Nitric oxide induces gelatinase A (matrix metalloproteinase 2) during rat embryo implantation

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Objective: To evaluate a reciprocal signaling interaction initiated by embryo-derived nitric oxide (NO) to facilitate implantation by increased production of gelatinase A (matrix metalloproteinase 2, MMP2) in uterine stroma.

Design: Experimental animal studies.

Setting: Reproductive-physiology research laboratory.

Animal(s): Female syngeneic Wistar rats aged 14 weeks.

Intervention(s): Vaginal smears to confirm pregnancy. Oviductal ligature to avoid the descent of blastocysts to the uterine lumen. Plasma exudation assays to locate uterine blastocyst implantation sites. Organ cultures treated with NO donors and nitric oxide synthase (NOS) inhibitors.

Main Outcome Measure(s): Expression of MMP2 and NO was assessed by Western blot and zymography of tissue extracts and by immunofluorescence of tissue sections.

Result(s): An increase in MMP2 activity was found in uterine extracts in early pregnant rats and was concentrated at implantation sites. Immunolocalization experiments showed that inducible NOS was expressed on the surface of the implanting blastocyst adjacent to the uterine epithelium at the sites of increased MMP2 expression. In organ culture experiments, NO donors were found to increase, whereas NOS inhibitors were found to decrease MMP2 activity in uterine tissue sections.

Conclusion(s): Blastocyst-derived NO contributes to the production of uterine-derived MMP2, an essential component of implantation and initiation of placentation. (Fertil Steril 2002;78:1278–87. ©2002 by American Society for Reproductive Medicine.)

Key Words: Decidualization, maternal–embryo interaction, pregnancy, rat uterus

The embryo implantation process requires extensive remodeling of the maternal extracellular matrix so that the embryonic cells can invade the uterus wall to make contact and merge with the maternal vascular network. Here, we provide experiments that demonstrate that nitric oxide (NO), the well-known regulator of vascular permeability, also controls the location of implantation by stimulating the expression of the matrix metalloproteinases (MMPs) enzyme, gelatinase A (MMP2). We have previously shown that nitric oxide synthase (NOS) activity peaks during the peri-implantation days of pregnancy. We now demonstrate a synchronized increase in MMP2 activity in uterine extracts from early pregnant rats and show that this activity is primarily localized to the implantation sites. Immunolocalization experiments show that inducible NOS (iNOS) is expressed on the surface of the implanting blastocyst adjacent to the uterine epithelium at the sites of increased MMP2 expression, suggesting that blastocyst-derived NO could be stimulating the production of uterine-derived MMP2, an essential component of implantation and initiation of placentation. This conclusion was validated by organ culture experiments in which NO donors were found to increase, whereas NOS inhibitors were found to decrease, MMP2 activity in uterine tissue sections.
Implantation is the physical attachment of the embryo to the uterus, and although it involves relatively few cell types, the reciprocal signaling relationships between maternal and fetal tissues can be very complex (1). Although many of the players involved in this process have not yet been identified, many of the targeted mutations in transgenic mice that result in embryonic lethality can be traced to incorrect coordination of implantation (2). In humans, implantation defects have been linked to the phenomenon of spontaneous, early-term abortions and to the development of pre-eclampsia, a serious disease of pregnancy, for both the mother and the fetus (3).

Implantation of rodent and human embryos involves penetration by embryonic trophoblastic cells through the uterine epithelial layer to make contact with the underlying basement membrane; subsequent degradation of this barrier allows the invasive trophoblasts to infiltrate the endometrial stroma, ultimately reaching the maternal vessels and establishing a vascular relationship with the mother (1). The invasion of the embryonic cells into the maternal tissue is facilitated by the production of MMPs, enzymes used for degradation of the intervening extracellular matrix (4). Matrix metalloproteinases function in the process of invasive- ness beyond clearing pathways, however, and a rapidly growing body of research has implicated MMPs in many aspects of intracellular signaling that are associated with the cellular invasion process (5).

