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A comparison of cell phenotypes in hemimegalencephaly and tuberous sclerosis

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Abstract Hemimegalencephaly, an uncommon sporadic nonfamilial congenital dysplastic abnormality of the central nervous system, constitutes a pathological spectrum of neuronal migration disorders, but consistently includes abnormal large neurons similar to those in the cortical tubers of tuberous sclerosis. Microscopically, there are also cells with homogeneous and weakly eosinophilic cytoplasm with a single eccentric nucleus, sometimes called balloon cells (likewise prominent in tuberous sclerosis). We looked for immunohistochemical and ultrastructural differences in the large neurons and balloon cells between hemimegalencephaly and tuberous sclerosis. Microtubule-associated protein 1B and 2, phosphorylated and non-phosphorylated neurofilament and synaptophysin identify the large neurons and distinguish them from balloon cells in both entities. Balloon cells in hemimegalencephaly showed no immunoreactivity for TSC2 gene product, tuberin, and vimentin, but similar cells in tuber tissue showed consistent immunoreactivity. Balloon cells in hemimegalencephaly showed no immunoreactivity for glial fibrillary acidic protein, but some cells in tubers showed such immunoreactivity. Ultrastructurally, balloon cells in hemimegalencephaly contained very few lysosomes, microfilaments, and microtubules, but abundant lipofuscin granules. Similar cells in tubers had prominent lysosomes, more microfilaments and microtubules, and very few lipofuscin granules. The resemblance between abnormal cells in hemimegalencephaly and tuberous sclerosis is superficial; their immunohistochemistry and electron microscopic profiles show distinct differences.

Key words Hemimegalencephaly · Tuberous sclerosis · Large neurons · Balloon cells · Immunohistochemistry

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Introduction

Hemimegalencephaly (HME) or unilateral megalencephaly is an uncommon sporadic nonfamilial congenital dysplastic abnormality of the central nervous system, of unknown etiology [3]. The clinical characteristics include asymmetric development of the skull due to overgrowth of one cerebral hemisphere, intractable seizures, and mental retardation [22]. Neuropathology consists of hemispheric enlargement, macro- and/or micropolygyria, cortical thickening with lack of neuronal lamination, blurred boundaries of gray and white matter, neuronal heterotopias, and prominent large cells [3]. The large cells are predominantly neurons, but non-neuronal so-called balloon cells are also readily identified [4]. The large neurons are bigger than Betz cells or comparable neurons in the unaffected hemisphere. They are characterized by increased DNA, expanded dendritic trees, enhanced dendritic branchings, and prominent perisomatic processes [16, 17]. The balloon cells are large, sometimes multinucleated, and may produce a fine fibrillary gliosis, especially in the subpial zone. Townsend et al. [19] reported a wide spectrum of pathological changes, ranging from mild disruption of cortical lamination, with small increases in neuronal size and number, to marked disorganization of the cortex, extending into the white matter. They initially suggested that HME was in a "border zone" between dysplasia and neoplasia [19]. These abnormal cells in HME are similar in appearance to the those observed in the cortical tubers of patients with tuberous sclerosis complex (TSC) [13, 15].

TSC is an autosomal-dominant condition associated with a spectrum of hamartomas involving almost every organ in the body [5]. The most common presenting symptoms are seizures and mental retardation. In the brain, the characteristic features are cortical tubers and subependymal giant-cell tumors [2]. Microscopically, tubers contain dense astrogliosis, irregular neuronal lamination, calcification, large neurons, and prominent balloon cells. Balloon cells in TSC have a propensity to cluster in

Table 1 Sources of neurosurgically resected samples studied (*HME* hemimegalencephaly, *TSC* tuberous sclerosis)

Patient no.	Age (years)	Tissue source
Patients with HME		
1	2	Left hemisphere
2	3	Right hemisphere
3	4	Left hemisphere
4	9	Left hemisphere
5	10	Left hemisphere
6	11	Left hemisphere
7	15	Right hemisphere
Patients with TSC		
8	2	Frontal lobe
9	3	Frontal lobe
10	4	Occipital lobe
11	7	Temporal lobe
12	9	Parietal lobe
13	10	Parietal lobe
14	11	Occipital lobe
15	13	Temporal lobe
16	17	Frontal lobe
17	18	Frontal lobe

subpial locations or around blood vessels, a tendency that is apparently lacking in HME. The junction between gray and white matter is indistinct, with diminished myelination.

