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Human Uterine Epithelial RL95-2 Cells Reorganize Their Cytoplasmic Architecture with Respect to Rho Protein and F-Actin in Response to Trophoblast Binding

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Key Words
Embryo implantation · Trophoblast binding · Uterine epithelium · Rho protein · Actin cytoskeleton

Abstract
Embryo implantation is initiated by interaction of trophoblast with uterine epithelium via the apical cell poles of both partners. Using spheroids of human trophoblastoid JAR cells and monolayers of human uterine epithelial RL95-2 cells to simulate this initial interaction, we previously demonstrated that formation of stable cell-to-cell bonds depends on actin cytoskeleton (F-actin) and small GTPases of the Rho family, most likely RhoA. In this study, we determined the apical as well as the basal distribution of these proteins by fluorescence confocal microscopy before and after binding of JAR spheroids. We focussed on changes in cytoplasmic organization with respect to apicobasal polarity of RL95-2 cells. Before binding of spheroids, significantly higher fluorescence signals of RhoA [37 ± 6 grey scale values (gsv)] and of F-actin (41 ± 3 gsv) were found in the basal region of RL95-2 cells as compared to the apical pole (RhoA: 24 ± 3 gsv, F-actin: 28 ± 2 gsv). After binding of JAR spheroids, this apicobasal asymmetry was inverted (RhoA: 55 ± 10 gsv apical vs. 25 ± 3 gsv basal; F-actin: 108 ± 17 gsv apical vs. 57 ± 7 gsv basal). Inactivation of Rho GTPases in RL95-2 cells by *Clostridium difficile* toxin A leads to a loss of their apical adhesion competence, as previously published. Here, we observed a uniform distribution of RhoA and F-actin between apical and basal region rather than an asymmetric one in toxin A-treated cells. These data suggest that activation of Rho GTPases and coordinated rearrangement of F-actin within uterine epithelial cells in response to trophoblast binding are part of a generalized structural and functional reorganization of the cytoplasm. This involves not only the immediate contact zone (apical) but also the opposite (basal) cell pole and may be a critical element of uterine epithelial reactions during transition between trophoblast adhesion and transmigration.

Abbreviations used in this paper

ATCC	American Type Culture Collection
BSA	bovine serum albumin
CLSM	confocal laser scanning microscopy
CMFDA	5-chloromethylfluorescein diacetate
EMT	epithelial-mesenchymal transition
FCS	fetal calf serum
gsv	grey scale value
PBS	phosphate-buffered saline
TRITC	tetramethylrhodamine isothiocyanate

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Introduction

Embryo implantation starts with adhesion of the blastocyst to the endometrial lining of the uterus. The endometrium has to acquire a specific functional state referred to as receptivity to enable trophoblast adhesion [reviewed by Denker, 1994; Carson et al., 2000; Denker and Thie, 2001]. Many data suggest that for the first steps of the implantation cascade the uterine epithelium is critical, and it has to modify its structural and functional organization in order to support trophoblast adhesion at the apical cell pole since this pole is – like in other simple epithelia – normally repellent so that except for the receptive phase it does not allow opposing cells such as trophoblast to adhere [Denker, 1986, 1990, 1993, 1994]. To mediate adhesive recognition and binding of trophoblast, molecular changes in composition of the apical plasma membrane of uterine epithelial cells occur [for example carbohydrates: Carson, 2002; integrins: Lessey and Castelbaum, 2002]. Additionally, changes in the overall organization of their cell architecture and their signaling systems seem to be essential [Denker, 1994; Thie and Denker, 1997; Bentin-Ley et al., 2000; Murphy, 2000; Nikas, 2000; Tinel et al., 2000; Denker and Thie, 2001]. Based on these data receptivity may critically depend on a change (destabilization and/or downregulation) of the polarized phenotype of uterine epithelial cells. This appears to lead to a functionalized cell type equipped with appropriate sets of adhesion molecules and signaling systems at the free surface [Tinel et al., 2000; Denker and Thie, 2001; Thie and Denker, 2002].

