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The regulatory function of the uterine epithelium for trophoblast attachment: experimental approaches

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Key words: Uterine Epithelium, Adhesion, Polarity, Trophoblast, Force Spectroscopy

SUMMARY

Embryo implantation in the mammalian uterus is initiated by the formation of a direct cell-to-cell contact between the trophoblast of the blastocyst and the uterine epithelium. This process is far from trivial since apical plasma membranes of epithelial cells are normally non-adhesive. The uterine epithelium has the remarkable ability to enter, under steroid hormone control, a specific state ("receptivity") at which it can down-regulate this repellent property and can finally become apically adhesive for trophoblast (probably aided additionally by local paracrine signals). Experimental data from recent years are beginning to shed some light on the involved cell biological/molecular events. They will be discussed on the basis of concepts concerning the regulation of epithelial cell polarity and with side views on epithelial-mesenchymal transformation. Recently developed experimental in-vitro systems have allowed to detect a remarkable degree of selectivity in the interaction of trophoblast and uterine epithelium, in contrast to stroma invasion. A new approach enables us to determine actual adhesive forces between living trophoblast and uterine epithelial cells with a special modification of the atomic force microscope (force spectroscopy). The potential use of such an approach is discussed.

INTRODUCTION

Failures in developing proper endometrial receptivity are assumed to be one major reason why the success rate of embryo transfer after in vitro fertilisation (IVF-ET) is still unsatisfactory worldwide. None of the many attempts at overcoming this with various regimes of hormone pretreatments and substitutions has so far led to a breakthrough, so that many authors assume that an endometrial factor is playing an important role here (Jones *et al.*, 1998; Lessey, 1998; Nikas, 1999b). The identity of

this endometrial factor remains a mystery, however; it may involve steroid hormone non-responsiveness of some so far unexplained type, or inability to respond properly to any postulated local signals from the blastocyst, but the cell biological basis of this lacking responsiveness is not understood. This may to a good part be due to our lack of understanding basic aspects of the cell biology of endometrial receptivity.

We are reporting here on model studies that we have performed in our laboratory, and in particular on certain methodological approaches that seem to promise to yield new insight into mechanisms involved. Of central interest is the ability of the uterine epithelium to develop an adhesion competence at its apical plasma membrane, a peculiarity in comparison with other simple epithelia which is receiving increasing interest recently since it was noticed that this phenomenon confronts us with a cell biological paradox (Denker, 1986, 1990, 1993). It must be pointed out that our concepts about the initial phase of embryo implantation, i.e. that adhesion is one of the first steps involved in the interaction between trophoblast and uterine epithelium, still remain not much more than a postulate, since these concepts are based primarily on morphological evidence from light and electron microscopical observations (reviewed in Denker, 1994, 1995). The actual adhesive forces that are thought to develop between these two partner tissues at the apical plasma membrane have until recently not been measured. This has led Lopata (1996) to even question the whole concept of adhesion as an initial step in the whole process.

In the first part of the present paper we will review experiments that show evidence for a central role of the uterine epithelium in acting as a barrier to trophoblast invasion into the endometrial stroma so that this epithelium can be expected to be critical for regulating implantation initiation. In the second part data will be discussed which give evidence for adhesive processes that are taking place at the apical plasma membranes of the uterine epithelium and the attaching trophoblast. In a third part we will discuss some novel experimental approaches that have been used for directly demonstrating the elaboration of adhesive forces at these apical plasma membranes, using uterine epithelial model systems, including evidence that an outside-in signalling can be elicited here and may be an important aspect of endometrial receptivity and the subsequent events of trophoblast attachment and invasion.

RESULTS AND DISCUSSION

Evidence for a critical role of the uterine epithelium in regulating implantation initiation

A number of observations suggest that the uterine epithelium plays an important role in regulating implantation initiation insofar as the trophoblast of the blastocyst cannot overcome this barrier (and not even attach to it?) except for this specific period of receptivity (reviewed in Denker 1993, 1994). Particularly impressive are the classical experiments of Cowell (1969) demonstrating that implantation is

possible outside receptivity if the uterine epithelium is simply removed experimentally. This ability to first restrict and then allow trophoblast penetration during a specific phase seems to be a peculiarity of the uterine epithelium and is not met by the tubal epithelium at least in experimental animals, perhaps in some contrast to the human (Tutton and Carr, 1984; Pauerstein *et al.*, 1990). In the pig with its epithelio-chorial placentation the trophoblast may nevertheless have some invasive properties but may be prevented from showing this ability in utero due to the presence of the uterine epithelial barrier (Samuel and Perry, 1972). The uterine epithelium may fulfill some signalling functions in this context. This has been shown specifically for the induction of decidualization (Ferrando and Nalbandov, 1968; Lejeune and Leroy, 1980).

More recently, the following experimental *in vitro* setups have given additional evidence for a critical role of the uterine epithelium in allowing or preventing attachment and invasion of invasive cells, and even provide evidence for a remarkable selectivity that points to the presence of highly specific recognition processes.

