Role of decidua in trophoblastic invasion

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Abstract

OBJECTIVE: Proliferation, migration and invasion of trophoblastic cells into the maternal endometrium are essential steps in human embryo implantation and placentation. Trophoblast invasion is normally limited in time, only during first and early second trimester of pregnancy, and in space, limited to the endometrium and the proximal third of myometrium. This process requires among other factors: the metalloproteinases (MMP) 2 and 9. Shallow trophoblast invasion is associated with pathologies including preeclampsia and fetal growth restriction whereas unlimited invasion is associated with hydatidiform moles and choriocarcinomas.

METHODS: In order to understand the role of decidua in this endometrial invasion by trophoblastic cells, we have developed a model of coculture of decidual and cytotrophoblastic cells in which we can evaluate the effect of each partner on the proliferative and invasive properties of the other.

RESULTS: Surprisingly, decidual cells secrete highest levels of MMPs, and their invasive potential seems to be increased in presence of cytotrophoblast (CTB). In contrast, invasive properties of CTB are not modified by decidual cells.

CONCLUSION: CTB secrete factors that favour invasion whereas decidua seems not to play a major role in regulating CTB invasion in vitro. Moreover, it is interesting to note that decidual cells could have potent invasive capacity which could explain, at least in part, endometriosis.

INTRODUCTION

Proliferation, migration and invasion of trophoblastic cells into the maternal endometrium are essential steps in human embryo implantation and placentation (Red-Horse *et al.* 2004). Cytotrophoblastic cells (CTB) proliferate and form columns of invasive CTB which invade the decidualized endometrial stroma, the spiral arteries and the inner third of the myometrium. The invasive behaviour of these cells is regulated in space but also in time (during first and early second trimester of pregnancy) and depends on both paracrine and autocrine factors such as growth factors, cytokines, hormones, prostaglandins, matrix metalloproteinases (MMPs) and other collagenases (Bischof *et al.* 2000). Secreted metalloproteinases, by their capacity to degrade extracellular matrix (ECM) allow the remodelling of endometrial tissue. These secretions are necessary for trophoblast invasion since phenanthroline, a non specific inhibitor of MMP, inhibits invasion of matrigel by cytotrophoblastic cells (Bischof *et al.* 1995). Endometrial cells also play a major role during the interaction with the invading trophoblast. Their morphology and secretion pattern are modified during the secre-

ORIGINAL ARTICLE

tory phase of the cycle in preparation to implantation (Gellersen *et al.* 2007). It is thus important to study trophoblast invasion in presence of decidua. Some in vitro models have already been developed to mimic embryo implantation or trophoblast invasion. After Kliman's description of matrigel invasion by CTB (Kliman *et al.* 1990) several in vitro models of coculture were developed to study the different factors involved in this process in vitro (Vicovac *et al.* 1988, 1995; Dunk *et al.* 2003; Popovici *et al.* 2006; Ntrivalas *et al.* 2006; Soghomonians *et al.* 2005). An in vitro model of embryo implantation using hatched blastocyst cultured on a confluent layer of stromal cells was described (Bentin-Ley and Lopata, 2000; Carver *et al.* 2003). Attachment and implantation of blastocyst into the stromal cells layer were also observed by microscopy (Carver *et al.* 2003). Then, Campbell *et al.* developed a bilayer coculture of CTB and decidual endothelial cells to study maternal-foetal cells interaction (Gallery *et al.* 2001). This study lead them to suggest that maternal cells could contribute to the control of endovascular CTB invasion by regulating migration of CTB and matrix metalloproteinase 9 (MMP-9) secretion (Campbell *et al.* 2003, 2004). Other studies use decidual conditioned media to study its effect on trophoblastic cell migration and/or invasion (Wright *et al.* 2006; Hannan *et al.* 2006 and Zhu *et al.* 2009). Here, using coculture of first trimester CTB and decidual cells purified from same abortion material, we can study the effects of one type of cells on proliferative and invasive properties of the other. Surprisingly, we observed a higher secretion of MMPs by decidual cells than by CTB, and the invasive properties of CTB were not modified in presence of decidual cells.

MATERIAL AND METHODS

Reagents

Dulbecco's modified Eagle's medium (DMEM), antibiotics mixture (penicillin, streptomycin), were products of Invitrogen (Basel, Switzerland). Fetal bovine serum was from Biochrom AG (Oxoid AG, Basel, Switzerland). Celltiter 96 Aqueous cell proliferation assay was purchased from Promega (Promega Corporation, Madison, USA). Collagen type I and collagenase were from Sigma (Sigma-Aldrich, Buchs, Switzerland).

