Regulation of matrix metalloproteinases in human endometrium

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Considerable evidence supports a role for matrix metalloproteinases (MMP) in menstruation, but their focal pattern of expression within perimenstrual and menstrual endometrium suggests local rather than hormonal regulation. Menstruation shares a number of features with inflammatory responses, with leukocyte infiltration, proliferation and activation, occurring in the endometrium prior to menstruation. We propose that the leukocytes release MMP at this time and also that interactions between leukocytes and the stromal and epithelial cells of the endometrium induce and activate MMP. Co-culture studies using mast cells or neutrophils with endometrial stromal cells support this hypothesis. How leukocytes enter the endometrium is not understood but a role for chemokines has been proposed. The expression patterns of eotaxin and its receptor CCR3 in endometrium support a role in chemoattraction of eosinophils but expression of monocyte chemotactic proteins 1 and 2 does not correlate with macrophage numbers. Nothing is known of how the leukocytes become activated. Nevertheless, the overall result is a tissue in which an inflammatory-type reaction occurs with release of a myriad of potent regulators. These induce production and activation of MMP and alter the ratio between these and their tissue inhibitors, resulting in tissue breakdown.

Key words: chemokine/leukocyte/mast cell/matrix metalloproteinase/neutrophil

Introduction

Menstruation is primarily an event of tissue destruction, resulting from partial breakdown of the functionalis layer of the endometrium at the end of a normal reproductive cycle in women. It follows and results from the fall in progesterone levels following the demise of the corpus luteum in the non-fertile cycle and the accompanying decline in oestrogen levels, and can be mimicked by withdrawal of exogenous hormones or by administration of progesterone antagonists such as mifepristone (Fraser, 1997). It is now clear that the tissue breakdown at menstruation arises as a result of the production and activation of a number of matrix metalloproteinases (MMP) immediately prior to and during menstruation and the subsequent disturbance in the balance between these enzymes and their natural inhibitors, the tissue inhibitors of metalloproteinases (TIMP) (Salamonsen and Woolley, 1996).

Matrix metalloproteinases in the endometrium

MMP are enzymes with a range of substrate specificities for collagens, proteoglycans and other matrix molecules, and are responsible to a large extent for the degradation of components of both interstitial and basement membrane extracellular matrix. Their synthesis is negligible in normal connective tissue and is generally associated with pathological conditions in which tissue breakdown is a feature. However, in the female reproductive tract, where tissue remodelling occurs normally on a cyclical basis during the reproductive years,
MMP are now known to play important roles (Hulboy et al., 1997). MMP can be divided into a number of subfamilies based on their domain structures. With the exception of the membrane-type (MT)-MMP which appear to be activated prior to insertion into the cell membrane, they are secreted from cells as latent enzymes which require activation extracellularly by a variety of natural proteases. At this time, it is difficult to determine the activation state of any enzyme detected within a tissue by immunohistochemical means, yet the state of activation is of critical importance. Once activated, the enzymes can be bound by one of the four members of the TIMP family and these are widely distributed in tissues including human endometrium (Zhang et al., 1998). If the balance between the active MMP and TIMP is sufficiently disturbed, degradation of specific extracellular matrix (ECM) substrates will occur and if uncontrolled, this will eventually result in destruction of the entire tissue. Thus it can be envisioned that a massive up-regulation of production of MMP and their concomitant activation must occur for the rapid and extensive tissue destruction seen at menstruation.

A large number of studies have now described the expression of mRNA and protein for MMP in human endometrium during the normal menstrual cycle and have described their cellular localization: those which appear to have a role at menstruation are summarized in Table I. Perimenstrually and during menstruation, most of the MMP are products of stromal cells, although MMP-7 is an epithelial cell product and MMP-9 is produced by eosinophils, neutrophils and macrophages within the tissue.

