in the subependymal nodule and white matter nodule may be more differentiated than those in the cortical tuber. The nature of the giant cells in tuberous sclerosis is discussed.

Key words: Tuberous sclerosis, Giant cells, Immunohistochemistry, Vimentin, Immature cells

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Introduction

Tuberous sclerosis (TS) was first reported in an infant with multiple cardiac rhabdomyomas and cerebral sclerosis by Von Recklinghausen (1862). Bourneville (1880) described its detailed neurological symptoms and cerebral lesions which he called «tuberous sclerosis of cerebral convolutions». TS is characterized clinically by adenoma sebaceum, mental retardation, and epilepsy.

The principal lesions in the central nervous system (CNS) are cortical tubers, subependymal nodules, and clusters of heterotopic cells in white matter (white matter nodules) (Donegani et al., 1972). These lesions all contain giant cells, the origin of which has been controversial (Arseni et al., 1972; Ribadeau Dumas et al., 1973; Stefansson and Wollmann, 1980, 1981; Bender and Yunis, 1980; Chou and Chou, 1989; Naramoto et al., 1989). In this study, the nature of the giant cells found in the principal lesions using histological and immunohistochemical methods was studied.

Materials and methods

A 19-year-old Japanese man, whose mother had a similar disease, was born at 40 weeks gestational age, weighing 2,810 g. The delivery was uncomplicated except for mild neonatal asphyxia. At 8 months, he developed his first attack of tonic clonic convulsions. These increased gradually in frequency and eventually occurred in series in spite of intensive treatment. At 3 years, he was diagnosed as having tuberous sclerosis. At 4 years, he was admitted to our hospital. Adenoma sebaceum on the face and severe mental retardation were evident. He could not speak except for babble and was restless and hyperactive. Seizures were resistant to the administration of valproic acid, phenobarbital, phenytoin, clonazepam and ACTH. At 12 years, bilateral renal tumors were detected. From 18 years, macroscopic haematuria appeared. Brain-computed tomography showed a few subependymal calcifications and massive calcification from the left frontal pole to the edge of the

left thalamus. Repeatedly recorded electroencephalograms demonstrated 3-6 Hz background activity with multifocal spikes and poly spike and wave complex, which is called hypsarhythmia. He died of renal failure on December 14, 1990.

General autopsy was performed six hours after his death. Markedly polycystic kidneys totally weighing 2,000 g and a fibrotic pancreas were found.

The brain was fixed in 10% formalin for three weeks, and embedded in paraffin. The specimens were coronally cut at 1 cm.

For light microscopic study, six μm sections were stained with hematoxylin-eosin (H-E), Cresyl violet (Nissl), Klüver-Barrera (K-B), Woelcke, and Holzer and Bodian stains.

For immunohistochemistry, three typical lesions were selected. The first was a cortical tuber in the left orbital frontal lobe, the second a subependymal nodule bulging into the left lateral ventricle, and the third a cluster of heterotopic cells in the left cerebral white matter. The studies were performed by the labelled streptavidin-biotin method (DAKO, USA). The primary antibodies used were mouse anti-human neurofilament (NFP, 70 and 200 Kd) (monoclonal, dilution 1:100), rabbit anti-human glial fibrillary acidic protein (GFAP) (polyclonal, dilution 1:1), mouse anti-vimentin (VM) (monoclonal, dilution 1:1), rabbit anti-human myelin basic protein (MBP) (polyclonal, dilution 1:1), which were all commercially available, (DAKO, USA). The secondary antibodies used were biotinylated anti-rabbit and anti-mouse immunoglobulins. 0.1% diaminobenzidine tetrahydrochloride (DAB) supplemented with 0.02% hydrogen peroxide solution was used for development. All sections stained for immunoreactivity were counterstained with Mayer's hematoxylin. The specificity of the immunostaining was confirmed by replacing the primary antibodies with non-immune rabbit serum or non-immune mouse serum.