The complex sequence of events of trophoblast-endometrial interactions is difficult to study in vivo, even in animals. Therefore, we have developed a cell culture model that provides information about basic processes during first steps of trophoblast adhesion, i.e. monolayers of human uterine epithelial RL95-2 cells cocultured with multicellular spheroids of human trophoblastoid JAR cells [John et al., 1993]. In contrast to other uterine epithelial cell lines originally tested, such as HEC-1-A cells, RL95-2 cells are adhesive for JAR spheroids. In ultrastructural studies monolayers of these cells show a reduced apicobasal polarity [John et al., 1993; Thie et al., 1995, 1996]. In contrast, polar uterine epithelial cells, i.e. HEC-1-A, resist trophoblast adhesion. Thus, RL95-2 cells have proven useful as a model for the receptive state of human uterine epithelium [reviewed by Thie and Denker, 2002].

New aspects gained from this cell culture model indicate that formation of cell-to-cell contacts between these uterine epithelial cells and trophoblast is a remarkably slow process that seems to include sequential steps of bond formation [Thie et al., 1998] as well as complex signal transduction cascades [Thie et al., 1997, 1998; Tinel et al., 2000]. In particular, Rho protein signaling has emerged as an important part of these cascades since inactivation of Rho GTPases by treatment of RL95-2 cells with *Clostridium difficile* toxin A largely suppressed adhesion of JAR spheroids [Heneweer et al., 2002]. Moreover, investigations by confocal microscopy showed a marked redistribution of small GTPase RhoA with a significant increase in intensity of fluorescence signal at the contact site between RL95-2 cells and JAR cells. These changes were also accompanied by a significant increase in signal of filamentous actin (F-actin) as well as an increased colocalization of RhoA with F-actin [Heneweer et al., 2002] corresponding to Rho being one of the major regulating proteins of the actin cytoskeleton [Etienne-Manneville and Hall, 2002]. Taken together with the fact that an unaltered organization of the actin cytoskeleton is indispensable for JAR spheroid binding [Thie et al., 1997], these data suggest that trophoblast binding to uterine epithelial cells and regulation of the actin cytoskeleton are linked by Rho signaling.

Here, we further investigated expression and distribution of the small GTPase RhoA and of F-actin in uterine epithelial RL95-2 cells before and after formation of cell-to-cell contacts to the trophoblastoid JAR cells. In addition to our previous observations, we now studied the intracellular distribution of both RhoA and F-actin along the apicobasal axis including reactions at the basal cell pole, i.e. opposite to the contact site with trophoblast. Intensities of RhoA and of F-actin signals in apical and basal regions of RL95-2 cells were compared by means of high resolution confocal laser scanning mícroscopy (CLSM). Indeed, we observed that JAR spheroid binding dramatically altered the concentration of Rho proteins as well as of F-actin at both apical and basal regions of RL95-2 cells. This resulted in an inversion of the original pattern of both of these proteins along the cells' apicobasal axis. These data suggest that trophoblast binding induces activation of RhoA and a coordinated apicobasal rearrangement of molecules, specifically F-actin, within uterine epithelial cells. These processes may be critical elements of a sequence of events that lead to acquisition of a new functional state in which uterine epithelial cells allow trophoblast adhesion and subsequent transmigration.

Materials and Methods

Antibodies and Fluorescent Dyes

The monoclonal mouse anti-human RhoA antibody 26 CH:sc-418 was obtained from Santa Cruz Biotechnology (Heidelberg, Germany) and used in 1:50 dilution in phosphate-buffered saline (PBS) supplemented with 0.5% bovine serum albumin (BSA). The secondary antibody, Alexa Fluor 633-conjugated goat anti-mouse IgG (A-21052) was obtained from Molecular Probes (MoBiTec, Göttingen, Germany) and used in 1:150 dilution in PBS-BSA. Tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin was obtained from Sigma-Aldrich (Deisenhofen, Germany) and used at a concentration of 25 µg/ml in PBS. The fluorescent vital dye 5-chloromethylfluorescein diacetate (CMFDA; Cell Tracker Green C-2925) was obtained from Molecular Probes (MoBiTec) and used at a concentration of 1 µmol/ml serum-free RPMI 1640 medium (Gibco-Life Technology, Eggenstein, Germany).

