INTRODUCTION

The basal lamina is a scaffold whose integrity is required for maintenance of ordered tissue structure and the regulation of cell growth within an epithelium. When tissue remodeling is to occur, it must undergo complex changes. The ability of invasive cells including those of malignant tumors to move across a basal lamina is thought to require the acquisition of specific properties (Vracko, 1974; England, 1982; Foltz et al., 1982; Gospodarowicz et al., 1982; Ingber and Jamieson, 1982; Bernfield et al., 1984; Liotta et al., 1983; Liotta et al., 1986; Gehlsen et al., 1988).

Implantation of the mammalian embryo in the uterus provides an example of invasion by a non-malignant type of cell, the trophoblast. The invasive properties of the trophoblast are very impressively shown when blastocysts are transplanted to ectopic sites, e.g., under the kidney capsule (Kirby, 1960). However, in the uterus, the process of trophoblast invasion is limited in time and space, so that the host organ avoids destruction. There is evidence that the process seems to be regulated from both sides, the trophoblast (invasiveness limited in time) and the uterus ("permissiveness" limited). Details of the interplay between both partners are, however, largely unknown.

Penetration of the trophoblast through the basal lamina has been studied in several species by electron microscopy (cf. Schlafke and Enders, 1975). In general, the morphological analysis of successive states has led to the conclusion that the trophoblast pauses, after penetration through the uterine epithelium, at the residual basal lamina before continuing with penetration into the stroma. However, one report gave surprising evidence that, in the rat, it is not the trophoblast but decidual cell processes that are the first to penetrate the basal lamina of the uterine epithelium (Schlafke et al., 1985).

The present communication describes observations on structural changes in the basal lamina of the uterine epithelium that can be observed in implantation chambers of the rabbit. Comparison is made between the changes that occur in the vicinity of the invading trophoblast, in parts of the implantation chamber which the trophoblast has not reached yet, and blastocyst-free segments of the uterus. It is found that considerable alterations of the uterine epithelial basal lamina do occur.

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even independent of contact with the invasive trophoblast, and they provide evidence for an active role of the uterine epithelium in remodeling this structure in preparation for embryo implantation.

MATERIALS AND METHODS

Female rabbits of mixed breeds were housed and mated as previously described (Denker, 1977). Two animals of each of the stages 6, 7, and 8 days post coitum (d p.c.; 6 d p.c. = 144 hours p.c.) were included in this investigation. For comparison, 2 animals of the non-pregnant, estrous stage were also studied, behavioral estrus being defined as lack of mating response after a couple of trials by two different vigorous bucks.

Perfusion fixation via the abdominal aorta was performed as previously described (Denker, 1977) using 0.1 M cacodylate buffer (pH 7.4) for the pre-rinse followed by a mixture of 2.5% glutaraldehyde and 2% formaldehyde in the same buffer (Karnovsky, 1965), at room temperature for about 10 minutes. Isoosmolarity of the buffer was obtained by addition of sodium chloride. The excised uteri were stored in the same fixative at 3-4°C for 24 hours. In order to detect morphological signs pointing to local influences of the blastocyst on the surrounding part of the endometrium, implantation chamber and blastocyst-free segments of the uteri were cut out in fixative and were investigated separately, from 6 d p.c. onwards. Uterine segments were rinsed in 0.1 M cacodylate buffer (with 5% saccharose), postfixed in 2% osmiumtetroxide and some of them additionally in 4% tannic acid in the same buffer, dehydrated in ethanol, bloc-stained with uranyl acetate, and embedded in araldite epoxy resin.

Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM 10 transmission electron microscope.

Figure 1. Uterine epithelium of a non-pregnant animal; the cylindric cells form plump foot processes anchoring in the stroma. X5700.

Figure 2. A well structured basal lamina with a lamina lucida and a lamina densa underlies the basal cell processes described in Figure 1. X45000.

Figure 3. Basal lamina of a uterine epithelium 6 d p.c.; the lamina lucida is not as clearly delineated, is reduced in width and electron dense material bridges the lamina lucida in many places. Notice higher magnification than in Figure 2. X60000.

Figure 4. Basal lamina region of the uterine epithelium in the blastocyst-free segments (8 d p.c.). Dark stained amorphous material (arrow) is accumulated in the region of the basal lamina. X38500.
RESULTS

Non Pregnant Animals

The epithelial lining of the rabbit endometrium consists, in the non-pregnant state, of columnar cells decorated with sparse microvilli, and of a few scattered ciliated cells. The epithelial cells are basally anchored in the stroma with plump foot processes (Figure 1). The whole basal surface of the cells including these foot processes is underlain by a well-developed, typically structured basal lamina with a distinct lamina densa (28 nm in diameter) and lamina lucida (30 nm in diameter) (Figure 2). The morphology of this basal lamina is the same in the different parts of the mucosa including luminal and cryptal epithelium. Foot processes, however, are not seen in the deepest parts of the crypts.