During embryo implantation, MMPs are expressed both by the trophoblast cells and by the endometrium (4). In particular, the endometrial stromal cells in the pregnant mouse (6, 7), rat (8, 9), and human (10, 11) have been found to express high levels of gelatinase A (MMP2) and gelatinase B (MMP9). Expression of these enzymes is critical for maintenance of the endometrial structure as well as for the early phases of deciduization and neovascularization required for placentation (4, 7). Accordingly, administration of MMP inhibitors to rodents affects many aspects of placental formation, including retardation of decidual remodeling and displacement of embryo implantation (8, 12). Although many aspects of MMP function during implantation have been elucidated, the triggers of MMP expression in endometrial, and trophoblastic cells during implantation remain largely unknown.

Nitric oxide, associated with inflammation and vasodilation (13), also plays a key role in rat embryo implantation (14–16). In rodents, NO signals for increased vascular permeability, vasodilatation, and blood flow in the uterus (17) and is a component of the decidual cell reaction (18, 19), and transgenic mice deficient for NOS show many reproductive defects (20, 21). Investigations in humans have also suggested that uterine NO may regulate vasodilation and myorelaxation aspects of embryo implantation (22, 23).

Three isoforms of NOS, the enzyme responsible for the synthesis of NO, have been described (13). The two constitutive isoforms of NOS that are Ca2+/calmodulin dependent are expressed in endothelial and nerve cells (eNOS and bNOS, respectively). The third isoform is cytokine inducible (iNOS), is Ca2+/calmodulin independent, and is produced by many types of cells. We previously showed that NOS activity is increased in the rat uterus at the initiation of implantation (evening of day 5 of pregnancy) through increases in the activity of both Ca2+/dependent and Ca2+/independent NOS isoforms (15) and that when the uterine synthesis of NO was blocked, the number of implanted embryos in the rat was significantly decreased (14).

We hypothesized that NO could function during the implantation process, beyond its properties as a vasoactive agent and inducer of angiogenesis in the placenta (24), by playing a positive regulatory role in the activation of MMPs that occurs during embryo implantation, thus linking trophoblast invasiveness to the permeability of the target vasculature. This hypothesis led to several specific predictions. First, the localization of iNOS during implantation should physically and temporally coincide with expression of functional MMP enzymes. Second, agents that increase NO levels should lead to increased MMP activity in uterine tissues, whereas agents that decrease NO levels should also decrease MMP activity. Here, we present experimental results that confirm these predictions for MMP2 but not for MMP9 (gelatinase B), showing that NO acts as a selective modulator of MMP activity during implantation.

**MATERIALS AND METHODS**

No institutional review board approval was required.

**Animals**

All research animals were used in compliance with the International Guiding Principles for Biomedical Research Involving Animals, Council for International Organization of Medical Sciences (CIOMS). Adult female Wistar rats were bred in our facilities and fed with Purina rat chow, and all animals were killed by cervical dislocation.

Estrus was determined by vaginal smears, and mating was confirmed by the presence of sperm in vaginal smears the morning after females had been caged overnight with males. These diagnostics defined the 1st day of gestation. Pregnant animals were killed in the morning (11:00 AM) of gestation days 3, 4, 5, and 6, as well as in the early evening (6:00 PM) of gestation day 5 (so as to cover peri-implantation days of pregnancy, because blastocyst implantation in rats begins late on day 5). Uterine horns (endometrium and myometrium) were surgically removed, the extraneous tissue was discarded, and uteri were flushed with phosphate-buffered saline (PBS; pH 7.4; 0.1 M). Female rats killed at estrus served as nonpregnant controls.

To obtain pregnant rats without embryos in the uterine lumen, bilateral oviductal ligation under ether anesthesia was performed on gestation day 2 to prevent the descent of
embryos to the uterine lumen, taking care not to disrupt the uterovarian vessels. A control group underwent sham operation. The retention of blastocysts in the oviductal tract was confirmed by microscopic observation on gestation day 5.