Endometrial Cell Line Cultures and Preparation of JAR Spheroids

All cell lines were purchased from the American Type Culture Collection (ATCC; Rockville, Md., USA). The nonpolar human uterine epithelial cell line RL95-2 [Way et al., 1983] (ATCC: CRL 1671) was maintained in a 1 + 1 mixture of Dulbecco's modified Eagle's medium (Gibco Life Technology, Eggenstein, Germany) and Ham's F12 medium (Biochrom, Berlin, Germany), supplemented with 10% fetal calf serum (FCS; Gibco), 10 m*M* HEPES pH 7.4 (Biochrom), 5 µg/ml insulin (Sigma-Aldrich, Deisenhofen, Germany), 100 U/ml penicillin (Boehringer, Mannheim, Germany) and 100 µg/ml streptomycin (Boehringer). For experiments, cells were cultured on poly-*D*-lysine-coated glass coverslips as described [John et al., 1993; Thie et al., 1995]. As an invasive trophoblast model, multicellular spheroids of human choriocarcinoma JAR cells [Pattillo et al., 1971] (ATCC: HTB 144) were allowed to attach to the free surface of endometrial cell monolayers (see below): JAR spheroids were prepared according to the procedure of John et al. [1993], i.e. a suspension of 450,000 JAR cells per 6 ml RPMI 1640 medium (Gibco) supplemented with 10% FCS was agitated at 37°C on a gyratory shaker (Certomat R; Braun, Melsungen, Germany) at 110 rpm in order to form multicellular spheroids 72 h after initiation of the culture.

Adhesion of JAR Spheroids to RL95-2 Monolayers

The JAR cell adhesion assays were performed as described by John et al. [1993] modified according to Heneweer et al. [2002]. In brief, JAR spheroids, prepared as described above, were traced with the fluorescent vital dye CMFDA for 45 min at 37°C and subsequently delivered onto confluent monolayers of endometrial RL95-2 cells grown on coverslips. After 60 min of coculture in JAR medium, spheroid adhesion to the monolayers was quantified by centrifugation of the coverslips with the cell-spheroid surface facing down at 12 *g* for 5 min. Attached spheroids were counted and the results expressed as the percentage of the number of spheroids seeded initially. For inactivation of Rho proteins, confluent monolayers of RL95-2 cells were treated for 24 h with *C. difficile* toxin A [Chaves-Olarte et al., 1999] at a concentration of 100 ng/ml before adhesiveness of RL95-2 cells was determined. Controls were cultured for the same time periods without toxin.

Fluorescence Staining

For immunostaining, samples were treated as described previously [Heneweer et al., 2002]. In brief, cells were fixed in a 1 + 1 mixture of ethanol-acetone for 10 min at room temperature, blocked by incubation with 0.5% BSA in PBS for 15 min and incubated with the primary antibody (see above) for 90 min at 37°C, which was omitted in control stainings. Thereafter, cells were rinsed in PBS/0.5% BSA, incubated with the corresponding fluorescence-conjugated secondary antibody (see above) for 90 min at 37°C and mounted in glycerol (90%)/PBS (9%)/*p*-phenylenediamine (1%). For actin staining, samples were fixed with 3% (para)formaldehyde for 15 min at room temperature, permeabilized by incubation with 0.05% Triton X-100 for 2 min, and incubated for 15 min with TRITC-phalloidin before mounting in PBS/glycerol/phenylenediamine. For combining immunofluorescence and F-actin staining, samples were fixed in (para)formaldehyde and permeabilized in Triton X-100 according to the actin staining protocol. Then, the primary antibody and the TRITC-phalloidin were applied simultaneously, while both were omitted in controls. Thereafter, the immunostaining reaction was performed as described above.

Laser Confocal Imaging, Image Analysis and Processing

Confocal microscopy was performed using a Zeiss Axiovert 100M microscope attached to a CLSM (model LSM 510; Carl Zeiss, Jena, Germany) as described previously [Heneweer et al., 2002]. The excitation source was an argon laser with output at 488 nm and two helium-neon lasers with output at 543 and 633 nm, respectively. CMFDA fluorescence was imaged with a 505 to 530 nm bandpass filter, TRITC fluorescence with a 560 to 565 nm bandpass filter, and Alexa Fluor 633 fluorescence with a 650 nm longpass filter. Optical tomography was performed at 0.5 µm intervals using a 40-fold oil immersion objective and a pinhole size corresponding to a value of 1.0 of the airy disk. Each slice was scanned 8 times followed by averaging in order to improve the signal to noise ratio. All measurements were performed on individual RL95-2 cells. In monolayers without contact to JAR cells, RL95-2 cells to be examined were selected randomly from the entire photographic field. In monolayers with contact to JAR spheroids, only RL95-2 cells with definite membrane contact to JAR cells were included. Mismatching with JAR cells was avoided by prelabelling these with CMFDA. For a semiquantitative determination of intensities of fluorescence signals, average grey scale values (gsv) of each channel were measured using the CLSM software (version 2.8 SR 1; Carl Zeiss). The apical-most and the basal-most slices of each stack were selected for analysis. For image enhancement as well as spatial measurements, Image Pro Plus software was used (version 4.5; Media Cybernatics, Crofton, Md., USA) which was additionally equipped with a Gaussian filter module [Adelmann, 1997] and a homomorphic filter plugin [Adelmann, 1998]. Adobe Photoshop software (version 7.0; Adobe Systems, San Jose, Calif., USA) was used for the arrangement of RGB-color images out of single grey scale images each representing the signal of one color channel.

