Ultrastructural and immunocytochemical evidence that an incompetent blood-brain barrier is related to the pathophysiology of cavernous malformations

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Abstract

***Objectives*—Cerebral cavernous malformations are linked to mutations of the KRIT1 gene at the CCM1 locus and to mutations at two other loci, CCM2 and CCM3, for which genes are not yet identified. There is little information regarding the function of KRIT1. Histological and immunocytochemical analysis of cavernous malformations have not shed much light on their pathophysiology.**

***Methods*—Morphological analysis of cavernous malformations was extended to the ultrastructural level by examining lesions from two patients by immunocytochemistry and electron microscopy.**

***Results*—The lesions consisted of endothelial lined vascular sinusoids embedded in a collagen matrix. Nuclei belonging to cells distinct from endothelial cells were rare. The basal lamina of the endothelial cells consisted focally of multiple layers. No tight junctions at endothelial cell interfaces were found; however, several examined endothelial cell interfaces demonstrated apparent gaps between endothelial cell processes where basal lamina was exposed directly to the lumen of the sinusoids. Heavy hemosiderin deposits were found underlying the vascular channels within microns of the basal lamina without evidence of disrupted vessels. No astrocytic foot processes were seen within lesions. Glial fibrillary acidic protein immunocytochemistry confirmed that astrocyte processes stopped at the border of the lesions.**

***Conclusions*—The absence of blood-brain barrier components may lead to leakage of red blood cells into these lesions and the surrounding brain in the absence of major haemorrhage, thus accounting for the propensity of cavernous malformations to cause seizures. These data also raise the possibility that KRIT1 plays a part in the formation of endothelial cell junctions and expression of a mature vascular phenotype.**

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Keywords: cavernous malformation; electron microscopy; glial fibrillary acidic protein; blood-brain barrier

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Cavernous malformations in the CNS consist of endothelial lined vascular sinusoids embedded in a connective tissue matrix. There is no normal nervous tissue within the lesion itself. The normal brain or spinal cord tissue at the periphery of the lesion is haemosiderin stained and gliotic. Blood flow within the channels is stagnant as evidenced by the presence of thrombus in various stages of organisation. These histological qualities lead to the classic mulberry appearance on gross examination and the diagnostic appearance of a reticulated core of mixed signal intensity surrounded by a hypointense rim on T2 weighted MRI sequences.[1] Despite their prominence on MRI, cavernous malformations are seen poorly, if at all, on conventional digital subtraction angiography. This is confirmation of the minimal blood flow through these vascular lesions.

Despite the fact that numerous studies have examined cerebral cavernous malformations using histology and immunocytochemistry,[2–9] to our knowledge, only one ultrastructural study of these lesions has been published.[10] There are reports of electron microscopic studies of cavernous haemangiomas in the retina[11] and orbit[12]; however, these lesions are distinct from cavernous malformations of the CNS. The previous ultrastructural analysis of cavernous malformations identified structural abnormalities of tight junctions between endothelial cells potentially related their pathophysiology.[10]

The blood-brain barrier consists of the interplay of three major microvascular components. The tight junctions between endothelial cell constitute the major permeability barrier, and the overall biology of the barrier is shaped by the interactions of the endothelium with the pericytes/smooth muscle cells and the astrocyte foot processes that cover most of the abluminal surface of the microvasculature.[13] The molecular constituents of the tight junction include the claudins, occludin, ZO-1, ZO-2, ZO-3, and a growing group of related proteins. The interaction of the adherans junction cadherin-catenin system proteins with tight junction proteins likely forms a functional system for signal transduction involved in endothelial cell-cell interactions, patterned vessel development, and tight junction formation.[14]

We report electron microscopic examination of two brain cavernous malformations. In addition, we include immunocytochemical data on the relation of astrocytes to these lesions. Our findings confirm and extend the previous findings suggesting that abnormalities of the blood-brain barrier are important in the pathophysiology of cavernous malformations.

Methods

TISSUE COLLECTION

Tissue from cavernous malformations was collected from two patients with sporadic disease at the time of surgical resection under an approved institutional review board protocol. These lesions had the characteristic appearance of type II cavernous malformations on MRI[15] (fig 1). One patient was a 28 year old Greek woman with an evolving left homonymous visual field defect and the other was a 35 year old white woman with medically intractable seizures. At the time of resection, lesion tissue was minced into cubes 1 mm in length on each side and placed immediately into 3% glutaraldehyde. Adjacent normal brain tissue was available from one case as a control and was similarly processed. Tissue was also sent for routine formalin fixation, paraffin embedding, and haematoxylin and eosin staining.