**Location of Embryonic Implantation Sites in Rat Uterine Tissue**

We used a plasma exudation assay (25) to locate uterine blastocyst implantation sites. Rats under ketalar anesthesia (Parke-Davis, Buenos Aires, Argentina) were injected intravenously with 0.5 mL of 0.5% Evans blue (Sigma, St. Louis, MO) in 0.9% NaCl (Sigma) at 6:00 pm on gestation day 5 and killed 15 minutes later. Then, uteri were removed and implantation sites were visualized as dyed areas, which correspond to areas with higher vascular permeability and coincide with the sites at which the blastocysts are implanting. By this method, we can differentiate implantation sites, such that each site corresponds to one implanting embryo. In the rat, those areas begin to be evident at 6:00 pm on day 5 of pregnancy (15, 25). Implantation regions were separated from interimplantation regions by careful dissection and were fixed for histology or were stored at −70°C for subsequent analyses.

Evans blue, at the concentrations found in the tissue extracts, did not interfere with other assays.

**Rat Uterine Tissue Extracts**

Frozen uterine tissues were homogenized in a Tissue-mizer (Ultraturrax) in 50 mM Tris buffer (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL of leupeptin and 1 mg/mL benzamidine (all from Sigma). The homogenate was centrifuged at 10,000 g for 10 minutes at 4°C, and the supernatant containing the cytosolic fraction was collected for detection of MMP2, MMP9, iNOS, and nNOS. The concentration of proteins in the pellet fraction was measured by Bradford (BioRad, Richmond, CA).

**Western Blots for iNOS, bNOS, MMP2, and MMP9**

Equal amounts of proteins of uterine extracts were size fractionated in 8% sodium dodecyl sulfate–polyacrylamide electrophoresis using a BioRad MiniProtean (BioRad) gel apparatus, and the resulting gel slabs were electrotransferred to an Immobilon-P (Millipore Corporation, Bedford, MA) membrane. After blocking the membrane with 5% nonfat milk in TTBS, pH 7.5 (100 mM Tris buffer with 150 mM NaCl and 0.1% Tween-20; Sigma), the blot was incubated with the specific primary antibodies. For NOS detection, we used monoclonal anti-iNOS or anti-nNOS antibodies (Transduction Laboratories, Lexington, KY) at a final concentration in blocking buffer of 1 μg/mL (1:1000 dilution). To detect MMP9 and MMP2, we used goat polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at a final concentration in blocking buffer of 1 μg/mL (dilution, 1/200). These antibodies recognize both latent (92 kDa) and active forms (83 kDa) of MMP9, as well as the latent (72 kDa) and active forms (62 kDa) of MMP2.

After washing the first antibody, membranes were incubated with the appropriate biotinylated secondary antibody (Vector, Burlingame, CA). The staining was performed with the Vectastain ABC kit (Vector) according to manufacturer instructions. Peroxidase activity was developed by a solution containing 5 mg of diaminobenzidine (Sigma) in 10 mL TTBS and 0.03% H2O2.

We used mouse macrophage lysates or rat pituitary extract as positive controls for iNOS or nNOS, respectively. The intensity of each band was quantified by Eagle Sight scanning densitometry (Stratagene, La Jolla, CA). In all cases the specificity of the bands was confirmed by performing an appropriate control without first antibody (data not shown).

**Zymography for Gelatinases**

Equal amounts of protein obtained from uterine tissue extracts prepared as described for Western blots were analyzed by substrate gel zymography by copolymerizing pig skin gelatin (Sigma) at a final concentration of 1 mg/mL in 8% polyacrylamide gel. Samples were equilibrated at room temperature for 30 minutes before loading in nonreducing gels. After running, gels were washed in 2.5% Triton X-100 (BioRad) for 30 minutes and then incubated for 24 hours at 37°C in 50 mM Tris buffer (pH 7.4) containing 0.15 M NaCl and 30 mM CaCl2 (Sigma). Then, gels were stained with Coomassie R-250 (Roche Diagnostic Corporation, Indianapolis, IN), and proteolytic activities were detected as colorless bands against the dark blue background. Because the sodium dodecyl sulfate activates the latent forms of the enzyme, both latent and active enzymes are detected by this method.

