Cells Tissues Organs

Cells Tissues Organs 2002;172:237–252
DOI: 10.1159/000066963

In vitro Studies on Endometrial Adhesiveness for Trophoblast: Cellular Dynamics in Uterine Epithelial Cells

Michael Thie Hans-Werner Denker

Institut für Anatomie, Lehrstuhl für Anatomie und Entwicklungsbiologie, Universitätsklinikum Essen, Germany

Key Words
Embryo implantation · Uterine epithelium · Surface adhesiveness · Cell polarity

Abstract
Initiation of embryo implantation involves adhesion of trophoblast cells to the epithelial lining of the endometrium. The mechanisms regulating the adhesive properties of the uterine epithelium for trophoblast during initiation of human embryo implantation, however, are still incompletely understood. We report here on model studies that we have performed in our laboratory, and in particular on certain methodological approaches that seem to yield new insight into basic mechanisms involved. Of central interest is the ability of the uterine epithelium to develop an adhesion competence at its apical cell pole. This confronts us with a cell biological paradox in that adhesion must be established at the pole which in simple epithelia is typically specialized to resist adhesion. Gain of apical adhesion competence by uterine epithelial cells should be related to cellular rearrangements, i.e. a modulation of their apicobasal cell polarity. Here, we used monolayer-cultured uterine epithelial RL95-2 cells as an in vitro model for the human receptive uterine epithelium. We demonstrated that formation of stable cell-to-cell bonds between the free (apical) pole of these cells and attaching trophoblast (modelled by JAr cells) depends on a number of structural and functional peculiarities that RL95-2 cells have in contrast to other uterine epithelial cells (HEC-1-A cells) which resist attachment via this cell pole. RL95-2 cells were shown to lack tight junctions and to exhibit only rudimentary adherens junctions and a non-polar organization of the actin cytoskeleton. Using the atomic force microscope in a force spectroscopy mode, we exactly defined the time dependence of adhesive interactions between RL95-2 cells and trophoblast, measured the pressure force needed to initiate this process, and screened the buildup of the adhesive forces between the binding partners. A dynamic interaction between the actin cytoskeleton and integrins (a prerequisite for functional activity of integrins) was shown to be an important aspect of the adhesive properties of RL95-2 cells. In addition, at least two types of calcium channels in the plasma membrane of RL95-2 cells seem to play a role in activation of a variety of calcium-sensitive re-

Abbreviation used in this paper

AFM atomic force microscope

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1422–6405/02/1723–0237$18.50/0

Accessible online at:
www.karger.com/cto

Michael Thie
Institut für Anatomie, Lehrstuhl für Anatomie und Entwicklungsbiologie
Universitätsklinikum Essen, Hufelandstrasse 55
D–45122 Essen (Germany)
Tel. +49 201 723 4389, Fax +49 201 723 5916, E-Mail michael.thie@uni-essen.de

sponse mechanisms including adhesiveness for trophoblast, i.e. diltiazem-sensitive channels seem to contribute to the initiation of JAr cell binding and SKF-96365-sensitive channels to participate in a feedback loop that controls the balance of bonds. By extrapolation, these data suggest an active role of the uterine epithelium in the process of embryo implantation which we are just beginning to understand in terms of its cell biology.

Epithelial Cell Polarity and Embryo Implantation

Embryo implantation is initiated in the human around days 6 and 7 after ovulation within the so-called implantation window. This window is defined by the fact that the endometrium must have reached a state of receptivity, and the blastocyst a state of attachment/invasion competence. Signaling events, systemic and local, by hormones and cytokines that may play a role here have recently received much attention and have been discussed in a number of reviews [Lopata, 1996a, b; Tabibzadeh, 1998; Lessey, 2000; Paria et al., 2000; Salamonsen et al., 2000; Bagchi et al., 2001]. However, identification of the signaling molecules involved does not necessarily shed light on the cellular processes that must be instrumental in establishing cell-to-cell and cell-to-matrix contacts and subsequent trophoblast invasion. These cell biological aspects of the initial phases of embryo implantation will be the focus of the present communication.

The epithelial lining of the uterine cavity forms the natural surface to which the trophoblast of the blastocyst has to adhere at the beginning of implantation (fig. 1). A fundamental property of simple epithelia like the uterine epithelium is to establish a polarized organization, a prerequisite for fulfilling their surface barrier functions. As one aspect of this, each epithelial cell exhibits three distinct membrane domains. While basal and lateral membranes are studded with adhesion molecules so that they can mediate cell-to-cell and cell-to-matrix adhesion, apical plasma membranes normally lack most of these molecules, are even armed with bulky and charged molecules which inhibit contact formation and, thus, lack adhesive properties [Nelson, 1992; Drubin and Nelson, 1996; Yeaman et al., 1999a, b; Wodarz, 2001].

The beginning of embryo implantation confronts us, therefore, with the perplexing fact that uterine and trophoblast epithelium make their first contact via their apical cell poles. Indeed, this phenomenon may be seen as a cell biological paradox [Denker, 1986, 1990, 1993, 1994]. This invites comparison with other cell systems in which contact formation via apical cell poles of epithelia is observed. Any basic aspects derived from this comparison might be relevant to our attempts at understanding the cell biological basis of the specific states which the two partner tissues have to attain at implantation: the adhesive/invasive phenotype of the trophoblast [Armant et al., 2000; Hohn and Denker, 2002; Wang and Armant, 2002] and the state of receptivity of the uterine epithelium [reviewed by Denker, 1994; Carson et al., 2000; Denker and Thie, 2001].

Molecular changes in the composition of the apical plasma membranes of the uterine epithelium at receptivity have been defined in several investigations, e.g. a reduction in the thickness of the glycocalyx of uterine epithelial cells and in cell surface charge [Enders and Schlafke, 1977; Anderson et al., 1990; Morris and Potter, 1990; Carson et al., 1998a, b; Aplin, 1999; Niklaus et al., 2001] and/or the biosynthesis and expression of new cell surface proteins as well as of cell surface-bound glycoconjugates [Lampelo et al., 1985; Anderson et al., 1988; Kimber and Lindenberg, 1990; Hoffman et al., 1996; Carson et al., 1998b; Olson et al., 1998; Illingworth and Kimber, 1999; Niklaus et al., 1999]. However, a number of findings suggest that much more than the expression of apical membrane-associated molecules is changed in the uterine epithelium at acquisition of receptivity, i.e. changes are seen in apical, lateral and basal features of these cells. Obviously, the conditioning of the uterine epithelium for trophoblast adhesion involves an extensive reorganization of the cell architecture that comprises many aspects of the apicobasal polarity and not only changes at the apical cell pole that have been addressed as the so-called apical plasma membrane transformation [Terry et al., 1996; Murphy, 2000]. These observations have led to the concept that receptivity represents a change and/or downregulation in the expression of the polarized epithelial phenotype of the uterine epithelial cells [Denker, 1986, 1990, 1994; Glasser and Mulholland, 1993].

This concept invites comparison with other cell systems in which cell adhesion is initiated via the apical pole of epithelial cells. This is the case (1) in development, i.e. in the course of the so-called epithelial fusion processes [reviewed by Denker, 1986, 1994], and (2) in adult life in the special situation of endothelium-leukocyte interactions [Kimber and Spanswick, 2000; Aurrand-Lions et al., 2002; Wild et al., 2002]. Interestingly, the embryological epithelial fusion processes are (in the same way as endometrial receptivity) combined with more extensive

changes in cell architecture and behaviour than required for attachment via the apical cell pole alone. They are (in most cases) associated with a process called epithelial-mesenchymal transition during which at least some of the epithelial cells attain a migratory (mesenchymal, fibroblastoid) phenotype. It is known that during development cells are able to switch several times from an epithelial to a mesenchymal phenotype and vice versa. This process profoundly influences structural gene expression and cell behaviour and is thought to be governed by master genes which still have to be identified [Hay, 1990, 1995; Thiery and Chopin, 1999; Boyer et al., 2000; Savagner, 2001].

However, it must be pointed out that the application of this concept to uterine receptivity is still hypothetical. Loss (at least partial) of polar organization along the apicobasal axis appears to be a common theme for all those systems. Changes in molecular parameters appear to be less consistent as far as data are available. As discussed previously [Denker, 1993, 1994] uterine epithelial cells do not downregulate their complete epithelial programme but only selected parameters. Although these cells continue to express most epithelial (in contrast to mesenchymal) genes, they show e.g. reduced or destabilized apicobasal polarity, i.e. one characteristic phenomenon connected with incipient phases of epithelial-mesenchymal transition as well as with epithelial fusion processes [Denker, 1993, 1994]. The need for continued expression of an epithelial phenotype is illustrated by findings from in vitro model studies (see below) showing that cells which lack epithelial-type adhesion molecules (dedifferentiated endometrial AN3CA carcinoma cells; fibroblasts) do not support attachment [Thie and Denker, 1997].

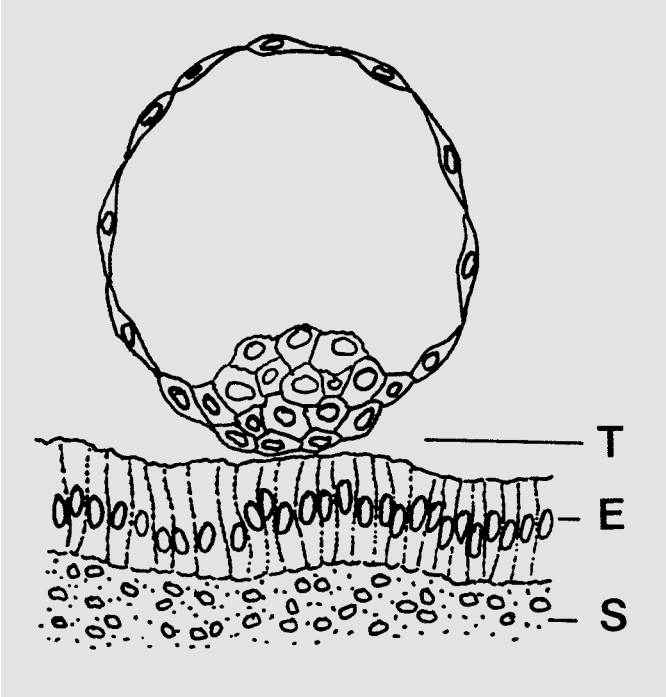


Fig. 1. Schematic drawing showing the cell-to-cell interaction between endometrium and blastocyst during the initiation of embryo implantation. Uterine epithelium (E) and blastocyst trophoblast (T) make their first contact via their free cell pole. S = Stroma [from Thie and Denker, 1997].