IMMUNOCYTOCHEMISTRY

For immunocytochemical studies, paraffin embedded specimens were sectioned at 4 μm, deparaffinised, and subjected to antigen retrieval by steaming (20 minutes at 80°C). Slides were then incubated at room temperature with antibodies directed against glial fibrillary acidic protein (GFAP) (polyclonal, 1:6000; Dako Co, Carpenteria, CA, USA). Antibodies were detected using the avidin-biotin complex (ABC) method (Vector Laboratories, Burlingame, CA, USA) with diaminobenzidine serving as the chromagen.

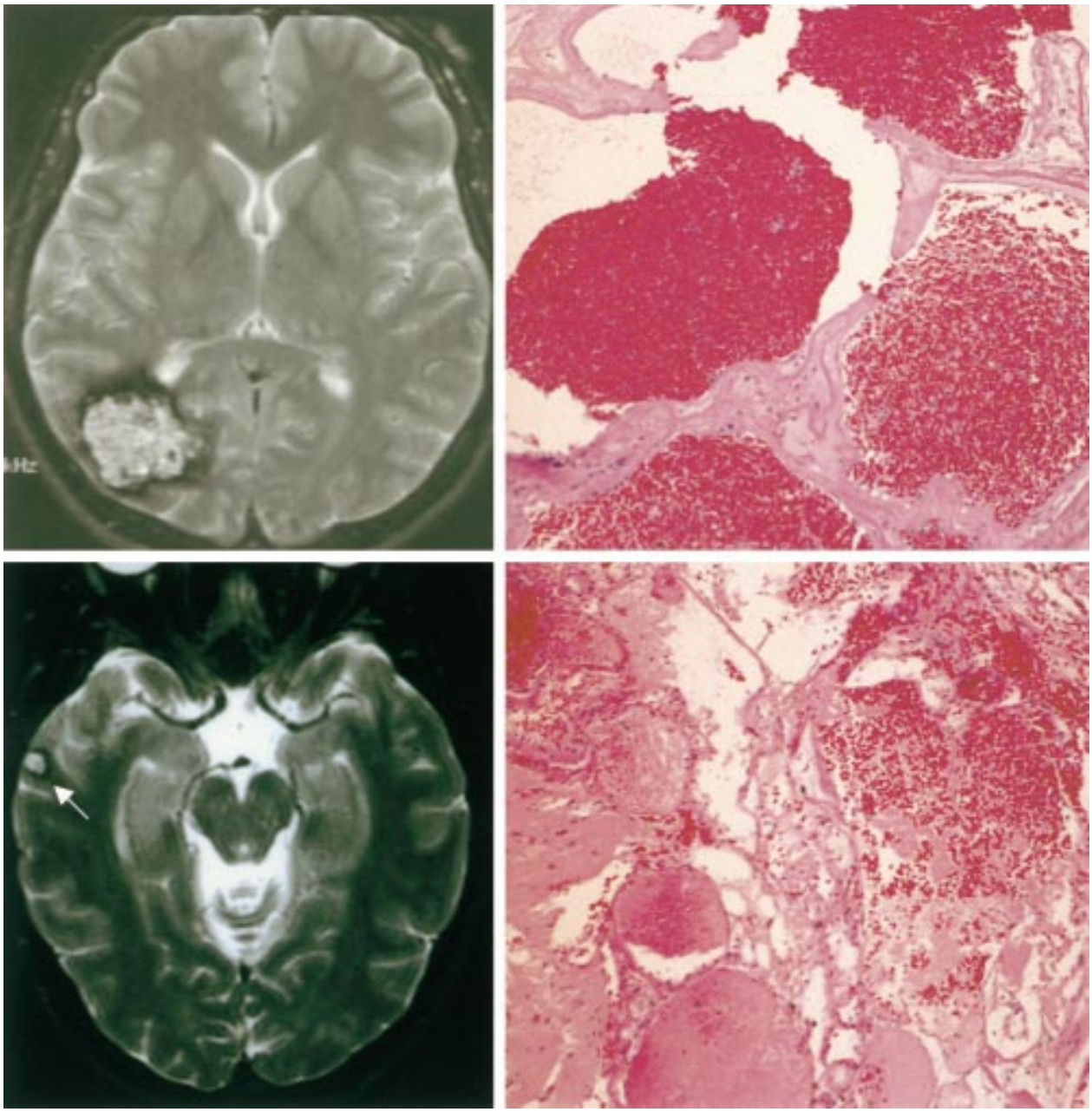

Figure 1 Axial T2 weighted MRI (on the left) and haematoxylin and eosin stained histological sections (on the right) demonstrating the cavernous malformations examined in this study. Original magnification of the histological sections×100.