Results

Gross pathology

The brain weighed 1,100 g. On its surface, an obvious cortical tuber was found in the left orbital frontal lobe. It was slightly granular and firm to the touch. Coronal sections in the left cerebral hemisphere showed a hard cavity from the orbital frontal lobe (the rectal and orbital gyri) to the rhinencephalon (the area subcallosa and post terminal gyrus). A few subependymal nodules bulging into the lateral ventricle were found. No so-called subependymal giant cell tumor was found. The corpus callosum was atrophic.

Histology

Many cortical tubers which could not be confirmed

visually were easily found in different regions of the cerebral hemispheres under Holzer preparations (Fig. 1). The lesions often extended beyond U-fibres to the cerebral white matter showing demyelination with fibrillary gliosis. The most conspicuous lesion was located on the basis of the frontal lobe to the rhinencephalon on the left side. A marked loss of neurons with intensive fibrillary gliosis and massive calcifications was found in the lesion. Massive giant cells with abundant eosinophilic cytoplasm were present and were variable in size and shape (Fig. 2). Most of them were gemistocytic, ovoid or polygonal-shaped, and some were fibrillated or spindle-shaped. Most of the giant cells did not have Nissl granules but were homogeneously impregnated. The giant cells occasionally had two nuclei, and peripheral vacuolation. Apart from the aggregation of the giant cells, a massive astrocytic proliferation was found. Other cortical tubers consisted of a small number of giant cells, showing moderate to severe neuronal loss without calcifications.

The subependymal nodule consisted of a large number of giant cells. Fibrillated or spindle-shaped giant cells were concentrated in the centre of the nodule, while gemistocytic, ovoid or polygonal giant cells were mainly located in the periphery (Fig. 3). Intense fibrillary gliosis and calcareous precipitation were also found in these lesions.

In the cerebral white matter, clusters of heterotopic cells (white matter nodules) were often found. There was a variety of cells including neurons with rounded cytoplasm, astrocytes and giant cells. Aggregations of gemistocytic, ovoid or polygonal giant cells were often found in the nodules.

The basal ganglia, thalamus, brain stem and

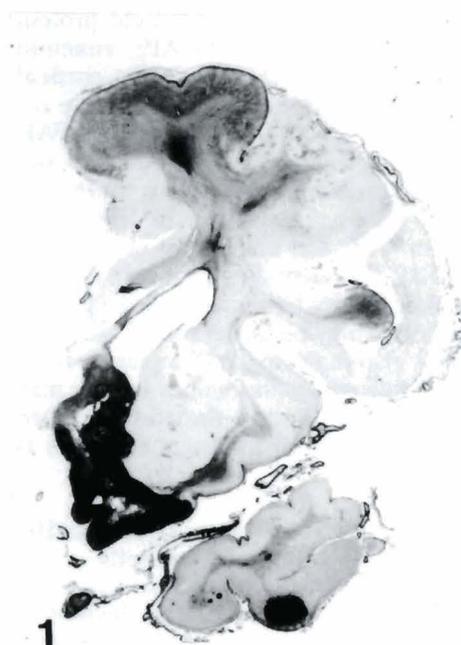


Fig. 1. Holzer stain demonstrates darkly-stained cortical tubers and white matter nodules. The orbital frontal lobe and rhinencephalon on the left side are most severely affected. x 1.0

cerebellum were spared.

Immunohistochemistry

Cortical tuber

Most giant cells were positive for NFP (Fig. 4A), and were more strongly positive for VM (Fig. 4B). They were not evenly immunostained and some cells negative for NFP or VM were randomly scattered among positive cells in the respective stains. A few giant cells were weakly positive for GFAP (Fig. 4C). A large number of astrocytes with many processes were strongly positive for GFAP (Fig. 4D) and some of them were positive for VM. Glial fibre bundles were also strongly positive for GFAP. No MBP-positive cells were found.

Subependymal nodule

Most fibrillated or spindle-shaped giant cells were strongly positive for VM (Fig. 5), and positive for NFP. Almost all gemistocytic, ovoid or polygonal giant cells were positive for NFP, while a few were positive for VM (Fig. 5). In the nodule, the giant cells showed neither

GFAP- nor MBP-immunoreactivity, but glial fibre bundles were intensely positive for GFAP.