The enzymatic activity was quantified by image analysis of the lysis bands. Conditioned media from the promyelocyte U-937 cell line were used as activity standards for MMP2 and MMP9 (data not shown).

To establish that the observed gelatinolytic activity was due to MMPs, the extracts were preincubated for 15 minutes in the presence or absence of 0.01 M EDTA (Sigma) before performing the electrophoresis, and gels were incubated in the presence or absence of ethylenediaminetetraacetic acid (EDTA) after electrophoresis; EDTA-dependent disappearance of the corresponding bands confirmed that the activity was due to metalloproteinases (data not shown).

**Immunofluorescence for iNOS, MMP2, and MMP9**

Uterine tissues corresponding to longitudinal segments containing one implantation site were removed from pregnant rats on the evening of day 5 of gestation. After surgery, tissues were fixed in 4% paraformaldehyde (Sigma) in PBS (pH 7.4; 0.1 M). Fixed tissues were embedded in OCT compound (Tissue-Tek, Miles, Elkhart, IN), sectioned by
cryostat (10-μm longitudinal slices), mounted on glass slides precoated with gelatin, and then stored at −20°C until further use.

To detect iNOS, MMP2, and MMP9, we used antibodies indicated above. After the first antibody, the slides were treated with the corresponding anti-mouse or anti-goat FITC conjugated antibody (1:50, Sigma), mounted in PBS–glycerol, and examined with Nikon-Microphot FX epifluorescence microscope with the appropriate filters. In some cases, sections were counterstained with hematoxylin and eosin. Control slides were performed by omitting primary antibody.

Incubation of Uterine Segments with NOS Inhibitors and NO Donors

We used implantatory uterine tissue (~80 mg) from pregnant rats on the evening of day 5 of gestation. We incubated the tissue sample in a metabolic shaker, under an atmosphere of 5% CO2 in 95% O2 at 37°C for 1 hour in 0.5 mL of Krebs Ringer Bicarbonate medium (ionic composition: Na+, 145 mM; K+, 5.9 mM; Ca2+, 2.2 mM; Mg2+, 1.2 mM; Cl−, 127 mM; HCO3−, 25 mM; SO42−, 1.2 mM; PO43−, 1.2 mM), as described elsewhere (26). We assayed the effect of the NOS inhibitor N_G-monomethyl-L-arginine, L-NMMA, the inactive enantiomer, D-NMMA (both used at 600 μM, Sigma), and the NO donors sodium nitroprusside (600 μM, Sigma) and spermine-NONOate (600 μM, Sigma). At the end of the incubation period, aliquots of the incubation medium were frozen at −70°C for subsequent determination of protein content by Bradford and for determination of MMP2 activity by zymography.

Statistics

All values presented in this study are means ± SE. Comparisons between groups were performed employing one-way analysis of variance, and differences between means were determined by Student-Newman-Keuls multiple comparison tests.

