Basal Lamina Formation by Astrocytes in Organotypic Cultures of Mouse Spinal Cord Tissue

HIROFUMI KUSAKA, M.D., ASAO HIRANO, M.D., MURRAY B. BORNSTEIN, M.D., AND CEDRIC S. RAINE, PH.D.

Abstract. The relationship between astrocytes, basal lamina and mesenchymal tissue was analyzed ultrastructurally in myelinated organotypic cultures of mouse spinal cord tissue grown in combination with its pia-arachnoid membrane. A discontinuous, well-developed basal lamina covered flat astrocytic processes which formed the basal layer of the explant opposing the pia-arachnoid membrane. Some astrocytic processes on the surface facing the pia-arachnoid membrane lacked basal lamina, had an irregular surface with microvillus-like protrusions but within the explant they formed intercellular chambers which were surrounded by basal lamina. Even in the presence of mesenchymal tissue which appeared to facilitate the formation of basal lamina in this system, the absence of basal lamina on some areas of the astrocytic plasma membrane suggests regional differences of the astrocytic processes and might reflect the epithelial nature of the astrocyte.

Key Words: Astrocytes; Basal lamina; Epithelial cells; Leptomeninges; Organotypic culture; Spinal cord, mouse.

INTRODUCTION

Organotypic central nervous system (CNS) tissue cultures provide a useful tool in developmental and experimental neuropathology (1-3). However, most studies using this system have focussed on neurons and myelin. In contrast, the astrocyte has been ignored to a great extent (4).

In a previous communication from these laboratories (5), the organization of astrocytes in mouse spinal cord cultures was systematically analyzed and reported as essentially identical to the situation in vivo. However, perhaps related to the lack of mesenchymal elements in vitro, these astrocytes did not possess well-developed basal laminae except for small fragments on the surface of the explant or in the minute intercellular spaces. As proposed by Lyser (6), mesenchymal tissue might exert an important influence upon the formation of basal lamina by astrocytes in vitro. In this paper, the organization of astrocytes in mouse spinal cord culture explanted together with pia-arachnoid membranes has been analyzed ultrastructurally with particular attention to the relationship between basal lamina, astrocytes and mesenchymal elements.

MATERIALS AND METHODS

Anterior portions of the spinal cords from 13- to 14-day-old mouse embryos were dissected together with the associated pia-arachnoid membrane and cultured by techniques described...
FIG. 1. a) A cross section of a mouse spinal cord explant. 20 div shows a loose layer of elongated cells (arrows) below the basal surface of the explant. col: collagen substrate. Pale staining neurons and densely staining oligodendroglia can be seen within the CNS tissue. Toluidine blue. ×240. b) Higher magnification of the basal part of the explant. Several elongated pia-arachnoid cells lie within the collagen substrate (col). Astrocytes (arrows) are seen in the basal part of the explant. Toluidine blue. ×1,200.

Previously (7), the explants were maintained in the usual nutrient medium (7), fixed after 20 days in vitro (div) by immersion for one hour in 2.5% glutaraldehyde buffered to pH 7.3 with phosphate and processed for electron microscopy (EM) as previously described (3).

Epon sections (1.0 µm) were cut from all explants and stained with 1% toluidine blue for light microscopy (LM). Selected areas were sectioned, double stained with uranyl acetate and lead citrate, and examined by electron microscopy (EM).

RESULTS

By light microscopy (LM), each explant which was dome-shaped in cross section and rested on the collagen substrate, consisted of two zones: a central and a peripheral zone (Fig. 1a). This has been described in previous studies (3, 5, 8). The central zone contained neurons with prominent nucleoli, oligodendroglial cells with a small amount of densely staining cytoplasm, and myelinated axons. The peripheral zone was composed mainly of astrocytes and their processes. Occasional macrophages were seen on the upper surface of the explant. In addition, elongated cells formed a discontinuous, two to three cell-thick layer between the spinal cord tissue explant and the collagen substrate (Fig. 1b). These cells had elongated, homogeneously staining nuclei and slender cytoplasmic processes which ran parallel to the surface of the explant.

