Matrix metalloproteinases and their specific tissue inhibitors in menstruation

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The timely breakdown of extracellular matrix is essential for menstruation. Matrix metalloproteinases, which are able to degrade virtually all components of the extracellular matrix, are spatiotemporally expressed in the cyclic endometrium. The expression of most matrix metalloproteinases is regulated transcriptionally and their proteolytic activities are precisely controlled. The balance between matrix metalloproteinases and their specific tissue inhibitors is believed to be crucial for menstruation. This review focuses on the roles of matrix metalloproteinases and their tissue inhibitors in the initiation of menstruation and on the regulation of matrix metalloproteinase expression and activation. For example, the function of matrix metalloproteinases and their tissue inhibitors in endometrial re-epithelialization and angiogenesis during endometrial regeneration, when cell migration is facilitated to ensure endometrial repair, is discussed. This and other processes, although not fully resolved, serve to illustrate the involvement of matrix metalloproteinases and their tissue inhibitors in the process of menstruation.

Human endometrium is a dynamic tissue that undergoes extensive cyclic degradation and renewal. It has been a target tissue for the study of sex steroid and morphophysiologic interaction. The functional layer of the endometrium is shed and discharged at menstruation as a result of declining steroid support (Fox, 1973). Menstruation is associated with damage to the epithelium, interstitium, endothelium and extracellular matrix (ECM), resulting in controlled bleeding, tissue dissolution and repair (Bullelli *et al.*, 1998). Despite the considerable progress made in understanding normal menstruation, the biochemical and cellular mechanisms involved remain poorly understood.

Matrix metalloproteinases (MMPs) form a family of homologous zinc-dependent enzymes collectively capable of degrading most proteins of the ECM at neutral pH. Several laboratories have identified MMP expression in the endometrium (Marbaix *et al.*, 1992; Martelli *et al.*, 1993; Rodgers *et al.*, 1994). A marked increase in endometrial expression of several MMPs occurs just before and during menstruation (Hampton and Salamonsen, 1994; Rodgers *et al.*, 1994). The specific spatial and temporal expression patterns of MMPs indicate their pivotal roles in the process of menstruation (Salamonsen and Woolley, 1996). As the activity of all MMPs can be inhibited by tissue inhibitors of metalloproteinase (TIMPs) in a 1:1 stoichiometry (Docherty *et al.*, 1985) and since TIMPs are also distributed in the

endometrium throughout the menstrual cycle (Rodgers *et al.*, 1994; Freitas *et al.*, 1999), the balance between MMPs and TIMPs is likely to be critical for menstruation (Hampton and Salamonsen, 1994).

Matrix metalloproteinases and their specific tissue inhibitors

MMPs form a multigene family of enzymes (Table 1) that share significant sequence arrangement and a common multidomain structure, but are glycosylated to different extents and at different sites. With the exception of membrane-type (MT)-MMPs, MMPs are extracellular enzymes that are secreted as inactive proenzymes (zymogens). These proenzymes consist of an N-terminal propeptide domain of about 80 amino acids followed by a catalytic domain of about 170 residues. With the exception of matrilysin (MMP-7) and MMP-26, all catalytic domains are bound covalently via a proline-rich linker of 10–70 residues to a C-terminal haemopexin-like domain of about 195 residues (Massova *et al.*, 1998; Bode *et al.*, 1999). The haemopexin-like domain may determine the substrate specificity of MMPs or play a functional role in their interaction with TIMPs (Massova *et al.*, 1998). In addition, MMP-2 and MMP-9 contain three repeats of a fibronectin type II-like domain that are inserted within their catalytic domain. In MT-MMPs, the polypeptide chain possesses an additional 75–100 residues, which form a transmembrane helix and a small cytoplasmatic domain (Sato *et al.*, 1996).

Zymogens become activated upon limited hydrolysis,

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Table 1. Classification of human matrix metalloproteinases (MMPs)