An in vitro Model for Simulation of Apposition and Adhesion of Trophoblast to Uterine Epithelium

The importance of certain functional elements of the complex process of implantation initiation is difficult to study in vivo, even in animal models. Moreover, these events are inaccessible to direct experimental investigation in the human for ethical reasons, in vitro as well as in vivo. We have, therefore, developed cell culture models that allow information about basic processes to be gained. Results obtained in these model studies give evidence for an active role of the uterine epithelium in the sense that it not only provides an adhesive surface for trophoblast attachment but also reacts in specific ways upon contact with trophoblast-type cells. These data are consistent with the view that, in vivo, the uterine epithelium is critical for regulating embryo implantation initiation [discussed in Denker and Thie, 2001].

In order to establish the in vitro models, our laboratory characterized parameters of the epithelial phenotype of a series of phenotypically different human endometrial cell lines and probed these for characteristics that mimic receptivity by monitoring adhesion of trophoblast-type cells. In these studies it was attempted to get insight into the programme underlying the regulation of apical adhesiveness of uterine epithelial cells. In brief, cell lines established from adenocarcinoma of human endometrium, i.e. RL95-2 cells [Way et al., 1983] and HEC-1-A cells [Kuramoto et al., 1972], were grown to confluence on poly-*D*-lysine-coated glass in medium supplemented with fetal calf serum [for additional details, see John et al., 1993; Thie et al., 1995]. These cell lines were selected from a larger pool of lines originally tested, as they stably express two phenotypes differing with respect to their cell polarity. In confluent HEC-1A monolayers (fig. 2A), the cells show a polarized epithelial phenotype with respect to the

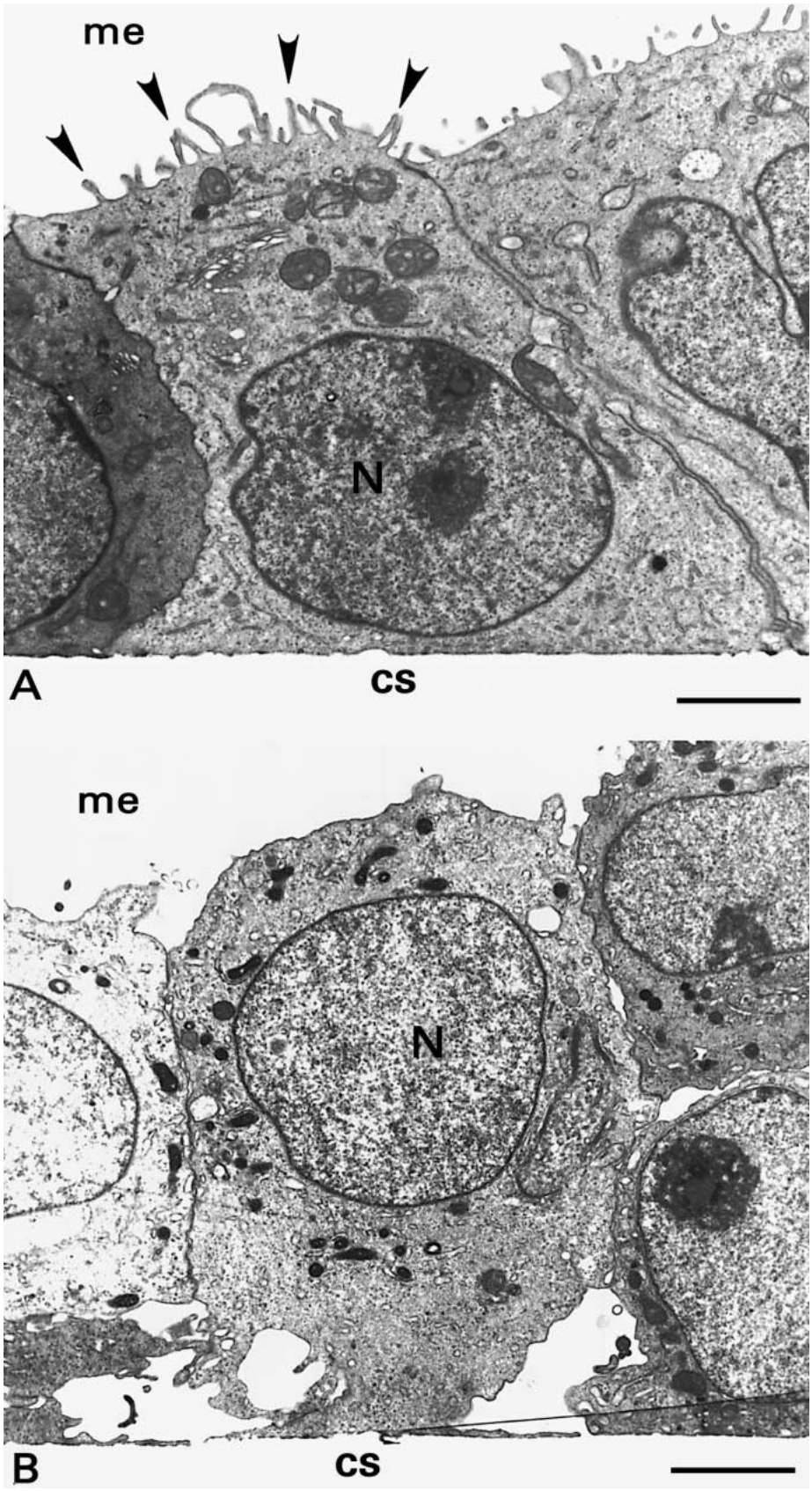
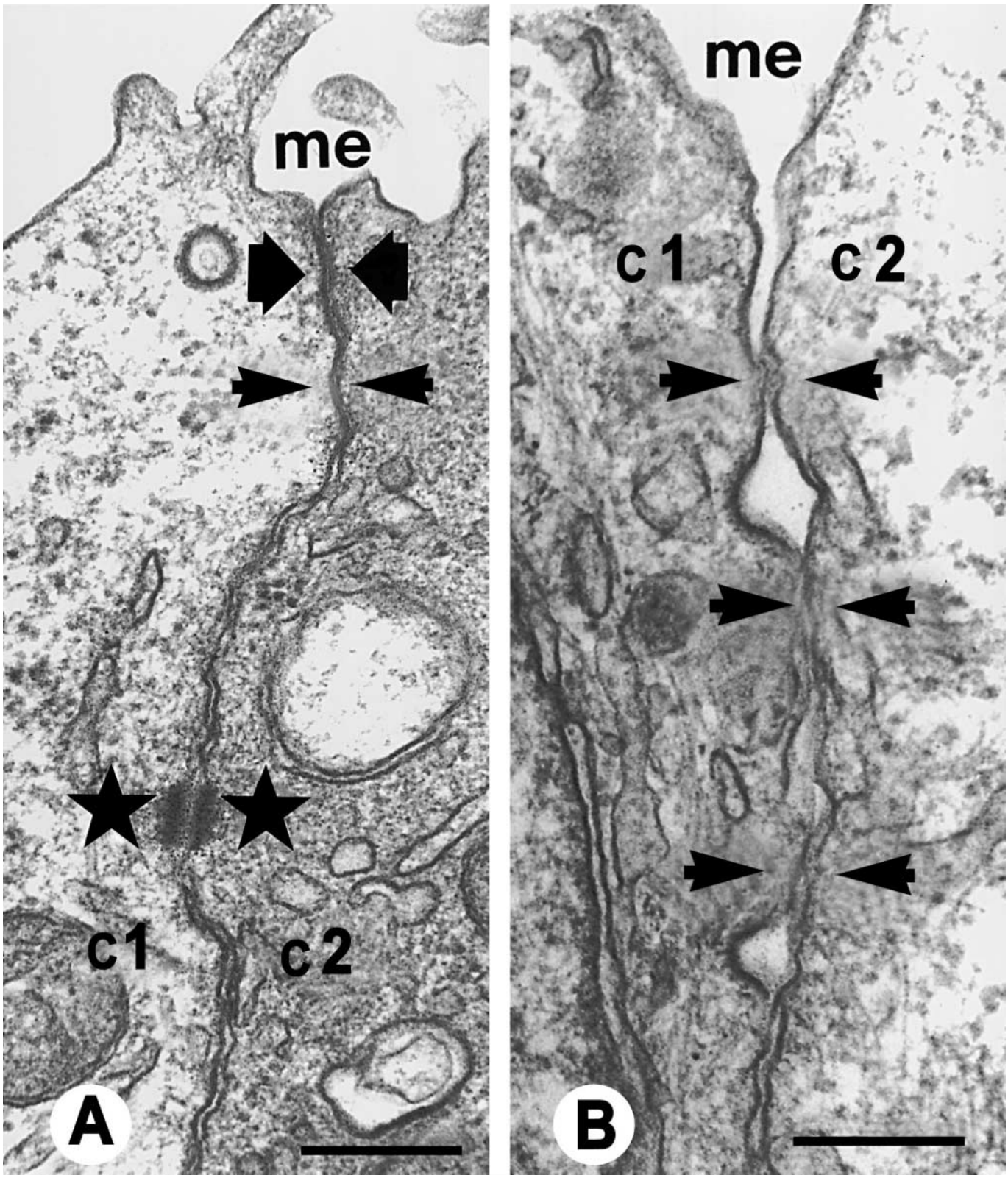


Fig. 2. Transmission electron micrographs of ultrathin sections of HEC-1-A cells (A) and RL95-2 cells (B), respectively, cultured on a poly-*D*-lysine-coated coverslip. HEC-1-A cells grow as an ordered monolayer exhibiting a highly polarized epithelial phenotype with numerous microvilli at the apical cell pole (arrowheads). RL95-2 cell monolayers show a lack of structural polarization and display only a few blunt microvilli at the upper cell surface. cs = Coverslip; me = growth medium; N = nucleus. Bars = 2 μm [from Thie et al., 1995].

Fig. 3. Membrane contacts of HEC-1-A cells (A) and RL95-2 cells (B), respectively, cultured on a poly-*D*-lysine-coated coverslip. HEC-1-A cells show closely apposed plasma membranes with properly formed tight junctions (thick arrows), adherens junctions (thin arrows) and desmosomes (asterisks). RL95-2 cells exhibit only primitive focal adherens junctions (arrows). me = Growth medium; c1 = cell 1, c2 = cell 2. Bars = 0.25 μm [from Thie et al., 1995].



