Scanning electron microscope study of human arachnoid villi

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The functional morphology of human arachnoid villi obtained from surgical biopsy specimens has been studied by scanning electron microscopy (SEM). On SEM examination, the villi appeared to be distended, as if functioning normally. The endothelial cells constituting the cerebrospinal fluid (CSF)-blood interface were covered by numerous microvilli, uniformly oriented along the major axis of the villus. Examination for cell-to-cell contact revealed only occasional areas of tight adherence between adjacent endothelial cells, while widened intercellular spaces were frequently observed. Generally corresponding to the apex of the villus, points of emergence of endothelium-lined hollow structures were identified; these may represent apical openings of open pathways from the subarachnoid space to the venous system. Ultrastructural arrangements consistent with a closed system of CSF reabsorption were also observed. Large cells maximally distended and protruding into the sinus lumen were commonly seen; these were interpreted as the result of the formation of giant vacuoles within the endothelium covering the villus.

This study has provided ultrastructural evidence for both closed and open systems of CSF reabsorption. Ultrastructural findings, such as gaps between endothelial cells and tubule-like endothelium-lined structures as previously identified in animals and observed in man by transmission electron microscopy, were demonstrated in human biopsy specimens by SEM.

**KEY WORDS**

- arachnoid villi
- scanning electron microscopy
- cerebrospinal fluid reabsorption

Arachnoid villi provide the main route for cerebrospinal fluid (CSF) reabsorption. However, the mechanism by which CSF traverses the villi to reach the venous system remains debatable. The concept that CSF reabsorption occurs by a simple process of membrane filtration has been supported by the electron microscopic investigations of many authors. It has been proposed that micropinocytotic vesicles or intracellular vacuoles within the endothelial cells covering the arachnoid villi could account for a "closed" system of CSF reabsorption. In contrast, some ultrastructural studies and most physiological investigations seem to demonstrate that gaps between endothelial cells lining the villi and tubule-like endothelium-lined structures may provide open communications between the subarachnoid spaces and the venous system.

It must be emphasized that all the above studies were carried out on various species of laboratory animals. Despite the extensive literature accumulated, until recently there have been few studies focused on the functional morphology of human arachnoid villi. Postmortem material is not suitable for ultrastructural study because the disappearance of the gradient of pressure between the subarachnoid space and venous system, to which the villi are physiologically subjected, may cause dramatic changes of their delicate structural organization.

The first electron microscopic study that focused on the functional ultrastructure of arachnoid villi obtained in vivo from human subjects provided ultrastructural evidence for both "closed" and "open" systems for CSF reabsorption. The same experimental strategy was used in the present study. This work was designed so as to investigate the morphology of arachnoid villi by scanning electron microscopy (SEM) using human villi carefully obtained by surgical biopsy during operations for intracranial tumors.
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Clinical Material and Methods

Arachnoid villi and granulations for SEM observations were removed from three patients, aged 37, 42, and 47 years, who were undergoing craniotomy for glial tumors. Institutional rules governing the protection of human subjects were followed in obtaining and studying these tissues.

In all cases computerized tomography (CT) scans showed normal ventricles, and preoperative measurement of intracranial pressure (ICP) did not reveal pressure fluctuations above physiological limits. Under these conditions, a normal gradient of pressure between the CSF and the superior sagittal sinus is expected, and a normal degree of distention of the villi can be reasonably assumed. Particular care was taken to avoid anesthesiological maneuvers that could influence ICP and central venous pressure. Possible effects of positional changes on ICP were excluded by carefully keeping the patients lying with the spine and sagittal plane of the skull horizontal during surgery.

In our cases, arachnoid granulations could be identified on the dura mater of the convexity corresponding to the middle meningeal vein or other large veins draining into the superior sagittal sinus. The granulations and a small amount of surrounding dura were easily and rapidly removed. No damage to the arachnoid tissue was evident macroscopically after excision. The anatomical specimens were immediately immersed in 5% glutaraldehyde in phosphate buffer, 0.1 M at pH 7.2 for 6 hours, washed in the same buffer for 30 minutes, dehydrated according to the critical-point method, coated by gold-palladium, and then observed with a JEOL 100-C scanning electron microscope.*

Results

With the aid of the dissecting microscope, single villi were readily identified within the granulation: they appeared macroscopically well distended and clearly bulging into the vessel lumen. Histological examination served to confirm the basic structural organization typical of a normally functioning arachnoid villus (Fig. 1).

Basic Architecture

Arachnoid cells herniating through small dural defects in the lumen of a vein or venous sinus form the neck and the stalk of the villus and subsequently enlarge, forming the stromal central core. At this level, collagen bundles and cytoplasmic processes of arachnoid cells form a spongy cellular and fibrous mesh through which CSF percolates freely in communication with the distal cranial subarachnoid spaces. A subendothelial space is finally interposed between the extracellular area of the core and the endothelial lining of the villus.

Arachnoid Granulation

A human arachnoid granulation, observed at low magnification, is shown in Fig. 2. The granulation has a typical cauliflower-like appearance; it is distended and projects into the venous lumen. The basal region of the granulation becomes narrower close to the neck, which can be followed until it penetrates the basal wall of the sinus. At least two single villi are identifiable in the

* JEOL 100-C scanning electron microscope manufactured by Japan Electron Optics Laboratory, Akishima City, Tokyo, Japan.

J. Neurosurg. / Volume 59 / October, 1983
granulation, separated by deep vertical fissures, and furrows of variable depth separate the villus into secondary lobules.