Subfamily	MMP	Other names	Molecular mass (Da) ^a	Location of gene	Substrates	Accession number
Collagenases	MMP-1	Interstitial collagenase Fibroblast collagenase	54 007	Chromosome 11	Col I, II, III, VII and X; gelatins; entactin; aggrecan; link protein	P03956
	MMP-8	Neutrophil collagenase PMNL collagenase	53 412	Chromosome 11	Col I, II and III; aggrecan; link protein	P22894
	MMP-13	Collagenase 3	53 819	Chromosome 11	Col I, II and III	P45452
Gelatinases	MMP-2	Gelatinase A 72 kDa Gelatinase	73 882	Chromosome 16	Gelatins; col I, IV, V, VII, X and XI; fibronectin; laminin; aggrecan; elastin; large tenascin C; vitronectin, β -amyloid protein precursor	P08253
	MMP-9	Gelatinase B 92 kDa Gelatinase	78 427	Chromosome 20	Gelatins; col IV, V and XIV; aggrecan; elastin; entactin; vitronectin	P14780
Stromelysins	MMP-3	Stromelysin-1 Transin-1	53 977	Chromosome 11	Aggrecan; col III, IV, IX and X; gelatins; fibronectin; laminin; large tenascin-C; vitronectin; elastin; casein	P08254
	MMP-7	Matrilysin PUMP-1	29 677	Chromosome 11	Aggrecan, IGFBP-1; fibronectin; laminin; gelatins; elastin; col IV; entactin; small tenascin C; vitronectin; casein	P09237
	MMP-10	Stromelysin-2 Transin-2	54 151	Chromosome 11	Aggrecan; col II, IV and V; fibronectin; gelatins; weakly col III, IV and V; activate procollagenase	P09238
	MMP-11	Stromelysin-3	54 595	Chromosome 22	Weak activity on fibronectin; laminin; col IV; aggrecan; gelatins; IGFBP-1; α 1-proteinase inhibitor	P24347
	MMP-12	Metalloelastase	54 001	Chromosome 11	Elastin; fibronectin	P39900
Membrane-type MMPs	MMP-14	MT1-MMP MMP-X1	65 883	Chromosome 14	Col I, II and III; fibronectin; laminin-1; vitronectin; dermatan sulphate proteoglycan; activates proMMP-2, proMMP-13	P50281
	MMP-15	MT2-MMP	75 807	Chromosome 16	Activates pro-MMP-2	P51511
	MMP-16	MT3-MMP	69 521	Chromosome 8	Activates proMMP-2	P51512
	MMP-17	MT4-MMP	67 123	Chromosome 12	FibrinQ9ULZ9	
	MMP-24	MT5-MMP	73 231	Chromosome 20	Activates proMMP-2	Q9Y5R2
	MMP-25	MT6-MMP	62 554	Chromosome 16	Activates proMMP-2	Q9NPA2
Others	MMP-19	MMPase RASI	57 357	Chromosome 12	Aggrecan	Q99542
	MMP-20	Enamelysin	54 413	Chromosome 11	Amelogenin	O60882
	MMP-23	MIFR/FEMAL-YSIN	43 934	Chromosome 1		Q9UBR9
	MMP-26	Matrilysin-2 Endometase	29 708	Chromosome 11	Gelatin; β -casein; fibronectin	Q9NRE1

Information from Swiss-Prot database at www.expasy.ch/sprot/

Col: collagen; IGFBP-1: insulin-like growth factor binding protein 1; MIFR: metalloproteinase in the female reproductive tract; RASI: rheumatoid arthritis synovial inflammation.

^aCalculated from the number of amino acids.

Table 2. Human tissue inhibitors of metalloproteinases (TIMPs)

TIMP	Other names	Molecular mass (Da) ^a	Location of gene	Matrix metalloproteinase (MMP) substrate	Accession number
TIMP-1	Metalloprotease inhibitor 1 Erythroid potentiating activity (EPA) Fibroblast collagenase inhibitor Collagenase inhibitor	23 171	Chromosome X	MMP-1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 16	P01033
TIMP-2	Metalloprotease inhibitor 2 CSC-21K	24 399	Chromosome 17	MMP-1, 2, 3, 7, 8, 9, 10, 13, 14, 15, 16, 19	P16035
TIMP-3	Metalloprotease inhibitor 3 MIG-5 protein	24 145	Chromosome 22	MMP-1, 2, 3, 7, 9, 13, 14, 15	P35625
TIMP-4	Metalloprotease inhibitor 4	25 502	Chromosome 3	MMP-1, 2, 3, 7, 9	Q99727

Information from Swiss-Prot database at www.expasy.ch/sprot/
CSC-21K: cystein-serine-cystein 21K; MIG-5: mitogen-inducible gene 5.

^aCalculated from the number of amino acids.

during which the propeptide is lost. Activation of the pro-MMPs into active MMPs can be reproduced *in vitro* by addition of different agents such as mercurial salts, plasmin or trypsin. Although the physiological activation of the different MMPs is poorly understood, plasmin (Eeckhout and Vaes, 1977) and MMP-3 (Birkedal-Hansen *et al.*, 1993) have been shown to be potent activators of several MMPs.