In-vitro studies using tissue explants and separated endometrial epithelial and stromal cells have demonstrated that both endocrine and paracrine mechanisms can regulate MMP in the endometrium. Progesterone has long been recognized as a regulator of collagenase activity (Jeffrey et al., 1971) and is a potential regulator of MMP in the endometrium. Physiological concentrations of progesterone applied to explants of human endometrium almost completely abolished the release of both latent and active MMP (Marbaix et al., 1992, 1995) while in a cell culture model for menstruation, withdrawal of progesterone from decidualized endometrial stromal cells resulted in increased production of MMP-1, -2 and -3 (Salamonsen et al., 1997). Progesterone also regulates the activation of MMP-2 via its action on MT1-MMP in endometrial stromal cells (Zhang et al., 2000a). Despite this in-vitro data, there are strong arguments against direct actions of progesterone withdrawal accounting for the up-regulation of MMP at menstruation, the most cogent being its endocrine rather than paracrine action, given that MMP production and actions in endometrium are very focal events. Local regulators produced by epithelial and stromal cells in the endometrium have also been demonstrated to regulate MMP production by adjacent cells. These include interleukin (IL)-1 (Rawdanowicz et al., 1994; Singer et al., 1997), transforming growth factor-β (Bruner et al., 1995) and tumour necrosis factor (TNF)-α (Rawdanowicz et al., 1994). However, the products of leukocytes, which are present in peri-menstrual endometrium in large numbers are likely to be of critical importance in stimulating the local production and activation of MMP which result in menstruation.

### Endometrial leukocytes

There are now many publications describing the dramatic increase in the numbers of lymphomyeloid cells in the endometrium immediately prior to menstruation (reviewed in Salamonsen and Lathbury, 2000). The relative numbers of the different cell types in the tissue on days 26–28 of the cycle can be summarized as follows: macrophages (6–15% of total endometrial cells), neutrophils (6–15%), endometrial granular lymphocytes (eGL, 6–15%), eosinophils (3–5%), T cells (1–2%), B cells (1–2%) and mast cells (3–5%) (Salamonsen and Woolley, 1999). Each of these cell types, when activated, has the potential to release a plethora of cytokines which could potentially stimulate the production of MMP by adjacent cells, and a number of proteases which could activate MMP (Salamonsen and Lathbury, 2000). Studies from our laboratory have recently examined the possible contributions of leukocytes to regulation of stromal cell production of MMP and the activation of these enzymes.
Mast cells are resident in the endometrial stroma in fairly constant numbers throughout the cycle and undergo extensive activation prior to menstruation as demonstrated by immunolocalization of the mast cell protease, tryptase, at extracellular sites (Jeziorska et al., 1995) (Figure 1A). Thus it is likely that mast cells assume important functional roles at menstruation. Mast cells contain secretory granules which store a heterogeneous range of regulatory molecules. Those with likely importance in this context include cytokines such as TNFα and IL-1 and the proteases, tryptase and chymase. There is substantial structural and functional heterogeneity in different mast cell populations with respect to both protease and cytokine production. In the endometrium the mast cells in the functionalis layer are predominantly of the tryptase-only phenotype whereas those in the basalis layer express both tryptase and chymase (Jeziorska et al., 1995). Tryptase is a known activator of proMMP-3, which is a key MMP as, in its active form, it can activate a number of other latent MMP, thereby establishing a cascade of MMP activation (Salamonsen and Woolley, 1996). Furthermore, tryptase can activate urokinase type plasminogen activator (uPA), which in turn can produce plasmin, a further activator of MMP (Stack and Johnson, 1994).

**Paracrine interactions between leukocytes and endometrial cells**

We examined the interaction between human mast cells and endometrial stromal cells with regard to MMP production and activation (Zhang et al., 1998). The human mast cell line, HMC-1, was used in these studies and shown to produce the enzyme tryptase but not chymase, and the cytokines IL-1 and TNFα but not granulocyte stimulating factor (G-CSF), granulocyte-macrophage stimulating factor (GM-CSF), IL-6 or MMP. In co-culture with endometrial stromal cells, the mast cells stimulated stromal cell proMMP-1 and proMMP-3, and to a lesser extent, proMMP-2 production (Figure 2), with increasing stimulation as mast cell

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**Table I.** Cellular localization of matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinases (TIMP) in peri-menstrual and menstrual human endometrium