Statistical Analysis

The data are represented as means ± SE with n denoting the number of experiments. Statistical analysis were performed by using the Kruskal-Wallis test for global differences between groups and the Wilcoxon signed rank sum test for their pairwise comparison. A value of $p < 0.05$ was considered significant.

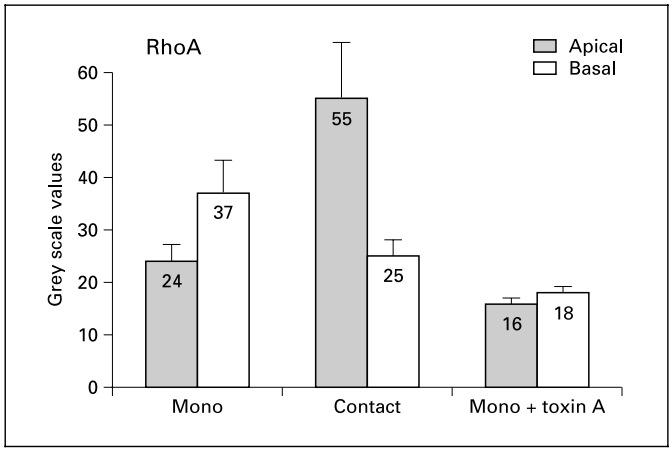

Fig. 1. Quantification of RhoA in the apex (Apical) and at the base (Basal) of monolayer-cultured RL95-2 cells before (Mono) and after (Contact) binding of JAR spheroids as well as after pretreatment with 100 ng/ml toxin A for 24 h (mono + toxin A). For semiquantitative evaluation of fluorescence, average gsv were determined. Stacks of six xy-sections at 0.5 µm intervals were measured with the first marked slice at the apical and the basal plasma membrane domain, respectively. Numbers of cells tested: n = 16 (Mono), n = 14 (Contact), n = 13 (Contact + toxin A). Values are mean ± SE. Mean gsv of the apex and of the base were compared. Values differ significantly ($p < 0.05$) between these groups as well as between apical and basal cell poles within groups except for toxin A-treated cells.

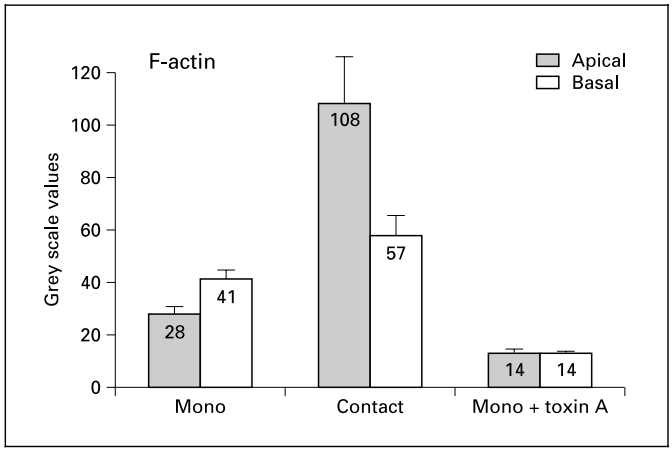

Fig. 2. Quantification of F-actin in the apex (Apical) and at the base (Basal) of monolayer-cultured RL95-2 cells before (Mono) and after (Contact) binding of JAR spheroids as well as after pretreatment with 100 ng/ml toxin A for 24 h (Mono + toxin A). For semiquantitative evaluation of fluorescence, average gsv were determined. Stacks of six xy-sections at 0.5 µm intervals were measured with the first marked slice at the apical and the basal plasma membrane domain, respectively. Numbers of cells tested: n = 16 (Mono), n = 14 (Contact), n = 13 (Contact + toxin A). Values are mean ± SE. Mean gsv of the apex and of the base were compared. Values differ significantly ($p < 0.05$) between these experimental groups as well as between apical and basal cell poles within groups except for toxin A-treated cells.