Cluster of heterotopic cells in the white matter

Most giant cells were positive for NFP (Fig. 6), and a few were positive for VM. No GFAP-positive giant cells were observed. A large number of GFAP-positive astrocytes were observed among the giant cells, and a small number of VM-positive astrocytes were also found. No MBP-positive cells were observed.

Discussion

Neuropathologically, the present case showed three typical lesions of TS (Donegani et al., 1972): cortical tubers; subependymal nodules; and clusters of heterotopic cells in the white matter (white matter nodules). All of these lesions contained giant cells with a variety of sizes and shapes.

In the immunohistochemical study, the majority of the giant cells in the cortical tuber showed positive immunoreactivity for both NFP and VM. In both the subcortical nodule and white matter nodule, the majority

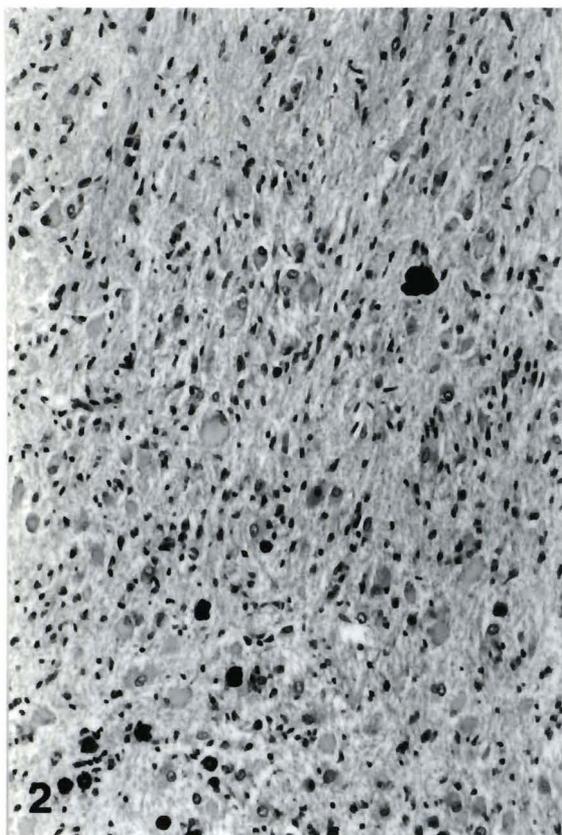


Fig. 2. Aggregation of giant cells with abundant eosinophilic cytoplasm in a cortical tuber of the left orbital frontal lobe. Size and shape of the cells are variable. Calcium deposits are also found. H-E stain. x 200

