Development of Reichert's membrane in the early mouse embryo

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Abstract Although the composition of Reichert's membrane, a thick multilayered basement membrane between the parietal endoderm cells and the trophoblast cells of rodents, has often been investigated, the site of its production remains a subject of controversial discussion. In particular, the role of the trophoblast cells is unclear. In the present work we examined the initial development of Reichert's membrane in the early mouse embryo, using glutaraldehyde fixation with tannic acid. In the early blastocyst the occurrence of a tannic-acid-positive layer located at the inner surface of the mural trophoblast indicated the onset of basement membrane formation by the trophoblast cells. In the peri-implantation phase, this basement membrane extended into lateral areas of the inner cell mass separating the newly differentiated ectoderm and endoderm cells from each other. In these lateral regions, where the recently formed primitive endoderm cells had been attached to the monolayered basement membrane of the mural trophoblast, the membrane began to reveal the typical multilayered structure of Reichert's membrane. Our findings indicate that the initial formation of Reichert's membrane begins with the formation of a basement membrane of the mural trophoblast cells, followed by an apposition of basement membrane material, probably synthesized by primitive endoderm cells, along this primary membrane.

Key words Basement membrane · Mouse embryo · Reichert's membrane · Tannic acid fixation

Introduction

Reichert's membrane is a basement-membrane-like layer composed of collagen and noncollagenous glycoproteins lying between parietal endoderm cells and trophoblast cells in rodent embryos. This membrane probably acts as a filter allowing free access of nutrients to the embryo while excluding maternal cells (Gardner 1983). It may also play a role in the migration of the parietal endoderm cells to form an inner lining next to the trophoeoderm cells (Carnegie and Cabaca 1991). The chemical composition of Reichert's membrane resembles that of other basement membranes, consisting of type IV collagen, laminin, nidogen, fibronectin and heparan sulphate proteoglycan (Inoue and Leblond 1988). Due to its thickness and its easy separation from surrounding embryonic and maternal tissues, this membrane has often been used as a model for studies concerned with basement membrane fixation, composition (Hogan et al. 1984) and pathological thickening (Timpl and Dziadek 1986).

For many years, there has been discussion as to whether Reichert's membrane, a multilayered basement membrane, is a product of the trophoblast cells or the endoderm cells of the early mouse embryo. In vitro studies clearly demonstrate that the parietal endoderm cells produce basement membrane components, which together build Reichert's membrane (Cockroft 1987; Fatemi 1987; Kurniken et al. 1983; Laurie et al. 1982; Mazariagos et al. 1987; Smith and Strickland 1981). Fowler et al. (1990) showed that mouse embryo-derivated parietal endoderm cell lines produce basement membrane components such as type IV collagen, laminin, nidogen, BM-40 and heparan sulphate proteoglycan and it is now generally accepted that Reichert's membrane is synthesized by parietal endoderm cells. However, some authors have discussed the contribution of the trophoblast cells to the production of this membrane (Carnegie 1991; Jollie 1968; Schlafke and Enders 1963). In a previous study dealing with the formation of Reichert's membrane in vitro, we found some evidence that not only the parietal endoderm cells but also the trophoblast cells take part in the formation of Reichert's membrane (Salamat et al. 1992).

Nadijcka and Hillman (1974) and Enders et al. (1978) had previously described patchy, amorphous material between the inner cell mass and the trophoblast cells. Wartiovaara et al. (1979) localized fibronectin in
the accumulating extracellular matrix material at the blastocoele side of the trophoblast. Leivo et al. (1980) described the appearance of collagen and laminin in the early mouse embryo, localizing these glycoproteins in early basement membrane material. Dziadek and Timpl (1985) localized nidogen and laminin in the early blastocyst.

Jollie (1968) had reported that an electron-dense layer about 75 nm wide with a 50-nm-thick electron-light area adjacent to the trophoblast cells formed a trophoblastic epithelial lamellar complex, resembling a basal lamina. Furthermore, he suggested that Reichert’s membrane might well represent the fused epithelial basal laminae of the two germ layers between which it arose. However, as he accurately pointed out, the problem was that the origin of the membrane could hardly be ascertained by observing its fine structure when it was already differentiated. To resolve this problem we investigated the development of Reichert’s membrane from the morula stage (72 h post-coitus) to the post-implantation stage (day 5 post-coitus) in vivo.

