Membranous Ultrastructure of Human Arachnoid Cells

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Abstract. The ultrastructure of arachnoid cell membranes was investigated by conventional transmission EM and by freeze-fracture techniques in human arachnoid granulations. Arachnoid cells showed widespread membrane specialization in the granulations including the formation of desmosomes, gap junctions, tight junctions, intermediate junctions, hemidesmosome-like structures, and micropinocytotic vesicles. However, the extent of the specialization varied from portion to portion; this was clearly shown on freeze-fracture replicas. Numerous extracellular cisterns were separated by cytoplasmic bodies or slender processes, joined by these junctional complexes. Uncoated and coated vesicles were abundant along the surface of extracellular cisterns representing the pathway of CSF. Complexes of branching tight junctions were comprised of 1–50 particle strands, which formed elaborate meshworks accompanied by numerous gap junctions and desmosomes. Micropinocytotic vesicles were often concentrated in the arachnoid cell cluster up to 40 per 1 μm², which is equivalent to the concentration in brain capillary endothelial cells. The results of this study clearly suggest that arachnoid cells in arachnoid granulations are not only tightly adherent to form a firm structure for the passage of CSF, but that the arachnoid cells lining the CSF pathway show intense cell-cell communication and pinocytic activity. This high transcellular activity probably reflects active transports or secretion of certain molecules by arachnoid cells.

Key Words: CSF drainage; Freeze-fracture; Gap junction; Human arachnoid granulation; Micropinocytotic vesicle.

INTRODUCTION

Since Weed’s classical work in dogs (1), there have been numerous reports illustrating the complex anatomical structure of arachnoid villi in various animals. It is obvious that arachnoid villi or granulations provide a major route for drainage of cerebrospinal fluid (CSF) into venous sinuses in humans (see review (2)), although other pathways of CSF and interstitial fluid drainage from the brains into cervical lymph nodes have also been documented in animals (3–7) and recently even in humans (8). Most of the studies so far have concentrated on the morphology and functions of the CSF-blood interface, i.e. the endothelial cells that cover the villi separate CSF within the villus from venous blood and act as a CSF-venous blood barrier. The main point of controversy has been whether 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 (9–10) (open channel theory), or whether CSF is transported across the endothelial lining of the villus by pressure-dependent pinocytosis or large fluid vesicles through the endothelial cells (9, 11) (closed channel theory). However, few studies have focused on the functions and biological activities of arachnoid cells, although arachnoid cells are the main component of the arachnoid villi and granulations (12–13). Furthermore, menigiomas and arachnoid cyst linings are derived from arachnoid cells, but the contribution of biological activities of arachnoid cells to cyst formation is still undetermined. Leptomeningeal cells contain catechol-O-methyltransferase (14), glutamine synthetase (15), and certain neurotrophic factors (16–17) that play a role in the regeneration of vasopressin nerves after hypophysectomy (18–19). In addition, human arachnoid cells have recently been reported to synthesize prostaglandin D synthase (20), although the details of the function and mechanism of secretion of the enzyme are still unknown.

Freeze-fracture techniques visualize extensive sheets of the plasma membrane and display the en face vista of the interior of cell membranes (21), where the tight junctions (TJs) appear as strands of particles, desmosomes as focal particle aggregates, pinocytotic vesicles as pits or concave depressions, and gap junctions (GJs) as densely packed particles and pits in a 2-dimensional plane. Recently, several types of water-selective membrane channels were discovered in a number of tissues and were named the “aquaporin family” (22–24); a relationship between water permeability and orthogonal arrays on the replicas has been proposed (25). Accordingly, freeze-fracture technique is well suited for the investigation of intramembranous specialization-rich arachnoid cells, but until now there has been no freeze-fracture study of arachnoid granulations. The aim of this study is to closely observe intramembranous structures in arachnoid cell membranes and to visualize the three-dimensional localization of TJ, GJs, desmosomes and vesicular structures in order to advance our understanding of the functions of arachnoid cells.
MATERIALS AND METHODS

Preparation of the Materials

Arachnoid granulations and their membranes were collected from 17 human adults (aged 18 to 65); in 11 cases, tissue was obtained postmortem, and in 6 subjects during surgery for intracranial disease. The autopsy cases comprised 4 cases of brain tumor, 1 cerebral contusion, 1 spinal cord injury, 1 acute pancreatitis, and 4 subarachnoid hemorrhages. The operated patients comprised 5 brain tumors and 1 unruptured cerebral aneurysm, in which the granulations were not affected and patients showed no apparent history of raised intracranial pressure. At operation or necropsy, performed within 3 hours (h) of death, arachnoid granulations were carefully dissected out and immediately immersed in fixative with minimal mechanical injury. For the light microscopic investigation, some specimens were fixed in 4% paraformaldehyde and embedded in paraffin. For the electron microscopic investigation by both ultrathin sectioning and freeze-fracture replicas, the specimens were submerged in 2.5% glutaraldehyde at 4°C and granulations were dissected from the surrounding tissue with the aid of a microscope.

