Secretion of matrix metalloproteinases by human endometrial cells in vitro

M. Martelli¹, A. Campana² and P. Bischof¹*

¹Department of Obstetrics and Gynecology, University of Geneva; and ²Clinic of Infertility and Gynecologic Endocrinology, Geneva, Switzerland

At each menstrual cycle, the uterine endometrium undergoes intense remodelling. Of the many factors implicated in tissue remodelling, the matrix metalloproteinases (MMPs) play a central role owing to their capacity to degrade the extracellular matrix. The aim of this study was to examine the nature and cellular origin of endometrial proteases as evaluated in culture systems with clearly characterized cell types. Endometrial cells from hysterectomy specimens were prepared using collagenase digestion. Bone-marrow-derived cells (a known source of proteases) were removed by immunopurification. Cells were cultured on different substrates (matrigel, agarose, glass or plastic). Purity of cell preparations was examined by immunocytochemistry, and proteases were characterized by zymography on SDS-PAGE containing gelatin. The cell phenotype in culture was largely influenced by the type of substrate. Gelatin-degrading enzymes detected in culture supernatants of stromal and epithelial cells had molecular masses ranging from 42 to 248 kDa, and were identified as metalloproteinases. We conclude that human endometrial stromal and epithelial cells express several matrix metalloproteinases, the expression of which clearly depended on the purity of cell preparation, on cell adhesion and on the nature of the substrate on which the cells grew. These enzymes might be involved in endometrium remodelling, blastocyst implantation and trophoblast invasion.

Introduction

The uterine endometrium, a well-known target for steroid hormones, is regularly shed and rebuilt throughout reproductive life, implying an active and continuous tissue remodelling. Uterine endometrial cells therefore provide an effective model for studying the role of enzymes implicated in extracellular matrix (ECM) degradation. A wide variety of factors participate in tissue remodelling including growth factors, cytokines and matrix metalloproteinases (MMPs or matrixins). MMPs are a group of highly homologous proteolytic enzymes believed to play a major role in the remodelling of connective tissues under various physiological and pathological conditions (Masure and Opotenakke, 1989; Woesner 1991). These enzymes participate in the breakdown of the major protein components of the extracellular matrix such as collagen, proteoglycans, fibronectin and laminin (Emonard and Grimaud, 1990; Van Wart and Mookhtar, 1990). These enzymes depend on Ca²⁺ and Mg²⁺ and are optimally active at neutral pH (Birkedal-Hansen, 1988).

Seven MMPs have been identified in mammalian cells (Woesner, 1991) and are grouped in three different classes, depending on their structural homology and substrate specificity, interstitial collagenases, gelatinases and stromelysins. The fibroblast-type interstitial collagenase (MMP-1) (Stricklin et al., 1977; Goldberg et al., 1986) and the neutrophil-type homologue (MMP-8) (Hasty et al., 1990; Mallya et al., 1990; Mookhtar and Van Wart, 1990) can degrade collagen type I, II and III. They are secreted as proenzymes (52 kDa) and are activated by trypsin, plasmin, kalikrein and chymotrypsin in vitro. The 72 kDa gelatinase (MMP-2) produced by proliferating fibroblasts (Sato et al., 1985; Hibbs et al., 1987; Wilhelm et al., 1989) and by tumour cells (Sato et al., 1983; Collier et al., 1988), and the 92 kDa gelatinase (MMP-9) produced by neutrophils (Hibbs and Bainton, 1989), macrophages (Hibbs et al., 1987) and by certain transformed cells (Wilhelm et al., 1989; Bernhard et al., 1990) have an identical substrate specificity degrading native collagen type IV, V and X (Wilhelm et al., 1989; Okada et al., 1990). These gelatinases contain a fibronectin-like domain which allows them to bind to gelatin (denatured type I collagen). The 72 kDa gelatinase is activated by organomercurial salts in vitro and not by plasmin or trypsin. Human fibroblasts also produce stromelysins (Okada et al., 1986; Whitham et al., 1986; Wilhelm et al., 1987; Saus et al., 1988), a group of three homologous enzymes (MMP-3, MMP-10 and MMP-7), that are also called proteoglycanases. These degrade type IV and IX collagens, laminin, fibronectin, elastin and proteoglycans (Chin et al., 1985; Okada et al., 1986, 1989). All MMPs are secreted as inactive proenzymes that are activated by limited proteolysis (He et al., 1989; Okada and Nkanishi, 1989) by other proteases, losing a 10 kDa peptide upon activation. In the active form, MMPs are regulated by inhibitors present in plasma and in the extracellular matrix. MMPs can be inhibited by α2-macroglobulin (Barret and Starkey, 1973) and α2-antitrypsin present in plasma and by tissue inhibitor of metalloproteinases (TIMP) located in the extracellular space (Cawston et al., 1981). The purification and isolation of TIMP has previously been described (Finn, 1986; Goldberg et al., 1989).

