Epithelial autolysis during implantation of the mouse blastocyst: an ultrastructural study

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SUMMARY

An ultrastructural investigation was made of cell death in the uterine epithelium during the implantation of the mouse blastocyst. Cell death occurs in two phases: (i) early individual cell death, and (ii) later general cell death.

Individual dead epithelial cells are phagocytosed and digested by trophoblast cells. At 95 h post coituim (p.c.) there are 1–4 dead cells; at 105 h and 113 h, 6–8 dead cells, and at 116 h, 2–3 dead cells. No lysosomal involvement could be identified in the death of these cells.

The general breakdown of the uterine epithelium around the implanting blastocyst is first recognizable at the ultrastructural level at 113 h p.c., and continues until the adjacent deteriorated epithelium has been phagocytosed by the trophoblast cells at 119 h p.c. Ultrastructurally, from 113 h p.c. cytoplasmic portions of epithelial cells are trapped within cytosegrosomes, and there is an increase in size of the dense lysosomal bodies found in viable epithelial cells at 105 h p.c. The dense bodies, which are positively stained for acid phosphatase enzyme at 105 h p.c., increase in diameter approximately 3–5 times between 105 h p.c. and 119 h p.c. These results provide evidence of intracellular digestion of small portions of the cytoplasm possibly through the formation of cytosegrosomes which then fuse with residual bodies already present. Thus the evidence favours a process of autolytic breakdown of the uterine epithelium around the implanting blastocyst.

INTRODUCTION

During formation of the ‘implantation chamber’ in rodents, the uterine epithelial cells adjacent to the blastocyst first deteriorate, and then die and are phagocytosed by trophoblast cells: in the mouse this takes place between 113 and 129 h p.c. (post coitum). Mesometrially, however, the epithelium remains intact. There have been few studies on the breakdown of the antimesometrial epithelium, and it is still not clear whether this takes place by the action of the blastocyst on the epithelium or by a process of autolysis or self-digestion (Boyd & Hamilton, 1952; Blandau, 1961).

‘Implantation chamber’ formation, whether in normal pregnancy or in the decidual reaction of the pseudopregnant uterus, may be regarded as a morpho-

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genetic process (Finn, 1971) and cell death is now recognised as making a positive contribution in certain morphogenetic processes (Saunders, 1966: Hinchliffe & Ede, 1973). Lysosomes and lysosomal enzymes such as acid phosphatase are known to play an important role in autolytic processes in cell death. The present study was designed to test the hypothesis of epithelial autolysis, and analyses the process of epithelial deterioration around the implanting blastocyst using thin sections for light and electron microscopy, and using acid phosphatase histochemistry. In particular, evidence was sought relating to the role of lysosomes in both trophoblast cells and dying cells of the uterine epithelium during implantation.

MATERIAL AND METHODS

Implantation sites identified by the pontamine sky blue reaction were obtained by the methods previously described (El-Shershaby & Hinchliffe, 1974). Sites were fixed at 105, 113, 116, and 119 h.p.c. and at least eight sites were examined for each stage. The sites were fixed in 4 % glutaraldehyde, post-fixed in 1 % osmium tetroxide, embedded in Araldite/epon mixture, and sections were cut at 1μm for light microscopy (stained with toluidine blue) and ultrathin sections were cut for electron microscopy as previously described (El-Shershaby & Hinchliffe, 1974).

Acid phosphatase staining at the light and electron microscope level was carried out at 105 and 113 h.p.c. using the Gomori (1952) method as described by Barka & Anderson (1963) and as modified for electron microscopy by Holt & Hicks (1962). Fixation was by 4 % glutaraldehyde at 0-4 °C for 3 h, followed by 24 h in cacodylate buffer at pH 7-4, and sections were cut on a freezing or cryostatic microtome. After incubation for 1 h in the Gomori medium and post-fixation in 1 % osmic acid for 1 h, the sections were embedded for light and electron microscopy as previously described. Difficulties encountered with this technique, particularly in embedding and due to damage during freezing, made it impossible to obtain a series of reliable results showing lysosomal changes in uterine epithelium and trophoblast cells over the whole period 95–119 h.p.c.

