A light and electron microscopic and immunohistochemical study of human arachnoid villi

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The structure of human arachnoid villi was investigated by light and electron microscopy with the aid of immunohistochemical techniques. The human arachnoid villi examined were basically composed of four portions: a fibrous capsule, an arachnoid cell layer, a cap cell cluster, and a central core. The arachnoid cell layer encompassing the central core was mostly covered by the thin fibrous capsule with an endothelial investment. However, the fibrous capsule was often absent at the apical portion of the villus and a factor VIII-related antigen stain failed to confirm the investment of endothelial cells. Instead, the arachnoid cell layer abutted directly upon the lumen of a lateral lacuna or the sinus. The arachnoid cell layer was thickened in places, forming cap cell clusters; it usually consisted of outer and inner zones. On vimentin staining, the former was slightly positive while the latter was strongly positive. The central core contained a network of arachnoid cells intermingled with connective tissue fibers and was in continuity with the cranial subarachnoid space. Electron microscopy showed that the arachnoid cells contained a larger number of intermediate filaments in the inner zone than in the outer zone. Ultrastructural immunohistochemical localization showed that vimentin was localized at the intermediate filaments and desmosomal plaques of the arachnoid cells. The arachnoid cells showed a marked variety in both the cell forms and the number of intermediate filaments or desmosomes, depending on their location.

KEY WORDS arachnoid villi • arachnoid cell • endothelial cell • morphological study • cerebrospinal fluid absorption • meningioma

It is generally accepted that arachnoid villi play an essential role in the absorption of cerebrospinal fluid (CSF) and form a site of origin for meningiomas. Since the classic work of Weed, arachnoid villi have been studied mainly in laboratory animals. The structure of human arachnoid villi has been depicted by diagrams of animal arachnoid villi, and only a few studies have focused on the functional examination of human arachnoid villi. Accordingly, the role played by these structures in CSF absorption and the cellular derivation of meningiomas still remains obscure. The present study was undertaken to investigate the structure of human arachnoid villi by light and electron microscopy and by immunohistochemical techniques.

Materials and Methods

Light and Electron Microscopy

Arachnoid villi were obtained post mortem from 18 subjects ranging in age from 7 to 86 years. At necropsy, performed within 3 hours of death, a block of tissue was resected including the superior sagittal sinus and the meninges with neighboring cortex, all with their relationship undisturbed. For the light microscopic investigation, half of each block was fixed in 4% paraformaldehyde and embedded in paraffin. Serial sections were made and stained with hematoxylin and eosin and elastica van Gieson. For the electron microscopic investigation, the other half of each block was submerged in 2.5% glutaraldehyde at 4°C, and specimens of arachnoid villi were excised from the lateral lacuna or superior sagittal sinus. These specimens were further fixed in 2.5% glutaraldehyde for 2 hours, postfixed in 1% osmium tetroxide for 1 hour, and embedded in Epon-Araldite. Ultra-thin sections of selected areas were stained with uranyl acetate followed by lead citrate, and were examined with a Hitachi H-600 electron microscope.

Immunohistochemical Labeling

Antibodies to factor VIII-related antigen for use as an endothelial cell marker and five intermediate fila-
FIG. 1. Schematic drawing of a human arachnoid villus as revealed in the present study.

ment proteins showing tissue specificity [13,17] were applied for immunohistochemical staining. Selected sections were stained with rabbit polyclonal antibodies to human factor VIII-related antigen, human epithelial keratin, chicken desmin, and bovine glial fibrillary acidic protein (GFAP) by the peroxidase-antiperoxidase method. [22] Other sections were stained with mouse monoclonal antibodies to human neurofilaments and human vimentin by the avidin-biotin peroxidase complex method. [10]

For immunohistochemical localization at the ultrastructural level, the postembedding protein A gold method [6,18] was used. Specimens of arachnoid villi were fixed in 0.4% paraformaldehyde for 30 minutes at 4°C and embedded in Epon-Araldite at room temperature for 3 weeks. Ultra-thin sections were stained with monoclonal antibodies against vimentin, diluted 1:10 at 4°C for 24 hours, and then stained with the protein A gold solutions (5 nm particles), diluted 1:10 for 12 hours. Immunostained sections were counterstained with uranyl acetate for 1 minute and with lead citrate for 15 seconds. Control preparations for immunohistochemical labeling were made by applying nonimmune serum or omitting the primary antibody.