MMPs play a critical role in many physiological processes such as wound healing, bone growth, nerve outgrowth, ovulation, embryo implantation, embryonic development, uterine involution and menstruation. Overexpression of MMPs is also linked to pathological processes such as tumour invasion and metastasis, arthritis and atherosclerosis.

TIMPs (Table 2) are the major endogenous inhibitors of MMP activities in tissues (Nagase and Woessner, 1999). Binding of TIMPs to the catalytic domain results in efficient inhibition of the enzymatic activity of MMPs (Massova *et al.*, 1998). The TIMP family includes four proteins, TIMP-1–4, which share some sequence identity (40, 30 and 37% between TIMP-1 and TIMP-2, -3 and -4, respectively) (Douglas *et al.*, 1997). Although TIMPs do not seem to differentiate much among the various MMPs, a certain degree of specificity has been observed. For example, TIMP-2 and -3, unlike TIMP-1, are effective inhibitors of MT-MMPs, whereas TIMP-1 preferentially binds MMP-9 (Strongin *et al.*, 1995).

TIMPs are multifunctional proteins. Besides inhibiting MMP, TIMPs have other functions such as growth factor-like and anti-angiogenic activity (Gomez *et al.*, 1997). TIMP-1 also stimulates fibroblasts to produce MMP-1 (Clark *et al.*, 1994). TIMP-1 and -2 bind not only to activated gelatinases but also to proMMP-9 and -2, respectively (Strongin *et al.*, 1995). The complex between MT1-MMP and TIMP-2 acts as a cell surface 'receptor' for proMMP-2 activation *in vivo* (Butler *et al.*, 1998).

Respective roles of MMPs and TIMPs in the process of menstruation

Role of MMPs and TIMPs in the onset of menstruation

Markee (1948) used intraocular endometrial transplants in rhesus monkeys to determine that menstruation was initiated by the rapid regression of endometrial thickness as a result of fluid loss and tissue lysis, which were followed by intense vasoconstriction of the spiral arterioles and subsequent bleeding from focal points. Regression of the endometrium was later observed in non-menstruating mammals. The endometrial wet mass and collagen content of cyclic rats decrease during metoestrus to 20% of their value at pro-oestrus (Yochim and Blahna, 1976), indicating that proteolysis of the ECM takes part in this regression (Eeckhout, 1990). Even before bleeding commences, small lesions in human endometrial luminal epithelium can be seen under an electron microscope (Ludwig and Spornitz, 1991); these lesions contribute to the very rapid but incomplete degeneration of the functional layer and exposure of open capillary vessels. Widespread degeneration of human endometrial stroma and basal lamina occurs at the end of the luteal phase (Roberts *et al.*, 1992). The classic view that menstruation is a process generated primarily by vascular events is thus challenged by the concept that breakdown of the ECM in the functionalis initiates menstruation.

A large number of studies strongly implicated MMPs and TIMPs in menstruation. Studies using immunocytochemistry (Martelli *et al.*, 1993; Rodgers *et al.*, 1993) or *in situ* hybridization (Hampton and Salamonsen, 1994; Rodgers *et al.*, 1994) showed that endometrial cells produce MMP-1, -2, -3, -7 and -9, and TIMP-1 and -2. Messenger RNAs for proMMP-1 and -3 are detected in normal endometrium only peri-menstrually and menstrually. Therefore, it is likely that both MMP-1 and -3 have

important roles in the tissue degradation that initiates menstruation (Hampton and Salamonsen, 1994). mRNAs encoding TIMP-1 and -2 are present throughout the cycle but increase to a lesser extent than do mRNAs encoding MMP on days 1 and 2 at menstruation (Hampton and Salamonsen, 1994). Given that the stability of mRNAs encoding MMPs and TIMPs is equivalent and that translation of the mRNAs results in the production of proteins in endometrial tissue, a major alteration in the balance between enzymes and their inhibitors is in favour of tissue degradation (Salamonsen and Woolley, 1996). Endometrial MMPs have different cellular origin. Observations *in vivo* and *in vitro* indicate that human endometrial stromal and epithelial cells, when cultured separately, can release several MMPs (Martelli *et al.*, 1993). Most MMPs are produced by stromal cells at menstruation (Rawdanowicz *et al.*, 1994; Rodgers *et al.*, 1994). MMP-2 and -9 have a stromal expression throughout the menstrual cycle. MMP-9 is detected in the glandular epithelium but only during the secretory phase (days 19–20) (Freitas *et al.*, 1999). An immunocytochemical study (Freitas *et al.*, 1999) showed that MMP-2, -3 and -9 were also detected in endometrial vascular structures, such as pericytes and smooth muscle cells. In particular, MMP-3 was expressed in vessels during the pre-menstrual and menstrual phases. The abundant expression of MMP-3 in the vessels situated in the superficial layer of the endometrium during menses indicates that it initiates degradation of the vascular wall during menstrual breakdown. The occurrence of MMPs in both stromal and vascular compartments at the beginning of menstruation indicates that bleeding results from degradation of both stromal components and vascular walls (Freitas *et al.*, 1999).

