Alterations in the Neuroepithelial Basal Lamina in a Neurological Mutant with Prenatal Hydrocephalus

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Abstract. The neuroepithelial basal lamina (BL) appears to be crucial in controlling cell-cell interactions during the early histogenesis of the nervous system. In this investigation we examined the changes in the BL in a neurological mutant of the rat previously characterized as having BL anomalies which progress to aqueductal stenosis and prenatal hydrocephalus. Embryos were obtained from matings of rats homozygous for the prenatal hydrocephalus gene or from controls, both originally derived from the same Wistar albino stock. On days 12 and 13 of development, embryos were processed for indirect immunofluorescence localization of BL components type IV collagen or laminin. Additional whole litters were processed for ultrastructural analysis of neuroepithelial and BL morphology. In control embryos, neuroepithelial BL components formed a smooth linear boundary to the basal surface of the neuroepithelium. This unbroken border was interrupted only in regions of active neural crest cell migration (day 12), and in areas of imminent vascularization (day 13). In hydrocephalic embryos on the 12th day there were gaps in the continuity of the BL and an apparent reduction in deposition of type IV collagen. By day 13, blood vessels had prematurely colonized the neuroepithelium, few BL breaks were observed, and deposition of type IV collagen appeared similar to that seen in control embryos. Ultrastructurally, a similar pattern of change was observed. The neuroepithelium of control embryos was uniformly bounded by an organized BL consisting of a lamina lucida subjacent to the plasma membrane, and a lamina densa which merged with scattered collagen fibrils in the mesenchymal compartment. On day 12, the BL of hydrocephalic embryos had large gaps through which neuroepithelial cells projected. In addition, areas of redoubled BL material were present. By day 13, the BL appeared similar to that of control embryos. The BL discontinuities and redoubling seen in hydrocephalic embryos are discussed in relation to the role of the BL in supporting and providing functional polarity to neuroepithelial cells.

Key Words: Basement membrane; Immunocytochemistry; Laminin; Neuroepithelium; Rat embryo; Type IV collagen; Ultrastructure.

INTRODUCTION

It has been suggested that embryonic basal laminae (BL) function both as passive support for an epithelium and also to control information transfer between epithelium and subjacent mesenchyme (1). Unlike BL of other epithelial organs which are compositionally well characterized, the structure and regional variations of the neuroepithelial BL have received little attention. Like other BL, it is known to contain hyaluronate (2), laminin (3, 4), fibronectin (5, 6), entactin (4), and heparan sulfate proteoglycan (3) during neurulation. However, possible regional variations in structure and composition as well as the role of the BL in later phases of development remain to be determined.

In this investigation, we examined the neuroepithelial BL in a neurological mutant.
of the rat in which discontinuities in the BL were correlated with neuroepithelial disorganization, and aqueductal stenosis leading to hydrocephalus (7). To examine the underlying basis for these anomalies, we carried out a detailed immunocytochemical study of the pattern of deposition of two structural BL components, laminin and type IV collagen, and examined the early alterations in the BL at the ultrastructural level in these embryos.

MATERIALS AND METHODS

Tissue

Embryos were obtained from matings of rats homozygous for a gene which results in prenatal hydrocephalus (7) or from rats of Wistar origin (controls). The mutation arose spontaneously in the 50th generation of consecutively brother–sister-mated, normal albino rats that originated from Wistar stock. The mutants were segregated to constitute a divergent line now nearing 50 generations of brother–sister mating. The normal, parent line (controls) has been continued and is now in its 100th generation.

Animals were mated and the day sperm was found in the vaginal smear was considered the first day of pregnancy. On the 12th or 13th day, decidual swellings were removed from females under ether anesthesia and embryos dissected from decidua and chorion. Embryos were examined for the presence of external anomalies and were then staged and fixed as described below.

For immunocytochemistry, embryos were fixed in a solution of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer for one hour (h) at room temperature. They were then washed in phosphate buffer, cryoprotected in 20% sucrose in phosphate buffered saline (PBS), and frozen in OCT (optimal cutting temperature) embedding compound (Miles) by immersion in hexane cooled over an acetone/dry ice slurry. Blocks were stored at −70°C before sectioning at 6–8 micrometers using an International cryostat. Transverse sections were collected on high molecular weight (350,000) poly-l-lysine (0.1%) coated glass slides and stored at −70°C before immunocytochemical staining.