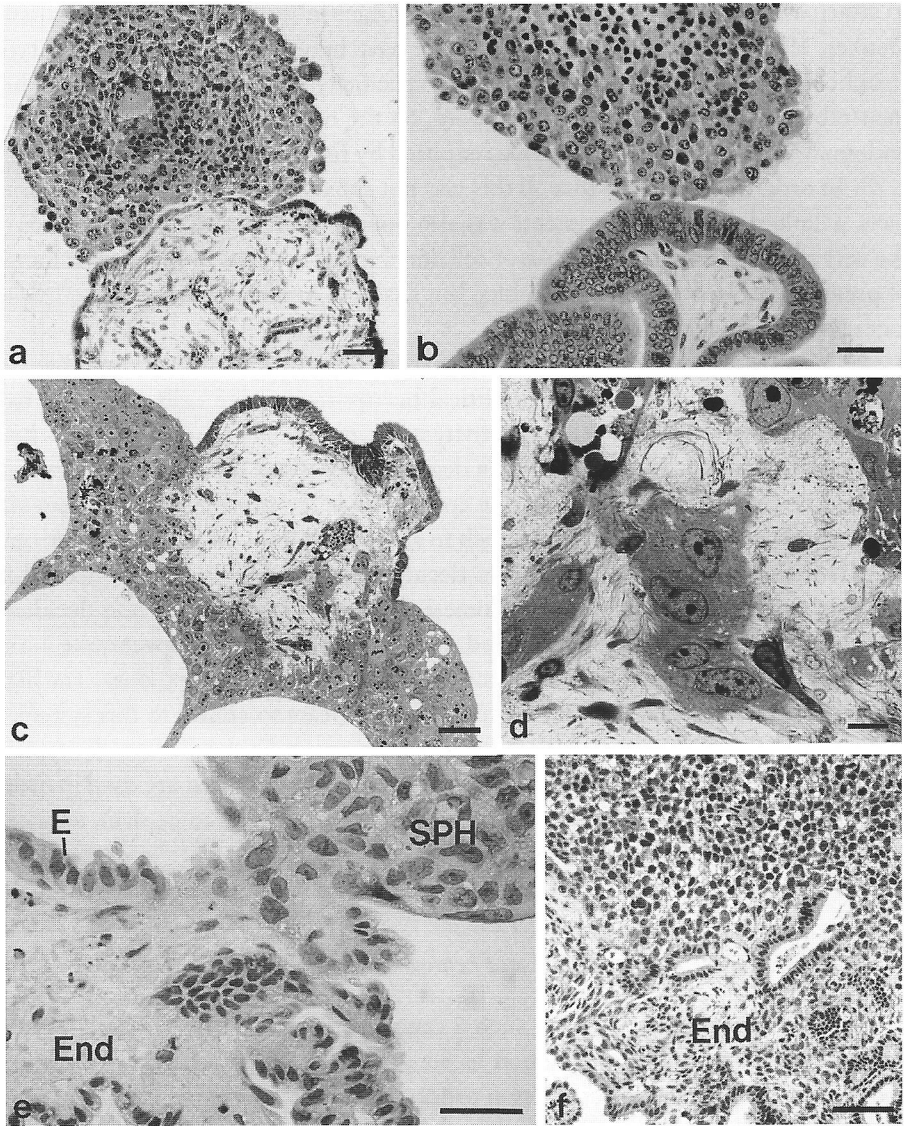
A model system has been developed in our laboratory in order to study certain aspects of the interaction between human trophoblast-type cells and various host tissues (non-physiologic host tissues or endometrium) in a three-dimensional culture system *in vitro*. The trophoblast type cells are being used in the form of multicellular aggregates (so-called spheroids) in order to have an alternative to using human embryos (blastocysts) (which in our opinion is not acceptable for ethical reasons). The trophoblast type cells used are either choriocarcinoma cell lines (BeWo, Jeg-3 and JAR) or normal trophoblast cells isolated from first trimester or mature placentae. These spheroids are confronted with host tissue models that are also kept as complex three-dimensional structures, i.e. fragments of embryonic chick heart (Mareel *et al.*, 1979) or fragments of endometrium (human, Grümmer *et al.*, 1994; rabbit, Hohn *et al.*, 1989; Donner, 1992). Before confrontation with trophoblast spheroids is started, the host tissue fragments are pre-cultured in order to allow degenerated cells to be shed so that they cannot influence the outcome of the experiment. In case of endometrium this pre-culturing of the host tissue has also the advantage to allow the epithelium to regenerate a complete lining around the tissue fragment (Hohn *et al.*, 1989; Donner, 1992; Grümmer *et al.*, 1994).