At the EM level, the organization of the explant observed by LM was confirmed and was essentially identical to that seen in cultures grown without adjacent mesenchymal tissue (3, 8, 9). Astrocytes had homogeneous nuclei and a pale cytoplasm containing glial filaments, glycogen granules, mitochondria, dense bodies, a few microtubules, and the other usual organelles (Fig. 2a), similar to mature astrocytes in vivo (10–12). Astrocytes and their processes, connected by punctate adhesions and gap junctions, formed a continuous layer around the entire perimeter of the explant, in direct contact with the culture environment. In the central zone, flattened astrocytic processes closely contacted and invested some neurons and encompassed occasional synaptic complexes, as in vivo (13, 14). Astrocytic processes with basal laminae occasionally surrounded a small intercellular space (Fig. 2b) (5). Membranous whorls of smooth endoplasmic reticulum (ER) and large mitochondria were also found in the astrocytic processes, especially near the margin of the explant (15).
Fig. 2. a) An astrocyte has a homogeneous nucleus and a cytoplasm containing glial filaments, mitochondria, dense bodies, a few microtubules and other usual organelles. ×5,600.
b) An astrocytic process containing glycogen granules surrounds a minute intercellular space lined with basal lamina (arrows). Beneath the basal lamina, the astrocytic membrane shows hemidesmosomes. ×30,000.
Fig. 3. Elongated cells with well-developed rough endoplasmic reticulum are seen between the collagen substrate and the explant. These cells do not possess basal lamina. Astrocytic processes, which are not covered by basal lamina, are connected to each other with junctional complexes, and from there extend microvillus-like processes. ×6,700.

Spindle-shaped cells with homogeneous nucleochromatin were aligned discontinuously between the explant and the collagen substrate (Fig. 3). These cells, also linked to each other by junctions, did not possess basal laminae (12). The cytoplasm contained well-developed and often distended rough ER, in addition to mitochondria and vesicles. Some of these cells were closely apposed to the surface of the explant, whereas others lay away from the surface like pial cells in vivo (12, 16–18).

Along most of the basal surface of the culture, a well-developed basal lamina was observed on the astrocytic processes which formed a continuous layer in contact with or facing the pia-arachnoid cells and the collagen substrate (Fig. 4a). The surface of astrocytic processes covered by basal lamina was usually flat, but sometimes had infoldings that were also covered by basal lamina (Fig. 4b), essentially the same as in vivo (16–18). However, the basal lamina was not continuous over the entire surface of the explant in contact with the pia-arachnoid membrane (Fig. 5a, b). When not covered by basal lamina, astrocytic processes usually had an irregular surface with many microvillus-like protrusions. Occasionally, along the same astrocytic process apposing the pia-arachnoid membrane, some portions were covered by basal lamina, while others lacked basal laminae and possessed microvillus-like processes (Fig. 5b). Although some astrocytic processes had irregular surfaces and microvillus-like pro-
Fig. 4. a) The basal surface of an explant faces the pia-arachnoid membrane (not shown) and consists of astrocytic processes linked by junctional complexes, which are covered continuously by a well-developed basal lamina (arrow). Glial processes contain abundant glial filaments, glycogen granules, large mitochondria and hemi-desmosomes. ×16,000. b) The basal surface of an explant facing the pia-arachnoid membrane shows infoldings of astrocytic processes, which are covered by basal lamina (large arrow). Although a portion of pial cell (small arrows) containing well-developed rough endoplasmic reticulum invaginates astrocytic processes, it is not covered by basal lamina. Several elongated pial cells with many vesicles lie in the collagen substrate below. ×6,700.
Fig. 5. a) Astrocytic processes from the basal aspect of the explant on the left are flat and covered by basal lamina. Astrocytic processes on the right have no basal lamina on the surface of the explant but have irregular surfaces with microvillus-like processes. A small intercellular channel is surrounded by astrocytic processes with basal lamina (arrow). ×6,000. b) The basal lamina, covering the flat surface of an astrocytic process, ends at a point (small arrow), from which astrocytic processes become irregular and have microvillus-like processes. Fragments of basal lamina are seen inside the explant (large arrows). Flat cells containing vesicles, well-developed rough endoplasmic reticulum and mitochondria lie below. ×13,000.
Fig. 6. The lower surface of an astrocytic process faces the pia-arachnoid membrane and has no basal lamina. The upper surface facing an intercellular space is lined by basal lamina (large arrow). Note the hemi-desmosomes. × 20,000.