Sections were air dried, exposed to 5% normal goat serum followed by the primary antibody for one h at room temperature. They were washed in PBS and exposed to the second antibody (goat anti-rabbit IgG) conjugated to FITC (1:50, Cappel) for 30 minutes at room temperature. Sections were washed extensively in PBS, coverslipped with glycerol containing 0.1% phenylenediamine and sealed with nail polish. Controls were incubated in preimmune serum or in PBS in place of the primary antibody. Sixteen hydrocephalic and 12 control embryos were processed for immunocytochemical staining.

Antibodies to laminin were obtained from Dr. J. Varani, University of Michigan; and antibodies to type IV collagen from Dr. H. Furthmayr, Yale University. Both antigens were isolated from EHS tumor, raised in rabbits, were affinity purified and exhibited no cross reactivity by ELISA. Anti-laminin was used at a concentration of 1:50 and anti-type IV collagen 1:100.

After staining, slides were viewed and photographed in a Leitz Dialux Orthoplan photomicroscope using ultraviolet light. Immunofluorescence was recorded using Kodak 2475 Recording film.

For light (LM) and transmission electron microscopy (TEM), embryos were fixed in 1% glutaraldehyde and 1% tannic acid (8) in 0.075 M phosphate buffer for one h at room temperature. Embryos were then osmicated, dehydrated and embedded in Araldite resin. Thick sections were cut, heat-affixed to glass slides, and stained with 1% toluidine blue for LM. Thin sections were collected on grids, double stained with uranyl acetate and lead citrate, and viewed and photographed on a Philips 400 transmission electron microscope. A total of 14 hydrocephalic and ten control embryos were examined using TEM.

RESULTS

By the 12th and 13th days of development, control embryos had developed 25–30 and 33–40 somites respectively, the neural tube had closed throughout its length.
and the facial region was forming. Optic pits and well-formed branchial arches could be seen on the surface of the cephalic region of these embryos. The external appearance of the hydrocephalic embryos has been described in detail (9). Briefly, they were characterized by a reduction in the midface and nasal region, with altered vibrissal patterns, a midbrain that was often very domed, and the eyes abnormally formed and positioned compared with controls.

Immunocytochemistry

In control embryos examined on the 12th day of development, laminin and type IV collagen outlined the smooth basal surface of the neuroepithelium (Fig. 1A, C). Staining was slightly more intense near the midline, becoming less intense in regions of neuroepithelial bending (near the optic vesicles) and in regions of neural crest migration. Laminin and type IV collagen also were found in BL of the surface ectoderm, otic vesicles and particularly in blood vessels forming in the region.

On the 13th day of development in control embryos, the pattern of deposition of laminin and type IV collagen similarly outlined the basal surface of the neuroepithelium and were found in BL of blood vessels, and surface ectoderm as well (Fig. 2A, C). Rather than the smooth linear pattern seen on day 12, staining of the basal surface of the neuroepithelium was occasionally interrupted by the presence of vessels next to but not yet within the neuroepithelium (Fig. 2C).

In hydrocephalic embryos, staining of the neuroepithelial BL components resulted in a pattern that was often broken and uneven. Large gaps in the BL were observed particularly on the 12th day of development (Fig. 1B, D), but were also present on the 13th day (Fig. 2B). On the 12th day blood vessels approached, but did not yet penetrate the neuroepithelial BL in hydrocephalic embryos (Fig. 1B). However, by the 13th day, blood vessels containing nucleated red blood cells were seen within the neuroepithelium of hydrocephalic (Fig. 2D) but not control embryos.

In day 12 embryos, staining for type IV collagen was less intense, although clearly present, in the neuroepithelial BL of hydrocephalic compared with control embryos (compare Fig. 1C and D). By day 13, however, fluorescence was similar to that seen in controls (Fig. 4C, D).

Microscopy

Histologically, the neuroepithelial cells from both hydrocephalic and control embryos exhibited their normal pseudostratified appearance, with cell processes typically contacting luminal and basal surfaces. Neuroepithelial cells were joined at their apices by highly developed junctional complexes, with occasional microvilli and microfilament bundles at the luminal surface. Interphase nuclei were located in the basal third of the cells, and a well developed Golgi complex and considerable rough endoplasmic reticulum were found throughout the cytoplasm.