All specimens were further fixed in 2.5% glutaraldehyde for 2 h, postfixed in 1% osmium tetroxide for 1 h, and embedded in Epon-Araldite. Semithin sections were cut with an LKB ultramicrotome and stained with toluidine blue. Ultrathin sections of selected areas were stained with uranyl acetate followed by lead citrate, and were examined with a Hitachi H-600 electron microscope. For freeze-fracture replicas, arachnoid granulations were placed perpendicularly on coverslips and cut into 50-μm sections. The rest of the tissue on coverslips was dehydrated and embedded in paraffin, and prepared for H-E staining to ensure adequate orientation of the structure in the adjacent Vibratome section (Fig. 1).

Freeze-fracture Technique

Details of the procedure for the freeze-fracture have been described elsewhere (26–27). Briefly, the Vibratome-sliced specimens were infiltrated in a graded series of glycerol-Ringer’s solution, placed between gold specimen discs of a Balzers double replica device, rapidly frozen in liquid nitrogen-cooled Freon 22, and stored in liquid nitrogen. These specimens were fractured in a Balzers 080 freeze-fracture apparatus at −110°C and were shadowed with platinum and carbon immediately after fracturing. Replicas were covered with 1% colloid solution in amyl acetate. After the tissue was completely dissolved with Clorox, the colloid was removed by soaking in amyl acetate for ultrastructural observation.

RESULTS

The Light Microscopic Observation

Human arachnoid granulations were composed of 3 types of cells: arachnoid cells, endothelial cells and fibroblasts. Almost all were arachnoid cells that form 3 different cell cluster patterns; arachnoid cell layer, cap cell cluster and central core. The other two cell types were endothelial cells, investing the surface of arachnoid granulations, and fibroblasts in the fibrous capsule. The localization of these cells was clearly recognized under a light microscope in sections cut perpendicularly to the axis of the granulation (Fig. 1).

Observations on Ultrathin Sections

The membrane structure of arachnoid cells was basically uniform: i.e. the cell bodies and processes were tightly juxtaposed and intermingled as interdigititation, and
linked by a number of junctional devices, including desmosomes, TJs, GJs, intermediate junctions, hemidesmosomes and subplasmalemmal linear densities (Fig. 2). The cluster of arachnoid cells was characterized by a number of extracellular cisterns that usually appeared to be electron-optically empty or contained fine collagenous or fuzzy material. There were many micropinocytotic vesicles, and they were recognized even at the membranes of fingerlike processes of arachnoid cells. Thin lamellae of cytoplasm were almost equal in thickness to the size of the vesicular profiles. These vesicles were more prominent along the cell surface toward cisterns, which are thought to be the channels of CSF drainage (Fig. 3). Arachnoid cells in the central core have thin cytoplasmic processes and showed fewer vesicular and junctional structures than those seen in arachnoid cell clusters (data not shown). The electron-dense arachnoid cells that formed the innermost layer of the fibrous capsule also showed many micropinocytotic vesicles, well-developed rough endoplasmic reticulum, and were connected to each other by junctional complexes (data not shown).

Freeze-fracture Study

Fracture planes in human arachnoid granulations usually passed through the cytoplasm and within the plasmalemma, and the fracture face of the plasmalemma clearly revealed intramembranous particles and the fine structure of intramembranous specializations of arachnoid cells.

Tight Junctions

Freeze-fracture electron micrographs revealed both the P- and E-face of arachnoid cell TJs (Fig. 4). A complex TJ is normally seen as the sealing strand particles associated with the P-face in replicas. In arachnoid cells, there was no polarity for location of the TJ as seen in epidermal tissue and epididymis, which show apical and basolateral localization of TJs. On the contrary, the distribution of the TJ meshwork was patchy, and there were areas without any junctions. This randomly located, junction-free area probably reflected the region facing the large extracellular cisterns. The E-face of the TJs consists of shallower grooves with intermittent particles, and these are complimentary to the cylindrical ridges on the P-fracture face (Fig. 5). The elaborate tight junctional meshworks were comprised of 1 to over 50 TJs, and associated with numerous other junctional devices (Fig. 4). The sealing strands were not parallel, but branched, sometimes discontinuous and anastomosed to form an elaborate meshwork.