With this model it was possible to detect a surprisingly high degree of selectivity in the interaction of the endometrium, and specifically the uterine epithelium, with the various types of invasive cells. For example, while all three choriocarcinoma cell lines (BeWo, Jeg-3 and JAR) proved to be highly invasive with respect to pre-cultured chick heart tissue (an non-specific host tissue that is highly useful in a general invasiveness assay, i. e. the Mareel assay; Mareel *et al.*, 1979), they showed clear differences in behaviour with respect to endometrium. JAR spheroids adhered very poorly to the epithelium of human endometrium and, as a consequence, did not invade (Grümmer *et al.*, 1994). On contrast, BeWo cells did adhere and integrate into

the uterine epithelial lining but they did not invade very deeply into the endometrial stroma but rather tended to stay at the surface of the endometrial fragment. Jeg-3 cells showed a very aggressive behaviour: They adhered easily to the uterine epithelium and invaded it very quickly and deeply so that the whole endometrial fragment was finally destroyed (Figs. 1 e, f). There was not much evidence for cell death (apoptosis or necrosis) at the invasion front.

It appears remarkable that this *in vitro* culture model with human endometrium as a host tissue seems to allow to monitor differences between the various choriocarcinoma cell lines with respect to their ability to attach to and to interact with endometrial host tissue (if confronted via an intact uterine epithelium). As mentioned above, this is in contrast to the Mareel assay (Mareel *et al.*, 1979) using embryonic chick heart as a host tissue where all three cell lines showed highly invasive behaviour. Interestingly, the selectivity seen in case of the endometrium appears to be mediated by the uterine epithelium, not the stroma. This is strongly suggested by the results of another series of experiments involving not human but rabbit endometrium (Donner, 1992; Hohn *et al.*, 1987; Donner *et al.*, 1991; Hohn *et al.*, submitted). In this case spheroids of various invasive cell lines were used (carcinoma, choriocarcinoma and sarcoma cell lines: MO₄, a mouse embryonic fibrosarcoma line; NBT II, a urinary bladder tumor of the rat; MCF-7, a human mammary carcinoma; 12R1-C-RK, a renal tumor of the rat; LLC-H61, a human lung carcinoma; V2, a rabbit carcinoma; Jeg-3, a human choriocarcinoma). None of these various tumor cell spheroids attached well to the epithelium of pre-cultured rabbit endometrial fragments *in vitro*, and there was no deep invasion through the epithelium into

Fig. 1 — Model studies showing a high degree of selectivity of the interaction between uterine epithelium and various types of invasive cells, *in vitro* and *in vivo*. There appears to be a specificity with respect to cell type as well as with respect to species. (a) *In vitro* confrontation of rabbit endometrium (below) (explanted at 4 2/3 days p.c. and pre-cultured for 2 days) with a spheroid of MO₄ (murine sarcoma) cells (above) for 24 hours. The spheroid is barely attached to the uterine epithelium and there is no sign of invasion. The same negative result was obtained with human choriocarcinoma and rabbit carcinoma cells, whereas rabbit blastocyst trophoblast is known to adhere and to invade in this system. (b) An MO₄ cell spheroid (above) transferred to the uterine lumen of a rabbit *in vivo* (stage shown is 8 days of pseudopregnancy, 32 hours after transfer of spheroids). Again no evidence for attachment to the uterine epithelium (below) nor invasion. (c, d) The result is different when the uterine epithelium is removed: In the same *in vitro* system as shown in (a) multicellular spheroids from various species can attach easily and invade if confronted not with epithelium but with exposed endometrial stroma. In the case shown here, human Jeg-3 choriocarcinoma cell spheroids (lower and left hand side) were combined with the denuded stroma surface of a pseudopregnant rabbit endometrial fragment. The stage shown is after 3 days of confrontation culture. The spheroids have flattened on the surface of the endometrial fragment. At the higher magnification (d) it can be seen that choriocarcinoma cells have invaded deeply into the endometrial stroma. (e, f) Human endometrium as a host tissue. Explants from the mid-secretory phase where pre-cultured as above and confronted with choriocarcinoma spheroids via the intact uterine epithelium. BeWo cells (e) tend to integrate into the uterine epithelium but show little tendency to invade into the endometrial stroma at 4 days of confrontation culture. In contrast, Jeg-3 cells (f) (above) behave very aggressively and destroy large parts of the endometrium (End) (below) already after 2 days. (spheroid = SPH; uterine epithelium = E) Bars: a, b, c, f: 50 µm; d: 10 µm; e: 25 µm (a-d: from Donner, 1992; e, f: from Grümmer, 1991. See also Hohn *et al.*, submitted; Grümmer *et al.*, 1994).



the endometrial stroma to any significant extent (Fig. 1 a). It appears remarkable that not even the human choriocarcinoma (trophoblast derivative) cell line Jeg-3 showed any aggressive behaviour with regard to rabbit endometrium via the epithelial surface, although, as described above, it does show such behaviour when confronted with human endometrium. Even cells from the same species, i. e. rabbit V2 carcinoma cells, did also not attach to and invade through rabbit uterine epithelium. However, it had been shown previously that trophoblast of rabbit blastocysts is able

to attach and to invade via the epithelial cell surface in the same system (Hohn and Denker, 1990). Therefore it appears that we have here a phenomenon of cell type (trophoblast) specificity as well as species specificity (human/human but not human/rabbit). All mentioned cell lines were, on the other hand, able to invade into endometrial stroma whenever this was exposed by removing the epithelium (Figs. 1 c, d). In order to make sure that all this was not simply due to *in vitro* culture artefacts, a limited series of experiments was also done *in vivo*. In this case, spheroids of the very aggressive MO₄ mouse tumor cell line were transferred to the uterine lumen of pregnant or pseudopregnant rabbits, but again no invasion was seen through intact uterine epithelium (Fig. 1 b).