To elucidate the differences between HME and TSC, we compared the ultrastructural and immunohistochemical appearance, focusing on the large neurons and balloon cells in the two diseases.

Materials and methods

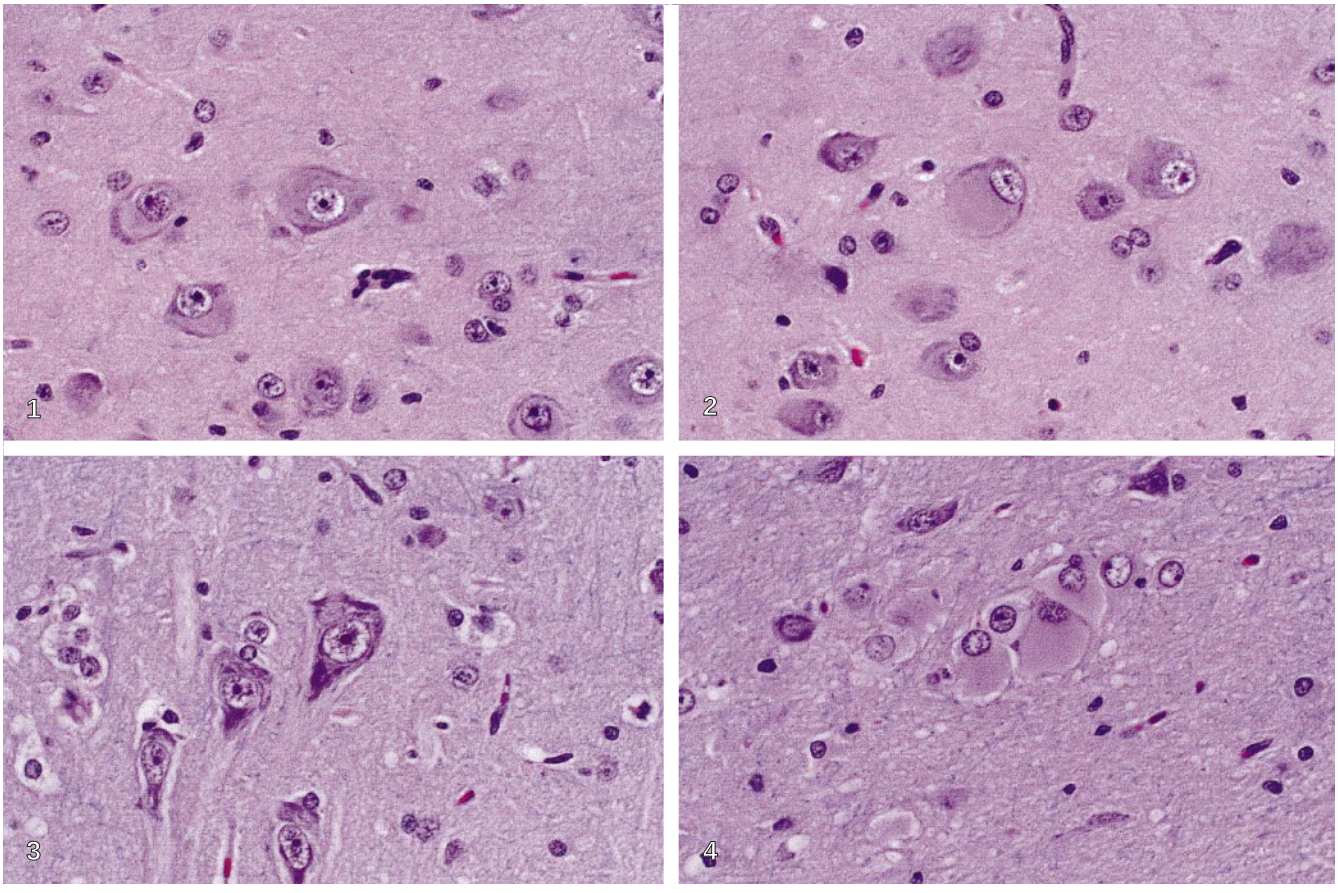
Between 1993 and 1997, surgical samples of HME tissue from seven children, 2–15 years of age, were retrospectively selected on the basis of the pathological diagnosis of HME (Table 1). Similarly, neurosurgically resected samples from 10 children with TSC, 2–18 years of age, were retrospectively selected on the basis of a diagnosis of cortical tuber using the criteria of Gomez [5]. All had been treated for intractable epilepsy at The Hospital for Sick Children, Toronto, Canada. All samples contained cerebral cortex and