MMP-7 is also clearly related to menses as mRNA encoding MMP-7 has been localized to endometrial glandular and luminal epithelial cells, and reaches a peak concentration in the pre-menstrual and menstrual phases (Rodgers *et al.*, 1993). However, the role of MMP-7 in the degradation of epithelial tissue remains to be investigated.

In addition to stromal cells, epithelial cells, endothelial cells and vascular smooth muscle cells (SMCs), the endometrium contains many different bone marrow-derived cells, including macrophages, T cells, B cells and granulocytes, which colonize the endometrium at each cycle. Leucocytes represent an increasing proportion of endometrial cells as the menstrual cycle progresses (Shi *et al.*, 1995). Large granular lymphocytes (LGLs), T cells, B cells and macrophages also secrete MMP-2 and MMP-9 (Shi *et al.*, 1995). LGLs release the highest gelatinolytic activity, followed by the macrophages, T cells, B cells and monocytes. Although gelatinolytic activity does not represent the total proteolytic activity of these cells, the contribution of these bone marrow-derived cells to the total proteolytic potential of the endometrium is quantitatively greater than the contribution of the epithelial and stromal cells after ovulation (Bischof, 1997).

The spatiotemporal expression and activation of MMPs

in the endometrium is closely associated with the process of normal menstruation (Hampton and Salamonsen, 1994; Rodgers *et al.*, 1994; Salamonsen and Woolley, 1996). The fact that synthetic MMP inhibitors completely inhibit the breakdown of menstrual tissue *in vitro* is taken as evidence for the instrumental role of MMPs in the onset of menstruation (Marbaix *et al.*, 1996).

Norplant is an effective and widely used progestin-only contraceptive, which has a high discontinuation rate owing to unpredictable uterine bleeding. Expression of MMP-9 (Vincent *et al.*, 1999) and MMP-1 (Vincent *et al.*, 2000) is significantly higher in endometrial tissues from users of Norplant as compared with non-users. Irregular bleeding in women treated with Norplant correlates with the appearance of MMP-1 and -3 and the increased production of MMP-2, the activation of proMMP-1, -2, -3 and -9 and the decreased secretion of TIMP-1 (Galant *et al.*, 2000; Marbaix *et al.*, 2000). These findings imply that irregular bleeding occurs when the concentration of active MMPs exceeds the inhibitory capacity of TIMP-1, so as to freely degrade the ECM.

Putative role of MMPs and TIMPs in endometrial regeneration

Endometrial regeneration usually begins by the second day after bleeding has started (Smith, 2001). Epithelial cell migration followed by replication characterizes the biodynamics of endometrial surface repair (Bulletti *et al.*, 1998). Bleeding stops when the surface has been completely re-epithelialized.

Heterogeneous expression of TIMP-1 and TIMP-2 occurs in arteriolar SMCs and in the stroma during menstruation, and staining occurs in non-necrotic tissue and is especially abundant in the arterioles, delimiting necrotic from non-necrotic areas. This expression pattern implies that TIMPs limit the action of MMPs in some areas, thus preserving the capacity of endometrial regeneration (Freitas *et al.*, 1999).

The roles of MMPs and TIMPs during endometrial repair have not been elucidated in detail. Most of our understanding derives from studies carried out on wound healing in skin.

It is generally accepted that migration and remodelling events during skin repair require both the activity of MMPs and their counter-regulation by TIMPs. After injury, an elaborate chain of events is triggered to ensure that basal keratinocytes migrate to re-epithelialize the wound (Martin, 1997). Increased MMP and plasmin expression is critical for the leading keratinocytes to dissolve the fibrin barrier around them, thereby facilitating their locomotion. Once the wound has been covered, the keratinocytes stop proliferating and switch off their MMP secretion (Martin, 1997). A similar expression pattern of MMPs in migrating epithelium has been found regardless of wound types and species (Saarialho-Kere *et al.*, 1994; Salo *et al.*, 1994; Kahari and Saarialho-Kere, 1997; Madlener *et al.*, 1998). Although endometrial regeneration differs from wound

healing because no scarring occurs, it is still reasonable to speculate that MMPs and TIMPs participate in the process of endometrial re-epithelialization.