Results

In order to obtain information on whether subcellular location of RhoA and F-actin may play a role in the binding of trophoblast we characterized the distribution of both proteins within RL95-2 cells (1) before and (2) after binding of JAR spheroids as well as (3) after pretreatment of RL95-2 cells with *C. difficile* toxin A. Although RL95-2 cells show little morphological evidence for a polar organization along an apicobasal axis, we define those parts of the cells that are adjacent to the substratum as 'basal' and the opposite free pole as 'apical'.

Before Binding of JAR Spheroids

Before binding of JAR spheroids, a markedly asymmetric distribution along the apicobasal axis of RL95-2 cells was found both for RhoA and F-actin. Staining of both proteins was significantly more concentrated in basal regions of cells (RhoA: 37 ± 6 gsv; F-actin: 41 ± 3 gsv) than in apical regions (RhoA: 24 ± 3 gsv; F-actin: 28 ± 2 gsv) (fig. 1, 2). As described in detail elsewhere [Heneweer et al., 2002], F-actin of the free apical region was found to form a dense network containing small aggregates of an irregular size (data not shown). F-actin staining at the base of RL95-2 cells was localized in spike-/filopodium-like structures [Adams, 2001] and in distinct patches close to the plasma membrane at places where the external face of the cell might be closely attached to the substratum (fig. 3). Typical stress fibers were rarely observed. A rather homogenous staining of Rho protein was found both in the apical as well as in the basal region of RL95-2 cells (fig. 3). In addition, small RhoA granules were present whose average diameters were significantly greater in the basal region of the cells (diameter: 410 ± 50 nm) than in the apical one (diameter: 380 ± 40 nm) ($p < 0.05$) (fig. 4).

After Binding of JAR Spheroids

After 1 h of confrontation culture, while cell-to-cell bonds between RL95-2 cells and JAR spheroids were formed [Thie et al., 1998], the distribution of RhoA as well as of F-actin within RL95-2 cells changed. In the apical region, staining of RhoA increased by ~2.3-fold (24 ± 3 vs. 55 ± 10 gsv; $p < 0.05$) (fig. 1) and of F-actin by ~3.6-fold (28 ± 2 vs. 108 ± 17 gsv; $p < 0.05$) (fig. 2) compared to levels in the apex of nonconfronted cells.