Changes in the number and size of the dense bodies or lysosomes in the uterine epithelium during normal pregnancy were quantified using the morphometric method of Weibel (1969). For morphometric analysis of dense lysosomal bodies within the uterine epithelial cells of the implantation sites, specimens were sectioned and about 15–21 cells, for each stage examined, were photographed at ×3000 magnification. The final magnification of the prints was ×1200. Further details in the application of this method are given in El-Shershaby (1974). Changes in the size of the dense lysosomal bodies in the epithelium during pregnancy were estimated by measuring the diameter along the longest axis of the dense body to the outside of the outer membrane, or to the edge of the body if no membrane was visible. All the recognizable lysosomal dense bodies in each cell were measured.
RESULTS

The overall changes at the light microscope level in the implantation site at 116-119 h p.c. during individual and general epithelial cell death and during trophoblast phagocytosis of epithelium are summarized in Figs. 1 and 2.

1. Viable epithelial cells

Viable epithelial cells were examined at 95, 105 h p.c., and, where viable patches continued to exist, at 113 and 116 h p.c. Initially the viable cells are tall and columnar at 105–113 h p.c. (Fig. 5), but later at 116–119 h p.c. they become much shorter. The general details of epithelial ultrastructure were similar to the description by Potts (1969), and the present account concentrates on the lysosomal dense bodies. The Golgi body does not appear to change between 95 and 105 h, but the number of Golgi vesicles has increased at 113 h and 116 h,
Fig. 2. Cell death processes in the 119 h.p.c. mouse blastocyst and adjacent uterine epithelium. Abbreviations as for Fig. 1.

Abbreviations for photographic figures

av, Autophagic vacuoles; db, dense body; dec, dead epithelial cell; ep, epithelial cells; Gb, Golgi body; Gv, Golgi vesicles; li, lipid droplets; mf, myelin figures; pn, pycnotic nucleus; t, trophoblast cells.

Figures 3–5

Single dead uterine epithelial cells (Figs. 3, 5) adjacent to the blastocyst at 105 h.p.c. Note chromatopycnotic nucleus in Fig. 3. Tall columnar viable epithelial cells containing residual dense bodies are also illustrated in Fig. 5, and are shown stained for acid phosphatase which is localized in dense lysosomal bodies in Fig. 4 (also 105 h.p.c.)
Fig. 6. Single dead epithelial cell in process of phagocytosis by a trophoblast cell at 113 h p.c. Note two 'arms' of trophoblast (ta) engulfing dead cell. Inset, light micrograph of the same area.
Epithelial autolysis during implantation

possibly indicating increased activity preparatory to autolysis. Many membrane-bound vacuolated and non-vacuolated dense bodies containing dense granulation are observed mainly in the apical region; some bodies showed membranous arrays and myelin figures (Fig. 5). The average size of these dense bodies is 0.20 \mu m at 95 h and 0.16 \mu m at 105 h p.c. (see Fig. 7). Acid phosphatase activity is localized at 105 h in bodies of the same number and size and in the same location as the dense bodies (Fig. 4), which can thus be identified positively as ‘dense lysosomal bodies’ (or ‘residual bodies’ or ‘cytosomes’). Dense lysosomal bodies have been reported in the viable epithelium by Potts (1969), and there is no evidence of any build-up of these bodies prior to commencement of cell deterioration: it is more probable that they are formed during the normal turnover of epithelial cell organelles.

2. **Death of isolated uterine epithelial cells**

A small number of isolated epithelial cells in various stages of deterioration or death is found in the ‘implantation chamber’ (but not away from it) between 95 and 116 h (Figs. 3, 5). The number of dead cells is as follows: at 95 h, 1–4 dead cells; at 105–113 h, 6–8 dead cells; and at 116, 2–3 dead cells. Fig. 3 shows a cell with a clearly identifiable chromatopycnotic nucleus. The ultrastructure of these cells is similar to that of the 95 h dead cells, which has already been described (El-Shershaby & Hinchliffe, 1974). Death takes place without any appearance of autophagic vacuoles or any increase in size of lysosomal dense bodies. Thus the mechanism of cell death in these cells is different from that occurring during the general breakdown of the whole antimesometrial uterine epithelium, which begins at 113 h p.c.

After the loss of the zona pellucida (ZP), stages in phagocytosis of these dead
Epithelial autolysis during implantation

1075
cells by the trophoblast cells are clearly seen beginning at 105 h p.c. Small pieces of dead cell may be phagocytosed (El-Shershaby, 1974, fig. 96), or a whole dead cell with clearly identifiable nuclear material may be surrounded by trophoblastic arms (Fig. 6). Trophoblast cells containing dead epithelial cells in vacuoles may be identified at 105 h p.c. and 113 h p.c. (El-Shershaby, 1974, fig. 32).