Mechanisms of endometrial repair include angiogenesis, the process of new blood vessel formation from pre-existing blood vessels (Folkman, 1997). Angiogenesis consists of a sequence of events, including dissolution of the basement membrane underlying the endothelial layer, migration and proliferation of endothelial cells, formation of the vascular capillary, and formation of a new basement membrane (Sang, 1998). Angiogenesis can occur by sprouting or non-sprouting mechanisms. The pleiotropic effects of most MMPs and TIMPs in the angiogenic process have been reviewed in detail by Sang (1998). For example, MMP-2 and -9 activities are important in the early steps of endothelial capillary formation. MMP-1 activity is responsible for endothelial cell migration through the interstitial spaces. TIMP-1, -2, -3 and possibly -4 inhibit neovascularization through anti-MMP-dependent or -independent pathways. Fibrinolysis by the plasma membrane-anchored MT1-MMP in endothelial cells is critical for neovascularization (Hiraoka *et al.*, 1998). Furthermore, mice made genetically deficient in MMP-2, although revealing no gross developmental abnormalities, have an impaired angiogenic response to tumour stimuli (Itoh *et al.*, 1998). Targeted inactivation of MMP-9 causes a transient delay in bone development because of defective vascular invasion (Vu *et al.*, 1998). Overexpression of the multipotent fibroblast growth factor 1 (FGF-1) stimulates endothelial cell migration by mediating MMP-1 gene transcription and collagenolytic activity (Partridge *et al.*, 2000).

Angiogenesis is an essential component of endometrial renewal, because new blood vessel formation is required to supply oxygen and nutrients. Disruption of angiogenesis in mice results in endometrial atrophy (Klauber *et al.*, 1997). Endometrial angiogenesis was characterized by non-sprouting mechanisms such as intussusception and elongation (Rogers and Gargett, 1999). In humans, growth of endometrial endothelial cells begins at menstruation and starts from the ruptured spiral arterioles and venules (Smith, 1998).

Vascular endothelial growth factor (VEGF) is the main angiogenic and permeability factor of the endometrium, and is involved in both physiological endometrial angiogenesis and endometriotic angiogenesis (Shifren *et al.*, 1996). VEGF expression is controlled by ovarian steroids in proliferative and secretory endometrium (Shifren *et al.*, 1996). During menstruation, local factors, such as hypoxia, can upregulate VEGF expression and this may play an important role in angiogenesis and endometrial repair after menstruation (Sharkey *et al.*, 2000). Emerging evidence demonstrates that VEGF can induce MMP expression and inhibit TIMP expression in endothelial cells (Lamoreaux *et al.*, 1998). VEGF can also stimulate MMP secretion from human aortic SMCs (Wang and Keiser, 1998). Both endothelial cell- and SMC-derived MMPs may facilitate degradation of vascular basement membrane, which is a

crucial stage of angiogenesis. Upregulated SMC-derived MMPs may also promote SMC migration in a later stage of angiogenesis (Wang and Keiser, 1998). Thrombospondin type 1 (TSP1) is thought to function as an inhibitor of angiogenesis (Bein and Simons, 2000) and is expressed in endometrial stromal cells (Iruela-Arispe *et al.*, 1996). TSP1 can prevent the activation of MMP-2 and -9 zymogens, which probably contributes to the anti-angiogenic activity of TSP1 (Bein and Simons, 2000). In this respect, it is perhaps significant that, in the endometrium, MMP-2, TIMP-1 and -2 are detectable in vessels throughout the menstrual cycle (Freitas *et al.*, 1999). MMP-2 is expressed intensely in capillaries and SMCs at the end of the luteal phase. MMP-3 is expressed markedly in SMCs and in the basal portion of endothelial cells during the pre-menstrual and menstrual period. In contrast, MMP-9 is detected in spiral arteries during the secretory phase and in vascular structures during the midfollicular and menstrual phases. Taken together, these observations indicate that MMPs and TIMPs actively take part in the vascular events accompanying endometrial regeneration. A delicate shift in the balance between MMPs and their inhibitors occurs probably within the capillaries or in their environment during endometrial angiogenesis.