RESULTS

Nitric Oxide Synthase Protein Levels Increase During Implantation

We have previously demonstrated that the activity of the uterine Ca2+/calmodulin-independent NOS increases at the beginning of rat embryo implantation (15). To determine whether this increase in NOS activity is due to an increase in protein levels, we analyzed by Western blot the levels of the iNOS isoform during early pregnancy (gestation days 3 to 6), using animals in estrus as nonpregnant controls. We found that the ~130-kDa iNOS band in the cytosolic fraction of uterine homogenates increases during early pregnancy to reach a plateau in the morning of day 5, immediately before the onset of implantation (Fig. 1; days 3 and 4 vs. estrus, P<.05; day 5 morning, evening and day 6 vs. days 4 and 3,
Dissection of uterine tissue at sites of implantation from the tissue between implantation sites (the intersite domains) revealed that the increases in uterine NOS protein levels were not dependent on the presence of the implanting embryo (Fig. 1B). Tissues were extracted on the evening of gestation day 5 and analyzed by Western blot for expression of both iNOS and bNOS (the NOS isoforms previously found to be increased in the pregnant rat uterus [27]). Levels of both isoforms were found to be higher on gestation day 5 of pregnancy as compared with control nonpregnant uterus (estrus; \( P < .01 \)), but no significant difference was found between sites of implantation and intersites. These results suggest that the increased uterine NOS at the beginning of the embryo implantation process is a more general response of the uterus that is not localized to the implantation sites.

**Rat Embryo Implantation Is Correlated With Increased Levels and Activity of MMP2**

To correlate NOS levels with MMP activity, we analyzed the protein level and activity of MMP2 and MMP9 during early pregnancy (gestation days 3 to 6) in rat uterine homogenates. By Western blot, we found that uterine MMP2 expression levels peak during evening of gestation day 5, persisting through gestation day 6 (\( P < .01 \), day 5 evening and day 6 vs. day 3 and estrus, Fig. 2A). The increase of MMP2 protein levels was accompanied by increased enzymatic activity, as measured by gelatin zymography (Fig. 2B). The protein levels and corresponding activity of MMP9 are increased in pregnant compared with nonpregnant uteri (\( P < .05 \), Fig. 2A and B) but did not significantly change during implantation.

By contrast with the nonspecific localization of NOS increases (Fig. 1B), both gelatinases, but particularly MMP2, showed higher activity at the implantation sites relative to the intersites (\( P < .001 \) by densitometric analyses), and we found that the local activity on the evening of gestation day 5 is higher than on day 6 (\( P < .01 \), Fig. 2C). Together, these observations suggest that implantation in the rat uterus is associated with increased levels of MMP2 but not MMP9.

**Expression of MMP2 Increases at Sites of Implantation**

Immunofluorescent staining of NOS, MMP2, and MMP9 in rat uterus sections, fixed in the evening of gestation day 5, revealed distinct staining patterns (Fig. 3). Uterine implantation sites could be localized by Evans blue staining, typical of the decidualization preparatory to implantation (see arrows in Fig. 3B and 3Cb).

Figure 3B and C depicts the localization of MMP2 and MMP9, respectively, using antibodies that recognize both the active and the latent form of the enzymes. Although MMP2 staining in the uterine epithelium is increased at the antimesometrial implantation sites (Fig. 3B), MMP9 is not (Fig. 3C). Higher magnification images suggested that MMP staining was localized to secretory granules, as expected (data not shown).

This evidence is consistent with our suggestion that embryo-derived NO could be increasing the expression of MMP2 at the sites of implantation. This effect appears to be highly site specific, for although local increases in MMP2 expression at implantation sites were substantial (Figs. 2 and 3), the overall levels of MMP2 are primarily controlled by maternal signals, as demonstrated by oviduct ligature experiments (Fig. 4). Measurement of MMP2 activity in uterine tissue from rats that have suffered ligature of the oviduct in day 2 of pregnancy (a procedure that prevents the access of embryos to the uterine cavity) revealed that overall levels of MMP2 do not substantially differ from those in intact uterus.

**Nitric Oxide Modulators Affect MMP2 Activity in Isolated Uterine Segments**

Although the previous observations had provided strong evidence that increased uterine MMP2 and embryo-derived NOS isoforms were coordinated with implantation, they did not conclusively reveal whether MMP2 expression was caused by, or just correlated with, NO signaling. The ideal experiment to differentiate these possibilities would be to treat pregnant rats with NO inhibitors and then follow expression of MMP2. However, because NO signaling is required for proper implantation (14–16), the process of implantation and the effects of NO are difficult to conclusively dissect in situ.