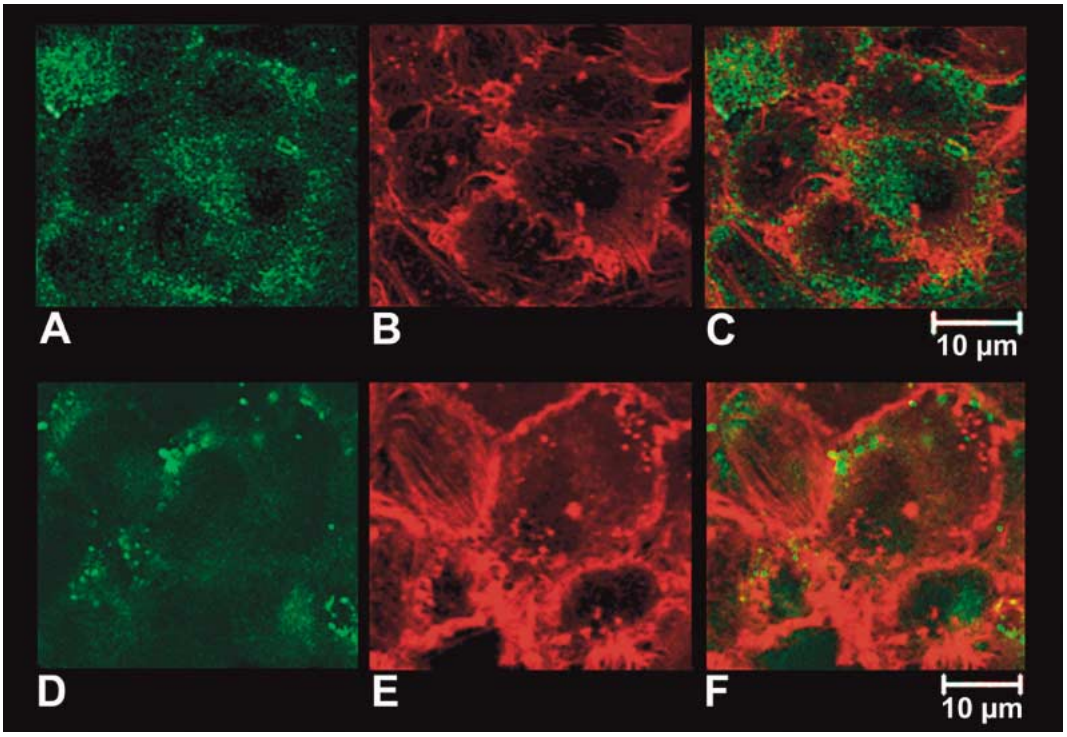

Fig. 3. Localization of RhoA (**A**, **D**) (green) and F-actin (**B**, **E**) (red) in the basal region of RL95-2 monolayers before (**A**, **B**) and after binding of JAR spheroids (**D**, **E**). xy-sections represent second slice from the basal cell pole. Merger of confocal images (**C**, **F**). Typical patterns are presented.

Quantification of RhoA and F-actin fluorescence in the basal region of RL95-2 cells revealed by ~0.7-fold lowered levels of RhoA fluorescence (37 ± 6 vs. 25 ± 3 gsv; $p < 0.05$) (fig. 1) and by ~1.4-fold raised levels of F-actin (41 ± 3 vs. 57 ± 7 gsv; $p < 0.05$) (fig. 2) after JAR spheroid binding. Again, there was a markedly asymmetric distribution both of RhoA and F-actin along the apicobasal axis of RL95-2 cells. But compared to nonconfronted cells, staining of proteins was now significantly ($p < 0.05$) more concentrated in the apical region of RL95-2 cells (RhoA: 55 ± 10 gsv; F-actin: 108 ± 17 gsv) than in their basal one (RhoA: 25 ± 3 gsv; F-actin: 57 ± 7 gsv) (fig. 1, 2). In addition, condensation of F-actin in big clumps was observed in the apical region confirming our results published previously [data not shown; see Heneweer et al., 2002]. Also the size of RhoA granules increased by ~2.0-fold (diameter: 380 ± 40 vs. 760 ± 150 nm; $p < 0.05$) (fig. 4). At the base of RL95-2 cells, F-actin staining was still seen within spike-/filopodium-like structures and small submembranous patches (fig. 3) but also as a network of fine filaments/stress fibers (fig. 3). The pattern of RhoA staining was dominated also in this basal region by the occurrence of RhoA-positive granules

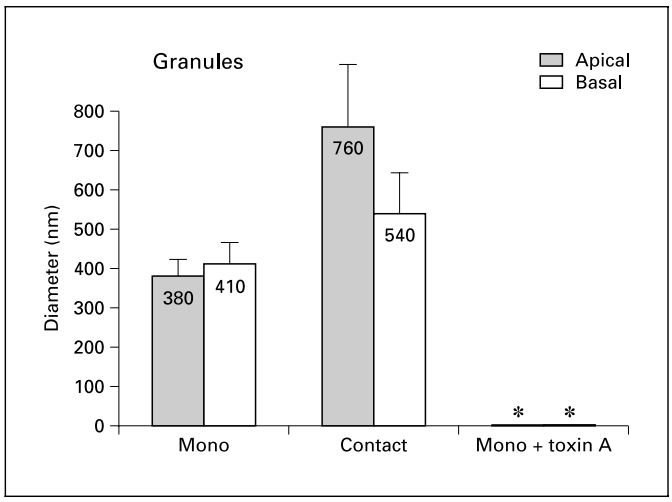

Fig. 4. Diameter of RhoA granules in the apex (Apical) and at the base (Basal) of monolayer-cultured RL95-2 cells before (Mono) and after (Contact) binding of JAR spheroids as well as after pretreatment with 100 ng/ml toxin A for 24 h (Mono + toxin A). Numbers of granules tested: n = 42 (Mono), n = 39 (Contact). Values are mean ± SE. Mean granule sizes at the apex and at the base differ significantly ($p < 0.05$). Corresponding granule diameters for each culture condition also show significant differences ($p < 0.05$). Asterisk indicates that diameters of granules are beyond detection.