**Endothelial Cells**

The villus is covered by typical endothelial cells, generally located uniformly along its major axis (Fig. 3). The cells appeared to be stretched and flatter at the neck and the middle of the villus, and larger and more convex at the apex. At medium-high magnification, a dense population of microvilli was shown homogeneously distributed on the surface of the cells. The size and number (five/sq μ) of the finger-like microvilli were comparable to those described by others in animals. A search for cell-to-cell contact revealed a predominant pattern of clearly recognizable spaces between the borders of adjacent cells, rather than the expected close apposition of cellular borders.

At the apex of the villus, the superficial lining cells presented other morphological arrangements. These cells partially lost the longitudinal orientation which is typical of the neck and the middle of the villus. They were more convex, the microvilli on their surface were decreased in number, and the intercellular spaces appeared to be irregular and narrower (Fig. 4 left). Large endothelium-lined cavities were often identified. Some of the endothelial cells around the cavities presented a characteristic club-shaped appearance (Fig. 4 right). Red blood cells were frequently identified within the cavities, despite their absence on the surrounding surface of the villus (Fig. 5). Enlarged intercellular spaces are occasionally seen at the apex of the villus, in which red blood cells are caught while escaping from the villus toward the venous lumen (Fig. 6).

**Giant Vacuoles**

Another structural arrangement observed in the upper half of the villus consisted of endothelial cells with a typical vesicular shape. Observation at high magnification disclosed the cellular size and clear outlines of these structures, and confirmed that they were giant vacuoles (Fig. 7 left). In contrast to these cells, which bulged and were smooth and maximally distended, other vacuoles appeared to be flattened and partially opened (Fig. 7 right).

**Discussion**

In this study the anatomical specimens were excised from subjects with normal CSF dynamics. The structural organization of the villi depends on the gradient of pressure between CSF and superior sagittal sinus. It has been demonstrated that the collapse of the villi which follows a sudden fall in the pressure gradient is completed within 3 to 5 minutes. In our cases, excision of the granulation took considerably less time.

As seen in histological preparations (Fig. 1) and in low-magnification SEM (Fig. 2), the villi appeared to be well distended, as though functioning normally with
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Fig. 4. Scanning electron micrograph of the apex of a human arachnoid villus. Left: These endothelial cells have a less obvious longitudinal orientation. × 1550. Right: Large endothelium-lined cavity with club-shaped cells. × 1700.

Fig. 5. Endothelium-lined cavity at the apex of a human arachnoid villus. Red blood cells are visible within the cavity. Microvilli are present at the orifice of the cavity, which is lined by regular endothelial cells. There is no evidence of fracture or cracking of the superficial cells. × 2000.

Fig. 6. Red blood cell caught between two cells while escaping from a villus. × 4400.
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a normal gradient of pressure. At high magnification, the surface of the endothelial cells covering the villus showed no signs of fracture, distortion, or disorganization (Fig. 5).

This fits with the general criteria by which the tissue fixation and preparation are judged to be satisfactory for SEM. Thus, it can reasonably be assumed that our specimens maintained their original anatomical and physiological relationship during excision and fixation.

The critical role played by arachnoid villi in the process of CSF reabsorption is generally accepted. However, the mechanism by which CSF traverses the villi so as to reach the venous system is still unclear. On the basis of ultrastructural and physiological studies, the theories that have been advanced broadly fall into the following categories: 1) CSF crosses by means of direct communication between the external surface and the extracellular spaces of the core of the villus via tubule-like structures or widened intercellular spaces ("open" theory); or 2) CSF is transported across the endothelial lining of the villus by pressure-dependent pinocytosis or large fluid vesicles through the endothelial cells ("closed" theory). The "open" theory is supported in the present study by the observation of widened intercellular spaces between the borders of adjacent endothelial cells lining the villus.

The large endothelium-lined cavities generally identified at the apex of the villus may represent the apical openings of pre-formed channels. Similar structures were described by SEM in experimental animals and were found to be linked with the subarachnoid space via endothelium-lined tubules.

The direct drainage of intact red blood cells from the arachnoid villi is generally denied in the literature; however, a recent SEM study demonstrated that red cells can pass through the endothelium intercellularly. In our specimens, red blood cells were identified and used as natural tracers in passage from the subarachnoid space to the venous system, showing the size and patency of pre-formed open channels. This interpretation is also supported by previously reported transmission electron microscopic observations of red cells within the subendothelium space in close proximity to the apical opening of endothelium-lined tubules in human biopsy specimens of arachnoid villi.

Conversely, the giant vacuoles identified in our investigation are consistent with the "closed" theory. The vacuoles were observed in two different forms, full and empty, perhaps representing the anatomical-functional substrate of the cyclic process of vacuolization within the cytoplasm of endothelial cells.

The morphological-functional arrangements previously demonstrated by SEM studies of arachnoid villi of experimental animals include microvilli, gaps between the endothelial cells, and openings of small vacuoles (2 to 3 μm in size) and pre-formed channels. The

FIG. 7. Left: Endothelial cell of human arachnoid villus with a giant vacuole. The cell is maximally distended, and there are no microvilli. × 2000. Right: Another giant vacuole, which appears to be opened and flattened. × 2000.
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The present study has reinforced all these SEM findings; moreover, we observed that in human arachnoid villi it is also possible to identify the following structures: 1) large endothelial-lined cavities, far larger than other types of communications described in experimental animals; and 2) giant vacuoles, as large as the entire surface of an endothelial cell.

On the basis of our observations, we suggest that two different and probably additional pathways are available for CSF transport in man. Neither of which rules out the other. The vacuolization mechanisms are consistent with the transport of protein and CSF across the cytoplasm of endothelial cells by an active process; the widened intercellular spaces and the pre-formed channels provide evidence for the passive and valve-like aspects of the function of arachnoid villi.

Acknowledgment

The authors are indebted to Dr. Richard Greenberg for his helpful criticism in reviewing the manuscript.

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Manuscript received December 2, 1982.
Accepted in final form April 1, 1983.

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