3. Autophagic processes in uterine antimesometrial epithelium

At 113 h p.c. most antimesometrial epithelial cells are found to contain autophagosomes or cytosegrosomes which are clearly distinguishable from the dense residual bodies found at 95 and 105 h p.c. These vacuoles are defined by an outer membrane and contain granular electron dense material, membranous structures (Fig. 8) and possibly deteriorated mitochondria. At 113 h p.c. enlarged (0·36 μm) dense bodies are found. The Golgi body appears to be active, and the cytoplasm contains increased numbers of Golgi vesicles, or primary lysosomes (Fig. 8). At 116 h p.c. autophagic vacuoles can no longer be distinguished separately from the dense bodies, which have become considerably enlarged (average size 0·56 μm at 116 h p.c. and 0·73 μm at 119 h p.c.) and contain some very dense patches, membranous arrays and sometimes lipid droplets (Fig. 9). These dense bodies which are fewer than in previous stages can no longer be regarded as 'residual bodies'. By this stage, degeneration changes are not limited to lysosomal bodies, but are taking place throughout the cytoplasm. These changes include accumulation of lipid droplets, swollen endoplasmic reticulum and Golgi body, accumulation of chromatin at the nuclear margin and appearance of a gap between nuclear membranes (Figs. 9, 10). The nucleus is sometimes fragmented, and the basement membrane is thickened, due to collagen fibre accumulation.

In some areas at 116 h and in most areas at 119 h, epithelial cells are in an advanced stage of deterioration. It has become difficult to identify organelles in such cells which have lost their polarity and are rounded. In many cases both nuclei and cells are in process of fragmentation (Fig. 11).

Figures 8 and 9

Fig. 8. Epithelial cell from the implantation chamber at 113 h p.c. showing autophagic activity. There are two large autophagic vacuoles containing dense bodies, vacuoles and myelin figures. The Golgi body is prominent, and Golgi vesicles are common in parts of the cytoplasm.

Fig. 9. Advanced degenerative changes in epithelial cells from the implantation chamber at 116 h p.c. The cells show large dense autophagic vacuoles, and in addition degenerative changes are visible in the cytoplasm and include lipid accumulation and vacuolation of the endoplasmic reticulum (ver).
4. Trophoblast phagocytosis and digestion of deteriorated uterine epithelial cells

The trophoblast cells were examined for evidence of a potential for lysosomal attack on the epithelium. There is no evidence of a build-up of Golgi vesicles within the trophoblast cell between 105 and 113 h. The conclusion of Smith & Wilson (1971) based on light-microscope histochemistry that a 'border of lysosomes' was formed at the time of trophoblast attachment to the epithelium could not be confirmed ultrastructurally. However, in later stages (116–119 h) there is an increased number of Golgi vesicles in the giant trophoblast cells, but this is probably related to digestion of the phagocytosed epithelial dead cells. Conclusive evidence as to the nature of trophoblast lysosomal activity during implantation must await a satisfactory study of the distribution of acid phosphatase (or other lysosomal enzyme) in trophoblast at the ultrastructural level.

Trophoblast phagocytosis of deteriorated epithelial cells begins at 116 h, when rounded whole epithelial cells are found in the trophoblast cytoplasm, representing the early stage of phagocytic digestion (Fig. 11). Late stages of digestion are also found (Fig. 10). Mesometrially, the epithelium adjacent to the blastocyst is still intact at this stage. At 119 h, most of the epithelium at the antimesometrial pole of the embryo has disappeared, and the trophoblast cells each contain up to six dead epithelial cells. By 129 h no epithelial cells adjacent to the embryo remain, although trophoblast cells still contain epithelial cells in various stages of digestion. The process of phagocytic digestion of epithelial cells by the trophoblast has not been described in detail here since accounts at the electron-microscope level have already been published (e.g. Smith & Wilson, 1974; Tachi, Tachi & Linder, 1970).