To circumvent this problem, we used an organ culture procedure in which implantatory segments of uterine tissue were isolated on the evening of gestation day 5 and then treated in culture with the NO donors spermine-NONOate and sodium nitroprusside, as well as the general NOS inhibitor L-NMMA and its inactive enantiomer D-NMMA. In these experiments, NO donors significantly increased MMP2 activity in implantatory uterine segments, whereas the NOS inhibitor had the opposite effect (Table 1).

**DISCUSSION**

This study defines an NO–MMP2 signaling pathway that activates tissue remodeling at the time of embryo implantation in rats. We show that during the early implantation period, iNOS and MMP2 enzymes are expressed proximally in the uterine epithelium and that the level and activity of iNOS are increased several hours before the increase in MMP2 activity in the uterus. Moreover, inhibitors of NOS diminished MMP2 activity, whereas NO donors enhanced it in isolated implantatory rat uterine tissue.
Matrix metalloproteinase 2 is increased at the time and sites of implantation. (A), Analysis of MMP2 and MMP9 in the same samples used in Figure 1A. The antibodies recognize both the latent proenzymes and the active forms, and the graphs depict total expression levels for both latent and active forms as the means ± SE of four gels (samples for each condition were obtained from four different animals). The densities of the bands were normalized using the estrus band (E) as baseline. *P<.05, day of pregnancy vs. estrus; **P<.01, day of pregnancy vs. estrus. Typical blots are shown below each graph. (B), Gelatinase zymography from the same samples analyzed by Western blot in A. Enzyme activity is manifested by loss of the substrate, shown as white bands in the gels below each graph. Data correspond to the means ± SE of four gels (samples for each condition were obtained from four different animals). The negative densities of the bands were normalized using the estrus band as baseline. The graphs represent total MMP9 and MMP2 activity, including bands for both latent and active forms. *P<.05, day of pregnancy vs. estrus; **P<.01, day of pregnancy vs. estrus. (C), Gelatinase zymography in uterine strips isolated from estrus (E) or implantation sites (S) and intersites (I) of pregnant rats at the evening of gestational day 5 or on the morning of gestational day 6. (a): The density of the MMP2 bands, normalized using the estrus band as baseline. Data correspond to the mean ± SE of samples obtained from four different rats for each condition. ***P<.001, S vs. I; **P<.01, day 5e vs. day 6. (b): Typical zymogram.

MMP2 shows increased expression at sites of NOS expression. (A), Staining for iNOS at a site of blastocyst implantation reveals expression in the uterine epithelium (ep) that faces the lumen (lu), as well as in the adjacent blastocyst (bl). Panel b shows a higher magnification of the field shown in panel a. (B), Staining for MMP2 shows increased expression at site of implantation (arrows; center of site was torn). (a), Immunofluorescence of MMP2. (b), Corresponding hematoxylin and eosin stained section. (C), Staining for MMP9 shows no increase at site of implantation (arrows). (a), Immunofluorescence of MMP9. (b), Corresponding hematoxylin and eosin stained section. Scale bars represent 40 μm.

In doing so, we have shown that NO, a signal associated with increased vascular permeability, can direct expression of MMP2, an enzyme associated with cellular invasiveness, in the initial steps of a process that results in the breaching of uterine epithelial and endothelial integrity and the ultimate apposition of the maternal blood supply with embryo-derived cells.

A number of previous studies have shown that NO derivatives can modulate MMP activity in culture, including human vascular smooth muscle cells (28), human neutrophils (29), human cartilage explants (30), and human term placentas (26), and in their isolated trophoblastic cells (31, 32), as well as during metastasis progression of carcinosarcoma (33). Here we present the first direct evidence of this reciprocal signaling relationship in situ, in which NO acts as an activating signal to promote expression of MMP2.