Results

Human arachnoid villi were basically composed of four portions (Fig. 1): the fibrous capsule, the arachnoid cell layer, the cap cell cluster, and the central core. [30]

FIG. 2. Photomicrograph showing that the arachnoid cell layer (A) encompassing the central core (CC) is covered by a fibrous capsule (F). This layer is thickened to form a cap cell cluster (C). Elastica van Gieson, × 320.

Fibrous Capsule

Except for the apical portion, the arachnoid villi were usually covered by a thin fibrous capsule reflected from the surrounding dura mater (Figs. 1 and 2). Immuno-
Morphology of human arachnoid villi

Fig. 3. Immunohistochemical staining for factor VIII-related antigen. Although the fibrous capsule has an endothelial investment (A, arrow) similar to the venous wall (A, arrowhead), the apical portion of an arachnoid villus (B, asterisk) has no factor VIII-positive endothelial cells. Peroxidase-antiperoxidase counterstained with hematoxylin, × 200.

Peroxidase stains for factor VIII-related antigen confirmed the presence of endothelial cells at the luminal surface of the fibrous capsule (Fig. 3A). The latter was composed of three layers (Fig. 4). The outermost endothelial cell lining rested upon an almost continuous basement membrane. The endothelial cells contained many micropinocytotic vesicles and a few cytoplasmic vacuoles up to 0.4 μm in diameter. The middle layer consisted of bundles of collagen fibers and elastins intermingled with tiers of fibrocytes. The innermost layer consisted of electron-dense cells which were quite similar to dural border cells15-19 (Fig. 5). Their cytoplasm contained numerous cytoplasmic filaments, small vesicles, rough endoplasmic reticulum, and an electron-dense nucleus. These cells were connected to each other and to the outermost tier of the arachnoid cell layer or the cap cell cluster by a small number of desmosomes. Large extracellular cisterns were irregularly separated by thin cytoplasmic bridges and sometimes contained a small amount of filamentous material.

Arachnoid Cell Layer

The arachnoid cell layer encompassing the central core was covered mostly by the thin fibrous capsule with an endothelial investment (Figs. 1 and 2). However, the apical portion had no fibrous capsule or endothelial cells positive for factor VIII stains (Figs. 3B and 6). Instead, the arachnoid cell layer abutted directly upon the lumen of a lateral lacuna or the sinus. In most specimens, the arachnoid cell layer consisted of an electron-lucent outer zone and an electron-dense inner zone (Fig. 7). The cytoplasm contained an intermediate filament 8 to 12 nm in diameter, pinocytotic vesicles,
FIG. 5. The electron-dense cells (D) contain numerous cytoplasmic filaments and well-developed rough endoplasmic reticulum. These cells are connected to each other and to the outermost tier of the arachnoid cell layer (A) by a small number of desmosomes. \( \times 17,500 \).

large vesicles, rough endoplasmic reticulum, glycogen-like granules, and lysosomal residual bodies. These cells had elongated cell processes interdigitated with each other and connected by a large number of desmosomes. There were also a small number of tight junctions, intermediate junctions, and subplasmalemmal linear densities. The extracellular cisterns measured up to 10 \( \mu m \) in maximum diameter adjacent to the arachnoid cells and usually appeared on electron microscopy to be empty. However, in the villi affected by subarachnoid hemorrhage, these cisterns contained erythrocytes extending from the cranial subarachnoid space (Fig. 8).

Cap Cell Cluster

The arachnoid cell layer was thickened in places, forming cap cell clusters in which whorls or psammoma bodies were occasionally seen (Figs. 1 and 2). Arachnoid cells of the cap cell cluster were polygonal and more compactly distributed than those of the arachnoid cell layer. These cells had many short thick cytoplasmic processes interdigitated with each other and connected by a series of desmosomes. Small extracellular cisterns containing collagen or fibrous long-spacing fibers were seen among these cells (Fig. 9). In several specimens, subplasmalemmal linear densities were observed at the cell membrane facing these extracellular cisterns.