3

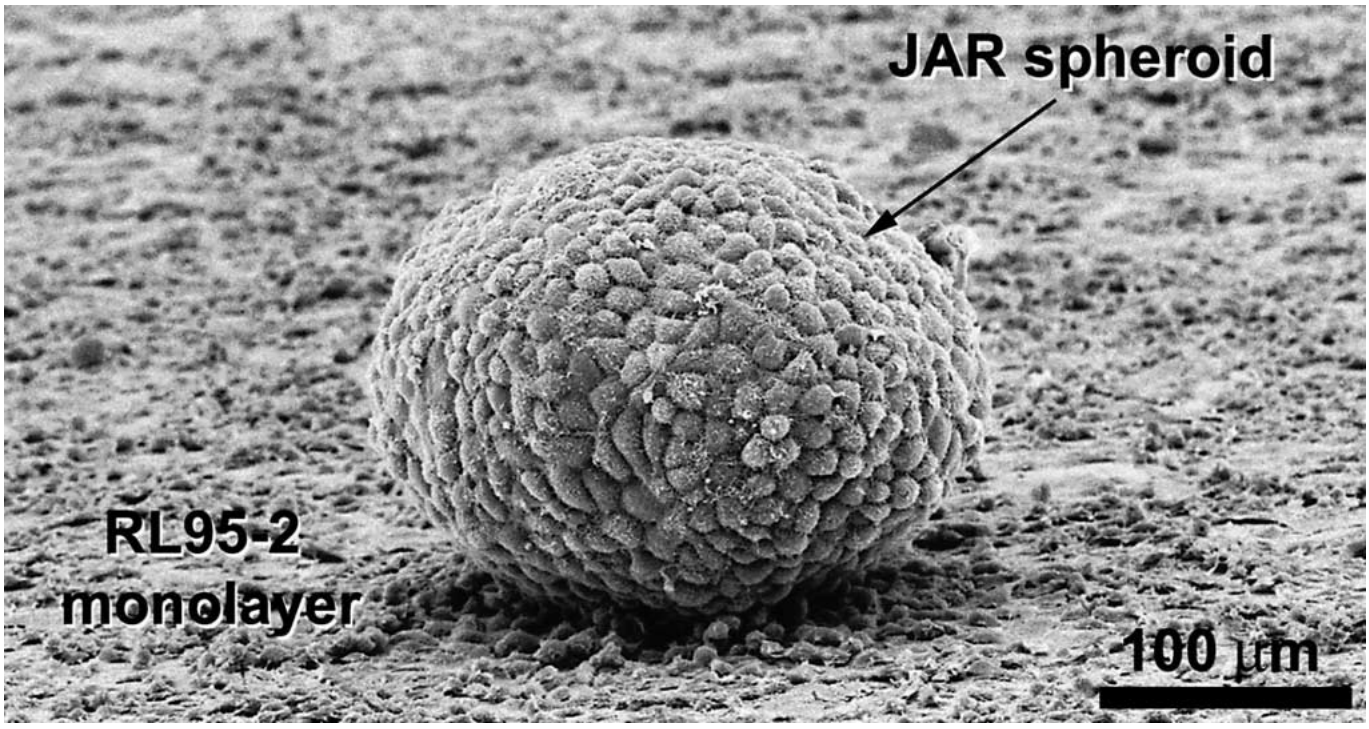


Fig. 4. Scanning electron micrograph of a single JAr spheroid adhering to the free surface of a RL95-2 cell monolayer 60 min after initial contact between the spheroid and the monolayer [from Tinel et al., 2000].

distribution of organelles and to membrane organization. Nuclei are situated at the base of the cells whereas mitochondria, endoplasmic reticulum and Golgi apparatus are located predominantly at the supranuclear region. The cells show closely apposed lateral plasma membranes with tight junctions in the subapical region as well as adherens junctions and desmosomes scattered along the lateral membrane (fig. 3A). The apical surface is covered with numerous short microvilli (fig. 2A).

In contrast, RL95-2 cells show ultrastructural features indicating lack of epithelial polarization (fig. 2B). Nuclei are located in the centre of the cell and organelles tend to pile up perinuclearly. Cells form primitive adherens junctions but no tight junctions (fig. 3B). The free surface of the cells appears dome-like and is largely free of microvilli (fig. 2B). Although RL95-2 cells exhibit a lack of structural polarization, these cells express proteins associated with the epithelial phenotype as do HEC-1-A cells. For example, RL95-2 cells express two different E-cadherin-catenin complexes and, with respect to the intermediate filament-desmosome system, both cell types express desmoplakin I and the same pattern of cytokeratin polypeptides (cytokeratin 7, 8, 18, 19) as well as vimentin. Thus, we have selected human endometrial cell lines which can be characterized as epithelial and polarized (HEC-1-A cells) versus epithelial and non-polarized (RL95-2 cells). As controls, non-polarized cells (fibroblasts; dedifferentiated endometrial cells lacking epithelial marker molecules, i.e. AN3CA cells) were also studied but will not be discussed in detail here [see Thie and Denker, 1997].

Functional analysis indicated that RL95-2 cells might be useful, in first order approximation, as an in vitro model for the receptive human uterine epithelium, and HEC-1-A for the non-receptive state. Apical adhesiveness of RL95-2 monolayers and HEC-1-A monolayers for human trophoblast-type cells was tested using an in vitro assay involving confrontation with multicellular spheroids of choriocarcinoma cells [routinely JAr cells; Pattillo et al., 1971]. In this system, JAr spheroids model the trophoblast of a blastocyst and RL95-2 or HEC-1-A monolayers represent the uterine epithelium (fig. 4; compare fig. 1 and 4). In brief, JAr spheroids were delivered onto confluent monolayers of endometrial cells. After 60 min of co-culture, spheroid adhesion to the monolayers was quantified using the centrifugal force-based adhesion assay [John et al., 1993]. Attached spheroids were counted,

and the results expressed as the percentage of the number of spheroids seeded initially. JAr cells attached with high efficiency to RL95-2 monolayers either in the presence or the absence of serum. In contrast, JAr cell attachment to HEC-1-A cells was low and comparable to attachment in the controls, i.e. poly-*D*-lysine-coated glass. Therefore, RL95-2 monolayers, but not HEC-1-A monolayers, were classified as adhesive for JAr cells when confronted from the luminal (apical) side. According to this, structural features of non-polarized epithelial cells were correlated with functional features of adhesiveness for trophoblast while structural features of polarized epithelial cells were correlated with non-adhesiveness.

Thus, RL95-2 cells mimic an important aspect of the in vivo situation at implantation initiation, i.e. apical adhesiveness, and might serve as an in vitro model for the receptive human uterine epithelium [Raboudi et al., 1992; John et al., 1993; Rhode and Carson, 1993; Thie et al., 1995, 1996, 1997, 1998; Liu et al., 1998; Chervenak and Illsley, 2000; Hohn et al., 2000; Martin et al., 2000; Tinel et al., 2000; Perret et al., 2001].

Cellular Dynamics in Receptive Uterine Epithelial Cells as Related to Trophoblast Binding Capacity

The RL95-2 cell culture model allowed us to systematically study certain mechanisms involved in formation of cell-to-cell contacts between uterine epithelial cells and trophoblast. New aspects derived from these investigations indicate that this cell-to-cell interaction is a remarkably slow process and that it includes sequential steps of bond formation as well as complex signal transduction cascades [Thie et al., 1997, 1998].

Role of the Actin Cytoskeleton

The actin cytoskeleton is known to respond to integrin signaling and to play an essential role in mediating cellular responses in various cell systems [Giancotti and Ruoslahti, 1999; Calderwood et al., 2000; Geiger et al., 2001; Geiger and Bershadsky, 2001]. Uterine epithelial cells have been reported to undergo a reorganization of their apical cortical actin network at receptivity in the rat [Luxford and Murphy, 1992]. In RL95-2 cell cultures, a prominent actin cytoskeleton is found in the cell cortex but actin-containing microvilli are largely missing at the apical membrane [Thie et al., 1996]. Likewise, various integrins (α6, β1, β4) are found evenly distributed along the entire plasma membrane of RL95-2 cells including its apical (free) aspect [Thie et al., 1995]. This is in marked contrast to HEC-1-A cells which show the pattern of distribution that is typical for a polarized epithelium, i.e. a specialized actin cytoskeleton at the apical pole with ordered insertions of the filament bundles in the microvilli, and integrins concentrating at the basolateral membranes but sparing the apical aspect. In our in vitro model we have used cytochalasin D, an inhibitor of actin polymerization [Cooper, 1987], in order to gain insight into the role of actin filaments (and integrins) in apical adhesiveness of RL95-2 cells for trophoblast.

When exposed to cytochalasin D, RL95-2 cells showed a loss of their apical adhesiveness for trophoblast. JAr spheroid attachment rates were found to be reduced to values which were comparable to those of attachment to poly-*D*-lysine-coated glass coverslips [this was true in the presence as well as in the absence of fetal calf serum; Thie et al., 1997]. α6, β1 and β6 integrin subunits remained evenly distributed over the whole cell periphery in cytochalasin D-treated cells. However, two main changes in microfilament architecture were observed: the microfilament system subjacent to the plasma membrane appeared diminished and/or disassembled and numerous coarse actin aggregates were present in varying locations within the cytoplasm.