Regulation of MMP gene expression

Acute progesterone withdrawal induces both secretion and activation of certain MMPs (Martelli *et al.*, 1993; Hampton and Salamonsen, 1994; Rodgers *et al.*, 1994; Lockwood *et al.*, 1998). In contrast, physiological concentrations of progesterone almost totally abolish MMP-1 release, as well as MMP-2 and -9 activity, in explant cultures of human endometrium (Marbaix *et al.*, 1992). Therefore, it may be hypothesized that the expression of several MMPs in the human endometrium is controlled ultimately by ovarian steroids. In parallel to endometrial tissue changes, the temporal and spatial expression pattern of MMPs indicates the involvement of autocrine–paracrine regulations. The only study *in vivo* of the effects of steroids on the expression of MMPs and TIMPs in the endometrium of a primate (Rudolph-Owen *et al.*, 1998) showed that, although endometrial MMPs were upregulated by withdrawal of progesterone in ovariectomized monkeys, many of the MMP transcripts decreased either abruptly or gradually after menstruation in the absence of progesterone. However, MMP expression was confined to the upper functionalis zone during menstruation, but after menstrual breakdown was complete, and expression of MMP-7 and TIMP-1 shifted from the functionalis to the basalis zone in the absence of both oestradiol and progesterone. Therefore, it seems unlikely that progesterone is the only hormone involved in regulating the expression, localization and function of endometrial MMPs.

Cell–cell interaction has an important role in mediating aspects of steroid action during growth and differentiation in the endometrium (McClellan *et al.*, 1986). After the

withdrawal of progesterone, there is a marked endometrial stromal compaction that increases cell–cell contacts. Regulation of MMP expression has been associated with changes both in the shape of cells and in cell contacts (Aggeler *et al.*, 1984; James *et al.*, 1993). Osteen *et al.* (1994) used isolated and recombined types of endometrial cell to demonstrate that isolated human endometrial epithelial cells require the presence of stromal cells to restore steroid suppression of MMP-7 secretion, confirming the necessity for stromal-derived factors as mediators in this suppression process. The number of lymphomyeloid cells (for example, eosinophils, macrophages and neutrophils) increases markedly in the endometrium during the pre-menstrual phase, and these cells synthesize a plethora of cytokines and proteases, including MMPs (Salamonsen and Woolley, 1999). Mast cell activation, which results in the release of potent regulators, such as tumour necrosis factor α (TNF- α), interleukin 1 (IL-1), histamine, heparin, tryptase and chymase (Salamonsen and Lathbury, 2000), increases immediately before menstruation (Jeziorska *et al.*, 1995).

Expression of endometrial MMPs can be regulated by locally produced cytokines and growth factors or by products of migratory or resident cells (Bischof and Campana, 2000). Transforming growth factor β (TGF- β) is produced by endometrial stromal cells in response to progesterone, and this cytokine can suppress expression of epithelial MMP-7 independently of progesterone. Indeed, antibodies against TGF- β abolish progesterone-induced MMP-7 suppression in stromal–epithelial cell co-cultures (Bruner *et al.*, 1995). IL-1 α derived from the epithelium is the key paracrine inducer of MMP-1 in endometrial fibroblasts in the absence of ovarian steroids (Singer *et al.*, 1997). IL-1 and TNF- α derived from human mast cells may stimulate production of stromal cell proMMP-1, -3 and, to a lesser extent, -2 (Zhang *et al.*, 1998). Several recombinant cytokines can induce the selective expression of MMPs in cultured human endometrial fibroblasts: MMP-1 was induced by epidermal growth factor (EGF), platelet-derived growth factor-BB (PDGF-BB), TNF- α and IL-1 α ; mRNA encoding MMP-11 was induced concomitantly on treatment with insulin-like growth factor II (IGF-II) or IL-6 and, particularly, with EGF and PDGF-BB; and mRNA encoding MMP-9 was induced quickly by TNF- α , but disappeared within 12 h despite continuing stimulation (Singer *et al.*, 1999).