*Reprint requests.
Received 6 April 1992.

© 1993 Journals of Reproduction and Fertility Ltd

Downloaded from Bioscientifica.com at 12/10/2018 11:02:10 PM
via free access
To our knowledge no MMPs have previously been described in the human endometrium. The present study reports the presence, nature and cellular origin of MMPs in human endometrial cell cultures. Cultures were prepared from hysterectomy specimens. In addition, epithelial and stromal cells were cultured separately on different substrates to investigate the role of cell adhesion on the induction of protease secretion.

Materials and Methods

Preparation of endometrial cells

Endometrial tissue was obtained from hysterectomy specimens performed for reasons other than malignancy. The phase of the endometrial cycle was determined on histological sections of the same endometrium. The nine endometria analysed were of the proliferative phase. Furthermore, in three cases, stromal and epithelial cells were obtained from early secretory endometrium bearing a Gravigard intrauterine device (IUD).

All manipulations were performed under sterile conditions. The cell preparation and separation techniques used were adapted from Tseng and Liu (1981) and were based on the fact that collagenase disrupts the stromal cells, leaving the endometrial glands intact. The tissue was minced and washed in Hank's balanced salt solution (HBSS, Sigma, St Louis), containing 200 U penicillin G ml⁻¹ (10⁶ IU), 200 µg streptomycin ml⁻¹ (Hoechst, Darmstadt) and 2.5 µg Fungizone ml⁻¹ (Gibco, Basel). The washed tissue was then incubated three times for 20 min without shaking in a water-bath at 37°C, in complete medium: RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (FCS; Amimed, Birsfelden), penicillin G (10⁶ IU), 200 µg streptomycin ml⁻¹, 2.5 µg Fungizone ml⁻¹, to which 1 mg collagenase ml⁻¹ (0.287 U ml⁻¹ from Clostridium histolyticum; Boehringer, Mannheim GmbH, Germany) was added. After each step, the collagenase digestion was stopped by the addition of complete medium to the supernatant.

The supernatants consisting of stromal cells (the endometrial glands sediments rapidly) were pooled, centrifuged at 700 g for 5 min, and the cells resuspended in culture medium consisting of complete medium in which the 10% FCS was heat inactivated (56°C for 30 min) and acid treated adjusted to pH 3 with 1 mol HCl l⁻¹, for 2 h at room temperature and neutralized with 1 mol NaOH l⁻¹. Cells were counted in a Neubauer cell in the presence of Trypan blue (0.5% Trypan blue, Serva, Feinbiochemica, Heidelberg, in normal saline 0.9% NaCl): half of the cells were placed in culture immediately and the other half were further immunopurified.

The remaining sediments glands were incubated with shaking at 37°C for 2 h, in complete medium with 300 mg collagenase ml⁻¹ (330 U ml⁻¹ from Clostridium histolyticum; Sigma, St Louis). After addition of complete medium, the tubes were centrifuged and the supernatant discarded. The pellet consisting of endometrial glands was cultured for 5–7 days at 37°C in complete medium. Once a monolayer of epithelial cells was obtained, the culture was trypsinized (trypsin (1:250) 0.05%, EDTA 0.02%, Boehringer Mannheim GmbH, Germany) washed with complete medium and the cells counted in the presence of Trypan blue. Half of the cells were put into culture and the other half was immunopurified.