The endometrial stroma is relatively rich in collagen and appears to have more collagen per unit volume than in the pregnant state.

Figure 5. Trophoblastic knob (T) attaching to the antimesometrial uterine epithelial surface 7 d p.c.; in this region the trophoblast has not yet penetrated the uterine epithelium. X4800.
Figure 6. Higher magnification of the basal lamina in a similar region as indicated in Figure 5 reveals a fuzzy lamina densa, whereas a lamina lucida is missing. X172000.

Periimplantation Phase

At 6 d p.c., i.e., one day before implantation starts, the rabbit blastocyst is immobilized in the forming implantation chamber but the trophoblast is still non-attached, the blastocyst coverings being still complete. Here as well as in the blastocyst-free segments most of the luminal cells of the endometrium show conspicuous apical protrusions which contain various cell organelles, and their apical plasma membrane is covered by sparse microvilli. The basal membrane underlying this epithelium follows a fairly smooth contour, since basal cell processes are now lacking in contrast to the non-pregnant state. The fine structure of the basal lamina is slightly changed. The lamina densa is thickened (40 nm) and does not appear as clearly delineated as previously (Figure 3). Electron dense material of the same texture as the lamina densa bridges the lamina lucida in many places. These discrete changes are seen at the uterine epithelium of the implantation chamber as well as in the blastocyst-free segments. Due to epithelial proliferation, the stromal space is now considerably reduced as compared to the non-pregnant state, and collagen fibers are less densely packed.

At 7 d p.c. when implantation starts in the abembryonic-antimesometrial region, more considerable changes in the morphology of the basal lamina are seen in the implantation chamber. In the antimesometrial region, the lamina lucida has totally disappeared in some places (Figure 6), and the remaining lamina densa is here in direct contact with the plasma membrane of the uterine epithelial cells. These remains of the lamina densa appear irregular in texture and outline. The described alterations are most typically seen in those parts of the uterine epithelium where trophoblastic knobs have attached or just fused but not reached the basal lamina yet (Figure 5). In contrast, in those regions where the trophoblastic knobs have already penetrated more deeply into the epithelium so that cytoplasm and nuclei of the trophoblastic type are seen adjacent to the basal lamina, cellular processes have penetrated the lamina densa and are not covered by a comparable structure. Next to them, a lamina densa may be seen that has detached from the epithelium so that a broad and irregular lamina lucida is found here (Figure 7). Further away from the points of attachment of trophoblastic knobs, but still within the implantation chamber, the basal lamina of the uterine epithelium keeps the
structure already described for the 6 d p.c. stage. This holds also true for the blastocyst-free segments.

Most striking alterations of basal lamina morphology are observed in the implantation chamber at 8 d p.c. at the uterine luminal epithelium of the placental folds. This is the stage just before attachment of the embryonic pole trophoblast which starts here after 8.5 d p.c. The luminal epithelium consists here, at this stage, of single-nucleated cells as well as of symplasms that are variable in size but contain considerably fewer nuclei than those seen simultaneously in the antimesometrial region. The apical plasma membrane shows numerous microvilli. The lateral plasma membranes are rich in complicated interdigitations. Most interesting are basal cell processes seen in this part of the epithelium. They are different from those found in the non-pregnant state, having a short stem and extending branches that are thin and irregular in shape giving the base of the cell a fringe-like appearance (Figures 9 and 10). These cell processes penetrate the basal lamina (Figure 10). In the basal portion of the epithelial symplasms numerous large vacuoles are found often linearly arranged. This is most typically seen in those cells which clearly show the described processes. The basal lamina consists of a thin and fuzzy lamina densa; the lamina lucida is mostly indistinct and has locally completely disappeared (Figure 11). The described changes in morphology of the basal lamina and penetration by basal processes are seen not only in the luminal epithelium but also in upper and middle parts of the crypts. In the same regions, the stroma is extremely edematous, and collagen fibers are rare. However, fibroblasts are still found here, in particular, close to the epithelium. The epithelium of the deepest parts of the crypts does not show the described changes: It remains without basal cell processes and stays clearly separated from the stroma by a continuous basal lamina as in the other stages.

Figure 7. In a region where a trophoblastic knob has fused with the antimesometrial uterine epithelium cytoplasmic processes penetrate the basal lamina (arrows) and extend into the stroma (7 d p.c.); the lamina lucida is relatively narrow and irregular in width. X44000.
Figures 8a and 8b. At the antimesometrial pole 8 d p.c., the mixed symplasm of uterine epithelial and trophoblastic origin shows a thin but continuous basal lamina which a) underlines numerous thin cell processes or b) is being penetrated by them. X21000.