The presence of integrins at the apical pole of RL95-2 cells suggests that signaling processes could be initiated here, in contrast to HEC-1-A cells where integrins are lacking in this specific membrane region. In order to investigate this experimentally we applied mechanical forces to the integrins and monitored changes in intracellular free calcium. Anti-integrin antibody-coated microbeads were loaded onto the free surface of RL95-2 monolayer cultures, followed by mechanical stimulation of the cells via application of defined forces in a magnetic drag apparatus [Thie et al., 1997]. It was possible to obtain typical responses of the cells that were characterized by increases in intracellular free calcium levels. Interestingly, calcium response patterns differed depending on the type of integrin subunit involved, e.g. while stimulation via integrin subunit α6 provoked a weak increase in intracellular calcium 50–150 s after starting mechanical stress, stimulation via integrin subunit β1 provoked a strong increase 200–300 s after starting stimulation (fig. 5). In contrast to untreated RL95-2 cells, cytochalasin D-treated cells showed no calcium signals after application of the same mechanical stress via any of the two integrin subunits. Thus, the loss of trophoblast binding competence of RL95-2 cells after cytochalasin D treatment and the block of integrin-mediated calcium signaling can be expected to

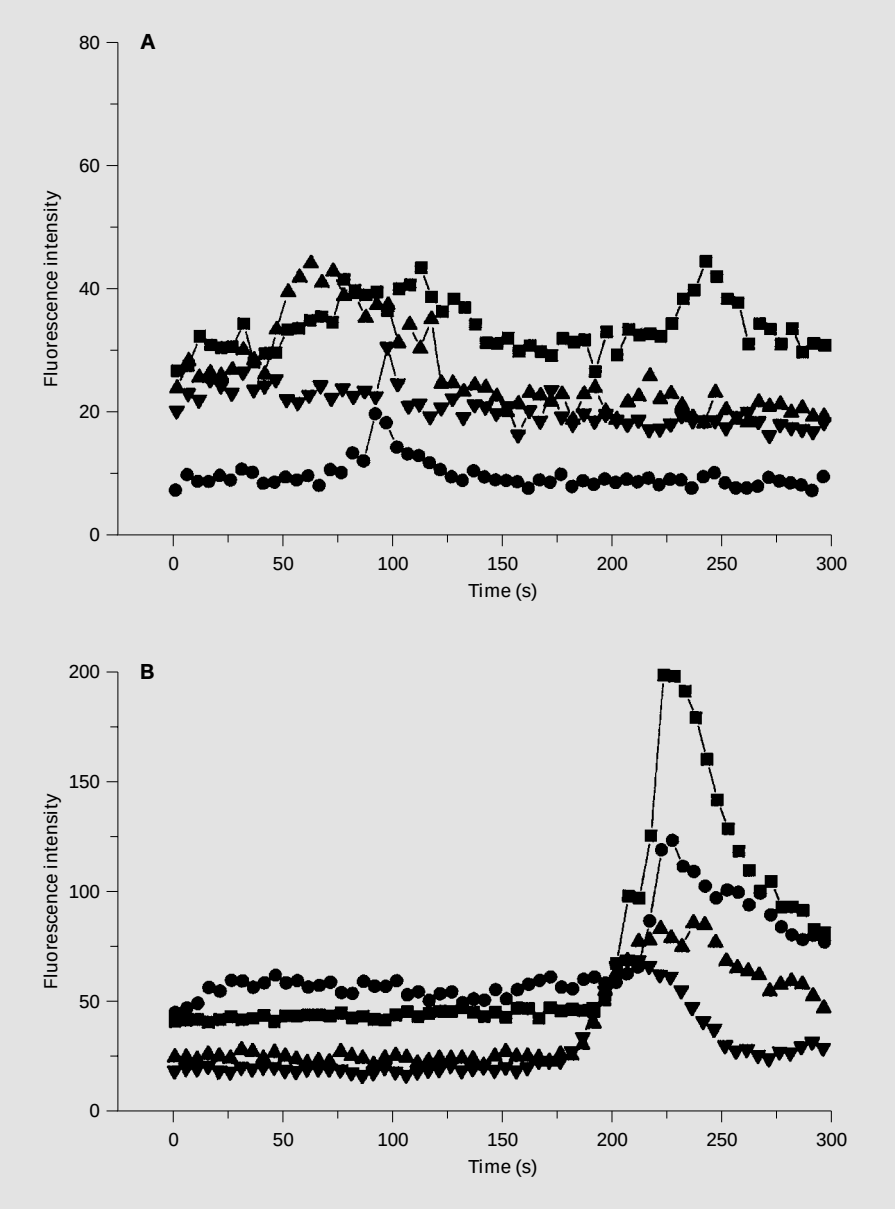


Fig. 5. Intracellular calcium signals of RL95-2 cells followed for 300 s after mechanical stimulation of the integrin subunit α6 (A) and the integrin subunit β1 (B). Calcium responses from four different cells (see symbols) were recorded [from Thie et al., 1997].

be causally connected, both requiring intact cytoskeletal links to integrins. These observations strongly suggest that not only proper organization of the actin cytoskeleton but also proper interaction between cytoskeleton and integrins (a prerequisite for functional activity of integrins) might be important aspects of the adhesive interaction between uterine epithelium and trophoblast.

Ca^{2+} as a Messenger

Calcium is well known to be involved in many cellular signal transduction pathways, and regulation of cell adhesion by calcium has been observed in many cell systems [Clark and Brugge, 1995; Sjaastad and Nelson, 1997; Evenas et al., 1998; for calcium responses in trophoblast cells, see Wang and Armant, 2002]. In a series of further experiments, we demonstrated that calcium signaling is elicited in RL95-2 cells not only via antibody-coated beads but also in a more physiological setting, i.e. upon contact with human trophoblast (JAr) cells and that this plays a role in their subsequent binding. The results suggest that this process seems to require the opening of at least two different types of calcium channels in the mem-

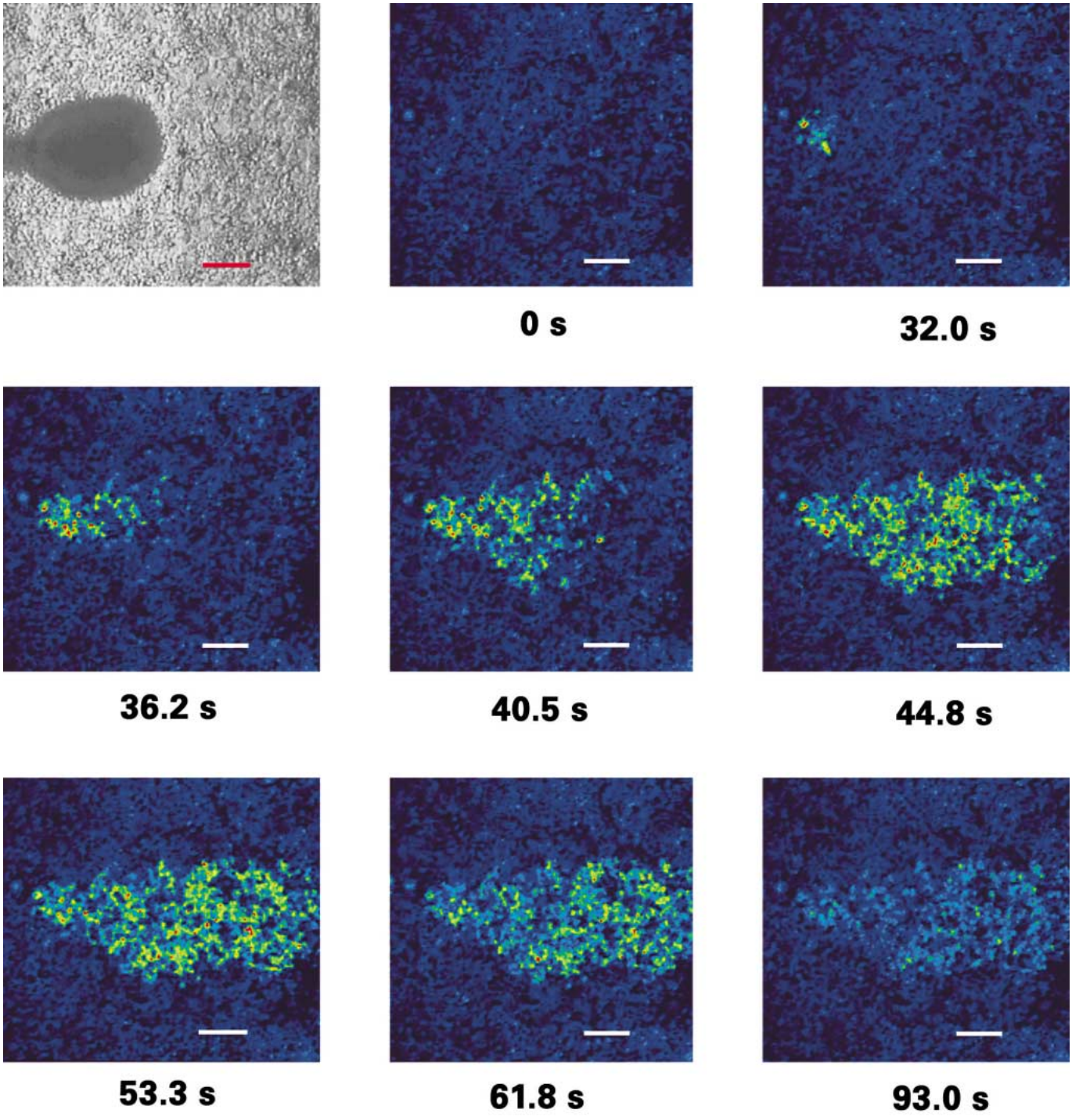
brane of RL95-2 cells and an influx of calcium from the extracellular fluid, transiently increasing the cytosolic calcium concentration.

In brief, impact of JAr spheroids upon monolayers of RL95-2 cells evoked a marked increase of intracellular calcium, i.e. the fluorescence ratio increased within 15 s after initial contact, reached a maximum and decreased towards the precontact level within 120 s [Tinel et al., 2000]. After recovery from this initial impact, no further changes in intracellular calcium were observed in the RL95-2 monolayers while JAr spheroids were kept stationary here. Movement of JAr spheroids across monolayers of RL95-2 cells, on contrast, again evoked changes in intracellular calcium, i.e. the calcium increased transiently as a response to spheroid movement (fig. 6). The separation of cell-to-cell bonds after prolonged contact between JAr spheroids and RL95-2 monolayers also led to calcium signals in the latter dependent on the duration of cell-to-cell contact. For example, the peak of fluorescence after 60 min of contact was 2.4-fold higher than the value after 10 min of contact [Tinel et al., 2000]. Further experiments showed that the calcium influx induced by separation of cell-to-cell bonds between JAr spheroids and RL95-2 cells consisted of two components. One part of the signal appeared to be basic calcium influx. The second part increased with the duration of contact and seemed to be based on receptor ligand interactions activating a receptor-mediated calcium channel. Using SKF-96365, an inhibitor of receptor-mediated calcium channels [Merritt et al., 1990], the influx of calcium could be reduced. Interestingly, the mechanism of calcium influx in RL95-2 cells evoked by movement of JAr spheroids appears to differ from that activated by separation of cell-to-cell bonds between spheroids and RL95-2 cells, i.e. pretreatment of RL95-2 cells with the calcium channel blocker diltiazem [Triggle, 1999] reduced the calcium increase in this case. SKF-96365 (but not diltiazem) also reduced adhesion of JAr spheroids to RL95-2 cells.