(fig. 3) with a diameter raised by ~1.3-fold (diameter: 410 ± 50 vs. 540 ± 90 nm; $p < 0.05$) (fig. 4) compared to nonconfronted cells.

After Pretreatment with Toxin A

If RL95-2 cells are treated with 100 ng/ml *C. difficile* toxin A for 24 h, Rho GTPases, including RhoA, are specifically inactivated accompanied by a loss of adhesiveness of RL95-2 cells for JAR spheroids as shown earlier [Heneweer et al., 2002]. Our present observations show a loss of the original asymmetric distribution of Rho protein and F-actin within these cells. A reduction of fluorescence intensities of Rho protein in the apical region by ~0.6-fold (24 ± 3 vs. 16 ± 2 gsv; $p < 0.05$) (fig. 1) and in the basal region by ~0.5-fold (37 ± 6 vs. 18 ± 1 gsv; $p < 0.05$) (fig. 1) was found compared to nontreated cells. Similarly, intensities of F-actin were reduced in the apical region by ~0.5-fold (28 ± 2 vs. 14 ± 0 gsv; $p < 0.05$) (fig. 2) and in the basal region by ~0.3-fold (41 ± 3 vs. 14 ± 0 gsv; $p < 0.05$) (fig. 2). Thus, in contrast to the asymmetric distribution of RhoA and F-actin along the apicobasal axis in untreated RL95-2 cells, we observed a uniform distribution in toxin A-treated RL95-2 cells as fluorescence staining of proteins in the apical region (RhoA: 16 ± 2 gsv; F-actin: 14 ± 0 gsv) was almost equal to that in the basal region (RhoA: 18 ± 1 gsv; F-actin: 14 ± 0 gsv) (fig. 1, 2). Moreover, the actin network now appeared disintegrated showing irregular clumps. Additionally, RhoA-positive granules were no longer observed neither in the apical nor in the basal region (data not shown).

Discussion

Adhesive interaction between trophoblast and uterine epithelium involves recognition phenomena on both sites which are still incompletely understood [reviewed in Kimber and Spanswick, 2000; Paria et al., 2002; Thie and Denker, 2002]. Monolayer-cultured endometrial RL95-2 cells have proven useful as an in vitro model for the human receptive uterine epithelium [John et al., 1993]. In combination experiments with human trophoblastoid JAR cells this model allows to study cell biological details of formation of stable cell-to-cell binding including signaling processes involved [Thie et al., 1997, 1998; Tinel et al., 2000]. We demonstrated previously that attachment depends not only on Rho GTPases, most likely RhoA, but also on remodelling of the actin cytoskeleton at sites of new cell-to-cell contact, i.e. in the apical region of uterine cells [Heneweer et al., 2002]. The results of the present study suggest that uterine epithelial RL95-2 cells respond to JAR cell contact with changes in subcellular distribution of RhoA and F-actin not only in the apical but also in their basal region, i.e. at the free cell pole as well as at the sites of cell-to-matrix contact. Interestingly, changes in both the basal and the apical region lead to an inversion of Rho protein and F-actin distribution along the apicobasal axis of RL95-2 cells during trophoblast binding. This suggests that changes seen in the uterine cells at attachment sites are of a more global nature, comprising general aspects of epithelial cell architecture and physiology.

Confluent monolayers of RL95-2 cells express a phenotype that is nontypical for simple epithelia, i.e. they show a reduced apicobasal polarity [Thie et al., 1995, 1996, 1997]. This may be relevant for adhesiveness of the free cell pole for trophoblast since well-polarized uterine epithelial cell lines (e.g. HEC-1-A) do not allow trophoblast to adhere [John et al., 1993; Thie et al., 1995]. Therefore, we have postulated that conditioning of uterine epithelial cells for appropriate trophoblast adhesion may involve extensive remodelling of cell architecture [Denker, 1986, 1990, 1994]. Indeed, the data reported in the present communication suggest that changes in apicobasal organization of uterine epithelial cells are a prerequisite for attachment of trophoblast via the apical cell pole. Although detailed mechanisms are still unknown, molecular principles may be simple: cellular changes of polar organization in uterine epithelial cells, i.e. phenotypic plasticity, are possibly related to well-known processes in ontogenesis called epithelial-mesenchymal transition (EMT) [Hay, 1990, 1995]. During these processes, epithelial cells are able to switch from an epithelial to a mesenchymal phenotype and vice versa including changes in structural gene expression and cell behavior. These changes are thought to be governed by master genes which still have to be identified [Hay, 1990, 1995; Thiery and Chopin, 1999; Boyer et al., 2000; Savanger, 2001]. A detailed comparison with complete EMT processes has revealed only partial similarities as uterine epithelium in vivo does not lose but maintains the majority of its epithelial characteristics. Nevertheless, the changes that were found to occur at receptivity particularly in the implantation chamber suggest that certain elements of EMT processes may indeed be involved in endometrial receptivity [Denker, 1993, 1994].