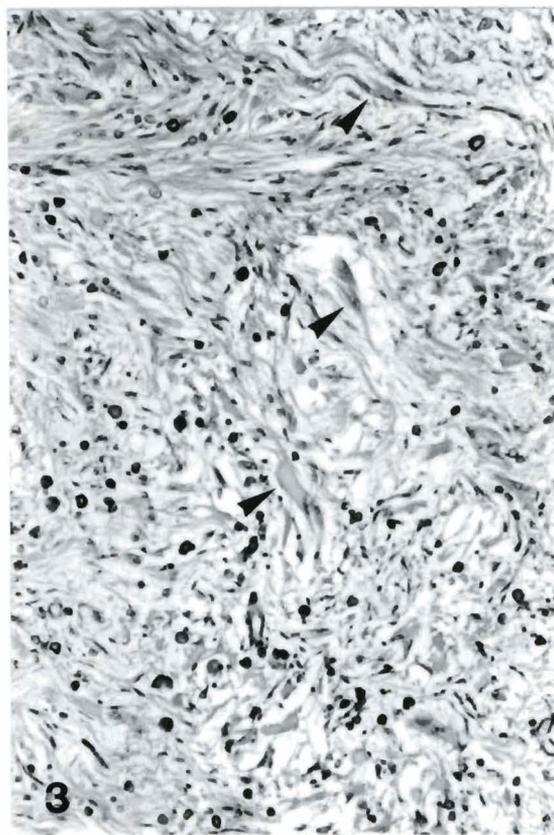
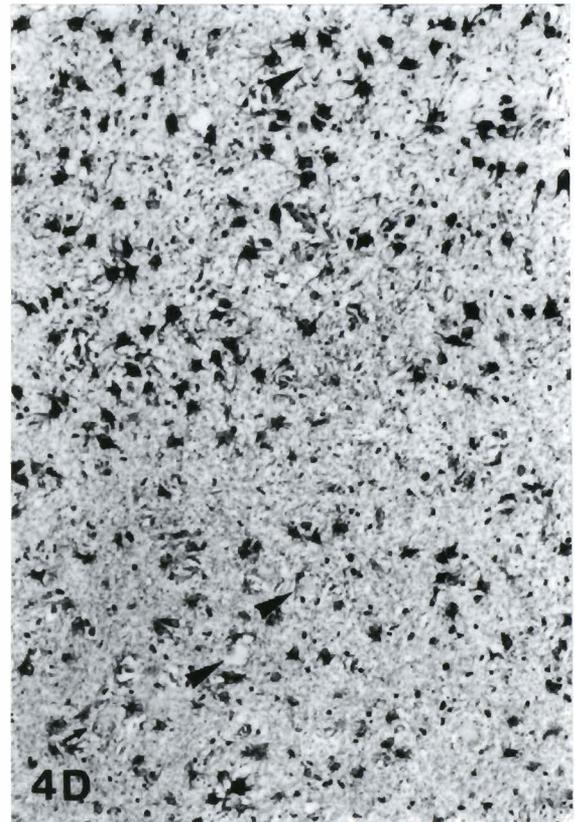
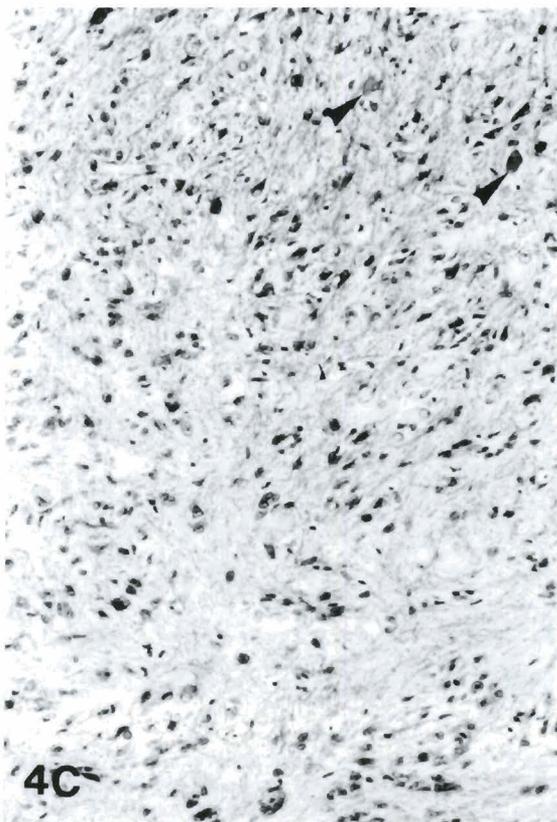
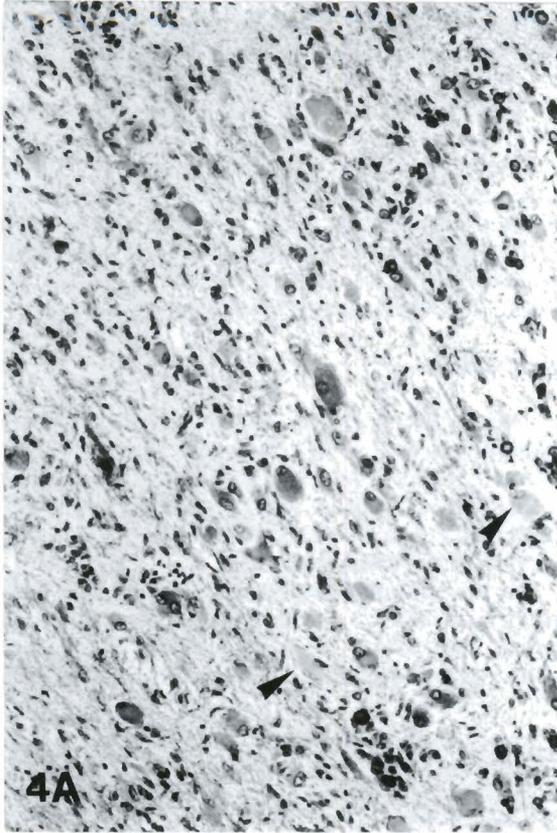