In the antimesometrial part of the implantation chamber, widespread fusion of uterine epithelial cells with each other and fusion of trophoblastic knobs with uterine epithelium have led to the formation of broad symplasms that already begin to express signs of degeneration. These large symplasms send small finger-like extensions of cytoplasm from their basal parts into the stroma (Figures 8a, 8b). In contrast to the placental folds described above, however, penetration of these cell processes through the basal lamina is seen more rarely here. Some of these processes touch the lamina densa more or less directly, i.e., the lamina lucida is locally absent or irregular in width (Figure 8a). This is similar to phenomena seen next to penetrating trophoblastic knobs at 7 d p.c. However, some of the processes are found to penetrate through the basal lamina in the same way and to branch as described for the placental fold region (Figure 8b).

In the blastocyst-free segments, electron microscopical evaluation of the basal lamina is very difficult at the same stage (8 d p.c.). A typical basal lamina cannot be recognized in any part of the epithelium, since there is an accumulation of amorphous material which is electron densely stained in tannic acid treated specimens. This material fills the whole stromal space underneath the epithelium reaching up to its base (Figure 4). Morphological criteria do not allow to decide whether this is basal lamina material or precipitated protein that possibly obscures the basal lamina structure.

The basal lamina of the epithelial cells of the deep parts of the crypts keeps its morphology throughout the periimplantation phase independent of the distance from the blastocyst.
DISCUSSION

A number of morphological and biochemical changes in the uterine epithelium that are elicited by maternal steroid hormones during the preimplantation phase have already been described in the rabbit and are thought to represent characteristics of the so-called "receptive phase" during which trophoblast attachment, adhesion, and implantation are possible (Davies and Hoffman, 1973, 1975; Beier and Kühnel, 1973).
The slight morphological changes in basal lamina structure which were observed during the preimplantation phase until 6 d p.c. and which are maintained in the blastocyst-free segments and in the deepest crypts, may be considered as an additional morphological marker for the pre-conditioning of the endometrium for implantation. The present study provides, in addition, data on more pronounced changes restricted to the implantation chamber. This latter phenomenon obviously needs the presence of a blastocyst since it was not observed outside the implantation chamber. However, it does not require any direct contact with the invasive trophoblast as seen clearly at the placental folds at 8 d p.c., i.e., half a day before the trophoblast attaches here. This suggests that the described changes in the implantation chamber are elicited or stimulated by diffusible signals provided by the blastocyst and which act at a distance, but that the immediate cause for the changes must be in the uterine epithelium.

The structural changes of the basal lamina observed in the implantation chamber are, in a number of details, different in the antimesometrial part of the endometrium (obplacenta, yolk sac placenta) as compared to the mesometrial part (where the chorioallantoic placenta is formed). Differences include the time course as well as the morphological patterns. Antimesometrially, structural changes of the basal lamina are only minor before the trophoblast has attached to the uterine epithelium. After trophoblastic knobs have attached and start to fuse with the uterine epithelium, however, the lamina lucida disappears leaving only remnants of the lamina densa exactly underneath the attachment/fusion site. The most basally located part of the cytoplasm of the trophoblast-uterine epithelial synctium still maintains structural characteristics of uterine epithelium. Later on, when cytoplasm of trophoblastic type has penetrated more deeply in this syncytium and has reached the basal lamina, cytoplasmic processes penetrate through the vestiges of the lamina densa to reach the stroma. Already one day later, however, at the time when the antimesometrial uterine epithelium has been transformed into broad syncytia containing a mixed population of uterine and
trophoblastic nuclei, a typical and nearly complete basal lamina is reestablished. Only rarely do processes of this syncytium penetrate the basal lamina. During the following two days, the syncitia will degenerate and will be sloughed off, and non-fused uterine epithelium will grow out of the crypts and regenerate a surface lining. Further investigations will be needed to understand the mechanisms involved in the termination of invasion.

At the placental folds, i.e., in the central part of the mesometrial endometrium, the basal lamina undergoes much more widespread and pronounced changes. Interestingly, these changes are seen here before the trophoblast has reached this scaffold, even before it has contacted the uterine epithelium at all. Penetration of the basal lamina before trophoblast invasion has already been observed in the rat; in this case, however, by decidual cell processes (Schlafke et al., 1985). This, on the other hand, cannot be a mechanism for erosion of the uterine epithelial cell basal lamina at this phase in the rabbit, since in this species decidualization starts about one day later, and not underneath the epithelium but around deeper blood vessels. Both studies are in agreement, however, in showing that in both species, uterine cells modify and even penetrate this barrier first. This suggests that the invasive trophoblast does not play the major role in penetrating or dissolving the basal lamina. This idea is supported by a recent publication (Roberts et al., 1988) where it has been shown that the human uterine epithelial cells including the basal lamina undergo the same morphological changes observed here. In the early secretory phase numerous epithelial cell projections extend through the basal lamina which has become focally diffuse. Interestingly these projections are often in close contact with underlying stromal cells. Since these phenomena are observed in cycling endometria, they seem to be regulated exclusively by maternal steroid hormones.