At the basal surface, the BL of control embryos consisted of a lamina lucida next to the plasma membrane and a thick lamina densa bordering the mesenchymal compartment (Fig. 3A). Collagen fibrils were often associated with the lower surface of the lamina densa. The BL formed a smooth, unbroken border to the neuroepithelium, except in regions of active neural crest migration present in the earlier embryos. There was little change between days 12 and 13 in control embryos except that by the 13th day, blood vessels often approached the BL, and the basal surface appeared less even, and more wavy. In addition, in day 13 embryos, the long collagen fibrils occasionally were positioned parallel to the BL (Fig. 4A), unlike on day 12 when they were more typically oriented at angles to it.
Fig. 1. Immunocytochemical demonstration of BL components laminin and type IV collagen in control (A, C) and hydrocephalic embryos (B, D) on the 12th day of development. A. Anti-laminin staining of the neuroepithelial basal lamina in a control embryo. Note its linear deposition in the basal lamina (arrowheads). Laminin is also associated with basal laminae of blood vessels and surface ectoderm. B. Unlike the even distribution of laminin in the neuroepithelial BL of control embryos, in hydrocephalic embryos there were numerous focal gaps (arrowheads) in the BL. C. Neuroepithelial BL (arrowheads) in a control embryo illustrating the pattern of deposition of type IV collagen. D. In mutant embryos on the 12th day, there were focal gaps in type IV collagen deposition (arrowheads), and overall, its distribution was sparse and patchy compared with controls. NE = neuroepithelium. Bars = 50 micrometers.

In day 12 hydrocephalic embryos, the basal surface of the neuroepithelium was considerably different. In many regions, the BL was pulled away from the NE cells, sometimes looping over itself, suggesting the presence of excess BL material (Fig. 3B). Alternatively, the BL sometimes appeared patchy and incomplete, or was totally absent (Fig. 3C). Often, few or no collagen fibrils (Type I) were associated with the BL, even in regions with seemingly excessive amounts of BL material. In regions lacking BL, the basal portion of the neuroepithelium, rather than remaining tightly apposed to adjacent cells, became disorganized. Large intercellular spaces were common, and portions of the basal cytoplasm projected into the mesenchyme. Occasionally, the atypical intercellular spaces were filled with ectopic cell processes (Fig. 3D). The actin filaments, normally oriented parallel to the basal plasma membrane, also were reorganized in regions of BL disruption and extended into the cytoplasmic processes.

Mesenchymal cells often made premature contact with the neuroepithelium both
Fig. 2. Transverse sections through the cephalic neuroepithelium (NE) of control (A, C) and hydrocephalic mutant (B, D) embryos on the 13th day of development. A. Anti-laminin immunoreactivity in the BL (arrowheads) of a control embryo. Its deposition is similar to that seen on the 12th day of development. M = mesenchyme. B. Similar region from a hydrocephalic embryo illustrating anti-laminin staining in the region. Focal gaps are still occasionally present in the BL (arrowheads). C. Anti-type IV collagen staining in a control embryo illustrating its even deposition except in regions of imminent blood vessel (b) invasion. D. Type IV collagen is more uniformly deposited in the neuroepithelial BL on the 13th day than at earlier stages. In many of these embryos, blood vessels (b) had already penetrated the NE, unlike controls. Bars = 50 micrometers.

in regions of disorganization as well as in areas in which the BL appeared ultrastructurally normal. Although the blood vessels which were forming often approached the neuroepithelium, they typically failed to penetrate it at this stage. In more advanced embryos (38–40 somites) the endothelial cells sent spike-like processes through the BL, between neuroepithelial cells as a prelude to overt invasion.

Surprisingly, by day 13 in hydrocephalic embryos, the neuroepithelial BL appeared very similar to that of control embryos; there were few focal breaks except in association with blood vessel colonization. The basal surface was occasionally slightly wavy (Fig. 4B) but the number and orientation of collagen fibrils was similar to that seen in controls (Fig. 4B–D). Intercellular spaces were slightly more common in hydrocephalic embryos than in controls and there appeared to be a small increase in the number of what has been termed “gap junctional vesicles” in the neuroepithelium of these embryos. Finally, in the neuroepithelium of hydrocephalic embryos examined on days 12 and 13, there was evidence of very active protein synthesis within neuroepithelial cells with ribosomes often clustered around pockets of secre-
Fig. 3. A. Transverse section through the base of the forebrain neuroepithelium of a day 12 control embryo. The basal surface is even, the BL (bracketed) is well formed and scattered collagen fibrils are associated with it. B. Transverse section through the neuroepithelium of a day 12 hydrocephalic embryo illustrating the retracted and doubled BL (arrowheads) often seen in these embryos. There is a considerable amount of BL material, but very few collagen fibrils in this region. There is also a dense mat of actin filaments just within the plasma membrane of this cell. C. Similar section through the forebrain region of a hydrocephalic embryo. There are large regions of BL discontinuity (between arrowheads) and an increase in intercellular space in the neuroepithelium of these embryos. Collagen fibrils were not present in the mesenchyme. D. Basal surface of the neuroepithelium in a hydrocephalic mutant illustrating breaks (arrowheads) in the continuity of the BL and the presence of many ectopic cell processes (p) between neuroepithelial cells. Bars = 0.5 micrometer.