Cell culture

Placental tissue was obtained from patients undergoing a legal abortion during the first trimester (7–12 weeks of gestation). Informed written consent was obtained from all patients before their inclusion in the study, for which approval was obtained from the local ethics committee.

Isolation and culture of CTB: CTB were isolated from first trimester placentas as described (Bischof *et al.* 1995). In brief, fresh tissue specimen were isolated and washed several times in sterile Hanks balanced salted solution (HBSS). Tissue was then enzymatically digested 4 times for 20 minutes at 37°C (0.25% trypsin, 0.25 mg/ml Dnase I). After incubation, the trypsin cocktail was neutralized with fetal bovine serum (FBS), and the cells resuspended in DMEM. This cell suspension was filtered (100 µm mesh), laid onto a Percoll gradient (70% to 5% Percoll diluted with HBSS) and centrifuged for 25 min at 1 200 × g. The 30–45% percoll zone containing CTB was collected, the cells washed and resuspended in DMEM. Cells were then immunopurified with immobilized anti-CD45 antibody according to a protocol published previously (Bischof *et al.* 1995).

Isolation of decidual cells: Decidua collected from the same abortion material as the one used to isolate CTB, was washed in sterile HBSS, minced under sterile conditions and subjected to two collagenase (3 mg/ml) digestions of 30 minutes at 37 °C. The resulting suspension was collected, neutralized with FBS, and centrifuged (1 000 g, 10 minutes). Cells were then resuspended in DMEM. This cell suspension was filtered (40 µm mesh), and seeded into flasks. The obtained cells were vimentin positive (>95%), prolactin positive (>95%), cytokeratin 7 negative and secreted prolactin in the culture medium.

Proliferation assay

CellTiter 96 Aqueous One solution cell proliferation assay was used according to the manufacturer's protocol. Absorbance was recorded at 490nm using a 96-well plate reader.

Invasion assay

Cell invasion assay was performed in an invasion chamber based on the Boyden chamber principle. Each insert is fitted with an 8 µm pore size polycarbonate membrane (Costar) precoated with rat tail collagen I (5 µg/cm^2). Inserts were washed in DMEM and incubated for 30 minutes at room temperature. For each well, 5×10^5 CTB or 2.5 ± 10^5 decidual cells (purified from same abortion material as CTB) in 100 µl of serum free media, in presence or not of respectively decidual cells (5×10^5 cells) or CTB (1×10^6 cells) in lower chamber, were added to the upper compartment of the transwell chambers. Cells were incubated for 48 h at 37 °C in a CO_2 (5%) incubator. After incubation, the culture supernatants were collected for zymography. Cells that did not invade but were attached to the collagen were sweeped away with a cotton swab. Viable cells that invaded collagen were stained with crystal violet cell stain (0.9% in ethanol). After washing the cells, the stain was extracted with a solution of 1% acetic acid: 50% ethanol for 15 min at room temperature. 100 µl of the dye mixture were transferred to a 96-well microtiter plate for colorimetric measurement at 560 nm. Data were expressed as the percentage of treated cells that invaded the collagen-coated membrane relative to the untreated (controls) cells. Collagen type I coated inserts incubated with medium without cells served as blank.

To test effect of mifepristone on decidual cells invasiveness, cells were cultured on collagen-coated mem-

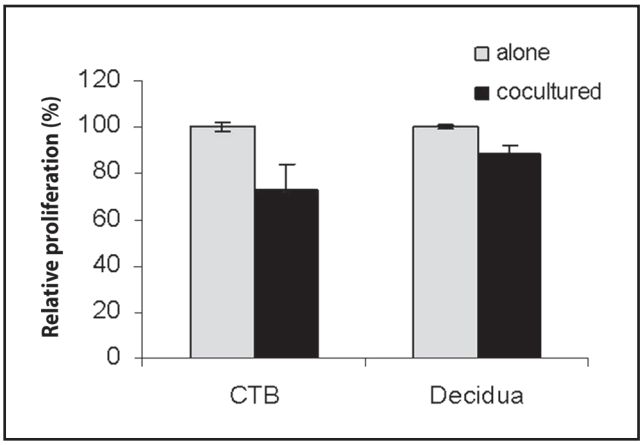

Fig. 1. Effects of coculture on proliferation of cells.