These results on one hand confirm earlier observations (see above) that the uterine epithelium appears to be a critical barrier to invasive cells including trophoblast. At the specific state of receptivity it down-regulates major parts of its barrier function, but not to zero, because a degree of selectivity (species specificity) appears to be retained. When the uterine epithelium is removed from the system virtually any type of invasive cells can attach to and invade into the endometrial stroma (maybe then depending on its state of decidualization which was not checked in these series of experiments). The endometrial stroma (at least in its non-decidualized state) appears to lack selectivity and seems to behave in this respect quite in the same way as a non-specific host tissue model (embryonic chick heart) does. The high degree of selectivity that the uterine epithelium appears to confer to this system is quite remarkable. It seems to include both a cell type and a species specificity, features that are not seen with most other *in vitro* model systems that are used in order to mimic invasive processes. A challenge for the future will be to explain, on a cell biological and molecular basis, this high degree of selectivity.

Apical adhesiveness: morphological and histochemical evidence

If the uterine epithelium has such a central and critical role in regulating the initial phase of embryo implantation as suggested by the observations discussed above, and if it is true that recognition processes of high selectivity are taking place here, we must ask what specific cellular and molecular events can be identified that may be crucial in this context. As mentioned in the beginning one important initial event is postulated to be adhesion of the apical plasma membrane of the trophoblast to the apical plasma membrane of the uterine epithelium. Since, as already mentioned, the whole concept that such an adhesion does at all take place has been questioned (Lopata, 1996) we must ask what data are available that may support the concept of apical adhesiveness. Morphological and histochemical findings which are supportive of this concept have been reviewed earlier (Denker, 1990, 1993, 1994, 1995). Further below we will in addition discuss new functional data on the measurement of actual adhesive forces.

In brief, changes seen in the uterine epithelium at receptivity (and particularly expressed in the implantation chamber, i. e. in the immediate vicinity of the blastocyst) involve all aspects of organisation of these epithelial cells, not only at the apical plasma membrane domain where the first contact with the trophoblast occurs (maybe beginning at the marginal parts of it, see Enders and Mead, 1996), but also at the lateral and basal plasma membranes and the cytoskeleton.

At the apical plasma membrane uterine epithelial cells develop the remarkable ability to form, at receptivity, junctions that are otherwise typically found only in the basolateral membrane compartments: "reflexive" gap junctions (Murphy *et al.*, 1982) and hemidesmosome-like junctions (with remnants of undissolved blastocyst coverings; for illustrations see Denker, 1977, 1995). In addition to these phenomena that had been seen with conventional electron microscopy, a number of histochemical changes have been reported to be detectable at the apical plasma membrane domain: changes in lectin binding properties, a reduction of the thickness of the glycocalyx, loss/downregulation of marker enzymes for the apical plasma membrane domain, increased density of intramembranous protein particles so that the values equal those typically found at the basolateral membrane domain, and an acquisition of receptors for matrix or cell surface molecules (for literature see Denker, 1990, 1994).

In the lateral plasma membrane domain, the original polar restriction of tight junctional strands to the subapical region is given up at receptivity (Murphy, Winterhager, for references see Denker, 1990) so that the strong polarity along the apico-basal axis is partially lost. This becomes even more obvious when the apical protrusions of the uterine epithelial cells (the so-called "pinopodes", an established characteristic of receptivity, see Martel *et al.*, 1991; Nikas, 1999 a and b) are formed. E-cadherin (uvomorulin) is also maximally concentrated in the subapical junctional belt region before receptivity but develops a more equal distribution over the whole lateral plasma membrane when receptivity is approached, in the rabbit; in certain areas of the implantation chamber it even becomes concentrated maximally at a very unusual location, i. e. at basal cytoplasmic processes that penetrate the basal lamina (Fig. 2; Donner *et al.*, 1992). The desmosome-associated protein desmoplakin shows the same type of changes (Classen-Linke and Denker, 1990 and unpublished results).

These phenomena may be extremes of more subtle changes taking place in other parts of the uterine epithelium of the rabbit implantation chamber and in cycling human endometrium around receptivity, i.e. more isolated basal cytoplasmic processes penetrating the basement membrane focally (Roberts *et al.*, 1988; Marx *et al.*, 1990).

Changes in the uterine luminal epithelium at receptivity involve, therefore, also the basal cell pole. A phenomenon that is known since a long time but is usually not seen in the context of apico-basal polarity is that in rats and mice the strength of adhesion to the basement membrane is considerably reduced (Tachi *et al.*, 1970; Schlafke and Enders, 1975; Chávez, 1990) so that one can push out practically pure epithelium from such uteri (Bitton-Casimiri *et al.*, 1977).