Immunopurification of endometrial cells

Cell suspensions were incubated with 50 µl of a mouse monoclonal antibody against human leucocyte common antigen (anti-LCA, anti-CD45, Dakopatts, Copenhagen) for 30 min at 4°C and shaken occasionally. After incubation, cells were washed with PBS containing: 137 mmol NaCl l⁻¹, 3 mmol KCl l⁻¹, 8 mmol Na₂HPO₄ l⁻¹, 1.5 mmol KH₂PO₄ l⁻¹ at pH 7.4 supplemented with 0.1% BSA (RIA grade, Sigma, St Louis). Prewashed (PBS–BSA 0.1%), magnetic particles (50 µl) coated with a second antibody, sheep anti-mouse IgG (Dynabeads M 280, Dynal, Milan Analytica, Switzerland), were then added to the cell suspension and incubated for 20 min at 4°C. A magnet was applied along the test tube to retain the particles bound to the cells expressing LCA. The supernatant was poured into a clean tube and the cells were washed with complete medium and counted.

Cell culture

One million epithelial or stromal cells per ml were routinely incubated in culture medium in a 12-well tissue culture plate (Costar, Cambridge, USA) and kept at 37°C under a 5% CO₂ atmosphere in air. Wells were either uncoated or precoated with basement membrane-like material (matrigel 300 µl per well: Collaborative Research, Inotech, Switzerland), agarose (300 µl per well: 0.5% in PBS, Agar Noble, Difco, Detroit) or glass (cover slips, Gribi AG, Bern). Medium was replaced every two days, centrifuged at 700 g and the unattached cells (if any) returned to their original wells. Aliquots of the supernatants were immediately frozen at −20°C.

Zymography

Zymography was performed according to Fisher et al. (1989) with some modifications. Gelatin (Merck, Darmstadt) at a final concentration of 1 mg ml⁻¹ was incorporated into the running gel containing 10% acrylamide (Fluka AG, Buchs, Switzerland), 25% 1.5 mol Tris(hydroxymethyl)-aminomethane l⁻¹ (Tris buffer, Merck, Darmstadt) pH 6.8, 0.4% SDS (Merck), 0.3% ammonium peroxodisulfate (APS; Merck). A stacking gel was layered containing 2% acrylamide, 0.05% N,N'-methylene-bisacrylamide, 25% Tris buffer (Tris 0.5 mol l⁻¹, pH 6.5, 0.4% SDS), 0.4% APS and 0.1% N,N,N,N-tetramethylmethylenediamine (TEMED).

The dimensions of the gel were 80 x 85 x 0.8 mm. Sample buffer (5 µl) consisting of 17.4% SDS, 7% sucrose (Fluka AG, Buchs) and 10 µl bromophenol in water (Siegfried, Zofingen) was added to 30 µl of the standardized (10⁶ cells ml⁻¹) cell supernatants to be analysed and 25 µl of this mixture applied to the gel. Electrophoresis ran for approximately 4 h at 7amp in 5–7°C in 25 mmol Tris–HCl l⁻¹ containing 0.19 mol glycine l⁻¹ and 0.1% SDS, pH 8.6. After electrophoresis, the gel was washed six times for 5 min at room temperature on a moving platform in 2.5% Triton X-100 in water (Sigma), and three times in PBS. The gel was then incubated overnight at room temperature in PBS, pH 7.4, containing CaCl₂ (0.9 mmol l⁻¹) and MgCl₂ (0.5 mmol l⁻¹), or EDTA (1, 5, 10 or 20 mmol l⁻¹; Fluka AG), 1,10-phenanthroline (0.2 or 2 mmol l⁻¹), 1 mmol iodoacetamide.
Table 1. Immunocytochemistry of human endometrial cells in culture