<table>
<thead>
<tr>
<th>MMP</th>
<th>Alternative name</th>
<th>Cellular location mRNA</th>
<th>Cellular location protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>Interstitial collagenase</td>
<td>Stroma, round arterioles and small vessels</td>
<td>Stroma, focal points</td>
<td>Rodgers et al. (1994); Salamonsen and Woolley (1996); Marbaix et al. (1996)</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatinase A</td>
<td>Stroma, epithelium in two cases</td>
<td>Stroma, endothelium some epithelium</td>
<td>Rodgers et al. (1994); Zhang et al. (1999a)</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Stromelysin 1</td>
<td>Stromata adjacent to epithelium, associated with basement membrane, structures of blood vessels</td>
<td>Stroma, focal points</td>
<td>Rodgers et al. (1994); Jeziorska et al. (1996)</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatinase B</td>
<td>NP, M</td>
<td>N, M, EO</td>
<td>Rodgers et al. (1994); Jeziorska et al. (1996)</td>
</tr>
<tr>
<td>MMP-10</td>
<td>Stromelysin 2</td>
<td>Stroma</td>
<td>N/A</td>
<td>Rodgers et al. (1994)</td>
</tr>
<tr>
<td>MMP-11</td>
<td>Stromelysin 3</td>
<td>Stroma</td>
<td>N/A</td>
<td>Rodgers et al. (1994)</td>
</tr>
<tr>
<td>MT1-MMP</td>
<td></td>
<td>N/A</td>
<td></td>
<td>Zhang et al. (1999a)</td>
</tr>
<tr>
<td>TIMP-1</td>
<td></td>
<td>Epithelium and periglandular stroma</td>
<td>Epithelium, epithelium, vascular smooth muscle</td>
<td>Rodgers et al. (1994); Zhang and Salamonsen (1997)</td>
</tr>
<tr>
<td>TIMP-2</td>
<td></td>
<td>N/A</td>
<td>Epithelium, epithelium, vascular smooth muscle</td>
<td>Zhang and Salamonsen (1997)</td>
</tr>
<tr>
<td>TIMP-3</td>
<td></td>
<td>N/A</td>
<td>Epithelium, epithelium, vascular smooth muscle</td>
<td>Zhang and Salamonsen (1997)</td>
</tr>
</tbody>
</table>

All data from in-situ hybridization and immunohistochemical studies. NP = neutrophils; M = macrophages/monocytes; EO = eosinophils; N/A = not examined. (Reproduced with permission from Salamonsen and Lathbury, 2000.)
Regulation of MMP in endometrium

Figure 1. Immunolocalization of (A) mast cells (B) neutrophils (C) eotaxin and (D) CCR3 in peri-menstrual human endometrium. Detection utilized antisera against tryptase (MAB1222, Chemicon Int., Temecula, CA, USA), elastase (clone NP 57, Dako, Glostrup, Denmark) eotaxin (a gift from Dr C. MacKay, Leukocyte Inc., Cambridge, MA, USA) and CCR3 (a gift from Dr B. Daugherty, Merck Research Lab., Rahway, NJ, USA) respectively. (For experimental details see Vincent et al., 1999; Zhang et al., 2000b.)

number increased (Zhang et al., 1998). Mast cell-conditioned medium also increased both protein and mRNA for stromal proMMP-1 and proMMP-3, demonstrating that the stimulatory molecule/s were soluble. These effects were abrogated (completely in the case of MMP-1 but only partially for MMP-3) by preadsorption of the conditioned medium with antisera against IL-1 and TNFα. When mast cell conditioned medium with added heparin (which stabilizes tryptase activity), was added to stromal cell culture medium in vitro, molecular weight forms indicative of active MMP-1 and MMP-3 appeared. No changes were seen in mRNA for TIMP-1, TIMP-2 or TIMP-3 in response to mast cell products. Hence, the selective effect of mast cell products on the production and activation of MMP but not TIMP would favour degradation of the ECM. Another important result from these studies was that the stimulatory effects of mast cell products were evident both in the presence and absence of progesterone. Therefore, different intracellular mediators may be involved in the inhibitory effects of progesterone and the stimulatory effects of the cytokines on MMP expression.