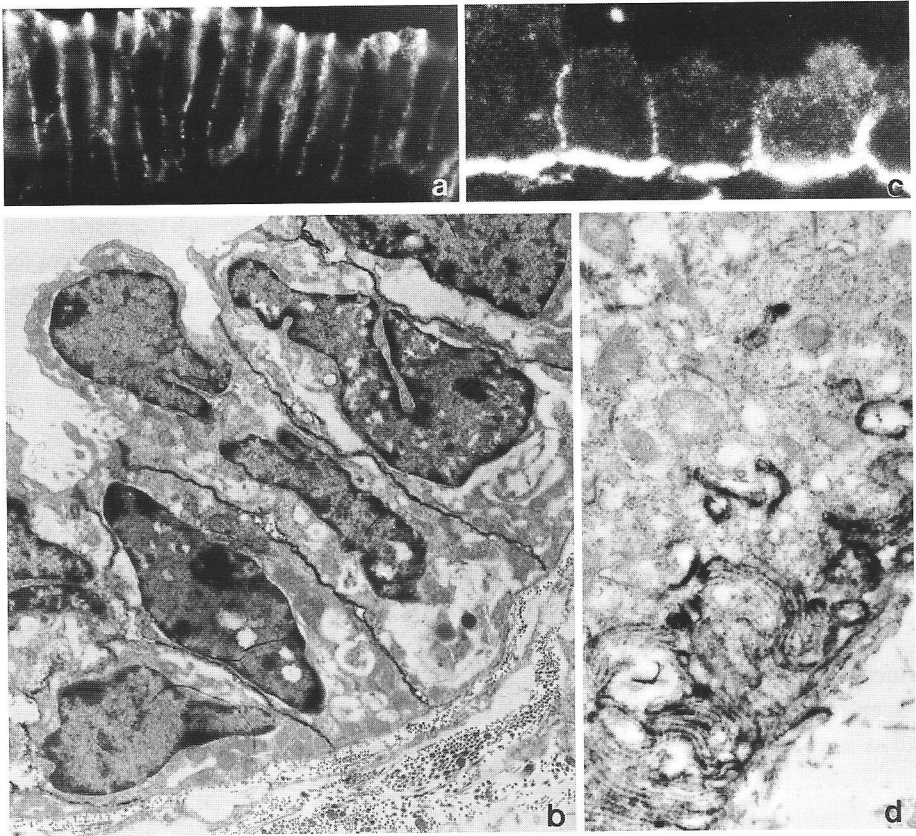


Fig. 2 — E-cadherin distribution in rabbit uterine epithelium. (a) In the non-pregnant (pre-receptive) state, maximal E-cadherin concentration is found in the subapical junctional belt region while less reaction is seen in the other parts of the lateral plasma membrane and none in the apical and basal domains. Light microscopical immunohistochemistry, FITC labelling, 680X. (b) The subapical maximum is largely lost at receptivity. Intermediate part of an endometrial crypt in the paraplacental region, 8 days p.c. TEM, horseradish peroxidase labelling, pre-embedding immunoreaction on a thick cryosection of PLP-fixed material. 3,500X. (c) Certain subpopulations of oligonuclear epithelial cells show extreme degrees of E-cadherin redistribution; endometrium of the rabbit placental fold, 9 days p.c. Here the maximal concentration of this adhesion molecule is found in a very unusual location, i. e. in the basal membrane region (below). The apical plasma membrane (above) remains free of E-cadherin. Light microscopical immunohistochemistry, FITC-labelling, 510X. (d) Electron microscopically, the basal plasma membrane region of this type of modified cells shows that E-cadherin is highly concentrated at the membranes of basal projections of these cells and seems to play a role in contact formation of these projections with each other. The stromal space is at the lower right hand corner. Labelling as in (b), 7,580X. (Fig. 2a, c from Denker, 1993; Fig. 2b, d from Denker, 1995).

In summary, the fact that changes in the uterine epithelium at receptivity are found not only at the apical plasma membrane where they would be needed for trophoblast attachment but also in the lateral and basal plasma membrane domains points to the fact that we obviously have to consider these changes to be of a global nature comprising the overall physiology of the uterine epithelial cell. In particular

the apico-basal polarity which was highly expressed in the prereceptive phase is partially lost (with respect to a number of parameters) at the receptive phase. The epithelial cell phenotype seems indeed to be changed more drastically than pure morphology suggests: Changes include organisation of the actin cytoskeleton (Luxford and Murphy, 1992, 1993; Murphy, 1995; Thie *et al.*, 1997) and even the intermediate filament system: In the rabbit implantation chamber, the density of vimentin filaments (which do occur in this epithelium in addition to cytokeratin) is strongly increased (Hochfeld *et al.*, 1990), and the intracellular distribution also changes from predominantly basal to predominantly subapical (Fig. 3). It was proposed that this surprising complexity of phenotypic changes that the uterine epithelium shows at receptivity points to a drastic change in the expressed genetic program and that it may bear some resemblance to the reprogramming of cells during epithelial-mesenchymal transitions (EMT) (Hay, 1995) (but only to a partial EMT, since the main epithelial characteristics are still maintained). Further it was hypothesised that these changes might be governed by certain master genes which may be on work in both systems although being controlled themselves in different ways (in the uterus by sex steroids in addition to various cytokines) (Denker, 1990, 1993, 1994).