Gap Junctions

In freeze-fracture electron micrographs, GJs are characterized by patches of densely packed particles of uniform size and shape in replicas. After membrane fracture, GJ particles, approximately 9 nm in size, or connexons, remained associated with the P-fracture face in arachnoid cells (Fig. 5). The E-fracture face of GJs appear as closely spaced pits, and these are complementary to the particles on the P-fracture face. Both the P- and E-fracture faces are often present over a single GJ, and in these cases the fracture plane has jumped between membranes (Fig. 6). The GJ was ovoid or polygonal in shape, mostly 0.5–2.0 μm in diameter, and sometimes circumferentially associated with TJ strands.

Desmosomes

In replicas, desmosomes appeared as focal particle aggregates on P-faces (Fig. 5). On E-faces, the desmosomes were recognized by a slight increase in small pits and in the number of particles compared with nonspecialized membrane. The desmosome was ovoid in form, mostly 0.2–0.6 μm in diameter. Numerous desmosomes were often surrounded by TJ strands (Fig. 5).

Micropinocytotic Vesicles

In replicas, micropinocytotic vesicles appeared as pits or concave depressions of approximately 50–100 nm in diameter. These circular depressions were concentrated up to 40 per μm² in some areas. At cisterns circumscribed by branching meshworks of TJ array, the membrane bulges slightly into the arachnoid cell and has many pits (Fig. 6). In some large areas without any junctional specializations, in which the membrane may be adjacent to large extracellular cisterns, many pinocytotic pits also could be seen.

Central Core

Arachnoid cells were interposed among the collagen fibrils in the region of the central core, and membrane specializations were less prominent.

Other Membranous Specializations

In no region of the replicas were other types of membranous specialization, such as orthogonal arrays, found in cytoplasmic membranes of the arachnoid cells.

DISCUSSION

Adhesion Mechanism between Arachnoid Cells

The present study clearly demonstrated the elaborate complexes of membrane specializations in human arachnoid granulations. In epithelial tissue, all components of junctional complexes are characteristically present at the apical portion of opposed cells, forming zonulae occludentes (28). The sealing strands of TJs tend to remain associated with the cytoplasmic membrane leaflet and appear on the P-fracture face in replicas (29). However, the TJs in the arachnoid cells were notable as sealing strand particles, remained both on the E and P-fracture faces,
Fig. 2. Transmission electron micrograph showing junctional complexes and abundant micropinocytotic vesicles that are prominent, especially along the extracellular cistern (some are indicated by curved arrows). Coated vesicles (arrows) are also noted. C; extracellular cisterns. ×20,000.

and were distributed in a patchy pattern (macula occludentes) without any polarity. These TJs consisted of more than 50 strands in some portions, and were accompanied by abundant desmosomes and junctions of other types, suggesting tight adhesion between adjacent cells. Intermediate filaments attached to the desmosomal plaques in arachnoid cells and meningioma cells, which are tumors derived from leptomeningeal cells, are known to be

Fig. 3. Transmission electron micrographs: a; abundant micropinocytotic vesicles (arrows) are prominent along an extracellular cistern (C) 11 × 7 μm in size. ×17,000. b; higher magnification of Figure 3a, which shows detail of micropinocytotic activity (arrows) in the arachnoid cell membrane. ×54,000.
vimentin-type filaments (30, 31); cytotkeratin-type filaments are also reported in some cultured conditions (32, 33). Vimentin connects the nucleus with the plasma membrane, contributing to the transport processes and signal transduction taking place between the cell surface and the nucleus (34). For example, overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation (35). Since arachnoid granulations form a bridge between the pulsatile brain surface and venous sinuses, which are rigidly fixed to the calvarium, mechanically strong adhesiveness among arachnoid cells would be indispensable to maintain the architecture. Recently, it has been reported that a calcium-dependent cell adhesion molecule, epithelial cadherin, is located in the plasma membrane of arachnoid cells, suggesting a role of this adhesion molecule in molecular adhesion mechanisms between arachnoid cells (36, 37). Accordingly, it is likely that the complex of junctional devices seen in this study plays a role in the junctional adhesion, while the Ca^{2+}-dependent adhesion molecule is another factor to support the complex architecture of arachnoid granulations.