<table>
<thead>
<tr>
<th>Antibody used</th>
<th>Cell detected</th>
<th>Supernatant Before purification (%)</th>
<th>Supernatant After purification (%)</th>
<th>Pellet Before purification (%)</th>
<th>Pellet After purification (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin</td>
<td>Epithelial</td>
<td>2.6 ± 2.1</td>
<td>2.5 ± 0.7</td>
<td>70 ± 18.3</td>
<td>76.7 ± 11.2</td>
</tr>
<tr>
<td>LCA</td>
<td>Bone-marrow-derived cells</td>
<td>20.4 ± 5.2</td>
<td>0.2 ± 0.4</td>
<td>2.5 ± 2.1</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Endothelial</td>
<td>1.4 ± 0.9</td>
<td>0.8 ± 0.8</td>
<td>0.2 ± 0</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Stromal bone-marrow-derived cells</td>
<td>93.6 ± 10.1</td>
<td>98 ± 2.64</td>
<td>12.5 ± 8.5</td>
<td>3.5 ± 0.7</td>
</tr>
</tbody>
</table>

Values given are means ± SD, n = 9 from proliferative phase and n = 3 secretory phase bearing a Gravigard intrauterine device.

Immunohistochemistry performed after trypsinization of endometrial monolayers obtained from intact glands grown in vitro for 5–6 days.

Antibody LCA: Leucocyte Common Antigen (CD45).

Results

Immunocytochemistry

The sedimentation technique used to separate stromal and epithelial cells from hysterectomy specimens was highly efficient (see Table 1). The supernatant contained between 80 and 100% stromal cells, whereas the pellet was rich in epithelial cells (77%). The major contaminant of the epithelial cell preparation was the stromal cells. After immunopurification the majority (more than 95%) of bone-barrow-derived cells were removed.

Phenotype of cells in culture

Stromal cells. Within 24 h of culture, proliferative endometrial stromal cells adhered to matrigel, glass and plastic, but not to agarose. The cells flattened out on the matrices, and grew into small colonies of aggregated cells. On glass and plastic, cells formed a monolayer (Fig. 1c, d, e and f), whereas on matrigel no monolayer was seen (Fig. 1a, c). On agarose, cells remained viable (negative with Trypan blue); however, they floated in the medium where they grew as aggregates (Fig. 1g, h). With time, these aggregates increased in size with no evidence of attachment. Irrespective of the substrate on which the stromal cells were grown, immunopurified cells grew in much larger colonies. This was particularly evident on matrigel where at day 9 the colonies of immunopurified cells were five times larger than the colonies of normal stromal cells (Fig. 1a, b).

Epithelial cells. After collagenase digestion, the glands remained intact. Glands from proliferative endometrium were shorter, thinner and less tortuous than those obtained during the secretory phase (Gravigard IUD bearing uteri). After 5 to 7 days, the glands were no longer recognizable and the epithelial cells formed a monolayer; these were then trypsinized and grown on different substrates.

Within 24 h of culture, epithelial cells adhered to matrigel (Fig. 2a, b) and formed a pattern consisting of epithelial cells
Fig. 1. Conventional microscopy of endometrial stromal cells obtained from a proliferative endometrium at 9 days of culture. Immunopurified (a, c, e, g) or non-immunopurified (b, d, f, h) cells were grown on matrigel (a, b); glass (c, d); plastic (e, f) and agarose (g, h). Scale bar = 100 µm.
obtained cells glandular-like Immunopurified be cells epithelial fixed cells cells floating (Fig. 2a). On agarose (Fig. 2c, d), cells remained viable and floating in the medium during prolonged culture. Aggregates of cells were seen only if the epithelial cells had been immunopurified (Fig. 2a, c). Minimal aggregation occurred in non-purified epithelial cell cultures on agarose. The phenotypes of the cells obtained from proliferative and secretory phase (Gravigard IUD bearing) endometrium were similar. The viability of endometrial cells grown on different matrices was the same at all incubation times, as analysed with Trypan blue.