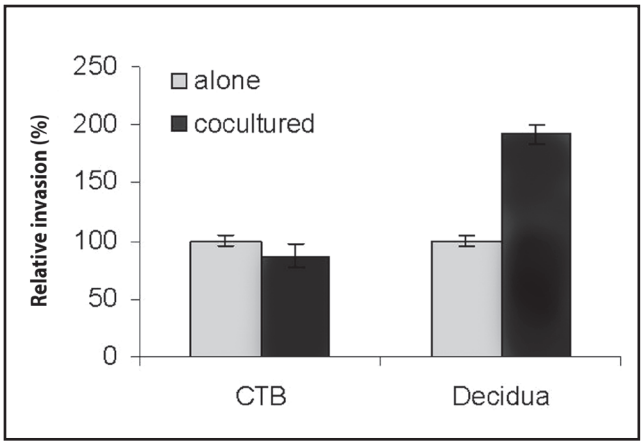

Fig. 2. Invasive properties of decidual and trophoblastic cells.

brane in presence or not of 20 µM of mifepristone for 48 h before measuring invasion.

Prolactin analysis

Culture supernatants were analysed for prolactin by an immunometric assay on the automate analyzer Kryptor (Brahms, Henningsdorf, Germany).

Zymography

Proteolytic activity of culture supernatants were assayed using gelatin-substrate gel electrophoresis as described previously (Martelli *et al.* 1999). Zymograms were scanned with an Epson Perfection 1 200 Photo scanner and the surface of the digestion bands measured by the Kodak 1D Image analysis software (Kodak, Rochester, NY).

Each experiment was run in triplicate, and done three times with different tissues.

RESULTS

Proliferation of cells

In parallel to the invasion assay, proliferative activity of each cells (coincubated or not) was studied. CTB cultured in vitro lost their capacity to proliferate, but this test allowed us to observe a slight negative effect on both CTB and decidua of coculture of these cells (Figure 1), and to correct our invasion assay.

Invasive properties of CTB and decidual cells

Invasive properties of first trimester CTB and decidual cells were studied on collagen I coated inserts, in presence or absence of the other type of cells in the lower chamber, thus without direct contact between CTB and decidual cells. Under these conditions, we observed that decidual cells are able to invade collagen I (Figure 2). Moreover, CTB significantly stimulate the invasive behaviour of decidual cells whereas decidual cells did not modify the invasiveness of CTB (Figure 2) despite the high amount of MMPs secreted by decidual cells (Figure 3).

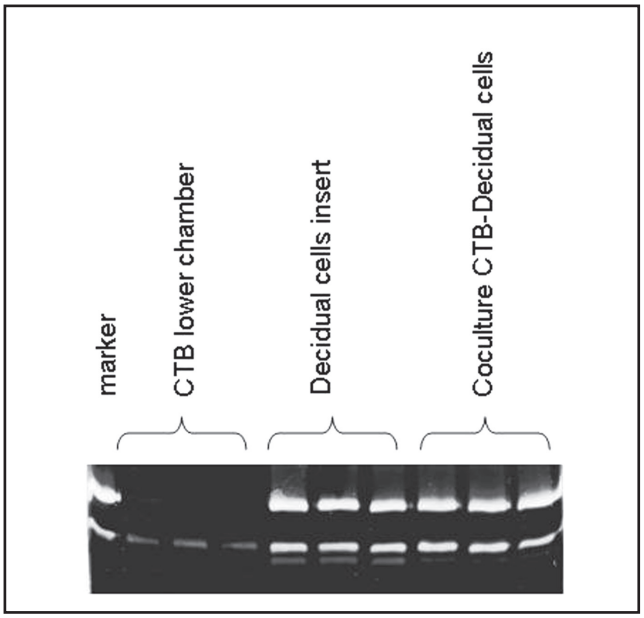

Fig. 3. Gelatinase activities in coculture supernatant.

MMPs activities

Figure 3 shows results of a representative zymogram obtained with cells at week 8. At this stage we can only observe MMP-2 activities in culture medium of CTB. We also observe intense activities of MMP-2 and -9 in decidual cells. These activities are much higher than in CTB, and seem to be not influenced by coculture with CTB and vice versa.

Effect of mifepristone on cellular invasiveness

In order to verify the effect of progesterone on MMP, and consequently, on invasion of decidual cells, we studied the effect of mifepristone on decidual cells invasiveness. As shown in figure 4, mifepristone significantly increased the invasiveness of decidual cells. This result confirms the importance of progesterone as an inhibitor of cellular invasion.