Apical adhesiveness: functional studies

The direct observation of bond formation between the free surfaces of uterine epithelium and the blastocyst is a major goal in the ongoing research of our group. Conventional cell-cell adhesion assays (e.g. John *et al.*, 1993) provide only very limited information on molecular processes of actual binding forces between uterine epithelium and trophoblast. However, a novel type of application of the atomic force microscope (AFM), the so-called force spectroscopy (Binnig *et al.*, 1986; Rugar and Hansma, 1990; Radmacher *et al.*, 1992) makes it possible to obtain insight into cellular and molecular dynamics of bond formation in real time.

In a first approach, we used human cell lines that had before been found to model certain aspects of the receptive vs. the non-receptive state of the uterine epithelium (Thie and Denker, 1997). A custom-made AFM (Florin *et al.*, 1994; Ludwig *et al.*, 1997; Rief *et al.*, 1997) and cantilevers coated with human trophoblast-type JAR cells (Patillo *et al.*, 1971) were used for these experiments (Fig. 4; cf. Thie *et al.*, 1998). JAR cell-coated cantilevers were brought into contact with monolayers of human uterine epithelial RL95-2 cells (Way *et al.*, 1983). RL95-2 is an uterine epithelial cell line that, in contrast to other endometrial cell lines like HEC-1-A (Kuramoto *et al.*, 1972) and AN3-CA (Dawe *et al.*, 1964), not only lacks epithelial polarity and shows loosening of lateral borders and changes in its actin cytoskeleton, but also exhibits adhesion competence of its apical pole for trophoblast-like cells (Thie *et al.*, 1995, 1996, 1997). In this respect, RL95-2 cells are used as a cell model system for the receptive human uterine epithelium.