Protease release

Conditioned medium of proliferative endometrial cells was analysed by zymography on polyacrylamide–gelatin gel electrophoresis. After 48 h of culture, stromal cell supernatants expressed four to eight digestion bands with molecular masses ranging from 42 to 248 kDa (Fig. 3). Four gelatinase bands were present on zymograms of immunopurified stromal cell supernatants (Fig. 3a, b and c; Table 2), whereas six to eight bands were seen with non-immunopurified cell supernatants (Fig. 3d, e and f). Two of these bands (89 and 64–58 kDa) appeared to be more intense than the others which had molecular masses of 113, 105, 82 and 53 kDa. The number of gelatinases with molecular masses below 53 kDa (49, 46, 43 and 42 kDa) ranged from two to four (Fig. 3). In the supernatants from purified endometrial epithelial cells after 48 h (Fig. 4) only two gelatinases were expressed (three gelatinases in non-immunopurified epithelial cell preparation), both of which had the same molecular mass as the most intense bands of the stromal cell supernatant (89 and 64 kDa). Culture medium that had not been exposed to cells was always negative during the zymography.

Effect of duration of culture. Gelatinases were analysed at day 2, 5 and 8 of culture. In stromal cell supernatants, the intensity of the gelatinases of higher molecular mass (248, 113, 105 and 89 kDa) reduced as duration of culture increases. The intensity of the 82 kDa gelatinase changed according to the substrate on which the cells were grown, as well as the incubation time, whereas the intensity of the 64–58 kDa gelatinase increased during incubation (Fig. 3a–c and d–f, respectively). For proteases of lower molecular mass (<53 kDa), the duration of incubation did not appear to affect their intensity. Except for a general decrease in intensity of all bands at day 5 of culture, no specific effect could be seen on the intensity of epithelial cell gelatinases (Fig. 4).

Effect of purification. The 248 and 82 kDa gelatinases of stromal cell preparations were clearly of lymphomyeloid origin since after immunopurification these bands could no longer be seen (Fig. 3a–c and d–f, respectively). When LCA-positive cells obtained from stromal cell preparation were cultured alone, they released only two gelatinases of 248 and 82 kDa, whereas cell-free medium incubated on the same substrates released no detectable proteases (results not shown).

Immunopurification reduced the intensity of the 113, 105 and 89 kDa bands indicating that were of stromal and lymphomyeloid origin. The 64–58 kDa gelatinase was always present

Fig. 2. Conventional microscopy of endometrial epithelial cells obtained from a proliferative endometrium at 9 days of culture. Immunopurified (a, c) or non-immunopurified (b, d) cells were grown on matrigel (a, b) and agarose (c, d). Scale bar = 100 μm.

surrounding an empty space, resembling a glandular lumen. Immunopurified epithelial cells (Fig. 2a) grew into larger glandular-like structures than normal epithelial cells. After 9 days, the glandular-like structures formed by immunopurified cells were about twice the size of those formed by normal cells (Fig. 2a, b).
Fig. 3. Zymograms of immunopurified (a, b, c) and non-immunopurified (d, e, f) stromal cell supernatants from a proliferative endometrium grown on M: matrigel; A: agarose; G: glass; and P: plastic for 0 to 2 days (a, d); 2 to 5 days (b, e) and 5 to 8 days (c, f).

Table 2. Comparison of metalloproteinases in different cell types

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Molecular mass (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>248</td>
</tr>
<tr>
<td>Embryonic cells</td>
<td>–</td>
</tr>
<tr>
<td>Rabbit corneal fibroblasts</td>
<td>–</td>
</tr>
<tr>
<td>Alveolar macrophages</td>
<td>–</td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>–</td>
</tr>
<tr>
<td>Stromal cells</td>
<td>+</td>
</tr>
<tr>
<td>Immunopurified stromal cells</td>
<td>–</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>–</td>
</tr>
<tr>
<td>Immunopurified epithelial cells</td>
<td>–</td>
</tr>
<tr>
<td>Cytotrophoblast cells</td>
<td>+</td>
</tr>
<tr>
<td>Immunopurified cytotrophoblast cells</td>
<td>–</td>
</tr>
</tbody>
</table>
with the same intensity irrespective of immunopurification, indicating an exclusively stromal origin of this protease (Fig. 3).