Fig. 1 Large neurons in cortex tissue from the left frontal lobe of a patient with HME (patient 3), showing Nissl substance around the edge of cytoplasm, stained with H&E combined with LFB (*HME* hemimegalencephaly, *LFB* Luxol fast blue). × 560

Fig. 2 Balloon cells in cortex tissue from the left frontal lobe of a patient with HME (patient 3), showing the nucleus located to one side, and abundant homogeneous and weakly eosinophilic cytoplasm devoid of Nissl substance, stained with H&E combined with LFB. × 560

Fig. 3 Large neurons in tuber tissue from a patient with TSC (patient 15), stained with H&E combined with LFB (*TSC* tuberous sclerosis). × 560

Fig. 4 Balloon cells in tuber tissue from a patient with TSC (patient 15), stained with H&E combined with LFB. × 560



white matter with identified abnormal cells, including large neurons and balloon cells (Figs. 1–4). Patients underwent thorough preoperative assessments according to published protocols [6].

Antibodies

Polyclonal rabbit anti-tuberin (anti-Tub-CT) was a gift from Masashi Mizuguchi, Jichi Medical College, Japan [10, 11]. Polyclonal antibodies against glial fibrillary acidic protein (GFAP) and chromogranin were purchased from Dako (Glostrup, Denmark). Monoclonal antibody against vimentin (V9) was purchased from Sigma (St. Louis, Mo.), against microtubule-associated protein 1B (MAP 1B), 2 and neurofilament (NF) (2F11 for 70 kDa and 200 kDa) from Sigma, against phosphorylated NF (pNF) from Sternberger (Lutherville, Md.) and against synaptophysin from Boehringer Mannheim Biochemica (Mannheim, Germany).

Immunohistochemistry

All surgical resections were fixed in 10% neutral buffered formalin for approximately 24 h at room temperature, cut into 3–25 pieces depending on the size of specimens, embedded in paraffin blocks, and sectioned serially at 5-µm thickness. After treatment with 0.5% H_2O_2 in methanol and blocking serum, these sections were incubated overnight at 4 °C with polyclonal antibody against tuberin (1 : 250), GFAP (1 : 1000), or chromogranin (1 : 800), or with monoclonal antibody against vimentin (1 : 100), MAP 1B (1 : 500), MAP 2 (1 : 1000), NF (1 : 400), pNF (1 : 1000) or synaptophysin (1 : 50). Subsequently, sections were stained with the avidin-biotin-peroxidase complex method (Vectastain ABC kit, Vector, Burlingame, Calif.). Application of chromogen 3,3′-diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin, allowed identification of immunoreactivity. Negative controls included omission of the primary antibody.

Electron microscopy

Samples were examined by electron microscopy from four cases with HME (patients 4–7) and two with TSC (patients 9 and 11). Tissue was fixed in universal fixative (equal parts of 4% formaldehyde and 1% glutaraldehyde) and postfixed in 1% osmium tetroxide, dehydrated in graded alcohols and propylene oxide, and embedded in Epon resin 812 (Fisher Scientific Company, Fair Lawn, N.J.). Sections, 1 µm thick, were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Philips 400 transmission electron microscope (Philips, Eindhoven, The Netherlands).

Results

Immunohistochemistry

In negative controls, immunoreactivity was not noted in astrocytes, large neurons, or balloon cells in either HME or TSC samples.

With tuberin antiserum, some glial cells and some large neurons showed no or only weak immunoreactivity in the cytoplasm in HME and TSC. Most of the weak immunoreactive cells were astrocytes. Tuberin immunoreactivity was not noted in balloon cells in HME, but was strongly and consistently noted in both processes and cell bodies in TSC (Fig. 5, Table 2).

With synaptophysin antiserum, astrocytes and balloon cells showed no immunoreactivity; large neurons, however, showed intense immunoreactive halos around cell borders in both HME and TSC cases (Fig. 6). With GFAP antiserum, all astrocytes in HME and TSC showed moderate immunoreactivity in cytoplasm and processes, but large neurons showed none. GFAP immunoreactivity was not noted in balloon cells in HME, but some of similar cells in TSC was strongly positive in processes and cell bodies (Fig. 7).