Gap Junctions

This study revealed that many gap junctions (GJs) were located between the adjacent arachnoid cells. The size, distribution, and irregularly ovoid or polygonal shape were similar to those observed in meningiomas (38), and those in arachnoid granulations were often accompanied by a meshwork of TJs, especially at the end.
Fig. 5. High-power view of a freeze-fracture electron micrograph reveals TJ strands associated with abundant GJs (arrows), and desmosomes (some are indicated by open arrows) on the P-face (P) of an arachnoid cell. Several TJ and desmosomes (curved arrows) on the E-face (E) are also illustrated. ×42,000.

of the TJ array. Gap junctions possess two main properties: intercellular adhesion as in adherens and occludens junctions, and the property of intercellular ionic and molecular transfer (39) associated with low resistance electrical communication between excitable cells (40). Gap junctions bypass the extracellular space by transporting messenger molecules and ions. Molecules larger than approximately 2000 daltons are not able to pass through GJs, whereas all molecules smaller than 1200 daltons readily move through these membrane specializations (29). The cell-to-cell communication that is made possible by GJs is dynamically regulated by several external factors, including calcium ion concentration. Cyclic AMP, which is one of a number of substances that can pass through GJs, is an intracellular messenger that regulates a number of important metabolic reactions in cells.
Inner fibers of the ocular lens, for example, contain large amounts of GJ membrane (41); they are not vascularized and demonstrate only limited glycolytic metabolism. The granulosal cell that is contained within a nonvascularized environment also possesses very large GJs. It has been suggested that the excessive junctional membrane in these cells may allow the intercellular diffusion of small metabolites and waste products through an intercellular circulation (42). The combination form of gap and tight junctional elements has been observed in various endocrine organs (43–45) and in the endothelium of vessels in various tissues (45–47). This combination is thought to be a precursor of both types of junction; these structures are sensitive to pH or Ca2+ concentration (48–50) and related to the contraction of the vessels (46). The fact that arachnoid granulations and membranes lack vascularization and are rich in GJs strongly suggests that there is rich intercellular circulation of some metabolites and ions through GJs, and that the intercellular communication through GJs might play a role in adhesiveness in response to changes of milieu such as ICP or circulating metabolites.

Extracellular and Transcellular Pathways

According to the studies of leptomeningeal development in murine and avian embryos, the compact layer around the neural tube, the meninx primitiva, shows the greatest proliferation of arachnoid cells during embryonic days (E) 12–13 (50), followed by the differentiation into inner and outer arachnoid layers by E 14 (51, 52). Subarachnoid space can almost be defined at E14–16 (50, 53), and the typical subarachnoid space of adult type is established by postnatal day 21 (53). GJs and desmosomes are notable in the inner layer at E14, and the TJ's later appear to undergo a process of maturation (52). In addition to the contribution to tight adhesiveness, the morphological complexity of TJ's, as reflected by the number and branching pattern of the sealing strands and associated junctions, correlates well with paracellular permeability; i.e., increased junctional complexity corresponds to decreased paracellular permeability (29). The branching TJ's and cohesion of cell membranes by various types of junctional apparatus made the extracellular cisterns, which act as the CSF pathway in human arachnoid granulations, not merely simple ducts, but two distinct extracellular spaces: continuous (barrier-free) and isolated (circumscribed by several to 50 strands of tight junctional arrays) space. The former (continuous cistern) probably freely communicates between the subarachnoid space along the surface of the brain to spaces beneath the endothelial cells covering the arachnoid villi (13). The large junction-free areas of membrane seen in replicas probably reflects membrane facing extracellular “channels,” and some authors suggest that these channels directly open into the venous sinuses (10). The isolated area showed highly concentrated openings of many pinocytotic vesicles. Tight junctions seal off the intercellular space and prevent the diffusion of molecules through the paracellular pathway (54, 55). The existence of transport function of the amphibian arachnoid has already been reported (56), and Zenker et al recently demonstrated that cationized ferritin can be transcellularly transported in rat spinal arachnoid cells by transcytotic mechanism (57). Although the distinct role of the pinocytotic vesicles relative to the transcellular permeability would only be clarified by specific tracer experiments, these concentrated vesicles in the isolated area do not merely indicate the high biological activity, but also represent the transcellular transport mechanism in human arachnoid cells.