MMPs are synthesized and released to the extracellular space as inactive proenzymes (pro-MMPs) and activated in a process that requires the proteolytic cleavage of their amino-terminal domains. In the inactive precursor, the Zn\(^{2+}\) atom at the active site is bound to an unpaired cysteine thiol group located in the pro-peptide domain, and disruption of this interaction by cleavage of the prodomain is believed to represent the critical step in initiating the process of MMP activation (5). Accordingly, although the observed stimulatory effect of NO on the MMPs could be due to an up-regulation of MMP gene expression (32, 34, 35), it may be that NO directly activates MMPs through oxidation or nitration of the thiol moiety of cysteine 71 in their pro domain, leading to the dissociation of this cysteine from the Zn\(^{2+}\) atom in the active site (28, 29, 36).

Increased understanding of the regulation of MMP2 production by NO in implantation has relevance to implantation failures that can lead to premature termination of pregnancies even if embryonic development proceeds normally. This may be why, even though many improvements in IVF techniques have been made in recent years, pregnancy success rates through these techniques have not substantially increased (1). Moreover, pre-eclampsia, a disorder caused by shallow and incomplete invasion of placental tissue into the maternal blood supply and that affects 7–10% of human pregnancies (3), has also been linked to response to NO production (37, 38). Consequently, our observations linking NO to production of MMP2 provide insight into pre-eclamptic syndromes, particularly because MT1-MMP, which activates MMP2, is specifically found to be down-regulated in pre-eclamptic tissues (39).

Immunofluorescent imaging of MMP9 and MMP2 uterine expression (Fig. 3) revealed a weaker and more generalized staining for MMP9 by comparison with the localized, intense reaction for MMP2 in the luminal and glandular epithelial cells of the rat uterus. Additionally, there was evidence of extracellular secretion into the glandular lumen, in agreement with observations that glandular secretion of MMPs and their presence in human uterine fluid is highest during the peri-implantation phase (11).

Our results also agree with those of Bany et al. (40), who reported that MMP9 mRNA is barely detectable in the endometrial stroma during implantation in mice, whereas...
MMP2 expression is abundant in the stroma subepithelium when the blastocyst is about to implant (day 4 in mice). However, they did not find a different pattern of mRNA expression for MMP2 between implantation and interimplantation areas days after implantation starts. The reason for this could be that the localized expression of MMP2 that we saw in implantation areas could be a transient effect corresponding to the beginning of implantation, or could be the result of a species-specific phenomenon.

Regulation of MMP expression is critical for many tissue-remodeling processes, ranging from embryonic development to cancer progression (5), although because the mechanisms that control MMP levels are often controlled by complex and reciprocal interactions between adjacent cell types, understanding of the processes that control MMP expression has lagged behind the recognition of their significance.

The immunolocalization experiments presented in this paper show that the MMP2 and iNOS enzymes are expressed in cells that lie in close proximity in the implanting rat uterus. Whereas the luminal epithelium stains positively for MMP2 at the site of implantation, the iNOS is highly expressed in the trophoblasts facing this epithelium, in some epithelial cells, and in the stroma. Considering that NO is a gas and can diffuse freely across cell membranes, it makes an ideal mediator of reciprocal interactions between the maternal tissue and the implanting blastocyst. The signal that stimulates NO synthesis is not known but could include ovarian steroids (41) or cytokines and growth factors as IL-1 and TNF-α, all of which are elevated at this time of pregnancy (42, 43); these same factors have also been found to modulate MMP activity in a number of tissues (5).

In summary, in this work we provide evidence suggesting that an increase in blastocyst-derived NO production at the time of implantation in rats may cause the activation and up-regulation of uterine-derived MMP2 seen at the specific sites of implantation, which is likely a component of the increase in tissue remodeling activity localized to the sites of implantation. Because endometrial cells and the microvascular wall act as an effective barrier to trophoblastic cell invasion, the intrinsic invasiveness of these cells is controlled, at least in part, by the specialized environment of the uterus.

References

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