Fig. 3. A subependymal nodule bulging into the left lateral ventricle. Fibrillated or spindle-shaped giant cells (arrowheads) and bundles of fibres are seen. Calcium deposits are also found. H-E stain. x 200

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of the giant cells were also positive for NFP, while the majority of gemistocytic, ovoid or polygonal giant cells were negative for VM. There were a few GFAP-positive giant cells only in the cortical tuber. There was no positive immunoreactivity for MBP in the giant cells.

It is well known that NFP, GFAP and MBP are specific markers for neurons, mature astrocytes and immature oligodendrocytes (Tanaka et al., 1988), respectively. VM was first established as a marker for mesenchymal cells, and later VM was found in immature cells of both neuronal and glial origin at the developmental stage (Dahl et al., 1981; Tapscott et al., 1981). In the developing neural tube, VM is present in all undifferentiated replicating neuroepithelial cells (Tapscott et al., 1981; Sasaki et al., 1988) and is gradually replaced by either NFP or GFAP as the cells specialize as neuron or glial cells (Schnitzer et al., 1981; Tapscott et al., 1981). In developing neuroepithelium,

VM coexist with NFP (Tapscott et al., 1981; Cochard and Paulin, 1984). In radial glial cells of the fetal brain, VM coexists with GFAP (Schnitzer et al., 1981; Lukáš et al., 1989). On the basis of above the knowledge, our results suggest that the majority of the giant cells in the cortical tuber are of neuronal origin, with a few of glial origin. It is likely that the giant cells in the subependymal nodule and white matter nodule more differentiate toward the neuron than those in the cortical tuber.

The origin of the giant cells in TS has been a matter of controversy. Arseni et al. (1972) concluded that all giant cells biopsied from a patient with TS fulfilled electron-microscopic criteria for neuron. However, Ribadeau Dumas et al. (1973) reported that, on electron microscopy, the giant cells of both cortical tuber and a periventricular tumor (subependymal giant cell astrocytoma) were identical, and astrocytic. Stefansson

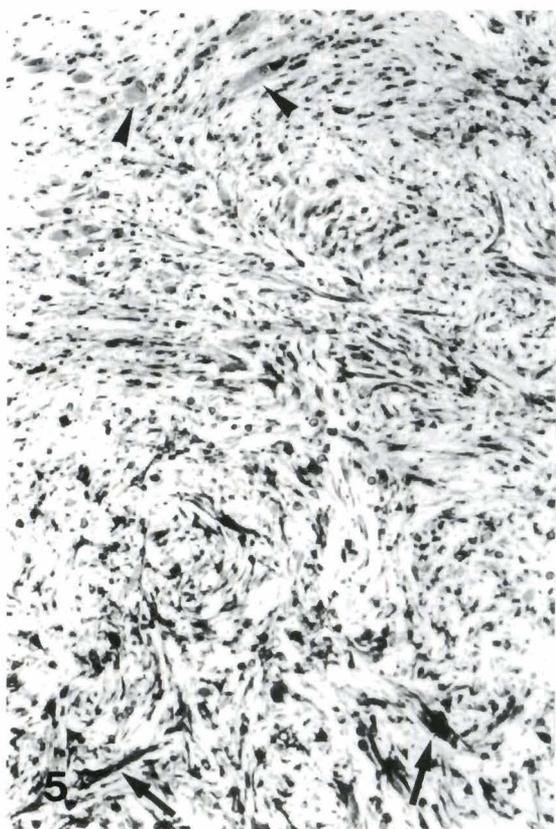


Fig. 5. A subependymal nodule showing VM-positive giant cells in the centre (arrows) and VM-negative giant cells in the periphery (arrowheads). VM with hematoxylin counter stain. x 200