The 82 kDa gelatinase from epithelial cell supernatants was not seen after immunopurification, whereas the intensity of the 89 kDa band was reduced and the 64–58 kDa band remained unchanged (Fig. 4). These results indicate a lymphomyeloid origin for the 82 kDa gelatinase, a dual origin, bone-marrow and epithelial, for the 89 kDa gelatinase and an epithelial origin for the 64–58 kDa gelatinase.

**Effect of substrates.** The pattern of expression of gelatinases secreted in the supernatant of cells growing on different substrates varied with cell adhesion and the nature of the substrates. Stromal cells grown on agarose did not adhere to this substrate and the gelatinases secreted in the supernatant produced less intense digestion bands than those secreted by cells grown on other substrates (Fig. 3a–c, d–f). After 2–5 days no digestion band could be seen on the zymogram of the supernatant of cells cultured on agarose indicating a strict adhesion dependence for the expression of the proteases (Fig. 3a–c). Immunopurified stromal cells grown for more than two days on matrigel strongly expressed the 82 and 89 kDa gelatinases, whereas the same cells grown on plastic expressed only the 89 kDa gelatinase and on glass none of these two bands was seen (Fig. 3a–c). The pattern of expression of the 68–54 kDa gelatinase did not vary with the nature of the substrate, but its expression increased with duration of culture.

**Variations during the endometrial cycle.** Numerous gelatinases were expressed by stromal cells obtained from secretory endometrium bearing a Gravigard IUD. After immunopurification, only two to six gelatinases was seen (compare Fig. 5a, b). The same digestion bands could be seen when stromal cell supernatants from early or late proliferative endometrium were compared with the previous phase, thus the intensity of the digestion band seemed to vary with phases of the cycle. During the early proliferative phase, the 64–58 kDa gelatinases were more intense than they were later in the cycle. In contrast, the 89 kDa band was apparently more intensely expressed during the proliferative phase than in the early proliferative or early secretory bearing a Gravigard IUD phases (Fig. 5).

**Effect of inhibitors and pH changes.** The effect of different inhibitors and pH changes were tested on stromal cell preparations obtained from a proliferative endometrium. When the gels were incubated overnight with 1, 10 phenanthroline (2 mmol l⁻¹), no digestion bands were visible. When the phenanthroline was replaced by EDTA (20 mmol l⁻¹) the intensity of the digestion bands was drastically reduced. No inhibition was observed with pepstatine A (1 μmol l⁻¹), PMSF (20 mmol l⁻¹) or iodoacetamide (1 μmol l⁻¹, results not shown). The endometrial gelatinases observed here were clearly neutral. Their highest activity was seen at pH 7.5 and bands were less intense when the gels were incubated at higher or lower pH values.

**Discussion**

Human endometrial cells isolated by enzymatic digestion and cultured in vitro are known to yield a mixed cell population. The endometrium is composed not only of stromal and epithelial cells (surface and glandular cells), but in the stroma, besides the stromal and endothelial cells of the blood vessels, many different bone-marrow-derived cells have also been identified. These include macrophages (Hunt, 1989), mast cells (Finn, 1986) and large granular lymphocytes (King and Loke, 1990). Different methods have been proposed for separating these endometrial cells from each other before plating (Lindenberg et al., 1984; Kirk et al., 1978; Satyaswaroop et al., 1979).