So, at least two types of calcium channels in the plasma membrane of RL95-2 cells seem to play a role in calcium influx and activation of a variety of calcium-sensitive response mechanisms including adhesiveness of uterine cells to trophoblast. In this context, diltiazem-sensitive channels seem to contribute to initiation of JAr cell binding and SKF-96365-sensitive channels may participate in a feedback loop that controls the balance of bonds. As shown by appropriate control experiments on extracellular calcium depletion, these processes seem to depend on calcium influx rather than on calcium release from intracellular stores in RL95-2 cells [Tinel et al., 2000]. As far as the cellular response to this calcium signaling is concerned, it is to be expected that a complex cascade of events is initiated at the free pole of uterine epithelial RL95-2 cells which culminates in the remodeling of this cell pole to allow firm adhesion of the trophoblast to take place. With regard to the above-mentioned data, at least some of the components involved in this process should contain elements regulated by calcium ions. The observed calcium dependence should be helpful in identifying these individual components.

Probing the Kinetics of Cell-Cell Adhesion by Force Spectroscopy

In order to gain more direct access to an analysis of the kinetics of the adhesive interactions, and to provide quantitative data on the adhesive forces measureable at the apical (free) pole of RL95-2 cells, we used a novel type of application of the atomic force microscope (AFM) [Binnig et al., 1986; Rugar and Hansma, 1990; Radmacher et al., 1992], i.e. force spectroscopy [for instrumentation and methodology of force measurements, see also Benoit and Gaub, 2002]. The experimental setup which we have designed for this purpose is schematically depicted in the upper inset of figure 7A. A microbead glued to the AFM cantilever was covered by a monolayer of JAr cells and served as a model for the implantation stage blastocyst/adhesion-competent trophoblast. This was brought into contact with a monolayer of RL95-2 cells (or HEC-1-A or other) under controlled conditions, and for defined periods of times [Thie et al., 1998]. The instrument used was a custom-made AFM [Florin et al., 1994; Ludwig et al., 1997; Rief et al., 1997]. Forces were measured first while lowering the JAr cells onto the free surface of endometrial cells and, thereafter, during separation of the two partner cell types. During the approach phase, two distinct types of repulsive interaction were distinguishable by recording the parameters of indentation: long-range soft repulsion followed by short-range hard repulsion [Thie et al., 1998]. The transition from the soft repulsion to the hard repulsion was continuous. With respect to the subsequent retraction part of the measuring cycle, distinct force versus distance curves were obtained which were consistent for the cell type used. Figure 7A shows force versus distance curves for non-adhesive HEC-1-A cells while figure 7B depicts the buildup of adhesive forces between JAr cells and the surface of RL95-2 monolayers. In the latter case, the first part of the separation curve reflected the reversal of the previous indentation of the JAr cells into the RL95-2 monolayers, which decreased with retraction until the point of zero applied force to the cantilever was



6

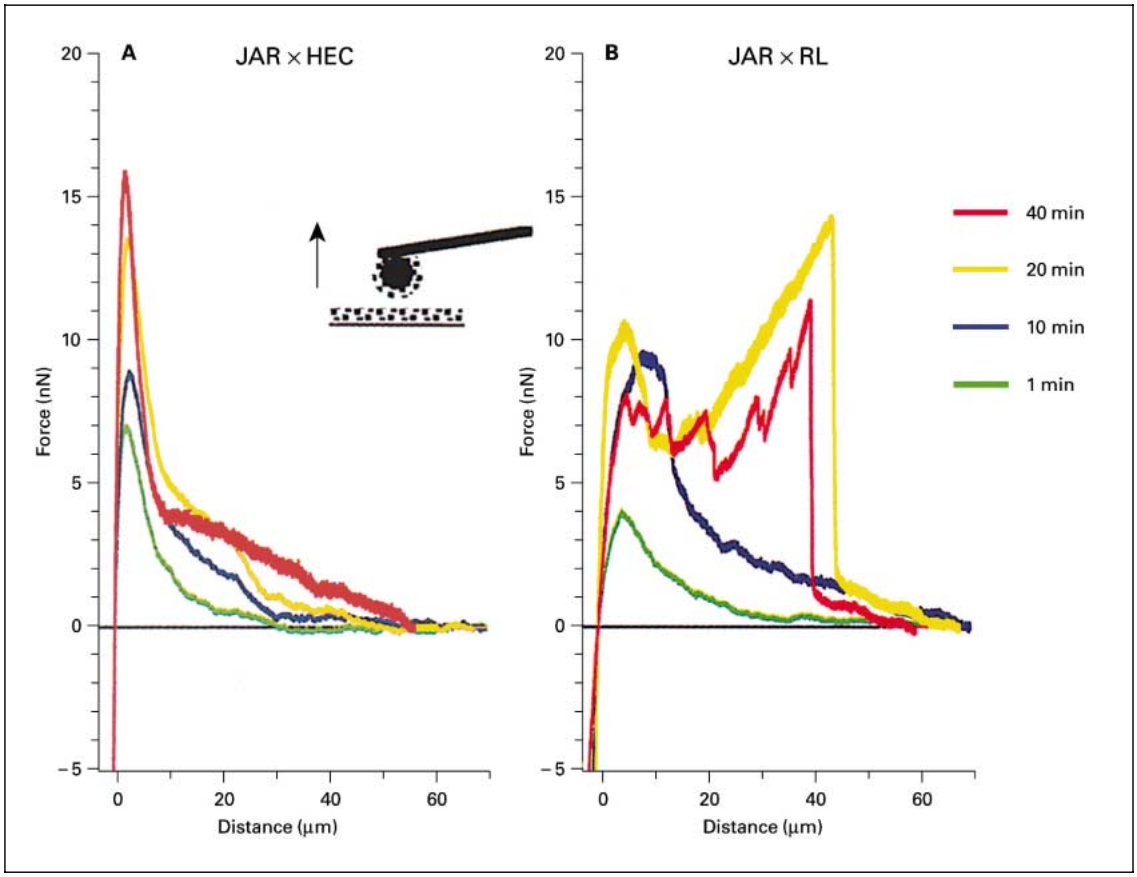


Fig. 7. Force spectroscopy experiments. Adhesive force curves for HEC-1-A cells (A) and RL95-2 cells (B) resulting when a JAr-coated microbead (JAR) was retracted after periods of 1–40 min of contact with the endometrial monolayer (JAR × HEC; JAR × RL). The horizontal axis shows the vertical movement of the cantilever; the vertical axis shows the force acting on the microbead. Note force rupture events when a JAr-coated microbead was retracted from RL95-2 cells after 20 or 40 min (B) [from Thie et al., 1998].

reached. In the case of adhesive interaction, there was a transition from the repulsive to the adhesive regime. The magnitude of the adhesion forces and the distance at which the surfaces finally separated completely depended on the duration of the contact. When JAr cells were separated from the monolayers within milliseconds after contact, no adhesion was observed. However, considerable adhesive forces were finally built up when the duration of contact was increased. One minute after initial cell-to-cell contact, the maximum force was around 4 nN. When the JAr cells were brought into contact for a prolonged time, i.e. 20 or 40 min, the adhesive maxima increased significantly. Characteristically, separation curves showed discrete force rupture events with increasing distance only after these prolonged interaction times. These probably represent the separation of individual membrane cell-to-cell contact areas, although the morphological equivalents still need to be identified.

Fig. 6. Pseudocolour images showing changes in intracellular calcium concentration in RL95-2 cells during movement of a JAr spheroid on the surface of an RL95-2 monolayer. The first image shows a transmitted light picture of the spheroid resting on the free surface of the RL95-2 monolayer. The following fluorescence ratio images illustrate changes in intracellular calcium concentration as seen at the indicated time points after the beginning of spheroid movement (direction from left to right). Before movement was initiated at 0 s, the spheroid had been kept resting on the monolayer for 20 s. Colour scale shows the fluorescence ratio range. Bars = 100 µm [from Tinel et al., 2000].

The major advantage of this experimental system is to exactly define the time dependence of adhesive interactions between trophoblast and uterine epithelium as well as to measure the pressure force needed to initiate the pro-

cess, and to screen the buildup of the adhesive forces. Further detailed analysis of the force distance curves, combined with appropriate experimentation, should allow the detection of finer details of the cascade of bond formation. For example, interactions with the glycocalyx (soft interaction) followed by more direct contact with the membrane-cytoskeleton complex as well as the contribution of the various types of intercellular junctions to the overall adhesive force could be studied. At the present state of analysis, available data already show that the adhesion of trophoblast to uterine epithelium appears to be a relatively slow process (when compared with endothelium-leukocyte interactions), possibly including signal transduction cascades and sequential steps of cell-to-cell binding. The experimental AFM approach allows us to identify the contribution of individual signaling events and the individual elements of the adhesion machinery under conditions that can be controlled in a relatively stringent manner.

The Cell Biological Basis of Embryo Implantation: Some New Ideas

Concepts concerning the cascades of events thought to be involved in the initial phase of embryo implantation have been largely derived from morphological observations. Electron microscopy suggests that direct cellular interactions begin with membrane contact at the apical cell poles of trophoblast and uterine epithelium. According to morphological criteria, this should lead to immobilization of the blastocyst within a part of the uterine lumen that will subsequently be addressed as the implantation chamber. This first process defines the individual cells of both partners that will remain adjacent to each other and that will interact more intensely. Membrane contacts between trophoblast and uterine epithelial cells then become more and more intimate suggesting that some degree of adhesive force increasingly builds up there [Schlafke and Enders, 1975]. Subsequently, the trophoblast overcomes the uterine epithelium in a process which appears to differ from one species to the other (intrusion; displacement and fusion type of implantation). At least in the intrusion type of implantation (which is thought to be the mode of operation in primates including the human [Bentin-Ley et al., 2000] although fusion does not seem to be totally excluded as a mode here) trophoblast and uterine epithelial cells keep a very close membrane-to-membrane contact while the trophoblast penetrates intercellularly, and the cytoplasmic architecture including the cytoskeleton of both types of cells displays marked alterations [Enders et al., 1983; Enders and Mead, 1996; Enders and Lopata, 1999]. These morphological observations strongly suggest that adhesive interactions via adhesion molecules like cadherins or integrins and an active participation of the actin cytoskeleton might be involved here. This remains true no matter whether the first contact of trophoblast with the uterine epithelium is made via the whole apical plasma membrane or via membrane stretches close to the lateral borders, i.e. to the junctional complex [Enders and Mead, 1996; Enders and Lopata, 1999; Bentin-Ley et al., 2000]. However, as pointed out by Lopata [1996a, b], actual adhesive forces that build up here during the initial phase of implantation have never been measured in vivo or ex vivo, not even in animal models, nor was it possible to study directly the interaction of these two partner cell types via the mentioned types of molecules and organelles. Due to this, it has even been questioned whether adhesion plays any role during this phase, and other authors favour the idea that programmed cell death rather than cell rearrangements plays the main role here [von Rango et al., 1998; Pampfer and Donnay, 1999; Galan et al., 2000a, b; Fei et al., 2001; Li et al., 2001; Selam et al., 2001; Simon et al., 2001].