The present ultrastructural investigation, using a glutaraldehyde fixation followed by tannic acid application, was carried out to clarify in vivo how trophoblast cells and/or parietal endoderm cells are involved in the onset of the formation of Reichert’s membrane. This period of development includes the steps from the first detectable electron dense extracellular matrix material outside a cell membrane to a monolayered basement membrane, and ends with the fully developed multilayered Reichert’s membrane.

Materials and methods

Preparation of embryos

Twenty NMRI (New Mexico Research Institute) mice were kept on a normal day/night cycle and received Altromin commercial food and water ad libitum. Day 0 of gestation was defined as starting at 11 a.m. of the day on which a vaginal plug was detected after a mating period of 3 h. On days 2–5 of gestation the pregnant mice were anaesthetized with ether, killed by cervical dislocation, and oviducts and uteri removed. The oviducts and uteri were transferred to 0.1 M phosphate-buffered saline (PBS) at 4 °C, the oviducts sealed with a clamp and buffer solution injected into the cæcum uteri from the vaginal side. When a high pressure had built up, the clamp was quickly opened and the embryos flushed out through the oviduct into the buffer solution.

Fixation of embryos

After collection with a glass pipette under a stereo-microscope, all embryos were fixed for 2 h in 1% glutaraldehyde in 0.1 M PBS supplemented with 1% tannic acid. The embryos were then post-fixed for 1 h in 1% osmium tetroxide, dehydrated in an ethanol series up to 100% and embedded in Epon (Herken and Barrach 1985). For orientation purposes, 1-μm-thick sections were cut and stained with toluidine blue. For electron microscopy, ultrathin sections were cut with a Reichert ultramicrotome and collected on formvar-coated copper grids, stained for 10 min with uranyl acetate and for 5 min with lead citrate and examined with a Zeiss EM 109 electron microscope.

Number of embryos

Some embryos were lost during the embedding procedure and in some cases there was insufficient penetration of the tannic acid fixation; the final results were based on: 12 embryos of the early morula stage, 14 embryos of the late morula stage, 16 embryos of the blastocyst stage with zona pellucida, 10 embryos of the blastocyst stage without zona pellucida, 18 embryos of the peri-implantation stage, and 12 embryos at day 5 (post-implantation).

Results

Morula stage (72–84 h)

No tannic-acid-positive structures bearing any resemblance to material of a developing basement membrane could be found in the early or in the compacted morula. Aggregations of granular tannic acid-positive structures with a diameter of 30 nm occur in extracellular spaces between the zona pellucida and the adjacent blastomeres.

Blastocyst stage (84–96 h)

In the early blastocyst, still surrounded by a zona pellucida, the first tannic-acid-positive structures which could be taken to be an early indication of the onset of the formation of a basement membrane (Fig. 1) were observed. These stained structures constituted an electron-dense layer of irregular thickness at the inner surface of the mural trophoblast cells that is oriented towards the blastocyst cavity (Fig. 2). In contrast to a typical basement membrane, the tannic-acid-positive layer was directly attached to the trophoblast cells and not separated from them by a lamina lucida or rara. Some short tannic-acid-positive fragments were also seen in the blastocyst cavity. At the border between the mural trophoblast cells and the inner cell mass, the stained layer ceased to exist. At the surface of the inner cell mass, oriented towards the blastocyst cavity, this tannic-acid-positive layer was no longer visible.

In contrast to the mural trophoblast cells, the tannic-acid-positive layer was not observed at the inner surface

Fig. 1 Early blastocyst of a mouse surrounded by a zona pellucida (arrowheads). ICM, inner cell mass. Square, area of the mural trophoblast from which Fig. 2 was taken. ×1,350

Fig. 2 Higher magnification of a mural trophoblast (T) with tannic-acid-positive layer at the inner surface (arrowheads). Z, zona pellucida. ×9,900

Fig. 3 Peri-implantation embryo of mouse with initial differentiation of the inner cell mass into ectoderm (Ec) and endoderm (En). Squares, areas of the embryo from which Figs. 4–6 were taken. ×980

Fig. 4 Higher magnification of the initial formation of the parietal endoderm (En). T Mural trophoblast cells. Arrowheads, basement membrane of the mural trophoblast. ×6,300

Fig. 5 Higher magnification of a mural trophoblast (T). Arrowheads, tannic-acid-positive basement membrane of the mural trophoblast. ×33,100