trusions without basal lamina on the basal aspect of the explant, the same processes possessed well-developed basal laminae on the surface surrounding small intercellular chambers within the explant (Fig. 6). Hemidesmosomes were always present on the region of plasma membrane which demonstrated basal lamina.

In contrast to the basal surface of CNS explants, the astrocytic processes which formed the continuous upper layer in contact with the nutrient medium did not possess basal laminae. These were swollen and clear in this study, a pia-arachnoid membrane was never seen on the upper surface of explants.

DISCUSSION

Our previous communication on organotypic mouse spinal cord cultures (5) reported that the cytoarchitecture of astrocytes in vitro was essentially identical to that seen in vivo. However, in contrast to astrocytes in vivo, the astrocytes described had only minute fragments of basal lamina on the surface of the tissue or around small intercellular spaces (5). In the present study, explants grown with associated pia-arachnoid membrane displayed the expected well-developed basal laminae between astrocytic processes and the pia-arachnoid cells, as reported by Lyser (6). Otherwise, the fundamental organization of astrocytes in the explant was the same as that in culture grown without pia-arachnoid membrane. However, not all astrocytes or their processes facing the pia-arachnoid membrane possessed basal laminae even though they were exposed to the same culture environment. Occasionally, even on the same astrocytic process apposed to adjacent leptomeninges, only some portions had basal lamina material.

As pointed out previously (5), basal laminae seen in this system at this stage of development (20 div) might represent persistent remnants of the original primitive
external glia limitans present when the tissue was explanted (19). On the other hand, since the elements of the explant had been reoriented and reorganized in the culture environment, the glia limitans had possibly become disrupted, thus producing the observed discontinuity in the basal lamina.

The explants used in the present study were obtained from embryonic mouse spinal cord. Since astrocytes in vivo continue to develop throughout the embryonic and early postnatal periods (20, 21) and since astrocytes develop asynchronously, the explants probably contain astrocytes at various stages of differentiation (21). Thus, the discontinuity of the basal lamina might reflect the developmental stage of astrocytes. However, consistent ultrastructural differences between astrocytic processes with and without basal lamina could not be found.

Since astrocytes are ectodermal in origin, it might be anticipated that they retain some epithelial cell characteristics (22) such as junctional complexes and continuous basal laminae on subpial and perivascular surfaces. In other words, the plasma membrane of astrocytes exhibit regional differences corresponding to the apical, lateral and basal surfaces of the epithelial cell, although not readily evident because of the complex architecture and absence of the apparent lumen surrounded by astrocytic processes in vivo. Accordingly, astrocytic processes in vitro presumably derived from the apical or lateral surface of the cell body, might not possess basal laminae even in the presence of mesenchymal tissue. Only those cell processes derived from the basal surface of the cytoplasm might have the ability to form or maintain basal lamina and this might be facilitated by the presence of mesenchymal tissue.

On the whole, astrocytes forming the basal layer in contact with the pia-arachnoid membrane and those forming the superficial layer in contact with the culture medium tended to show polarity. In addition, individual astrocytes in the basal layer also possess cytological polarity in regard to the presence of basal lamina, and together, these appearances probably reflect the epithelial nature of the astrocyte.

REFERENCES


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