Vestatory material. These features were also observed in controls but not with the same frequency.

Striking regional variations in BL structure were not noted except related to active neural crest migration from the neuroepithelium as mentioned above.

DISCUSSION

These results support and extend studies of the critical role of the neuroepithelial BL in the organization of the developing nervous system. They also highlight the remarkable degree of recovery possible within the neuroepithelium.

A number of neurological mutants of the mouse are characterized by anomalies of basal lamina composition (10, 11), or structure (12–14). Although these studies have focused on the period of neurulation, it has been suggested that at later stages of development, alterations in BL composition might be involved in neuritic out-
growth, in regulating regional patterns of proliferation and possibly cell migrations within the central nervous system (CNS). To date, there have been no compositional studies of the neuroepithelial BL in later phases of normal or in abnormal development.

In the hydrocephalic mutant described here, focal discontinuities in the BL appear to result in neuroepithelial disorganization, overgrowth and finally aqueductal occlusion with prenatal hydrocephalus (7). Irradiation of similarly staged embryos also produces a similar spectrum of BL breakdown, neuroepithelial disorganization and hydrocephalus (15). These observations suggest a high regional specificity, and also suggest that the neuroepithelial cells themselves may be the source of the BL anomalies.

As in neoplastic metastasis where BL rarefaction and breakdown is a prelude to overt invasion (16), neuroepithelial cells became disorganized, and reorganized their basal cytoskeleton in the absence of a complete BL. Several alternative explanations
might be invoked to explain these features. Most simply, since BL, particularly
the neuroepithelial BL, may provide a rigid substrate for the neuroepithelium during
development, focal gaps in its continuity would deprive cells of a stable base. Al-
ternatively, because many extracellular matrix and BL components (e.g. laminin,
fibronectin, heparan sulfate proteoglycan) are in transmembrane linkage with the
actin cytoskeleton (17, 18), the focal lack of BL components might also alter cellular
polarity and junction formation, with resulting local disorganization. Finally, the
neuroepithelial cells themselves may be abnormal, i.e. they may be synthetically
compromised, as reflected in an inability to produce a functional BL, or affecting
the secretion of junctional or other proteins required to maintain epithelial cell
polarity.

Similarly, the basis for discontinuities in the BL is uncertain. By analogy to met-
astatic cells, one would predict a lack of synthesis of BL components, which is
supported somewhat by the lag in type IV collagen deposition in hydrocephalic
embryos. Alternatively, synthesis may be normal but linkage to the cytoskeleton,
binding to the cell surface, or targeting to the baso-lateral surface may be impaired.
Synthesis, to judge by the number of protein–ribosomal aggregates observed, is, if
anything, stimulated in the hydrocephalic embryos. However, the presence of dou-
bled BL would seem to indicate an inability of the material to interact normally
with the plasma membrane. As in muscle regeneration (19) the presence of multiple
layers of BL may mark areas of earlier cell death. However, serial section analysis
of these embryos has indicated there is little neuroepithelial cell death.

There is an overall developmental delay of 12–24 h in hydrocephalic embryos as
compared with controls. A lack of synchrony between mesenchyme and neuroepi-
thelium resulting in the untimely absence or presence of a particular BL component
would alter the developmental program and could result in the premature contact
of the neuroepithelial BL by mesenchyme which we observed. Finally, premature
invasion of the neuroepithelium by capillary-forming cells could result because of
an abnormal BL configuration, or in response to an abnormal cellular product re-
leasing phagocytic cells into the region. These possibilities form a series of hypotheses
testable using a combined approach of in situ hybridization, electron microscopic
immunocytochemistry, and synthetic studies may be useful.

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