No astrocytes, large neurons, or balloon cells showed immunoreactivity with chromogranin antiserum. With vimentin antiserum in both HME and TSC, some astrocytes showed no or only weak immunoreactivity; large neurons showed none. Vimentin immunoreactivity was not noted in the balloon cells in HME, but was strongly and consistently present in the processes and in the cell bodies in patients with TSC (Fig. 8).

With MAP 1B or MAP 2 antiserum, astrocytes and balloon cells showed no immunoreactivity; some large neurons, however, showed variable immunoreactivity for MAP 1B and for MAP 2 in both HME and TSC cases (Figs. 9, 10). With NF or pNF antiserum, astrocytes and balloon cells showed no immunoreactivity; some large neurons, however, showed variable immunoreactivity for NF and for pNF in both HME and TSC cases (Figs. 11, 12).

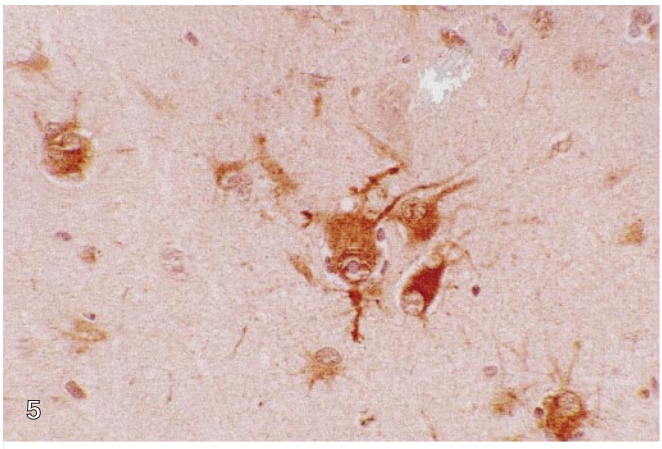
Table 2 Histochemical comparisons of unusual cell types in HME versus TSC (*GFAP* glial fibrillary acidic protein, *MAP* microtubule-associated protein, *NF* neurofilament, *pNF* phosphorylated neurofilament, *HME* cortex from patients with hemimegalencephaly, *TSC* cortical tubers from patients with tuberous sclerosis. *0* negative, + weakly positive, ++ strongly positive, *0+* negative to weakly positive, *0++* negative to strongly positive)

Antiserum	Astrocytes		Large neurons		Balloon cells	
	HME	TSC	HME	TSC	HME	TSC
Tuberin	0+	0+	0+	0+	0	++
Synaptophysin	0	0	Halo	Halo	0	0
GFAP	+	+	0	0	0	0++
Chromogranin	0	0	0	0	0	0
Vimentin	0+	0+	0	0	0	++
MAP 1B	0	0	0+	0+	0	0
MAP 2	0	0	0++	0++	0	0
NF	0	0	0++	0++	0	0
pNF	0	0	0++	0++	0	0

Electron microscopy

Balloon cells in HME were large cells with round to oval eccentric nuclei, occasional prominent nucleoli, and copious cytoplasm containing mitochondria, Golgi complexes, short strands of rough endoplasmic reticulum, prominent lipofuscin granules, and some microfilaments and microtubules, but few lysosomes (Fig. 13 a).