Cultured human endometrial stromal cells mimic menstruation-related events with remarkable fidelity. For example, Lockwood *et al.* (1998) demonstrated changes *in vitro* in MMP-1 and -3 mRNA and protein and an absence of effect on MMP-2 and TIMP-1 expression consistent with the results of the *in situ* hybridization study of Rodger *et al.* (1994). These results are consistent with the notion that some MMPs (MMP-1, -3 and -9) are regulated whereas others are constitutively expressed (MMP-2). Indeed, MMPs can be divided into two groups according to their regulation of gene expression: most MMPs exhibit inducible

expression and a small number are produced constitutively and are not inducible (Borden and Heller, 1997). The promoter regions of inducible MMP genes (MMP-1, -3, -7, -9, -10, -12 and -13; Fig. 1) have a remarkable conservation of important regulatory *cis* elements, such as activator protein 1 (AP-1) and polyomavirus enhancer element (PEA3) (Westermarck and Kahari, 1999). Most of these genes are not expressed in unstimulated cells, and their expression is induced markedly by growth factors, cytokines, chemical agents (for example, phorbol esters and actin stress fibre-disrupting drugs), physical stress, and oncogenic cellular transformation. In addition, the enhanced MMP gene expression may be downregulated by suppressive factors (for example, TGF- β , retinoic acid and glucocorticoids) (Nagase and Woessner, 1999). AP-1 is a homo- or heterodimeric DNA-binding protein composed of either two proteins of the Jun family or one protein of the Jun family and one protein of the Fos family (Westermarck and Kahari, 1999). In general, the AP-1 element seems to be important in initiating transcription of these genes, whereas PEA3 and possibly other sites contribute to the fine tuning mechanisms that modulate these genes (Matrisian, 1992). In contrast, MMP-2 is constitutively expressed. The promoter region of the MMP-2 gene (Fig. 1) contains an AP-2 binding site and two specificity protein 1 (SP-1) sites but lacks a TATA box and AP-1- and PEA3-binding sequences (Huhtala *et al.*, 1990). The MT1-MMP promoter shows similarity with that of MMP-2 (Lohi *et al.*, 2000).

Progesterone clearly acts to suppress endometrial MMP expression, but the mechanism of its regulation on transcription of specific MMP genes is not yet understood. The fact that the promoter region of most MMPs (both inducible and non-inducible MMPs) lacks traditional progesterone responsive elements indicates that progesterone may regulate certain MMP genes via nonclassical DNA sequences (Hulboy *et al.*, 1997). In addition, ligand-activated progesterone receptor (PR), like other activated nuclear receptors, such as glucocorticoid receptor (Yang-Yen *et al.*, 1990), retinoic acid receptor (Schule *et al.*, 1991) and thyroid receptor (Zhang *et al.*, 1991), may decrease the amounts of transcription factors (such as fos–jun) by a direct protein–protein interaction, resulting in a decreased binding of the transcription factors on the AP-1 site of MMP promoters. When human PR (hPR) was transfected into hPR-devoid human endometrial (HEC-1-B) cells, AP-1 activity was stimulated by the mere presence of the receptor in the absence of progesterone or of any other stimulus, whereas addition of the ligand (progesterone) reversed the effect (Bamberger *et al.*, 1996). Moreover, the influx of leucocytes (which are known to produce MMPs) into human endometrium coincides with progesterone withdrawal (Salamonsen and Lathbury, 2000), and so the progesterone decline may also provide cell–cell communication among the various migratory and resident cells and further influence local expression of MMPs.

Fig. 1. Human matrix metalloproteinase (MMP) activity is controlled by transcriptional regulation, pro-enzyme activation and inhibition by specific tissue inhibitors (TIMPs). With respect to gene expression, MMP-1 and -2 represent examples of an inducible and an uninducible MMP, respectively. Human MMP-1 promoter region contains binding sites (activator protein 1 binding site (AP-1) and polyomavirus enhancer A binding protein 3 responsive element (PEA3)) that are responsive elements for several growth factors and cytokines. ProMMP-1 (like most MMPs) is activated extracellularly in a stepwise manner. Human MMP-2 promoter region lacks AP-1- and PEA3-binding sequences. ProMMP-2 is activated on the cell surface. This activation process requires both active membrane-type 1 (MT1)-MMP- and TIMP-2-bound MT1-MMP. The TIMP-2 in this complex binds proMMP-2 to generate a ternary complex, which is assumed to bring the zymogen close to the active MT1-MMP. ProMT1-MMP is activated intracellularly by furin. TIE: TGF- β inhibitory element; SBE: STAT binding element; SP-1: specificity protein 1 binding site; sil: silencer sequence.