The mechanism of mast cell activation in the endometrium is currently unknown. Candidates for this action include corticotrophin-releasing hormone which is present in endometrial epithelial cells (Mastorakos et al., 1996) and which has a proinflammatory role with activation of mast cells as one response (Karalis et al., 1991). Likewise, endothelin-1, which is produced maximally by endometrial epithelial and decidual cells peri-menstrually (Marsh et al., 1996), is a potent inducer of histamine release from mouse peritoneal mast cells (Yamamura et al., 1994).
Neutrophils are the most abundant leukocytes in the human immune system and, like mast cells, contain specific secretory granules, the contents of which differ with the stage of neutrophil maturation. Neutrophils have been identified in endometrial tissue by their morphology and also by immunolocalization of the neutrophil-specific protease, elastase (Figure 1B). Endometrial neutrophils have also been defined as CD11bright, CD66b+ and CD16+ (Yeaman et al., 1998). The activation state of endometrial neutrophils in the endometrium appears to vary as some, but not all of the neutrophils are immunopositive for MMP-9 and/or MT1-MMP (Vincent et al., 1999; Zhang et al., 2000a). Interferon-γ has also been identified in intraepithelial neutrophils in human endometrium (Yeaman et al., 1998). Both elastase and interferon-γ can be identified in extracellular locations prior to menstruation, suggesting neutrophil activation at this time.

Neutrophils have the potential to release a large number of regulatory molecules (Table II). This provides a number of means for contributing to tissue degradation at menstruation. Firstly, they can synthesize MMP, including MMP-8, MMP-9 and MT1-MMP (Zhang et al., 2000a). Neutrophils also synthesize uPA de novo and store it as proenzyme (Owen and Campbell, 1995). Secondly, they produce elastase (Figure 1B) and cathepsin G, proteases whose substrates include elastin, proteoglycans and collagens III and IV, and heparanase which degrades heparan sulphate proteoglycans. Thirdly, elastase activates proMMP-3 and degrades TIMP-1 to inactive fragments while MT1-MMP activates proMMP-2 and uPA converts plasminogen to plasmin, which in turn activate a number of MMP. Furthermore, regulatory cytokines can regulate MMP expression by stromal/epithelial/endothelial cells, while the chemokines act as chemoattractants to other leukocytes, thus expanding the inflammatory cascade.

Studies in our laboratory have examined interactions within co-cultures of freshly prepared peripheral blood neutrophils and endometrial stromal cells (Lathbury and Salamonsen, 1998). Conditioned medium from neutrophils alone contained proMMP-9 and elastase, but not other MMP, while conditioned medium from co-cultures also contained active MMP-9 and elastase, but not other MMP, while conditioned medium from co-cultures also contained active MMP-9 and MMP-3 and fragments of degraded MMP-2. TIMP were also degraded. MMP-3 activation and MMP-2 degradation were not observed when the neutrophil-conditioned medium was pre-treated with anti-elastase. Therefore, it is likely that elastase is involved in the activation of MMP and alteration of the MMP:TIMP ratio in the endometrium in the vicinity of activated neutrophils.

There is now evidence that both MMP and

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**Table II. Some secretory products of neutrophils, of possible relevance in the endometrium**

<table>
<thead>
<tr>
<th>Matrix degrading enzymes:</th>
<th>MMP-8, MMP-9, heparanase, uPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other proteases:</td>
<td>Elastase, cathepsin G</td>
</tr>
<tr>
<td>Chemokines:</td>
<td>IL-8, Gro-α, -β, -γ, NAP-2, ENA-78, GCP-2</td>
</tr>
<tr>
<td>Cytokines:</td>
<td>IL-1β, TNFα, TGFβ, IFN-γ (after stimulation)</td>
</tr>
<tr>
<td>Reactive species:</td>
<td>Reactive oxygen, HOCl</td>
</tr>
</tbody>
</table>

MMP = matrix metalloproteinase; uPA = urokinase plasminogen activator; IL = interleukin; Gro-α, β, γ = growth-related gene α, β, γ; NAP-2 = neutrophil activating protein 2; ENA-78 = epithelial-derived neutrophil attractant 78; GCP-2 = granulocyte chemotactic protein 2; TNF-α = tumour necrosis factor-α; TGF-β = transforming growth factor-β; IFN-γ = interferon γ; HOCl = hypochlorous acid.
leukocytes are involved in the menstrual bleeding disturbances associated with the use of progestin-only contraceptives (Marbaix et al., 2000; Vincent et al., 2000). MMP-1 and -3 expression, activation of mast cells, and numbers of MMP-9 positive eosinophils, neutrophils and macrophages, all demonstrate similarities in endometrial pattern in women using these contraceptives whose endometrium shows shedding morphology, with the patterns seen in menstrual phase women (Vincent et al., 1999, 2000a). Thus, MMP and leukocytes appear to be implicated in the pathogenesis of abnormal uterine bleeding as well as in normal menstruation.