In TSC, these large balloon cells contained round to oval eccentric nuclei; the abundant cytoplasm also con-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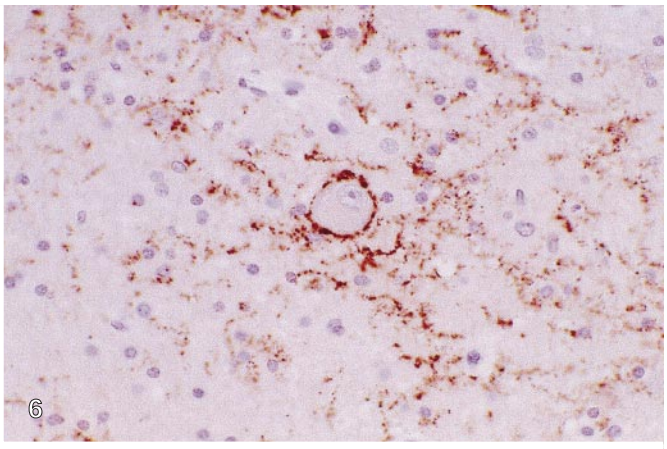
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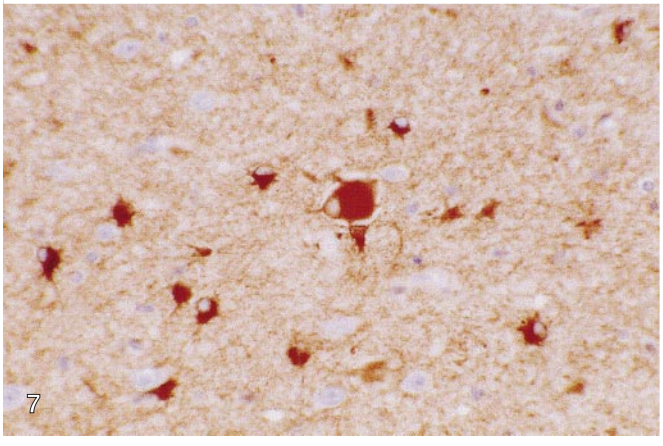
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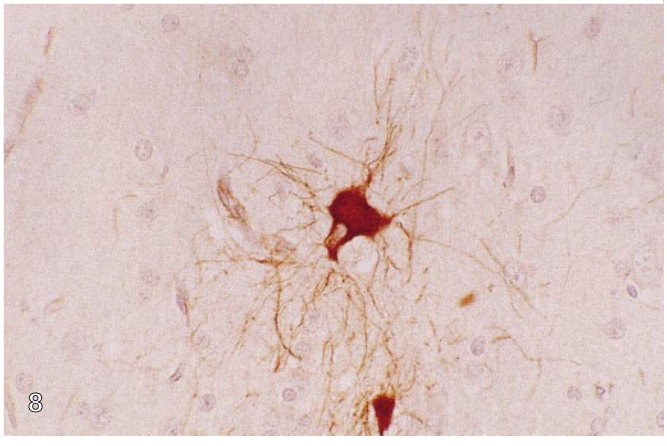
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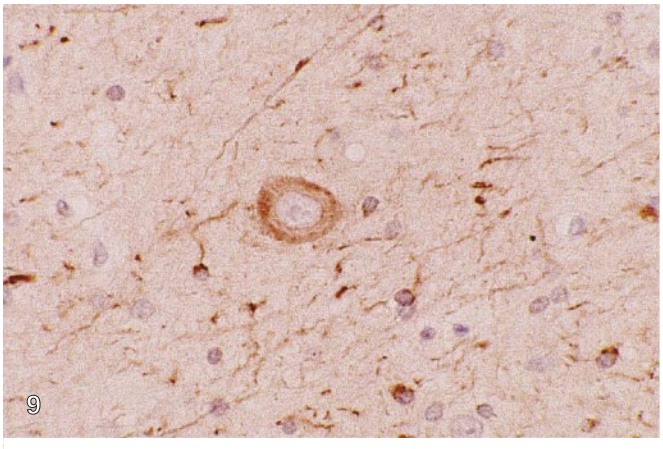
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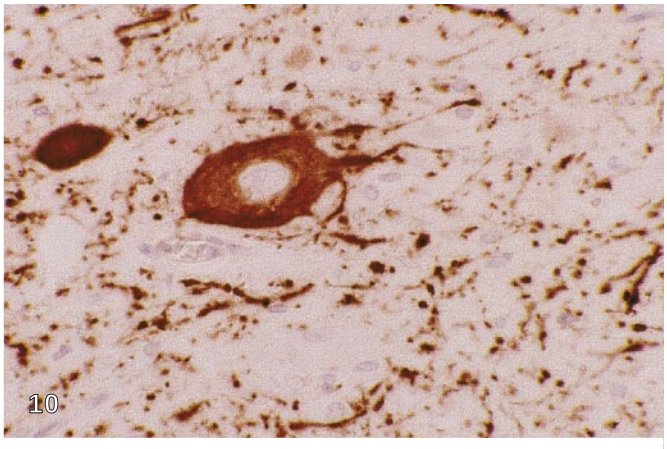
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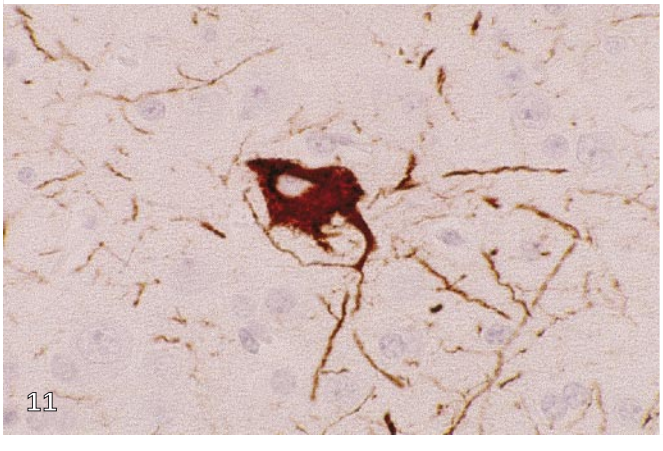
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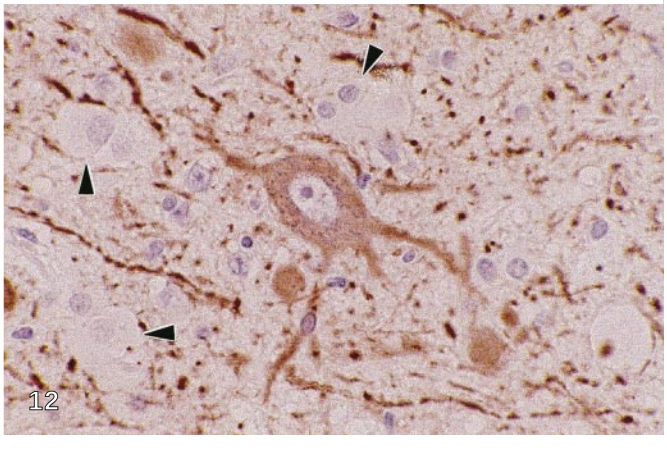
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◀ **Fig. 5** Tuberin immunoreactivity is strongly expressed in both cell bodies and cytoplasmic processes of balloon cells in tuber tissue from a patient with TSC (patient 12). × 560