Before characterizing the proteases secreted by endometrial cells, we chose to develop a technique for eliminating the bone-marrow-derived cells from the endometrial cell suspension because these cells are known to produce proteases in other tissues (Shapiro et al., 1990). Since leucocyte common antigen (LCA or CD45) is expressed on all bone-marrow-derived cells including those of the human decidua (Starkey et al., 1988) and endometrium (King et al., 1989), we used an antibody to this...
surface antigen to immunopurify the endometrial cell suspension before plating. This immunopurification procedure was very efficient since very few LCA-positive cells could be seen after immunopurification. This procedure yielded a stromal cell culture of over 95% purity. The epithelial cell culture, however, was still contaminated by 3–4% stromal cells.

As reported previously (Schatz et al., 1990; White et al., 1990), the phenotype of endometrial cells changes with the nature of the substrate on which they grow. Epithelial cells grew into glandular structures when cultured on matrigel (White et al., 1990; the present report) and stromal cells organized themselves into clusters (Schatz et al., 1990; this study). To our knowledge, the study reported here is the first attempt to grow human endometrial cells on agarose on which neither stromal nor epithelial cells attached. The inability of cells to attach to agarose serves as a good control to appreciate the effect of cell attachment on cell growth and protease production. Irrespective of the nature of the substrate on which the cells were cultured, immunopurified stromal or epithelial cells grew into much larger colonies than when bone-marrow-derived cells were present in the culture together with the other endometrial cells. This observation indicates that LCA-positive cells produce a factor or factors that inhibit endometrial cell–cell association. If this is also true in vivo, it would attribute to the bone-marrow-derived cell an important paracrine role in the remodelling of the endometrium.

Epithelial and stromal cells in culture expressed gelatinases as shown by zymography. Since serum (including FCS) contains well-known protease inhibitors such as α2-macroglobulin and α1-antitrypsin, the FCS added to our culture media were heat inactivated and acid treated to denature those inhibitors. This cell-free medium did not have intrinsic gelatinase activity even when incubated for 2 days over matrigel. The observed gelatinases were thus cell specific. These were active at neutral pH and were inhibited by EDTA or phenanthroline but not by other protease inhibitors, indicating that the observed gelatinases were metalloproteinases. Zymography, as used here, has its limitations which should be kept in mind before discussing the metalloproteinases observed. First, the molecular mass of the digestion bands is an approximation of the true molecular mass of the enzyme because the substrate included in the gel (gelatin) influences the electrophoretic behaviour of the enzymes. Second, SDS has to be used for the electrophoresis to be performed, but since this compound artificially activates the proenzymes into active enzymes, it is not possible to determine whether the metalloproteinases released in the culture medium are activated or not. Finally, the use of gelatin instead of collagen or casein or other substrates limits our observations to

---

Fig. 5. Zymograms of stromal cell supernatants from endometria obtained at different phases of the menstrual cycle, cultured on matrigel, agarose and glass (days 0–2). (a) Non-immunopurified cells; (b) immunopurified cells: early proliferative endometrium (lane 1); early secretory endometrium bearing a Gravipad IUD (lane 2) and late proliferative endometrium (lane 3).
the gelatinases only. With these limitations in mind, it was very interesting to note that the molecular mass of the endometrial metalloproteinases corresponded to those of the MMP's described in other cell types (Fisher et al., 1989; Adler et al., 1990; Fini and Girard, 1990; Sang et al., 1990; Sato and Mori, 1990; Shapiro et al., 1990; Welgus et al., 1990; Bischof et al., 1991).

Comparison between immunopurified and non-immunopurified endometrial cell cultures showed that the stromal cells released four gelatinases, two of which were also produced by bone-marrow-derived cells (113, 105 kDa): one (89 kDa) was produced by stromal, lymphomyeloid and epithelial cells, whereas the 64–58 kDa band was seen only in purified stromal and epithelial cell supernatants. This last gelatinase corresponds to the 72 kDa collagenase (MMP-2) which is known to migrate with a molecular mass of 66 kDa on gelatin-containing gels when activated (Fini and Girard, 1990). This band was also clearly expressed by cytotothrophoblast cells (Bischof et al., 1991). The intensely expressed 89 kDa gelatinase probably represents the 92 kDa collagenase (MMP-2) that degrades collagen type IV and V and which is also expressed in leucocytes, monocytes, macrophages (Welgus et al., 1990) and cytotothrophoblast cells (Bischof et al., 1991). The characterization of these gelatinases will require the use of antibodies before their identity can be assessed.