The mechanisms of change in the uterine epithelial basal lamina can only be speculated on at present. The basal lamina is linked by proteins and proteoglycans to the basal cell membrane (Bernfield et al., 1984) which is directly or indirectly coupled with the cytoskeleton. Therefore, changes in the basal lamina must be linked to profound changes in the cell biology of the uterine epithelium at implantation. A reciprocal relationship appears to exist between epithelial cells and their basal lamina. Maintenance of an ordered basal lamina structure requires physiological integrity of epithelium, in particular a functioning apico-basal polarity (e.g., intracellular sorting and transport processes). Therefore, changes in the physiological state of epithelial cells must result in changes in basal lamina structure. On the other hand, cells respond to basal laminae in various ways, and a changed basal lamina or its loss must be of relevance for the behavior of the uterine epithelium. If the basal lamina is destroyed, cells have been found (in other systems) to lose functional differentiation, and this leads to a disorder in organotypic architecture (Vracko, 1974). For isolated thyroid follicles and for corneal epithelial cells it was shown that cells lose polarity and produce cytoplasmic processes when the basal lamina is removed (Hay, 1977; Greenburg and Hay, 1988). Implications of this concept for understanding the mechanism of endometrial "receptivity" are presently discussed more in details elsewhere in this volume (Denker, 1990).
Figures 11a and 11b. Basal lamina of the epithelium of an endometrial crypt 8 d p.c., placental fold. a) The basal lamina is focally missing. b) This vestigial basal lamina is penetrated by a basal cell projection. X60000.

During tumor invasion, the extracellular matrix is often modified in a localized region (Liotta et al., 1979, 1986). Studies of the enzymes involved are still in their infancy. Collagen type IV is degraded by a specific type of collagenase, while laminin can be degraded by a number of different proteinases (Liotta et al., 1979, 1986). Various glycosidases and proteinases have been detected in the uterine secretion as well in implanting blastocysts. As far as their function is concerned, what has been proven experimentally so far is only a role in the dissolution process of the blastocyst coverings while effects on components of the endometrium, in particular during stromal invasion, need to be defined (Denker, 1977, 1983). Even though uterine epithelial cells appear to destroy their own basal lamina as suggested by results shown in the present paper, this seems to be under the influence of the blastocyst since it is observed only in the implantation chamber. It remains to be seen what components of the basal lamina are being altered during trophoblast invasion and which have already been altered in the preimplantation phase.

**SUMMARY**

In order to shed light on the function of the uterine epithelial basal lamina, its fine structure has been studied during the perimplantation phase (6-8 days post coitum, d p.c.) in the rabbit. The endometrium of the blastocyst-free segments and
the implantation chamber were investigated separately and compared to the situation in the non-pregnant animal.

Slight morphological changes are already observed from 6 d.p.c. onwards independent of the different parts investigated. The lamina densa ceases to be clearly delineated, expanding partly into the lamina lucida. This phenomenon is enhanced 7 d.p.c. in the implantation chamber, at a time when the trophoblastic knobs start to penetrate the antimesometrial uterine epithelium. Here, locally, a lamina lucida is totally missing.

The most obvious changes in basal lamina structure are observed at the mesometrial side 8 d.p.c., i.e., before trophoblast invasion has started. At the epithelium of the placental folds the lamina densa appears fuzzy and the lamina lucida is thin or even missing. In some places the basal lamina completely disappears. The uterine epithelium forms numerous cell processes at the basal side which penetrate the residual basal lamina and extend into the stroma. At the same time big symplasmas have formed at the antimesometrial side by fusion of the uterine epithelial cells with each other and with the trophoblast. Here, similar basal processes extend into the stroma partly penetrating an otherwise relatively typically structured basal lamina. In contrast, the blastocyst-free segments of these uteri reveal an intact basal lamina structure with a lamina lucida and a lamina densa in all stages up to 7 d.p.c. On 8 d.p.c. an accumulation of amorphous material often obscures the basal lamina structure.

In conclusion, remarkable changes are found in the fine structure of the basal lamina of the rabbit uterine epithelium in the implantation chamber starting even before trophoblast invasion. Basal cell processes of uterine epithelium or of symplasmas penetrate this scaffold independently of trophoblast attachment, thus facilitating trophoblast invasion. These findings suggest that the uterine epithelial basal lamina, at least in the receptive state of the endometrium, should not be considered as an effective barrier for the invasive trophoblast.

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