Fig. 6 Large neurons showing an intense synaptophysin-immunoreactive halo around cell borders in cortex tissue from the left frontal lobe of a patient with HME (patient 4). × 560

Fig. 7 Glial fibrillary acidic protein immunoreactivity is expressed in both cell bodies and cytoplasmic processes of some of balloon cells in tuber with TSC (patient 12). × 560

Fig. 8 Vimentin immunoreactivity is strongly expressed in both cell body and cytoplasmic processes of balloon cells in tuber tissue from a patient with TSC (patient 12). × 560

Fig. 9 MAP 1B immunoreactivity is weakly expressed in both cell body and cytoplasmic processes of large neurons in cortex tissue with HME (patient 5) (*MAP* microtubule-associated protein). × 400

Fig. 10 MAP 2 immunoreactivity is strongly expressed in both cell body and cytoplasmic processes of large neurons in tuber tissue from a patient with TSC (patient 9). × 560

Fig. 11 NF immunoreactivity is strongly expressed in both cell body and cytoplasmic processes of large neurons in cortex tissue with HME (patient 6) (*NF* neurofilament). × 560

Fig. 12 Phosphorylated NF immunoreactivity is weakly expressed in both cell body and cytoplasmic processes of large neurons, but not in balloon cells *(arrows)* in tuber tissue from a patient with TSC (patient 14). × 560

tained mitochondria, Golgi complexes, rough endoplasmic reticulum, microfilaments, and microtubules. In these cells, however, there were very prominent pleomorphic lysosomes and very few lipofuscin granules (Fig. 13b).