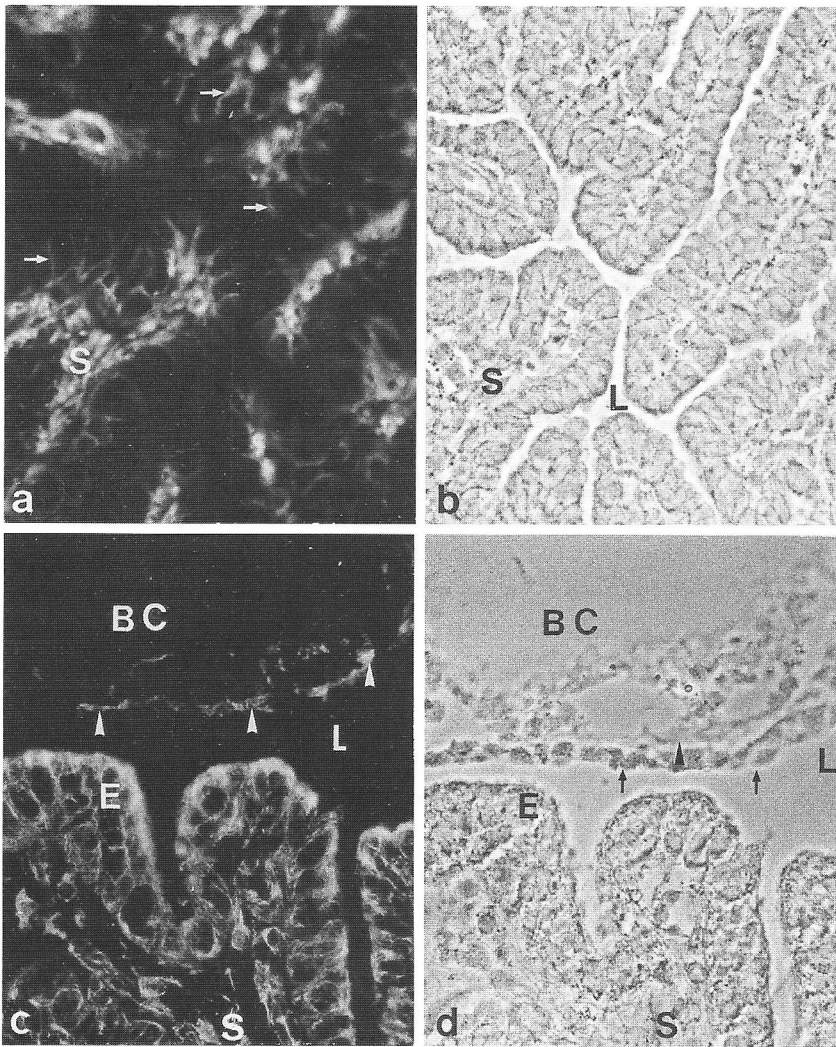


Fig. 3 — Changes in the uterine epithelium at receptivity, particularly expressed in the implantation chamber, comprise even the vimentin cytoskeleton. The micrographs show rabbit luminal epithelium at 9 days p.c. In interblastocyst segments of the uterus (a, b) the pattern of the non-pregnant state is largely maintained, i. e. discrete vimentin immunoreactivity (arrows in Fig. a) is found in the basal part of the epithelial cytoplasm (in addition to the usual strong reaction of cells in the endometrial stroma). In the implantation chamber (c, d), however, a very prominent vimentin reaction is seen in the luminal epithelium with maximum staining at the apical cell pole. BC: blastocyst cavity; E: epithelium; L: uterine lumen; S: stroma; arrowheads: mesoderm; arrows in Fig. d: trophoblast. (a, c: immunofluorescence; b, d: corresponding phase contrast) 320X (from Hochfeld *et al.*, 1990).

The AFM (force spectroscopy) approach permits to obtain quantitative data on the adhesive forces measurable at the apical pole of the cells. All experiments were performed in cell culture medium. Forces were measured first while lowering the JAR cells onto the free surface of endometrial cells. Forces were then continu-

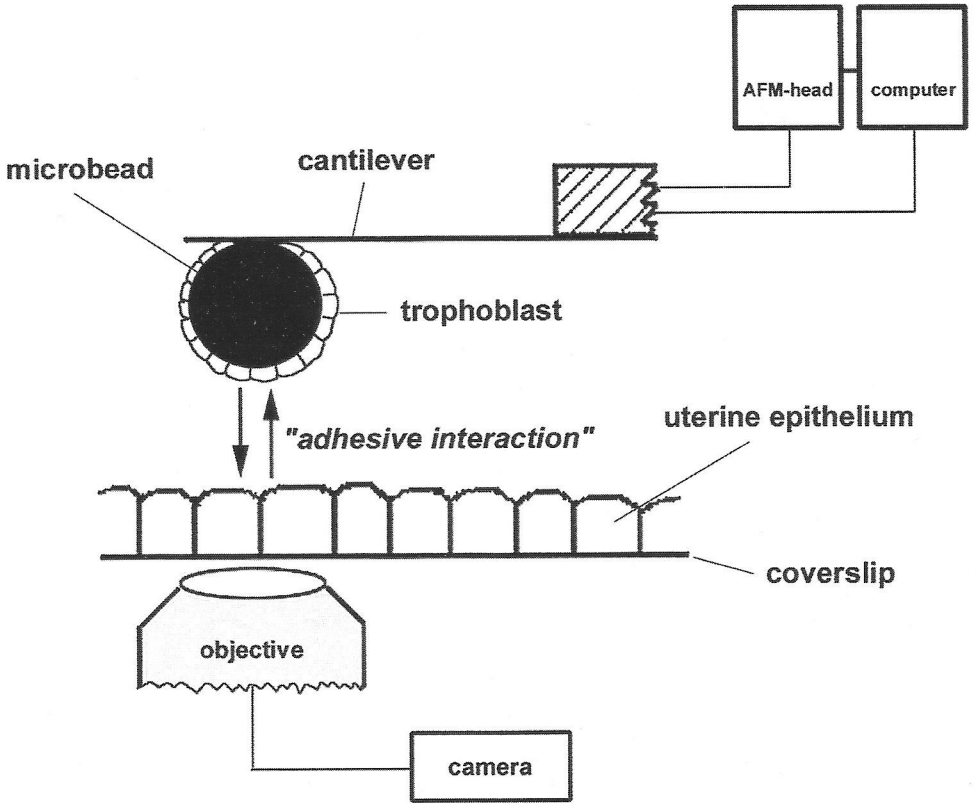


Fig. 4 — Schematic of atomic force microscope (AFM) operation showing the microbead-mounted cantilever in contact with the uterine epithelial monolayer. The microbead, glued to the AFM cantilever, is coated with human trophoblast-type JAR cells. Forces between the cell-coated microbead and the confluent endometrial monolayer are measured by cantilever deflection during the approach and separation (= adhesive interaction) of the cantilever. An inverted optical microscope is mounted under the fluid chamber of the AFM allowing examination of both cell systems.

ously recorded during several cycles of approach and separation. It was thus possible to identify and measure repulsive forces exerted during the initial contact, followed by adhesive interactions developing slowly thereafter. Force curves were measured by cantilever deflection during the approach and the separation of the cantilever; the contact was kept for variable periods of time.