The cell line-based in vitro models that have been discussed in the previous sections are certainly quite artificial so that data obtained in this way must be interpreted with caution. To what extent these observations can be extrapolated to the in vivo situation must be checked in future experiments, e.g. involving freshly explanted endometria. With this caution in mind it appears possible, however, to derive some ideas on individual cellular processes that may play a significant functional role in this initial phase of trophoblast-to-endometrial interactions.

RL95-2 cells which have been employed here as an in vitro model for the receptive uterine epithelium express a phenotype of epithelial cells that lack a pronounced apicobasal axis [Thie et al., 1995, 1996, 1997]. These characteristics are relevant for the adhesiveness of the free pole of RL95-2 cells for trophoblast, as uterine epithelial HEC-1-A cells, which exhibit a well-developed epithelial polarity [Drubin and Nelson, 1996; Yeaman et al., 1999a, b], do not allow trophoblast to adhere [John et al., 1993; Thie et al., 1995]. Thus, our data support the idea of phenotype modulation of uterine epithelial cells as a prerequisite for adhesiveness for trophoblast. The acquisition of adhesion competence of the apical cell pole should be part of complex cell biological transformations that uterine epithelial cells undergo [Denker, 1993, 1994]. These transformations may be under the control of sex steroids as well as

cytokines and other locally acting short-range signals emitted by the blastocyst.

The first adhesive interactions between trophoblast and uterine epithelium should involve peripheral proteins and carbohydrate groups extending far from the membrane (glycocalyx). These interactions are expected to firstly slow down the movement of the blastocyst within the uterine lumen so that the implantation chamber becomes defined. This might occur somewhat in analogy to the rolling of leukocytes at the surface of the blood vessel endothelium [Kimber, 2000; Kimber and Spanswick, 2000; Aurrand-Lions et al., 2002; Wild et al., 2002]. Indeed, in species in which the blastocyst coats (zona pellucida) are shed late the immobilization of the blastocyst can even involve these extracellular structures, specifically modifications of and interactions via their carbohydrate side chains [Böving, 1963; newer data e.g. on the horse discussed in Denker, 2000]. After shedding of the coats, a carbohydrate side chain-mediated interaction at this initial phase of apposition may also directly involve the cell surfaces of both partner epithelia, as discussed already at an early time point [Denker, 1970] and more recently [Kimber et al., 1995; Kimber, 2000; Kimber and Spanswick, 2000]. One would speculate that trophoblast adhesion to the uterine epithelium is reinforced subsequently by other types of cell surface molecules, e.g. integrins, and by the formation of discrete junctions. For the JAr-RL95-2 system we have found evidence that this time-dependent process includes sequential steps of bond formation [Thie et al., 1998]. In the AFM experiments, the initiation of trophoblast binding seemed to depend on a preparative phase of pressing the probe against the uterine RL95-2 cell monolayer. It was interesting to see that, in order to overcome the repulsive forces between the trophoblastic cells and the uterine epithelial cells, the cell-to-cell contact must occur with a distinct pressure. Thereafter, it is important that the contact must be secured for a certain time. If the duration of contact time and the pressure (force) of impact is insufficient, no strong binding will be established. This biological phenomenon might explain the necessity for immobilization of the blastocyst during the apposition phase.

During consolidation of the cell-to-cell contact, the phenotypic changes seen in the uterine epithelial cells appear to be progressing as electron microscopy of in vivo specimens suggests. It can be asked what directionality the causal interconnections might have. Does the membrane contact with trophoblast cause the uterine epithelial cells to continue with their initiated partial loss of apicobasal polarity and with the cytoskeletal modifications, or does the continuing modification of the uterine epithelial cells permit the trophoblast to continue with its maximization of membrane contacts and its penetration? Perhaps we are dealing with a phenomenon of reciprocity here. Whatever the causal interrelationship is, at the end of this phase, the blastocyst will adhere to a less-polarized uterine epithelial cell in a strong manner. When the first phase was successful, a cascade of reactions will start, which lead to further structural and functional modulation of the receptive uterine epithelial cell as electron microscopy of epithelial cells situated next to the penetrating trophoblast suggests. In the course of this process, properly localized adhesion molecules will interact with the cytoskeleton. As shown here, perturbation of the actin cytoskeleton decreased adhesive properties of the apical pole of RL95-2 cells, indicating that an unaltered organization of the actin cytoskeleton is indispensable for JAr spheroid adhesion [Thie et al., 1997]. Moreover, certain integrin receptor complexes on the apical (free) aspect of these cells seem to play a major role in trophoblast adhesion to RL95-2 cells. However, the molecules that mediate trophoblast adhesion to uterine epithelial cells in vivo are not well defined. Possible candidates are certain glycoconjugates [Carson et al., 2000; Kimber, 2000], trophinin [Aoki and Fukuda, 2001], cadherins [Thie et al., 1995] and integrins [Albers et al., 1995; Lessey et al., 1995; Thie et al., 1995, 1997; Lessey, 1998; Burghardt et al., 2002]. In addition, antiadhesion systems may modulate the first steps of this cell-to-cell interaction [Chervenak and Illsley, 2000; Aplin et al., 2001; Meseguer et al., 2001].

We have shown that the mechanical stimulation of RL95-2 cells via apical membrane-bound integrins (using beads or JAr cells), can elicit intracellular calcium waves which appear to be one aspect of mechanical/chemical signaling. Moreover, in RL95-2 cells, diltiazem-sensitive calcium channels were involved in initiating trophoblast binding, and SKF-96365-sensitive calcium channels seemed to participate in a feedback loop that controls the balance of bonds [Tinel et al., 2000]. So far, no further details are known about this signaling pathway in RL95-2 cells and how the signal is networked. Although not yet studied in detail, small GTPases of the Rho family might represent one missing link in these signaling cascades [Thie et al., 2002]. It is well known that Rho GTPases regulate various aspects of cell adhesion, including formation of focal contact sites and stress fibres [Bishop and Hall, 2000]. Rho GTPases play an essential role in membrane receptor signaling, including integrin receptors [Aplin et al., 1998]. All these data strongly suggest that Rho GTPases might regulate properties of the apical pole

of uterine epithelial cells that are crucial for adhesion competence for trophoblast binding.

Several lines of evidence from in vivo and in vitro experiments demonstrate that the epithelial lining of the uterine cavum plays a crucial role in regulating implantation initiation, acting as an effective barrier for trophoblast invasion outside receptivity [discussed in Denker and Thie, 2001]. The barrier model, however, does not seem to describe the role of the uterine epithelium completely. The data discussed above strongly suggest that the luminal epithelial lining of the endometrium should not be seen just as a passive firewall but as an active partner for trophoblast adhesion and invasion. Our data are consistent with the concept that uterine epithelial cells in the receptive state possess a cytoplasmic/membrane architecture of a modified type with reduced/downregulated apicobasal polarity. According to this, the apical cell pole has developed a new reactivity and is equipped with appropriate sets of adhesion molecules and signaling systems. There should be a first line of molecules responsible for weak adhesion and signaling followed in an overlapping way by a second/third line of other partner molecules and signaling events. Indeed, an increasing number of data suggests that in conjunction with trophoblast binding a cascade of signaling events must be initiated in the apical (sub)membrane region of uterine epithelial cells (maybe first involving membrane stretches close to the junctional complex as mentioned above) and that these signaling events are an essential element of adhesion of trophoblast and of embryo implantation initiation. Apparently not only the apical cell pole of the uterine epithelium is being reorganized but the lateral plasma membrane domains are changed also, leading to alterations in the junctional complex that facilitate the transmigration of trophoblast cells over the epithelial barrier that follows initial attachment to the free cell surface. Detailed studies (e.g. involving confocal laser scanning microscopy of attachment and invasion sites) presently being performed will hopefully shed light on any interrelationships that may exist between the initiation of cellular interactions at the apical cell pole and the subsequent interactions with the lateral plasma membrane during transmigration.

Acknowledgements

We wish to thank our colleagues A. Albers, M. Benoit, S. Butz, W. Dettmann, F. Dürr, P. Fuchs, H.E. Gaub, B. Harrach-Ruprecht, C. Heneweer, P. Herter, H.P. Hohn, H. Hoschützky, R. Kemler, L.H. Kruse, M. Ludwig, B. Nebe, H. Pommerenke, R. Röspel, J. Rychly, H. Sauer, M. Schmidt, F. Sieckmann and H. Tinel for their support during the course of these studies. The skillful technical assistance of B. Gobs, B. Maranca, D. Schünke and U. Tlolka is gratefully acknowledged. Permission to reproduce illustrations was granted by Antonio Delfino Editore, Roma (fig. 1), Urban & Fischer Verlag, Jena (fig. 2 and 3), Oxford University Press, Oxford (fig. 4–7).

References

Albers, A., M. Thie, H.P. Hohn, H.-W. Denker (1995) Differential expression and localization of integrins and CD44 in the membrane domains of human uterine epithelial cells during the menstrual cycle. Acta Anat *153:* 12–19.

Anderson, T.L., S.M. Sieg, G.D. Hodgen (1988) Membrane composition of the endometrial epithelium: Molecular markers of uterine receptivity to implantation; in Iizuka, R., K. Semm (eds): Human Reproduction. Int Congr Ser No 768. Amsterdam, Excerpta Medica, pp 513–516.

Anderson, T.L., J.A. Simon, G.D. Hodgen (1990) Histochemical characteristics of the endometrial surface related temporally to implantation in the non-human primate *(Macaca fascicularis)*; in Denker, H.-W., J.D. Aplin (eds): Trophoblast Invasion and Endometrial Receptivity. Novel Aspects of the Cell Biology of Embryo Implantation. Trophoblast Research. New York, Plenum Medical Book, vol 4, pp 273–284.