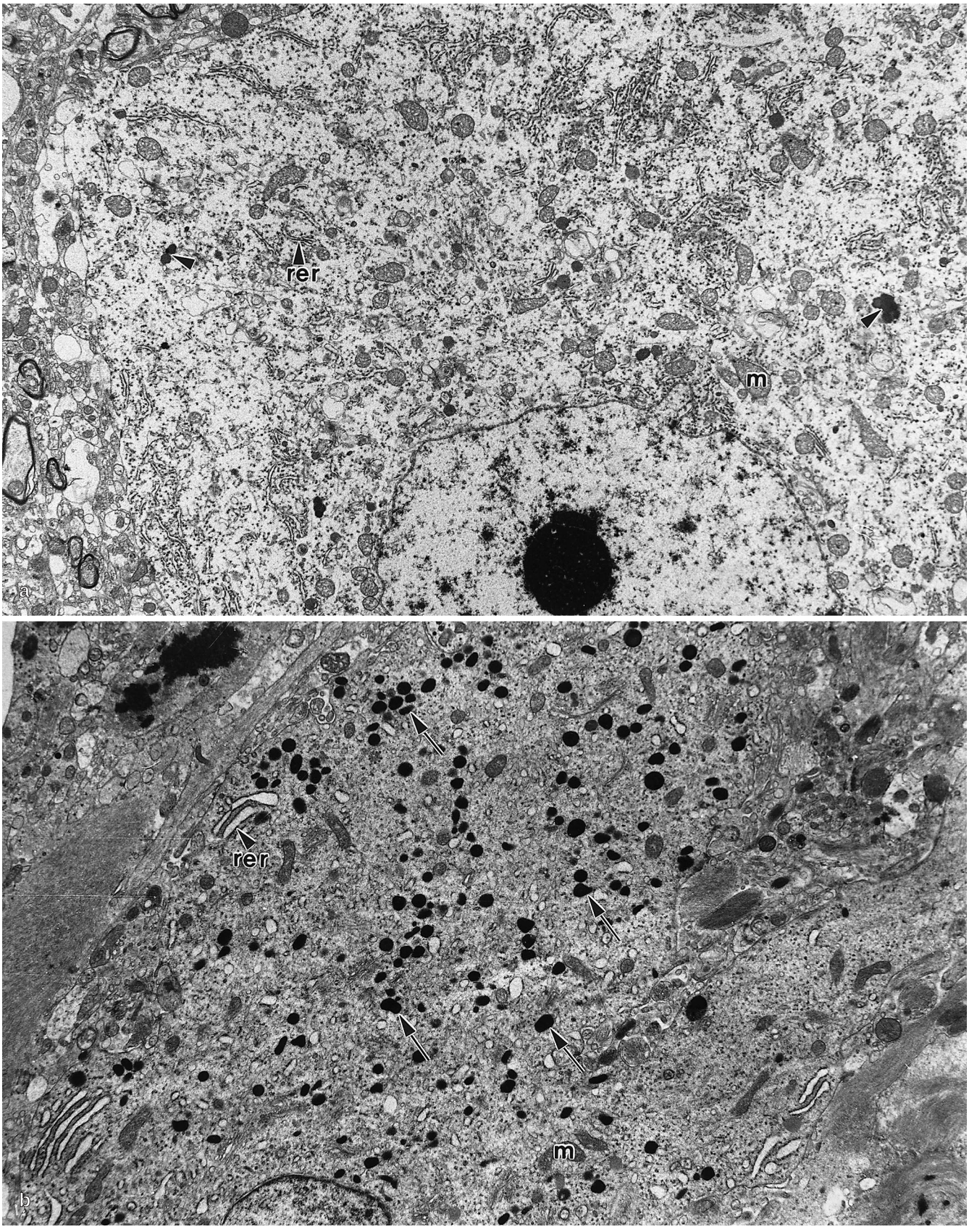
Discussion

The pathological spectrum in the hemisphere affected by HME includes significant abnormalities of neuronal morphology and orientation, with large neurons and balloon cells akin to those seen in the cortical tubers of TSC [6]. The designated large neurons are five to ten times the size of normal cortical neurons and have Nissl material concentrated around the outer edge of the cytoplasm [25]. The balloon cells have an eccentric nucleus, and abundant homogeneous and weakly eosinophilic cytoplasm devoid of Nissl substance. Large neurons in HME contain abnormal neurofilamentous accumulations similar to neurofibrillary tangles [6], and show immunoreactivity for neuronal markers only [3]. In comparison, large neurons in TSC show consistent immunoreactivity for MAP 1B, an immature cell marker, while balloon cells showed no immunoreactivity [25]. MAP 1B is the earliest MAP expressed by differentiating neurons [8], so that large neurons in TSC are thought to have features of neuronal immaturity [25]. In our immunohistochemical study with antiserum to synaptophysin, the large neurons of both HME and TSC cases showed intense immunoreactive halos around the cell borders, but balloon cells showed no such immunoreactivity. With antiserum to MAP 1B or MAP 2 and NF or pNF, some of the large neurons of both HME and TSC cases showed variable immunoreactivity in cytoplasm, but balloon cells showed no such immunoreactivity. These are useful markers to distinguish between the large neurons and the balloon cells, in both disease states. In previous studies, pNF expression has been observed in swollen cortical neurons in Creutzfeldt-Jacob disease [12], Pick bodies [21] and neurofibrillary tangles [21]. In normal neurons, pNF is present exclusively in axons; however, nonphosphorylated NF is widely distributed in perikarya, dendrites and axons [12]. The role of phosphorylation in neurofilament has not yet been determined; however, it has been suggested that it is a hallmark for functional impairment of neurons [12, 21]. These data and our results suggest large neurons of HME have failed to mature and have an appearance of potential functional impairment as do similar cells of TSC.