One major part of the processes that establish the apically adhesion-competent phenotype of uterine epithelial cells during trophoblast binding may be Rho GTPases. As shown here, the original asymmetric distribution of RhoA of RL95-2 cells changes upon formation of cell-to-cell

contact with JAR cells. Rho proteins increase in the apical region and decrease in the basal one, thereby reflecting an inverted asymmetric distribution within RL95-2 cells. After inactivation of Rho GTPases, RL95-2 cells lose adhesion competence for trophoblast binding [Heneweer et al., 2002]. Interestingly, they show additionally a uniform distribution of Rho proteins between apical and basal regions, as demonstrated in the present study. These data point to a dependence of uterine adhesiveness on Rho GTPases, most likely RhoA. All these changes were accompanied by reorganization of the actin cytoskeleton within RL95-2 cells supporting our previous observation that F-actin is essential for JAR spheroid adhesion [Thie et al., 1997]. This complies with the known role of Rho GTPases in regulating the actin cytoskeleton [Etienne-Manneville and Hall, 2002]. Moreover, Rho GTPases participate not only in regulation of the actin network but do also influence cell polarity [Braga, 2000, 2002]. For example, Rho proteins are shown to play a role in polarization of epithelial cells by formation and organization of actin filaments as well as in localization and functional regulation of actin-associated membrane proteins in the apex of these cells [Nusrat et al., 1995; Jou and Nelson, 1998]. In addition, Rho GTPases regulate the morphological transition of epithelial cells to a fibroblastoid phenotype [see EMT concept, above; Sander et al., 1999; Zondag et al., 2000]. Thus, it is reasonable to assume that activated RhoA may indeed play a major role in the remodelling of the uterine epithelial phenotype during acquisition of receptivity, but probably even more in the subsequent local processes of trophoblast binding and transmigration.

As electron microscopy of implantation in vivo suggests [Enders and Mead, 1996; Enders and Lopata, 1999], attachment of trophoblast to the surface of the uterine epithelium may initially involve patches of membranes at the borders to neighboring cells. The subsequent process of intrusion between these cells involves interaction with the lateral membranes and accompanying reactions of the underlying cytoskeleton. Extremely little is known about the cell biology of this process of trophoblast transmigration through the uterine epithelium. On the basis of our observations made in the RL95-2 cell model we suggest that trophoblast binding to the apical cell pole – no matter whether involving lateral or central membrane patches – may erase or even invert the intracellular asymmetry (which is already destabilized) between the apical and the basal cell pole. Although consequences for the basal cell pole of RL95-2 cells have not yet been investigated, rearrangement of GTPases-dependent pathways and subsequent reorganization of the actin cytoskeleton in this region can be expected to alter cell-to-matrix interactions. This might occur through interactions between extracellular matrix and basally localized integrins [Albers et al., 1995] which are linked to the cytoskeleton. Indeed, integrins function as transmembrane linkers that mediate bidirectional interactions between extracellular matrix and actin cytoskeleton [Burridge et al., 1988; Turner and Burridge, 1991; Sastry and Horwitz, 1993; Defilippi et al., 1999; Schoenwaelder and Burridge, 1999; Zamir and Geiger, 2001; Arthur et al., 2002]. In this manner, one can postulate a signaling pathway from the apex to the base of the trophoblast-attached uterine epithelial cells. This may enable them to control transmigration of trophoblast across the epithelial barrier by regulating the adhesive interaction of uterine epithelial cells with their own basement membrane. Obviously, further detailed studies are needed before the process of trophoblast adhesion and subsequent transmigration through the uterine epithelium can be completely understood on a cellular level.

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