Central Core

The central core was in continuity with the cranial subarachnoid space. Within the arachnoid villus there was a network of arachnoid cells intermingled with connective tissue fibers (Figs. 1 and 2). Many elongated cytoplasmic processes interdigitated with each other, surrounding bundles of collagen fibers, resulting in a trabecular structure (Fig. 10). Arachnoid cells in the

FIG. 6. Photomicrograph at the apical portion of an arachnoid villus (arrow). The arachnoid cell layer abuts directly upon the lumen (L) of a lateral lacuna. Elastica van Gieson, \( \times 160 \).

FIG. 7. Electron micrograph showing the arachnoid cell layer at the apical portion. The arachnoid cell layer consists of an electron-lucent outer zone (O) and an electron-dense inner zone (I). \( \times 5700 \).
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FIG. 8. Electron micrograph of a specimen with subarachnoid hemorrhage. The extracellular cisterns of the arachnoid cell layer are packed with red blood cells extending from the cranial subarachnoid space. × 2900.

central core contained a variable number of intermediate filaments; some were electron-dense while others were extremely electron-lucent. No blood vessels or nerves could be found within the central core.

Immunohistochemistry of Arachnoid Cells

The presence of intermediate filaments was a characteristic electron microscopic feature of the arachnoid cells. Although staining of arachnoid cells was negative for cytokeratins, desmin, GFAP, and neurofilaments, it was always positive for vimentin. However, the intensity of cytoplasmic staining varied from cell to cell. The outer zone of the arachnoid cell layer was slightly positive while the inner zone and some core arachnoid cells were strongly positive (Fig. 11). On immunoelectron microscopic examination, vimentin was localized at the intermediate filaments and desmosomal plaques (Fig. 12).

Discussion

From the ultrastructural study of the dura-arachnoid interface, Nabeshima, et al.,12 and Schachenmayr and Friede19 demonstrated the absence of the so-called “subdural space.” Instead of the subdural space, there is a complex tight layer of cells, termed “the interface layer,” which consists of both the innermost portion of the dura mater (“dural border layer”) and the outermost portion of the arachnoid (“arachnoid barrier layer”). Our studies confirmed the fusion of two layers in human arachnoid villi: the innermost portion of the fibrous capsule and the outermost portion of the arachnoid cell layer. The fine structure of the interface between the fibrous capsule and the arachnoid cell layer showed features quite similar to those of the dura-arachnoid interface in man. It is suggested from the present findings that the human arachnoid villus is a protrusion of the arachnoid through the wall of a lateral lacuna or the sinus into the venous lumen with the well-preserved dura-arachnoid relationship.

It is generally accepted that animal arachnoid villi are completely invested with an endothelial lining which is continuous with or indistinguishable from the

FIG. 9. Electron micrograph showing arachnoid cells in the cap cell cluster. They are characterized by numerous interdigitations, desmosomes, intermediate filaments, and subplasmalemmal linear densities (arrow). × 14,600.

FIG. 10. Electron micrograph of the central core showing scattered arachnoid cells with many elongated processes interdigitated with each other, × 3000.
FIG. 11. Immunohistochemical staining for vimentin. The outer zone of the arachnoid cell layer is slightly positive, while the inner zone and core arachnoid cells are strongly positive. Avidin-biotin peroxidase complex counterstained with hematoxylin, x 240.

Arachnoid cells are widely distributed within the subarachnoid space, the arachnoid barrier layer, the central core, the cap cell cluster, and the arachnoid cell layer. The common ultrastructural features of arachnoid cells are vimentin-positive intermediate filaments and interdigitating cytoplasmic processes which are connected by desmosomes as junctional devices. However, the number of these structures varied remarkably according to the location of arachnoid cells. Furthermore, arachnoid cells showed a marked variety of features on both light and electron microscopic examination, depending on their location.

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