Aoki, R., M.N. Fukuda (2000) Recent molecular approaches to elucidate the mechanism of embryo implantation: Trophinin, bystin, and tastin as molecules involved in the initial attachment of blastocysts to the uterus in humans. Semin Reprod Med *18:* 265–271.

Aplin, A.E., A. Howe, S.K. Alahari, R.L. Juliano (1998) Signal transduction and signal modulation by cell adhesion receptors: The role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. Pharmacol Rev *50:* 197–263.

Aplin, J.D. (1999) MUC-1 glycosylation in endometrium: Possible roles of the apical glycocalyx at implantation. Hum Reprod *14(suppl 2):* 17–25.

Aplin, J.D., M. Meseguer, C. Simon, M.E. Ortiz, H. Croxatto, C.J. Jones (2001) MUC1, glycans and the cell-surface barrier to embryo implantation. Biochem Soc Trans *29:* 153–156.

Armant, D.R., J. Wang, Z. Liu (2000) Intracellular signaling in the developing blastocyst as a consequence of the maternal-embryonic dialogue. Semin Reprod Med *18:* 273–287.

Aurrand-Lions, M., C. Johnson-Leger, C. Lamagna, H. Ozaki, T. Kita, B.A. Imhof (2002) Junctional adhesion molecules and interendothelial junctions. Cells Tissues Organs *172:* 152–160.

Bagchi, I.C., Q. Li, Y.P. Cheon (2001) Role of steroid hormone-regulated genes in implantation. Ann NY Acad Sci *943:* 68–76.

Benoit, M., H.E. Gaub (2002) Measuring cell adhesion forces with the atomic force microscope at the molecular level. Cells Tissues Organs *172:* 174–189.

Bentin-Ley, U., T. Horn, A. Sjögren, S. Sörensen, J. Falck Larsen, L. Hamberger (2000) Ultrastructure of human blastocyst-endometrial interactions in vitro. J Reprod Fertil *120:* 337–350.

Binnig, G., C.F. Quate, C. Gerber (1986) Atomic force microscope. Phys Rec Lett *56:* 930.

Bishop, A.L., A. Hall (2000) Rho GTPases and their effector proteins. Biochem J *348:* 241–255.

Böving, B.G. (1963) Implantation mechanisms; in Hartmann, C.G. (ed): Mechanisms Concerned with Conception. New York, Pergamon Press, pp 321–396.

Boyer, B., A.M. Valles, N. Edme (2000) Induction and regulation of epithelial-mesenchymal transitions. Biochem Pharmacol *60:* 1091–1099.

Burghardt, R.C., G.A. Johnson, L.A. Jaeger, H. Ka, J.E. Garlow, T.E. Spencer, F.W. Bazer (2002) Integrins and extracellular matrix proteins at the maternal-fetal interface in domestic animals. Cells Tissues Organs *172:* 202–217.

Calderwood, D.A., S.J. Shattil, M.H. Ginsberg (2000) Integrins and actin filaments: Reciprocal regulation of cell adhesion and signaling. J Biol Chem *275:* 22607–22610.

Carson, D.D., I. Bagchi, S.K. Dey, A.C. Enders, A.T. Fazleabas, B.A. Lessey, K. Yoshinaga (2000) Embryo implantation. Dev Biol *223:* 217–237.

Carson, D.D., M.M. DeSouza, R. Kardon, X. Zhou, E. Lagow, J. Julian (1998a) Mucin expression and function in the female reproductive tract. Hum Reprod Update *4:* 459–464.

Carson, D.D., M.M. DeSouza, E.G. Regisford (1998b) Mucin and proteoglycan functions in embryo implantation. Bioessays *20:* 577–583.

Chervenak, J.L., N.P. Illsley (2000) Episialin acts as an antiadhesive factor in an in vitro model of human endometrial-blastocyst attachment. Biol Reprod *63:* 294–300.

Clark, E.A., J.S. Brugge (1995) Integrins and signal transduction pathways: The road taken. Science *268:* 233–239.

Cooper, J.A. (1987) Effects of cytochalasin and phalloidin on actin. J Cell Biol *105:* 1473–1478.

Denker, H.-W. (1970) Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung und Implantation des Kaninchens. I. Allgemeine Lokalisierung und Charakterisierung hochmolekularer Kohlenhydratsubstanzen in frühen Embryonalstadien. II. Beiträge zu entwicklungsphysiologischen Fragestellungen. Zool Jb *75:* 141–308.

Denker, H.-W. (1986) Epithel-Epithel-Interaktionen bei der Embryo-Implantation: Ansätze zur Lösung eines zellbiologischen Paradoxons. Verh Anat Ges 80, Anat Anz Suppl *160:* 93–114.

Denker, H.-W. (1990) Trophoblast-endometrial interactions at embryo implantation: A cell biological paradox; in Denker, H.-W., J.D. Aplin (eds): Trophobloast Invasion and Endometrial Receptivity. Novel Aspects of the Cell Biology of Embryo Implantation. Trophoblast Research. New York, Plenum Medical Book, vol 4, pp 3–29.

Denker, H.-W. (1993) Implantation: A cell biological paradox. J Exp Zool *266:* 541–558.

Denker, H.-W. (1994) Endometrial receptivity: Cell biological aspects of an unusual epithelium. Ann Anat *176:* 53–60.

Denker, H.-W. (2000) Structural dynamics and function of early embryonic coats. Cells Tissues Organs *166:* 180–207.

Denker, H.-W., M. Thie (2001) The regulatory function of the uterine epithelium for trophoblast attachment: Experimental approaches. Ital J Anat Embryol *106(suppl 2):* 291–306.

Drubin, D.G., W.J. Nelson (1996) Origins of cell polarity. Cell *84:* 335–344.

Enders, A.C., A.G. Hendrickx, S. Schlafke (1983) Implantation in the rhesus monkey: Initial penetration of endometrium. Am J Anat *167:* 275–298.

Enders, A.C., A. Lopata (1999) Implantation in the marmoset monkey: Expansion of the early implantation site. Anat Rec *256:* 279–299.

Enders, A.C., R.A. Mead (1996) Progression of trophoblast into the endometrium during implantation in the western spotted skunk. Anat Rec *244:* 297–315.

Enders, A.C., S. Schlafke (1977) Surface coats of the mouse blastocyst and uterus during the preimplantation period. Anat Rec *180:* 31–46.

Evenas, J., A. Malmendal, S. Forsen (1998) Calcium. Curr Opin Chem Biol *2:* 293–302.

Fei, G., W. Peng, C. Xin-Lei, H. Zhao-Yuan, L. Yi-Xun (2001) Apoptosis occurs in implantation site of the rhesus monkey during early stage of pregnancy. Contraception *64:* 193–200.

Florin, E.L., V.T. Moy, H.E. Gaub (1994) Adhesive forces between individual ligand-receptor pairs. Science *264:* 415–417.

Galan, A., R. Herrer, J. Remohi, A. Pellicer, C. Simon (2000a) Embryonic regulation of endometrial epithelial apoptosis during human implantation. Hum Reprod *15(suppl 6):* 74–80.

Galan, A., J.E. O'Connor, D. Valbuena, R. Herrer, J. Remohi, S. Pampfer, A. Pellicer, C. Simon (2000b) The human blastocyst regulates endometrial epithelial apoptosis in embryonic adhesion. Biol Reprod *63:* 430–439.

Geiger, B., A. Bershadsky (2001) Assembly and mechanosensory function of focal contacts. Curr Opin Cell Biol *13:* 584–592.

Geiger, B., A. Bershadsky, R. Pankov, K.M. Yamada (2001) Transmembrane crosstalk between the extracellular matrix – cytoskeleton crosstalk. Nat Rev Mol Cell Biol *2:* 793–805.

Giancotti, F.G., E. Ruoslahti (1999) Integrin signaling. Science *285:* 1028–1032.

Glasser, S.R., J. Mulholland (1993) Receptivity is a polarity dependent special function of hormonally regulated uterine epithelial cells. Microsc Res Tech *25:* 106–120.

Hay, E.D. (1990) Epithelial-mesenchymal transitions. Semin Dev Biol *1:* 347–356.

Hay, E.D. (1995) An overview of epithelio-mesenchymal transformation. Acta Anat *154:* 8–20.

Hoffman, L.H., V.P. Winfrey, G.L. Blaeuer, G.E. Olson (1996) A haptoglobin-like glycoprotein is produced by implantation-stage rabbit endometrium. Biol Reprod *55:* 176–184.

Hohn, H.-P., H.-W. Denker (2002) Experimental modulation of cell-cell adhesion, invasiveness and differentiation in trophoblast cells. Cells Tissues Organs *172:* 218–236.

Hohn, H.-P., M. Linke, H.-W. Denker (2000) Adhesion of trophoblast to uterine epithelium as related to the state of trophoblast differentiation: In vitro studies using cell lines. Mol Reprod Dev *57:* 135–145.

Illingworth, I.M., S.J. Kimber (1999) Demonstration of oestrogenic control of H-type-1 carbohydrate antigen in the murine endometrial epithelium by use of ICI 182,780. J Reprod Fertil *117:* 89–95.

John, M.J., M. Linke, H.-W. Denker (1993) Quantitation of human choriocarcinoma spheroid attachment to uterine epithelial cell monolayers. In Vitro Cell Dev Biol *29A:* 461–468.

Kimber, S.J. (2000) Molecular interactions at the maternal-embryonic interface during the early phase of implantation. Semin Reprod Med *18:* 237–253.

Kimber, S.J., I.M. Illingworth, S.R. Glasser (1995) Expression of carbohydrate antigens in the rat uterus during early pregnancy and after ovariectomy and steroid replacement. J Reprod Fertil *103:* 75–87.

Kimber, S.J., S. Lindenberg (1990) Hormonal control of a carbohydrate epitope involved in implantation in mice. J Reprod Fertil *89:* 13–21.

Kimber, S.J., C. Spanswick (2000) Blastocyst implantation: The adhesion cascade. Semin Cell Dev Biol *11:* 77–92.

Kuramoto, H., S. Tamura, S.Y. Notake (1972) Establishment of a cell line of human endometrial adenocarcinoma in vitro. Am J Obstet Gynecol *114:* 1012–1019.

Lampelo, S.A., A.P. Ricketts, D.W. Bullock (1985) Purification of rabbit endometrial plasma membranes from receptive and non-receptive uteri. J Reprod Fertil *75:* 475–484.