DISCUSSION

Isolated dead epithelial cells, previously described at 95 h.p.c., before ZP loss (El-Shershaby & Hinchliffe, 1974), are also found at 105 h–116 h.p.c. adjacent to the implanting blastocyst. Fig. 6 shows unequivocally that the basophilic or

Figures 10 and 11

Fig. 10. Degenerative changes in epithelial cells from the implantation chamber at 116 h p.c. at a more advanced stage than Fig. 9. The cells are rounded up and are losing their polarity, and in addition to autophagic vacuoles show chromatin condensation and a 'gap' between nuclear membranes. One cell is in the final stage of deterioration, with a fragmented pycnotic nucleus. The trophoblast cell contains heterophagosomes with contents at late (dv) stages of digestion.

Fig. 11. A giant trophoblast cell from the antimesometrial pole of the mouse blastocyst at 116 h p.c. containing three deteriorated epithelial cells with recognizable but sometimes fragmented nuclei and lipid droplets. Epithelial deterioration has reached its final stage; the cells have lost their polarity, their nuclei are fragmented, large lipid droplets have accumulated, and many of the cells are fragmenting. Note thickened basement membrane (bm).
electron-dense bodies previously described as 'primary invasive cells' (Wilson, 1963) or 'W-bodies' (Finn & McLaren, 1967) are in fact dead epithelial cells in process of trophoblastic phagocytosis, as suggested by Finn & Lawn (1968). As discussed in more detail previously (El-Shershaby & Hinchliffe, 1974), many problems remain to be solved as to the significance of these cells: they appear to be specifically associated with implantation (McLaren, 1968), and the cause of death, whether due to trophoblast activity or autolytic processes, remains obscure.

The evidence presented here favours the hypothesis that the process of deterioration of the antimesometrial epithelium of the uterus is mediated by self-digestion, rather than by trophoblast attack or secretion (Finn & Hinchliffe, 1964, 1965). There is no evidence for a 'border of lysosomes' in the trophoblast cells in early implantation prior to epithelial deterioration. These findings are in agreement with Bergstrom's conclusion (1970) that the implanting mouse blastocyst shows only faint proteolytic activity. In the rabbit also, acid phosphatase remains at a constant low level in trophoblast cells throughout implantation (Abraham et al. 1970). In the guinea-pig, however, trophoblast cells show high levels of proteolytic activity which may play a role in the epithelial deterioration immediately adjacent to the blastocyst during implantation (Blandau, 1949; Owers & Blandau, 1971).

Beginning at 113 h p.c., the uterine epithelial cells show clear signs of progressive autolytic changes at the ultrastructural level. At 113 h p.c. epithelial cytoplasm contains both dense residual bodies and newly formed autophagic vacuoles containing sequestered portions of the cytoplasm. Subsequently the dense bodies increase in size but their number decreases over the same period. This decrease may be attributed to fusion between newly formed autophagosomes and already formed residual bodies, resulting in digestion of autophagosome contents as suggested by Ericsson (1969). These changes take place at 116–119 h p.c. At the same time the epithelial cells decrease in size, lose their polarity, lose contact with each other and show other characteristic ultrastructural evidence of deterioration.

Trophoblast phagocytosis of the whole uterine epithelium begins only at 113–116 h p.c., after the epithelial cells have undergone considerable deterioration. The deterioration appears to stimulate the phagocytic activity of the trophoblast cells since this activity is not shown, except in relation to isolated dead epithelial cells, between 105–113 h p.c. This mode of removal of deteriorating epithelium is different from that during epithelial deterioration in the oil-induced decidual reaction, where macrophage cells and polymorphonuclear leucocytes clear up the deteriorated epithelium (Hinchliffe and El-Shershaby, 1975).

The process described here of autophagic breakdown of uterine epithelium is consistent with Finn & Bredl's hypothesis (1973) that epithelial cell death is 'programmed', as in other examples of morphogenetic cell death (Saunders, 1966). In the rabbit, Abraham et al. (1970) found increased acid phosphatase
activity within the deteriorating uterine epithelium and concluded that the removal of the epithelial cells in implantation is mediated through autophagic activity. Similar autophagic activity was reported in other regressing epithelial tissues, e.g. in mammary gland and prostatic involution (Helminen & Ericsson, 1968, 1971). The results reported here are in agreement with Ericsson’s conclusion (1969) that, in general, involuting epithelial cells undergo autophagic breakdown of the cytoplasm. However, the question of the precise mechanism by which the ‘cell death programme’ of the epithelial cells is triggered still remains to be solved.

REFERENCES


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