Before discussing the quantitative changes of MMPs, it must be said that zymography is only a semi-quantitative technique and that the apparent increases or decreases in MMPs on the zymogram might be due to changes in cell number rather than changes in production rates since samples have been standardized according to the number of plated cells.

The expression of the endometrial MMPs seemed to be regulated by cell attachment and by the nature of the matrix on which the cells were grown. In the absence of cell–substrate adhesion (cultures on agarose) the expression of all gelatinases was very weak or absent in comparison to cells grown on glass, plastic or matrigel. Although zymography is only a semi-quantitative technique, it seems that the 64–58 kDa gelatinase secreted by stromal but not epithelial cells depends strictly on adhesion, as it was never expressed by cells grown on agarose. This observation also suggests that there is a cell-specific regulation of the MMPs. The fact that expression of MMPs also depends on the nature of the matrix is illustrated by the fact that stromal cells grown on plastic or glass did not express the 82 kDa gelatinase, whereas this enzyme was strongly expressed when the same cells were grown on matrigel. Besides these in vitro effects on protease activity, ovarian hormones appear to influence metalloproteinase expression. The 64–58 kDa gelatinase appeared to be expressed more during the follicular phase, whereas the 89 kDa gelatinase was more active during the luteal phase of endometrium bearing IUDs. It cannot be ruled out that this effect is due to the IUD rather than to the presence of progesterone at this stage of the cycle. In the cases where an IUD was present, the number and activity of the gelatinases was increased but this was essentially attributable to proteases secreted by bone-marrow-derived cells, as this was no longer after immunopurification.

In conclusion, this study shows for the first time that human endometrial cells release MMPs, the expression of which seems to be regulated by cell attachment and by the nature of substrates as well as other factors. We postulate that endometrial MMPs are implicated in uterine tissue remodelling after menses or parturition, and in the regulation of blastocyst hatching and implantation as well as trophoblast invasion.

The authors thank L. Haenggeli for her skilful assistance. This work was supported by Swiss National Fund for Scientific Research, grant number 32-29806.90.

References


Bernhard EJ, Muschel Rj and Hughes EN (1990) Mr. 92,000 gelatinase release contents with the metastatic phenotype in transformed rat embryo cells Cancer Research 50 3872–3877


Finn CA (1986) Implantation, menstruation and inflammation Biology Letters 61 312–328


Goldberg GI, Marmer BL, Grant GA, Eisen AZ, Wilhelm S and He C (1989) Human 72-kilodalton type IV collagenase forms a complex with a tissue inhibitor of metalloproteinase designated TIMP-2 Proceedings of the National Academy of Sciences, USA 86 8207–8211


Hibbs MS and Bainton DF (1989) Human neutrophil gelatinase is a component of specific granules Journal of Clinical Investigation 84 1395–1402


Downloaded from Bioscientifica.com at 12/10/2018 11:02:10PM via free access


Sato T, Ito A and Mori Y (1990) Interleukin 6 enhances the production of tissue inhibitor of metalloproteinases (TIMP) but not that of matrix metalloproteinases by human fibroblasts *Biological and Biophysical Research Communication* **170** 824–829


Stricklin GP, Bauer EA, Jeffrey JI and Eisen AZ (1977) Human skin collagenase: isolation of precursor and active forms from both fibroblast and organ cultures *Biochemistry* **16** 1607–1615


Wilhelm SM, Collier IE, Marler BL, Eisen AZ, Grant GA and Goldberg GI (1990) SV40-transformed human lung fibroblasts secrete a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages *Journal of Biological Chemistry* **264** 17 213–17 221