ELECTRON MICROSCOPY

Tissues for electron microscopy were fixed in 3% glutaraldehyde for 24 hours, then stained with 2% osmium tetroxide for 1 hour, and dehydrated in graded alcohols. Tissue was then embedded in plastic (Araldite based). Semithin sections were examined and representative areas containing cavernous malformations and adjacent cortex were selected. Thin sections were made from these blocks and stained with uranyl acetate/lead citrate. Sections were placed on copper grids and imaged using a JEOL JEM-100S electron microscope.

Results

HISTOLOGY

Review of the histological sections of these lesions disclosed the typical characteristics of cavernous malformations (fig 1). They consisted of vascular sinusoids lined by endothelial cells. A dense connective tissue matrix separated these sinusoids. Scattered nuclei of other cells were noted, probably representing rare pericytes or fibroblasts. The channels contained red blood cells and foci of organising thrombus. Within and surrounding these lesions was prominent haemosiderin staining. Reactive gliosis was noted at the periphery of the lesions.

IMMUNOCYTOCHEMISTRY FOR GFAP

Sections stained with immunocytochemistry for GFAP showed dense labelling of reactive astrocytes at the border of the cavernous malformations (fig 2). The GFAP staining in the processes of reactive astrocytes reached the border of the lesions and stopped abruptly at the interface of brain tissue with the collagenous matrix of the cavernous malformations. No staining could be seen reaching between the vascular channels. Although some sections demonstrated isolated islands of GFAP stained cells surrounded by vascular channels, these probably represent finger-like extensions of brain tissue at the periphery of the lesion between serpiginous vascular loops. Sections through this interface could then give the false impression of brain tissue trapped within the interstices of the lesion.

ULTRASTRUCTURE OF CAVERNOUS MALFORMATIONS

One of the most remarkable aspects of the ultrastructure of these lesions is the paucity of cell profiles with the exception of endothelial cells. Red blood cells and white blood cells were occasionally noted within the vascular sinusoids. Dense connective tissue bundles consisting mainly of collagen separated these endothelial lined channels (fig 3 A). No perivascular ensheathing cells or astrocytic foot processes were seen. The basal lamina underlying the endothelial cells was often multilayered (fig 3 C). Haemosiderin was scattered throughout the lesion, often within several microns of the basal lamina (fig 3 E) or even within endothelial cell profiles (fig 3 D). No tight junctions were identified between endothelial cells in the cavernous malformations, although sampling was too limited to exclude their presence. Several examples were found in which a gap was noted between the adjacent endothelial cells of almost a micron. At these gaps the lumen of the vascular sinusoid appeared to be in direct contact with the basal lamina (fig 3 B), an arrangement commonly seen outside of the CNS. Vessels in adjacent brain tissue from one specimen showed several endothelial-endothelial cell contacts with the appearance of tight junctions (fig 3 F and G). Rare structures similar but not identical to the endothelial-endothelial cell gaps in the cavernous malformations could be seen in adjacent normal tissue. However, these vessels had a single layer of basal lamina, perivascular cells including pericytes and smooth muscle cells, and related astrocytic foot processes (fig 3 F and H) typical of cerebral microvasculature.

Discussion

Examination of the ultrastructure of cavernous malformations shows endothelial lined vascular sinusoids embedded in a dense collagenous matrix and a paucity of any other cell type. These vascular sinusoids differ from normal cerebral microvessels in important ways. The basal lamina underlying the endothelial cells is