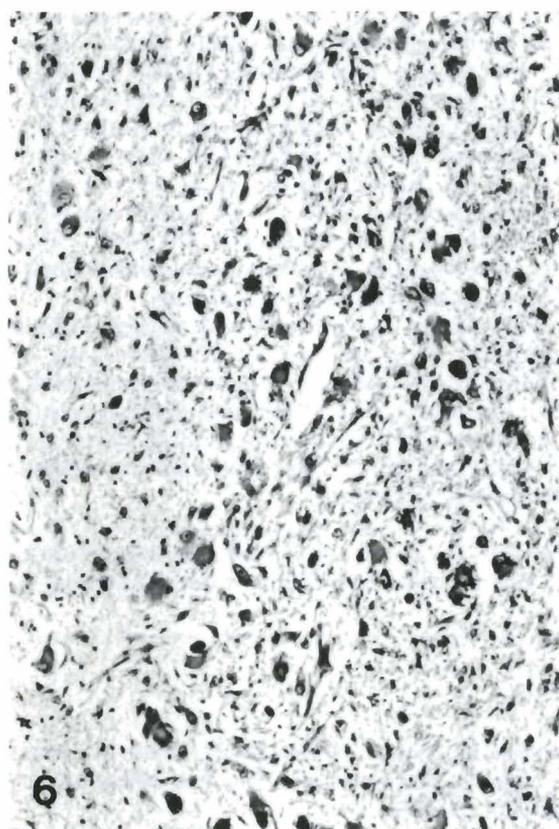


Fig. 6. Almost all giant cells are positive for NFP in a cerebral white matter nodule. NFP with hematoxylin counter stain. x 200

Fig. 4. A. A cortical tuber of the left orbital frontal lobe showing clusters of NFP-positive giant cells. Note a few NFP-negative giant cells (arrowheads). NFP with hematoxylin counter stain. x 200. **B.** Consecutive section from the same specimen as Fig. 4A, showing aggregation of VM-positive giant cells. Note a few VM-negative giant cells (arrowheads). VM with hematoxylin counter stain. x 200. **C.** Consecutive section from the same specimen as Fig. 4B, showing a few GFAP positive giant cells (arrowheads). Most of the giant cells are not stained for GFAP. GFAP with hematoxylin counter stain. x 200. **D.** Many GFAP-positive astrocytes are found in a cortical tuber of the left orbital frontal lobe. GFAP-negative giant cells (arrowheads) are scattered among GFAP-positive astrocytes. GFAP with hematoxylin counter stain. x 200

and Wollmann (1980, 1981) reported that the majority of the cortical giant cells in TS were negative for GFAP but positive for neuron-specific enolase (NSE), and concluded that such giant cells were of neuronal rather than astrocytic origin. Bender and Yunis (1980) found that one third of the giant cells in TS were immunostained with GFAP antiserum, with the degree of immunoreactivity varying from cell to cell. On the basis of further light and electron microscopic studies, they concluded that the giant cells of TS had features of both neurons and astrocytes. Recently, Chou and Chou (1989) demonstrated in an immunohistochemical study that the majority of giant cells in the cortical tuber were positive for both GFAP (over 85%) and NFP (over 80%), with a few positive for VM. Furthermore, in the deep subcortical layer they found the giant cells immunostained with GFAP, NFP, galactocerebroside, and occasionally with VM. They concluded that the giant cells in the periventricular, subcortical and cortical regions represented hamartomatous pluripotential cells expressing both neuronal and glial phenotypes or premature neuroglia retaining multipotential phenotypic expressions. Naramoto et al. (1989) investigated a cortical tuber and a subependymal nodule by immunohistochemical staining for NSE, GFAP and S-100 protein. They found that the giant cells in these regions were negative for GFAP, S-100 protein and NSE. They concluded that the cells negative for NSE, GFAP or S-100 protein might be undifferentiated, and might be intermediate cells between neurons and glia.

In the light of the above-mentioned studies and our present findings, the giant cells in TS brains may be undifferentiated cells and may remain ontogenetically immature. The immunoreactive and ultrastructural differences in the giant cells in individual cases may depend on the stage of the impairment during cellular differentiation and maturation.

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