DISCUSSION

The endometrial extracellular matrix (ECM) composition reflects a balance between new basement membrane and matrix proteins and activity of ECM-degrading protease. MMPs are involved in tissue remodelling. They are expressed by human endometrial stromal/decidual cells and by CTB (Bischof *et al.* 1991; Martelli *et al.* 1993). In stromal/deciual cells, their role is associated with menstruation (Salamonsen and Woodley 1996; Salamonsen *et al.* 1997; Dong *et al.* 2002), decidualization (Jones *et al.* 2006), and implantation where the balance between tissue inhibitors of MMP (TIMP-1 and TIMP-2) and MMP expressions regulates CTB invasion (Seval *et al.* 2004). Indeed, invasive behaviour of trophoblastic cells depends on MMP activities (Cohen *et al.* 2006), and particularly, MMP-9 and MMP-2 (Fisher *et al.* 1985; Librach *et al.* 1991; Staun-Ram *et al.* 2004). Their secretion profile in CTB is dependent on gestational age. Indeed, no MMP-9 secretion is observed before week 6 of pregnancy, but from 7 to 11 weeks MMP-9 secretion increases gradually (Xu *et al.* 2000; Staun-Ram *et al.* 2004). In contrast, MMP-2 production decreases from week 6 to week 11. This observation was confirmed in 8 weeks CTB supernatant. However, it seems paradoxical to observe that decidua, a tissue in which CTB migrate and invade, produces more active MMP-2 and -9 than CTB. Moreover, despite this important secretion of MMP-2 and -9 by decidual cells, we previously showed that decidual coculture of CTB did not induce an increase of CTB invasiveness. The increase of MMP-9, and mainly MMP-2, activities were also described in supernatants of CTB cultured in conditioned medium of in vitro decidualized stromal cells (Bischof *et al.* 1998). Under these conditions, an increase of TIMP-1 secretion was also observed (Bischof *et al.* 1998), and could explain, at least in part, why decidual coculture of CTB did not induce an increase in CTB invasiveness. The fact that CTB invasiveness is not modulated by the presence of decidual cells is somewhat surprising since CTB is known to be regulated by paracrine pathways (Bischof *et al.* 1998, 2000). From this observation, we could suggest that paracrine regulation of CTB invasion is negligible compared to its autocrine regulation under these conditions of culture.

It is never reported that first trimester decidual cells could have invasive property in pregnant uterus. We showed that decidua in culture can secrete high level of MMP-2 and -9 which could be responsible of its invasive property in vitro. This property is probably negatively regulated in uterus by progesterone but could play important role outside the uterine cavity, notably in endometriosis. We thus suggest that decidual cells could have potent invasive capacity outside uterine cavity, in an environment which does not contain progesterone, as it could be observed in endometriosis (May and Becker, 2008).

In conclusion, these experiments using cocultures of CTB and decidual cells purified from same abortion material lead us to confirm that CTB secrete factors that favour invasion whereas decidua seems not to play a major role in regulating CTB invasion in vitro. This observation is in agreement with a study that suggested that decidua is not necessary to trigger extravillous CTB (EVT) invasion, but that it could limit proliferation of trophoblast cell column and to accelerate the onset of EVT migration (Goffin *et al.* 2003). Moreover, it is interesting to note that decidual cells could have potent invasive capacity which could explain, at least in part, endometriosis.

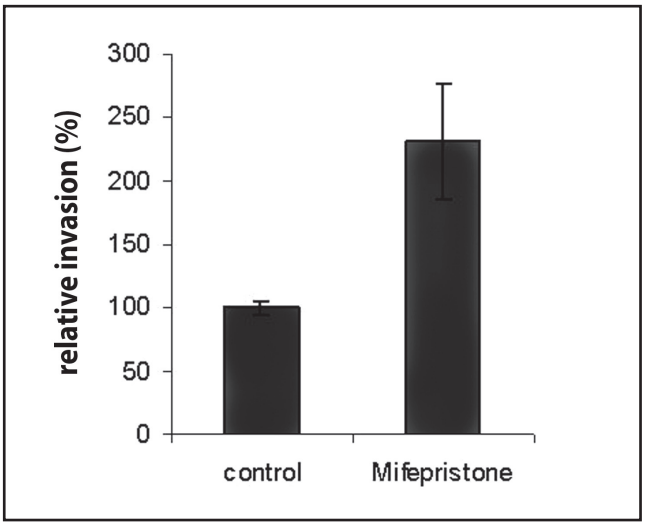

Fig. 4. Effect of mifepristone on invasive properties of decidual cells.

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