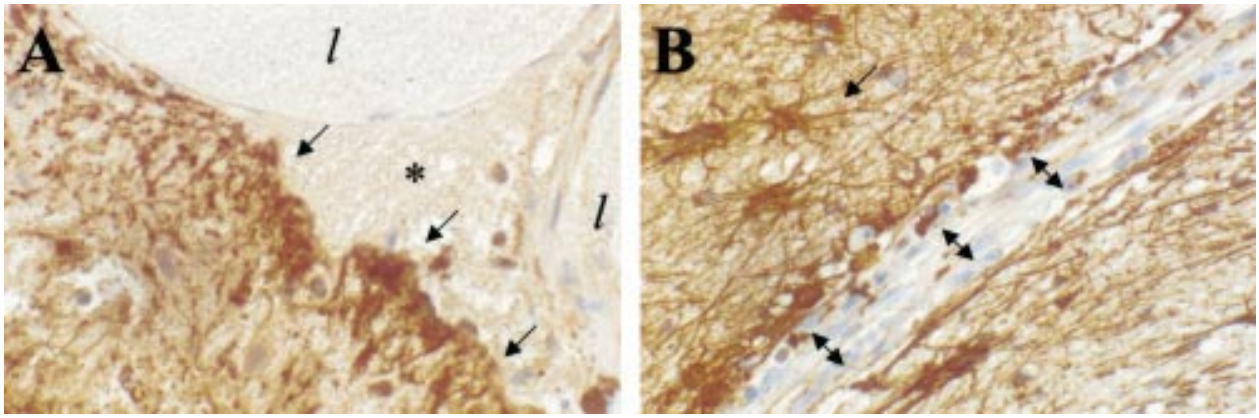

Figure 2 Immunocytochemistry using an antibody directed against glial fibrillary acidic protein. This is the same lesion shown in the lower panel of figure 1. (A) The border of the lesion with the surrounding brain is sharply demarcated by the line of densely stained GFAP positive astrocyte foot processes (arrows). The collagenous trabeculae (asterisk) between vascular channels (l) within the lesion are devoid of GFAP positive astrocyte processes. (B) The normal relation between astrocyte processes and brain microvessels can be seen in normal brain tissue adjacent to the cavernous malformation. A group of astrocytes (single arrow) can be seen with processes reaching the adjacent microvessel (double headed arrows). Original magnification×400.

often multilaminar and borders the dense collagenous matrix directly. There are no ensheathing pericytes, smooth muscle cells, or astrocytic processes bordering the microvascular units. Immunocytochemistry for GFAP confirms that astrocytic processes reach the border of these cavernous malformations but do not extend beyond the brain-cavernous malformation interface. Finally, gaps are noted between endothelial cells lining the channels of the cavernous malformations.

In the prior report of the ultrastructure of these lesions, the authors examined three cavernous malformations and compared them with normal tissue from two epilepsy resection specimens.[10] They reported poorly formed tight junctions with gaps between endothelial cells in the cavernous malformations. The vascular channels were embedded in an amorphous matrix lacking organised collagen. Subendothelial cells were also noted and thought to be pericytes. They concluded that the gaps between endothelial cells and the poorly organised "cavern" walls contribute to microhaemorrhages. Our observations support their findings of tight junction abnormalities in cavernous malformations. In addition, our findings include apparent abnormalities in the basal lamina that were not described in their specimens. The lesions examined here also