**Regulation of endometrial leukocytes**

The factors that regulate the presence and activity of leukocytes within the endometrium at menstruation have not yet been identified. There is some evidence for proliferation of these cells within the tissue. In particular, eGL express the proliferation markers Ki67 and/or BrdU throughout the menstrual cycle with a marked increase in the secretory phase (Pace et al., 1989; Tabibzadeh, 1990; Jones et al., 1998). However, in perimenstrual tissue eGL undergo morphological changes indicative of apoptosis and their major function appears to be related to pregnancy where they are particularly numerous in decidual tissue (Ritson and Bulmer, 1989; King et al., 1991). Cell culture studies show that eGL proliferate in the presence of endometrial stromal cells and progesterone (Inoue et al., 1996) while proliferation of peripheral blood mononuclear cells can be stimulated by soluble factors produced by endometrial epithelial cells treated with cytokines (Prabhala et al., 1998). These data suggest that proliferation in situ may account for at least some of the premenstrual increase in endometrial leukocytes.

Endometrial leukocytes may also enter the tissue from the blood perimimenstrually and presumably in response to the falling progesterone concentrations. However, progesterone receptors are not expressed by uterine CD45+ leukocytes (eGL, macrophages and T cells) (King et al., 1996; Stewart et al., 1998) suggesting indirect actions of progesterone on these cells. Whether neutrophils, eosinophils or mast cells express progesterone receptors is not known. However, there is some evidence that leukocyte trafficking into the endometrium may be regulated by chemokines, and that these in turn may be under the control of progesterone.

Chemokines are potent chemoattractant cytokines that promote the recruitment of multiple lineages of leukocytes and act via specific receptors on attracted cells (Kunkel et al., 1995). There are a very large number of chemokines and receptors, most of which have overlapping functions, and only a few of these have been examined in human endometrium (reviewed in Salamonsen and Lathbury, 2000).

Monocyte chemotactic proteins (MCP) recruit and activate monocytes. In an immunohistochemical study we examined the cellular location of endometrial MCP-1 and MCP-2 across the normal menstrual cycle. Both chemokines were detected in the endometrium although there was considerable variability between individuals. MCP-1 was present in the epithelium in 77% of tissues, in the stroma in 52% and in the vasculature in 34%. However, there was no correlation with the stage of the cycle in any cellular compartment, and hence no apparent correlation with the increased numbers of macrophages late in the cycle. MCP-2 staining was predominantly epithelial and was present in 42% of tissues but little immunoreactive protein was found in stroma or vasculature. As with MCP-1, there was no cyclical variation in MCP-2 expression (A.Hampton and L.A.Salamonsen, unpublished data).

Eotaxin is an unusual chemokine in that it has a relatively high degree of specificity for eosinophils. As eosinophils are present in human endometrium only immediately prior to and during menstruation, we postulated a role for eotaxin in their recruitment at this time (Zhang et al., 2000b). Eotaxin was immunolocalized to perivascular cells in the late secretory phase of the cycle (Figure 1C) and its receptor, CCR3, was strongly expressed by eosinophils in this tissue. This expression of eotaxin correlates with the presence of eosinophils in the tissue and hence supports the original hypothesis. However, the highest level of eotaxin expression was in luminal and glandular epithelial cells, in both proliferative and secretory phase tissue. CCR3 was also expressed by these cells (Figure 1D) suggesting that the epithelial eotaxin may have an autocrine function.
The overall picture emerging from such studies of chemokines in the endometrium suggests that while these pleiotropic molecules may play a role in leukocyte trafficking in the tissue, they are also likely to have additional functions. These may include the regulation of precursor cell cycling and differentiation, and growth factor or angiogenic properties (Schall, 1994).

Conclusions

Leukocyte differentiation and activation are likely to be critical for menstruation but at this time virtually nothing is known of how these changes occur in the endometrium. Furthermore, the functional studies required to provide evidence for a causative role for leukocytes and their products in the regulation of MMP and hence of menstruation are difficult to conceive or carry out, given that there are no laboratory animal models for studies of menstruation. Appropriate experimentation on human tissue is nevertheless ultimately required to provide answers relevant to women’s menstrual health.

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