Lessey, B.A. (1998) Endometrial integrins and the establishment of uterine receptivity. Hum Reprod *13:* 247–258.

Lessey, B.A. (2000) Endometrial receptivity and the window of implantation. Baillières Best Pract Res Clin Obstet Gynaecol *14:* 775–788.

Lessey, B.A., A.J. Castelbaum, S.W. Sawin, J. Sun (1995) Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil Steril *63:* 535–542.

Li, H.Y., S.P. Chang, C.C. Yuan, H.T. Chao, H.T. Ng, Y.J. Sung (2001) Nitric oxide induces extensive apoptosis in endometrial epithelial cells in the presence of progesterone: Involvement of mitogen-activated protein kinase pathways. Mol Hum Reprod *7:* 755–763.

Liu, S., J. Julian, D.D. Carson (1998) A peptide sequence of heparin/heparan sulfate (HP/HS)-interacting protein supports selective, high affinity binding of HP/HS and cell attachment. J Biol Chem *273:* 9718–9726.

Lopata, A (1996a) Implantation of the human embryo. Hum Reprod *11(suppl 1):* 175–184.

Lopata, A. (1996b) Blastocyst-endometrial interaction: An appraisal of some old and new ideas. Mol Hum Reprod *2:* 519–525.

Ludwig, M., W. Dettmann, H.E. Gaub (1997) AFM imaging contrast based on molecular recognition. Biophys J *72:* 445–448.

Luxford, K.A., C.R. Murphy (1992) Changes in the apical microfilaments of rat uterine epithelial cells in response to estradiol and progesterone. Anat Rec *233:* 521–526.

Martin, J.C., M.J. Jasper, D. Valbuena, M. Meseguer, J. Remohi, A. Pellicer, C. Simon (2000) Increased adhesiveness in cultured endometrial-derived cells is related to the absence of moesin expression. Biol Reprod *63:* 1370–1376.

Merritt, J.E., W.P. Armstrong, C.D. Benham, T.J. Hallam, R. Jacob, A. Jaxa-Chamiec, B.K. Leigh, S.A. McCarthy, K.E. Moores, T.J. Rink (1990) SK&F 96365, a novel inhibitor of receptor-mediated calcium entry. Biochem J *271:* 515–522.

Meseguer, M., J.D. Aplin, P. Caballero-Campo, J.E. O'Connor, J.C. Martin, J. Remohi, A. Pellicer, C. Simon (2001) Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. Biol Reprod *64:* 590–601.

Morris, J.E., S.W. Potter (1990) An in vitro model for studying interactions between mouse trophoblasts and uterine cells; in Denker, H.-W., J.D. Aplin (eds): Trophoblast Invasion and Endometrial Receptivity. Novel Aspects of the Cell Biology of Embryo Implantation. Trophoblast Research. New York, Plenum Medical Book, vol 4, pp 51–69.

Murphy, C.R. (2000) The plasma membrane transformation of uterine epithelial cells during pregnancy. J Reprod Fertil Suppl *55:* 23–28.

Nelson, W.J. (1992) Regulation of cell surface polarity from bacteria to mammals. Science *258:* 948–955.

Niklaus, A.L., C.R. Murphy, A. Lopata (1999) Ultrastructural studies of glycan changes in the apical surface of the uterine epithelium during pre-ovulatory and pre-implantation stages in the marmoset monkey. Anat Rec *255:* 241–251.

Niklaus, A.L., C.R. Murphy, A. Lopata (2001) Characteristics of the uterine luminal surface epithelium at preovulatory and preimplantation stages in the marmoset monkey. Anat Rec *264:* 82–92.

Olson, G.E., V.P. Winfrey, P.E. Matrisian, S.K. NagDas, L.H. Hoffman (1998) Blastocyst-dependent upregulation of metalloproteinase/disintegrin MDC9 expression in rabbit endometrium. Cell Tissue Res *293:* 489–498.

Pampfer, S., I. Donnay (1999) Apoptosis at the time of embryo implantation in mouse and rat. Cell Death Differ *6:* 533–545.

Paria, B.C., H. Lim, S.K. Das, J. Reese, S.K. Dey (2000) Molecular signaling in uterine receptivity for implantation. Semin Cell Dev Biol *11:* 67–76.

Pattillo, R.A., A. Ruckert, R. Hussa, R. Bernstein, E. Delfs (1971) The JAR cell line – continuous human multihormone production and controls. In Vitro *6:* 398.

Perret, S., P. Dockery, B.J. Harvey (2001) 17β-Oestradiol stimulates capacitative Ca^{2+} entry in human endometrial cells. Mol Cell Endocrinol *176:* 77–84.

Raboudi, N., J. Julian, L.H. Rohde, D.D. Carson (1992) Identification of cell surface heparin/heparan sulfate binding proteins of a human uterine epithelial cell line (RL95). J Biol Chem *267:* 11930–11939.

Radmacher, M., R.W. Tillmann, M. Fritz, H.E. Gaub (1992) From molecules to cells – imaging soft samples with the AFM. Science *257:* 1900–1905.

Rhode, L.H., D.D. Carson (1993) Heparin-like glycosaminoglycans participate in binding of a human trophoblastic cell line (JAR) to a human uterine epithelial cell line (RL95). J Cell Physiol *155:* 185–196.

Rief, M., M. Gautel, F. Oesterhelt, J.M. Fernandez, H.E. Gaub (1997) Reversible unfolding of individual titin Ig-domains by AFM. Science *276:* 1109–1112.

Rugar, D., P.K. Hansma (1990) Atomic force microscopy. Phys Today *43:* 23–30.

Salamonsen, L.A., E. Dimitriadis, L. Robb (2000) Cytokines in implantation. Semin Reprod Med *18:* 299–310.

Savagner, P. (2001) Leaving the neighborhood: Molecular mechanisms involved during epithelial-mesenchymal transition. Bioessays *23:* 912–923.

Schlafke, S., A.C. Enders (1975) Cellular basis of interaction between trophoblast and uterus at implantation. Biol Reprod *12:* 41–65.

Selam, B., U.A. Kayisli, N. Mulayim, A. Arici (2001) Regulation of Fas ligand expression by estradiol and progesterone in human endometrium. Biol Reprod *65:* 979–985.

Simon, C., F. Dominguez, J. Remohi, A Pellicer (2001) Embryo effects in human implantation: Embryonic regulation of endometrial molecules in human implantation. Ann NY Acad Sci *943:* 1–16.

Sjaastad, M.D., W.J. Nelson (1997) Integrin-mediated calcium signaling and regulation of cell adhesion by intracellular calcium. Bioessays *19:* 47–55.

Tabibzadeh, S. (1998) Molecular control of the implantation window. Hum Reprod Update *4:* 465–471.

Terry, V., T.J. Shaw, C.D. Shorey, C.R. Murphy (1996) Actin-binding proteins undergo major alterations during the plasma membrane transformation in uterine epithelial cells. Anat Rec *246:* 71–77.

Thie, M., H.-W. Denker (1997) Endometrial receptivity for trophoblast attachment: Model studies using cell lines; in Motta, P.M. (ed): Microscopy of Reproduction and Development. A Dynamic Approach. Rome, Antonio Delfino Editore S.r.l., pp 241–249.

Thie, M., P. Fuchs, S. Butz, F. Sieckmann, H. Hoschützky, R. Kemler, H.-W. Denker (1996) Adhesiveness of the apical surface of uterine epithelial cells: The role of junctional complex integrity. Eur J Cell Biol *70:* 221–232.

Thie, M., B. Harrach-Ruprecht, H. Sauer, P. Fuchs, A. Albers, H.-W. Denker (1995) Cell adhesion to the apical pole of epithelium: A function of cell polarity. Eur J Cell Biol *66:* 180–191.

Thie, M., C. Heneweer, L.H. Kruse, M. Schmidt, H.-W. Denker (2002) Function of Rho protein in the process of trophoblast adhesion – in vitro studies on signalling mechanisms in human uterine epithelial RL95–2 cells. Verh Anat Ges 97, Ann Anat Suppl *184:* 172–173.

Thie, M., P. Herter, H. Pommerenke, F. Dürr, F. Sieckmann, B. Nebe, J. Rychly, H.-W. Denker (1997) Adhesiveness of the free surface of a human endometrial monolayer for trophoblast as related to actin cytoskeleton. Mol Hum Reprod *3:* 275–283.

Thie, M., R. Röspel, W. Dettmann, M. Benoit, M. Ludwig, H.E. Gaub, H.-W. Denker (1998) Interaction between trophoblast and uterine epithelium: Monitoring of adhesive forces. Hum Reprod *13:* 3211–3219.

Thiery, J.P., D. Chopin (1999) Epithelial cell plasticity in development and tumor progression. Cancer Metastasis Rev *18:* 31–42.

Tinel, H., H.-W. Denker, M. Thie (2000) Calcium influx in human uterine epithelial RL95-2 cells triggers adhesiveness for trophoblast-like cells. Model studies on signalling events during embryo implantation. Mol Hum Reprod *6:* 1119–1130.

Triggle, D.J. (1999) The pharmacology of ion channels: With particular reference to voltage-gated Ca^{2+} channels. Eur J Pharmacol *375:* 311–325.

von Rango, U., I. Classen-Linke, C.A. Krusche, H.M. Beier (1998) The receptive endometrium is characterized by apoptosis in the glands. Hum Reprod *13:* 3177–3189.

Wang, J., D.R. Armant (2002) Integrin-mediated adhesion and signaling during blastocyst implantation. Cells Tissues Organs *172:* 190–201.

Way, D.L., D.S. Grosso, J.R. Davis, E.A. Surwit, C.D. Christian (1983) Characterization of a new human endometrial carcinoma (RL95-2) established in tissue culture. In Vitro *19:* 147–158.

Wild, M.K., K. Lühn, T. Marquardt, D. Vestweber (2002) Leukocyte adhesion deficiency II: Therapy and genetic defect. Cells Tissues Organs, *172:* 161–173.

Wodarz, A. (2001) Cell polarity: No need to reinvent the wheel. Curr Biol *11:* R975–978.

Yeaman, C., K.K. Grindstaff, M.D. Hansen, W.J. Nelson (1999a) Cell polarity: Versatile scaffolds keep things in place. Curr Biol *9:* R515–517.

Yeaman, C., K.K. Grindstaff, W.J. Nelson (1999b) New perspectives on mechanisms involved in generating epithelial cell polarity. Physiol Rev *79:* 73–98.