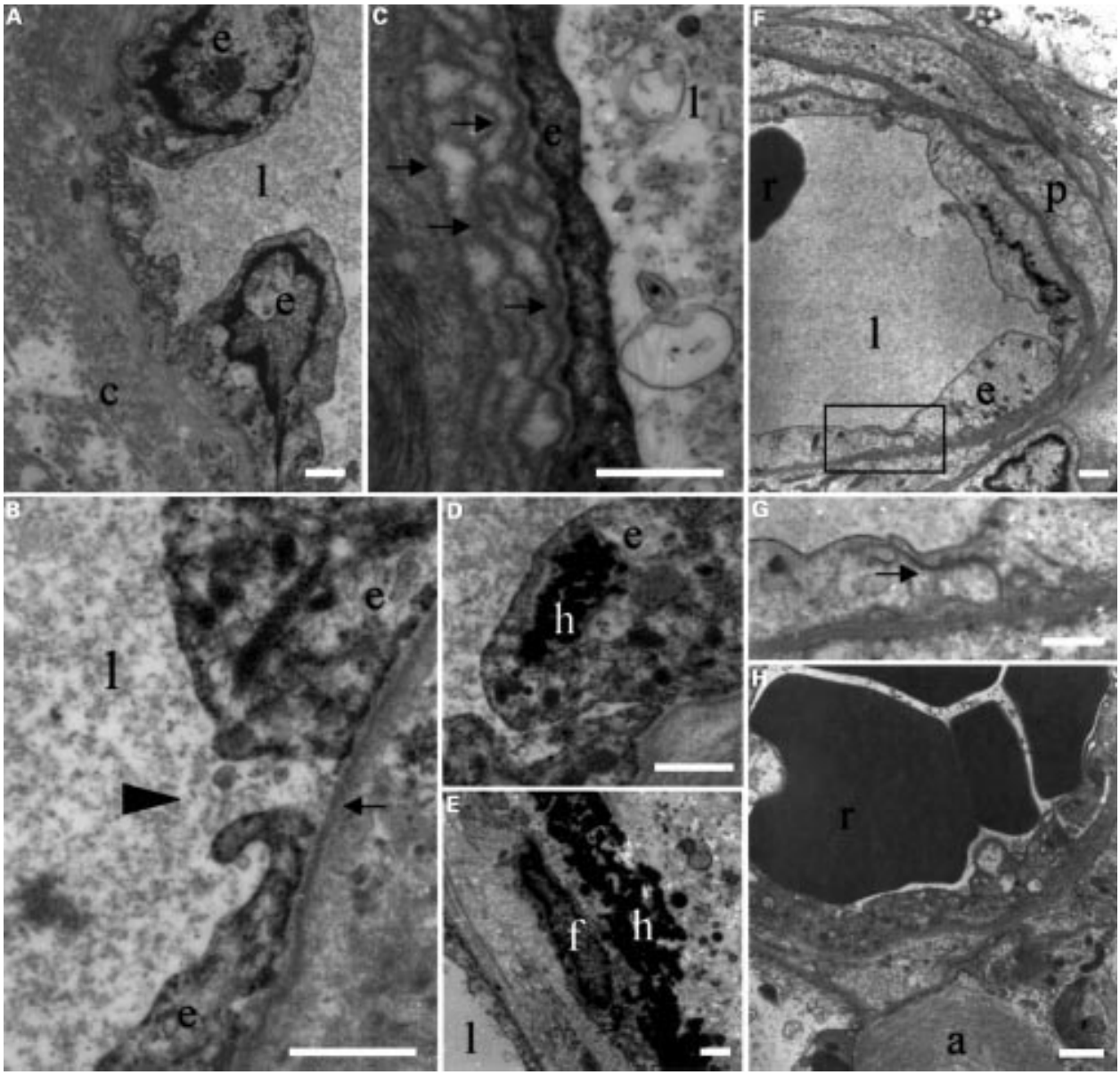

Figure 3 Electron micrographs of cavernous malformations (A-E) and microvessels in adjacent normal brain (F-H). (A) Ultrastructurally, these lesions consist of endothelial cells (e) lining vascular sinusoid lumens (l) and surrounded by a dense collagenous matrix (c). No perivascular cells were seen and the endothelial basal lamina was in direct contact with the collagenous matrix. (B) Gaps (arrowhead) between adjacent endothelial cells were seen in cavernous malformations where the lumen (l) was exposed directly to the basal lamina (arrow). (C) In focal areas, the basal lamina (arrows) demonstrated multiple abnormal layers. (D,E) Haemosiderin (h) could be seen within endothelial cells (D) and within microns of the lumens of the vascular sinusoids (E). A rare fibroblast profile (f) is seen in (E) in the connective tissue matrix. (F) A cerebral microvessel from brain tissue adjacent to a lesion demonstrates typical encircling pericytes (p) separating the endothelial cell basal lamina from the neuropil. A red blood cell (r) is noted in the lumen. (G) Magnification of the boxed area in F demonstrates a tight junction (arrow) between adjacent endothelial processes. (H) Another microvessel from the surrounding brain is being contacted by an astrocyte foot process (a) containing abundant intermediate filaments. Scale bar shown in all images represents 1 μm.

exhibited a more organised collagen matrix. Such lesion to lesion variability is not unexpected, and some aspects of cavernous malformation morphology might be linked to the specific genetic abnormality responsible for a given lesion. However, further ultrastructural examinations of cavernous malformations will be needed to understand the constancy and relevance of these findings as they are based on few observations.

The marked departure in brain cavernous malformation architecture from normal brain microvasculature architecture may play a part in the pathophysiology of these lesions. The apparent lack of appropriate junctional complexes and the absence of ensheathing cells likely translate into an incompetent blood-brain barrier and structurally weakened vessels. The haemosiderin seen deposited within microns of much of the basal lamina (in the absence of clinically significant haemorrhage) suggests a chronic leak of red blood cells into the interstices of the lesion and the surrounding brain. In addition, exposure of the basal lamina to the lumen of the vessel may (in conjunction with slow, low pressure flow) promote thrombosis within the vascular channels of these lesions.

We now know that mutations in the KRIT1 gene are responsible for a subset of familial cavernous malformations.[16–19] It is possible that cavernous malformations are all caused by genetic defects in KRIT1 and related genes encoding proteins that lie in a single signal transduction pathway. This pathway may be important in the normal formation and function of microvasculature. Formation of competent cerebral microvasculature relies on appropriate signalling between adjacent endothelial cells and between endothelial cells, pericytes, astrocytes, and the extracellular matrix. A disorder in such signalling could lead to the aberrant architecture seen at the ultrastructural level, a glomerulus of cerebral microvessels lacking an adequate blood brain-barrier.

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