A typical force curve is shown in Fig. 5. When the JAR-coated cantilever was separated from RL95-2 monolayers within milliseconds after contact, no adhesion was observed. However, considerable adhesive forces were measured when the duration of contact was increased. For a contact of 20 min (or 40 min) the force versus distance curves showed force rupture events due to the rupture of relatively stable bridges between confronted cells. Our observation that there are distinct rupture events upon separation of RL95-2 and JAR cells, but not upon separation of HEC-

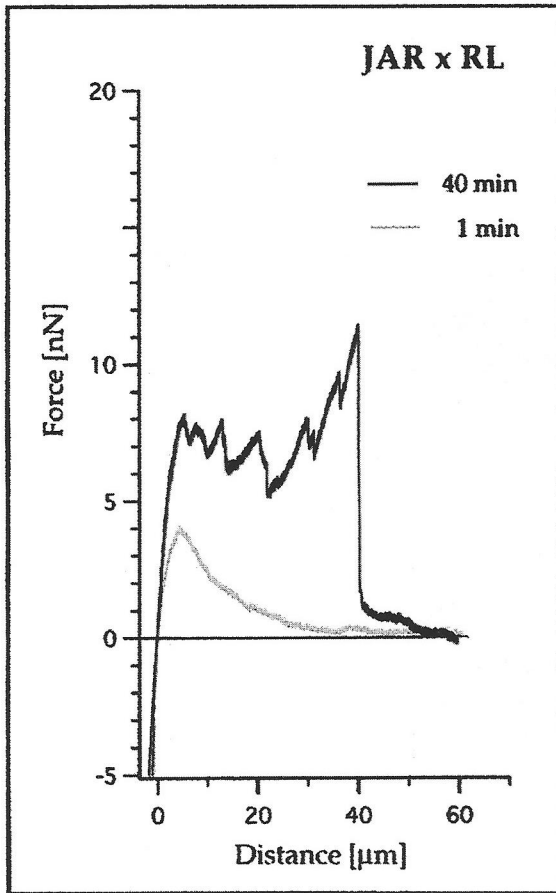


Fig. 5 — Typical adhesive force curves for RL95-2 cells (= RL) resulting when a JAR-coated microbead (= JAR) was retracted after periods of 1 or 40 min of contact. The horizontal axis shows the vertical movement of the cantilever; the vertical axis shows the force acting on the microbead. Note force rupture events (= force-induced bond dissociation) when a JAR-coated microbead was retracted from RL95-2 cells after 40 min of contact; JAR and RL95-2 cells formed membrane cylinders (= tethers) up to 40 μm .

1-A or AN3-CA and JAR cells (data not shown), indicates specific features of cell-to-cell bonds between RL95-2 (but not HEC-1-A or AN3-CA) and JAR cells (Thie *et al.*, 1998).

In conclusion, the force spectroscopy approach has allowed us to define features of adhesive interactions as well as of their time-dependent development between uterine epithelial RL95-2 cells and trophoblast-type cells via their apical (free) cell poles. This can be correlated with cell behaviour, i.e. the phenotype that mimics either receptivity (adhesion competence and permissiveness for invasion, RL95-2) or non-receptivity (repellent properties, HEC-1-A).

These findings open new ways how to explain mechanisms behind the phenomenon that the apical membrane surface of epithelial RL cells is somehow predisposed for trophoblast adhesion in contrast to other cells. This predisposition includes the ability to transmit mechanical signals to the cell interior, via membrane-bound integrins and an appropriately rearranged actin cytoskeleton (Thie *et al.*, 1995). Our data suggest that adhesion of trophoblast to uterine epithelium might be a relatively slow process (see time dependence, above), possibly including complex signal transduction cascades and sequential steps of bond formation. Under physiological conditions, the arrest of the blastocyst on the uterine epithelium may involve the transition from weak to strong binding, perhaps in a way that is somewhat similar to the rolling, arrest and penetration cascades in leukocyte-endothelial cell interactions (Butcher and Picker, 1996). The proper regulation of this complex cascade of events might determine whether the blastocyst will finally adhere and implant or fail.

On the basis of these observations we re-define the receptive state of the endometrium as a specific condition of this tissue in which the luminal epithelium is the critical effector (probably regulated by the stroma) and exhibits for a limited time period peculiar properties, not met by epithelia of other organs. Of central importance appears to be that the uterine epithelium acquires, at receptivity, competence of the apical cell pole to initiate specific cell-cell adhesion and signalling processes which we are currently studying in detail.

ACKNOWLEDGEMENTS. Many of the results reported here are based on collaboration with a number of colleagues whose input is greatly appreciated: Dr. A. Donner, PD Dr. R. Grümmer, PD Dr. H.-P. Hohn, Dorothea Schünke. We like to thank Gudrun Hellmund-Risse for typing the manuscript and D. Kittel and P. Babioch for the help with the illustrations. Permission to reproduce illustrations was granted by the following publishers: Wiley-Liss, a subsidiary of John Wiley and Sons, Inc., New York (Figs. 2a and c); TÜBITAK (Figs. 1a-f; 2b and d); Plenum Publishing Corp., New York (Figs. 3a-d).

These investigations were supported by Deutsche Forschungsgemeinschaft (DFG), Ministerium für Wissenschaft und Forschung Nordrhein-Westfalen, and Mildred-Scheel-Stiftung für Krebsforschung.

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