The precise origin of the balloon cells in HME and TSC remains undetermined. De Rosa et al. [4] reported that GFAP and vimentin were co-labeled in balloon cells in one of three HME cases. Vinters et al. [23] showed that balloon cells in cortical tubers of TSC showed variable immunoreactivity for glial and neuronal markers. Ultrastructural studies have led to diverse interpretations of the nature of balloon cells. Some reports concluded that these cells were astrocytic [20], and others that they were neuronal [1] or had both astrocytic and neuronal features [2]. Under a light microscope, balloon cells in HME are similar to those in the cortical tubers of TSC. In TSC these cells consistently show nestin or vimentin immunoreactivity [6, 25]. In our study, balloon cells in HME showed no immunoreactivity for vimentin, whereas those in TSC showed consistent immunoreactivity. Vimentin is one of the first intermediate filaments to appear in embryonic development, regardless of cell type, and is later replaced by the intermediate filament specific for that particular cell type (such as GFAP) [8]. In this study, some balloon cells in TSC showed immunoreactivity for GFAP. These observations suggest that markers of ”immaturity” and of astrocytic cells are frequently coexpressed in balloon cells in TSC. In HME, no balloon cells showed immunoreactivity for vimentin or GFAP.

Recently, tuberin, the TSC2 gene product, has been identified [18] and thought to function as a tumor growth suppressor [24]. In a previous study [11], neurons or glial cells in cortical tubers with TSC showed no or weak immunoreactivity for tuberin compared with those of controls without neurological disorders; balloon cells showed moderate or intense tuberin immunoreactivity. In our study, balloon cells of HME cases showed no immunoreactivity for tuberin, whereas those with TSC showed strong and consistent immunoreactivity. The significance of tuberin expression in balloon cells with TSC is unclear.

Ultrastructurally, balloon cells in HME display very few lysosomes, microfilaments, and microtubules, but contain lipofuscin granules. In comparison, balloon cells in TSC have prominent lysosomes, abundant microfilaments and microtubules, and very few lipofuscin gran-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◀ **Fig. 13 a, b** Low-power electron micrographs of balloon cells. **a** Cell from a patient with HME (patient 7) surrounded by a neuropil of cell processes, and is much larger than the other cells shown in the field. Note the copious cytoplasm that contains mitochondria *(m)*, rough endoplasmic reticulum *(rer)*, and lipofuscin granules *(arrowheads)*. **b** Cell from a patient with TSC (patient 11) with abundant cytoplasm containing mitochondria *(m)*, rough endoplasmic reticulum *(rer)*, and prominent pleomorphic lysosomes *(arrows)*. **a, b** Stained with uranyl acetate and lead citrate; × 6000

ules. In previous studies [7, 14] using immunogold techniques, intermediate filaments were labeled with GFAP or vimentin. These immunogold and electron microscopic findings are consistent with the results of our immunohistochemical and ultrastructural study.

The pathogenetic mechanism of HME is unknown. However, Manz et al. [9] reported an increase in DNA, RNA, and protein content in the affected hemisphere, thus implicating heteroploidy of chromosomal DNA and increased transcription and translation. The coexistence of a disorder of cell migration, proliferation, and hypertrophy may imply a growth factor disturbance that controls cell proliferation and growth. In this study, we found immunohistochemical and ultrastructural differences in abnormal cells between HME and TSC. These differences suggest distinctly different pathogenetic pathways despite similar phenotypical pathology.

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