Fig. 6. Freeze-fracture electron micrographs revealing a small cluster of TJ array 3.7 × 2.3 µm in size. a: highly concentrated pinocytotic vesicles (approximately 40 per µm²) are aggregated at this region. Arrows indicate GJs with regularly-arranged pits on E-fracture face. A large gap junction (*) is shown highly magnified in the lower left inset (b) ×35,000. b: a GJ 0.6 × 0.6 µm in size. The fracture plane has jumped between membranes, and both the P- and E-fracture faces are present over a single GJ. The junctional particles are associated with P-face and E-face are marked by arrays of complementary pits. ×60,000.

Microinocytotic Vesicles and Unknown Function of Meninges

Abundant microinocytotic vesicles were observed in the membrane both along large extracellular cisterns and morphologically isolated areas circumscribed by TJ arrays. Two types of vesicles occur: uncoated and coated vesicles. The latter have delicate radial bristles and contain predominantly dissolved proteinaceous material; they are formed by the pinching off of microinocytotic vesicles, budding from ribosome-free areas of rough endoplasmic reticular cisternae, or budding off from Golgi apparatus. These vesicles fuse with one another, form larger vacuoles, and play an important role in transcytosis of macromolecules including transportation of polypeptide products. Nabeshima et al demonstrated that numerous pits are attached to the plasmalemma of arachnoid barrier cells in the rat spinal and cerebellar meninges (54), but they did not define the function of the vesicular structures. Meningioma cells and the lining of arachnoid cysts are thought to be derived from arachnoid cells, since these cells show an almost identical ultrastructure to arachnoid cells, including fine cytoplasmic processes, desmosomes, TJ's, cisternlike extracellular spaces, and
abundant pinocytotic vesicles (13, 38, 58–59). These morphological characteristics have also been linked to explain the mechanism of the formation of cisterne-like structure or intratrumoral cyst formation in arachnoid cysts and meningiomas. In addition, morphological and enzyme-electron cytochemical evidence of secretion of fluid has been reported in arachnoid cysts (60). Other cystic tumors, such as low-grade astrocytomas, infrequently show multiple cystic changes, and pinocytotic activities in the tumor cells are rarely seen. Leptomeningeal cells have been known to contain catechol-O-methyltransferase (14), glutamine synthetase (15), and neurotrophic factors under certain conditions (16–19). Furthermore, Yamashima et al (20) recently demonstrated that human arachnoid cells synthesize prostaglandin D synthase (β-trace), which is the second abundant protein in the CSF. Although the details of the mechanism of secretion of these enzyme is as yet unknown, the many micropinocytotic vesicles observed in this study may contribute to the secretion of such enzymes in addition to some metabolites. It is known that after subarachnoid hemorrhage, erythrocyte blockage, rounding-up of leptomeningeal cells, and remarkable micropinocytosis of arachnoid cells are observed (61, 62). This vesicular formation may also serve as an alternative CSF transport mechanism, such as when the cistern, as a direct channel, is obstructed by cloggings by inflammatory cells or fibrotic and hyperplastic changes of arachnoid villi, in several pathologies including inflammations, meningitis, or chronic hydrocephalus (63–66). The increasing demands of CSF drainage might result in the transcellular transportation of the fluid between adjacent arachnoid cells. Since the details of the morphological changes of junctional complex and pinocytosis of arachnoid cells in pathology are still poorly understood, further study must be carried out.

CONCLUSIONS

In conclusion, the observation of freeze-fracture replicas of human arachnoid granulations clearly revealed the localization and relationship of junctional complexes and vesicles on arachnoid cell membranes. Arachnoid cells were firmly attached to each other by various types of junction to form complex CSF pathways. High transarachnoidal permeability through abundant GJs and micropinocytotic vesicles indicates functions of the regulation of the secretion of enzymes and growth factors and active transport of some metabolites and possibly CSF in normal and certain pathological conditions.

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REFERENCES

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40. Bennett MVL. Physiology of electrotonic junctions. Ann NY Acad Sci 1966;137:509–939


49. Young JD, Cohn ZA, Gilula NB. Functional assembly of gap junction conductance in lipid bilayers: Demonstration that the major 27 kD protein forms the junctional channel. Cell 1987;48:733–43


52. Van RJ, Low FN. Intercellular junctions in the developing arachnoid membrane in the chick. J Comp Neurol 1982;204:32–43


64. De RJ, De CW, Vender EH. Communicating hydrocephalus in treated leukemic patients. Eur Neurol 1979;18:8–14


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