Control of MMP activation

Apart from MMP-11 and most MT-MMPs, which are activated intracellularly by the Golgi-associated proteinase furin (Pei and Weiss, 1995, 1996), most MMPs are secreted from cells as inactive zymogens. In all cases, activation requires the disruption of the Cys-Zn²⁺ (cysteine switch) interaction, and the removal of the propeptide proceeds thereafter often in a stepwise manner (Nagase, 1997). Most pro-MMPs are likely to be activated by tissue proteinases or opportunistic bacterial proteinases *in vivo* (Nagase and Woessner, 1999). Studies in transgenic mice indicate that the urokinase plasminogen activator (uPA)-plasmin system is a key activator of pro-MMPs (Carmeliet *et al.*, 1997). Studies *in vitro* using human endometrial stromal cells indicated that PA activity is regulated primarily by changes in the expression of plasminogen activator inhibitor 1 (PAI-1) (Lockwood *et al.*, 1995; Schatz *et al.*, 1995), which is different from the regulation of expression of MMP, brought about primarily by changes in transcription of MMP that are independent of TIMP expression (Lockwood *et al.*, 1998). Progesterone inhibits the secretion of PAs by cultured endometrial explants (Casslen *et al.*, 1986) and stimulates the expression of PAI-1 by cultured endometrial stromal cells (Casslen *et al.*, 1992), indicating a control of this system by sex steroids during the menstrual cycle and its release before menstruation (Marbaix *et al.*, 1996).

Secreted pro-uPA binds to, and is activated at, specific cell membrane receptors, where it converts plasminogen to plasmin. Plasmin may degrade matrices that undergo rapid turnover but also may exert its prime impact on ECM proteolysis by activating MMP zymogen (Eeckhout and Vaes, 1977).

Both mast cell tryptase and chymase may play roles in MMPs activation at menstruation. MMP-3, which initiates a cascade of MMP activation (Birkedal-Hansen *et al.*, 1993), is activated by tryptase in human endometrial stromal cells (Zhang *et al.*, 1998). In addition, chymase can activate latent MMP-1 (Saarinen *et al.*, 1994).

MMP-2 is an exception. In tumour cells, endometrial neutrophils and endometrial granular leucocytes, activation of proMMP-2 has been shown to take place on the cell surface. MT1-MMP has been postulated to activate proMMP-2 in endometrium (Zhang *et al.*, 2000). This activation process requires both active MT1-MMP and TIMP-2-bound MT1-MMP. The TIMP-2 in the latter complex binds proMMP-2 to generate a ternary complex, which is assumed to bring the zymogen close to the active MT1-MMP (Butler *et al.*, 1998). On angiogenic endothelial and melanoma cells, MMP-2 may be localized in a proteolytically active form on the cell surface through binding to the integrin receptor $\alpha\beta3$ (Brooks *et al.*, 1996). Meanwhile, the uPA-plasmin system associated with the cell surface can also activate surface-bound proMMP-2,

Fig. 2. During the secretory phase, endometrial matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are secreted by several cells. It is likely that TIMPs override the activity of MMPs so that the net proteolytic activity is nil. During the first 2 days of the menstrual phase, leucocytes are attracted into the endometrium, a response that is triggered by progesterone withdrawal. These white cells, together with stromal (SC), endothelial (EnC) and epithelial cells (EC) produce important quantities of different MMPs, whereas production of TIMPs remains low, producing an increased proteolytic activity in the endometrium, and leading to destruction of the extracellular matrix and disruption of blood vessels, interstitium and epithelium. During the last 2 days of menses, MMP secretion is probably used for tissue repair, allowing keratinocytes to re-epithelialize the endometrial surface and helping endothelial cells to form new blood vessels. Eosin: eosinophils; Ma: macrophages; Neutr: neutrophils.

whereas soluble plasmin degrades it (Mazzieri *et al.*, 1997). In addition, MMP-2 can be activated through auto-lysis (Atkinson *et al.*, 1995).

Active MMPs are inhibited by α_2 -macroglobulin or by TIMPs, which are both present in human endometrium throughout the menstrual cycle, and protect the endometrium from excessive tissue degradation.

Conclusion

Matrix metalloproteinases have diverse substrate specificity, ranging from multiple ECM components to growth factors, cytokines and other proteinases. Recent studies have increased our understanding of the significance of MMPs and TIMPs in the process of menstruation (Fig. 2). It is becoming clear that MMPs play a key role in the cyclical breakdown of ECM that ultimately leads to menstruation. Although circulating concentrations of progesterone affect the expression pattern of several endometrial MMPs, local factors can enhance or override this regulation. Although the cellular and molecular processes of endometrial repair are still unclear, MMPs cleave structural substances and fulfil additional regulatory roles (such as the processing of inactive MMP and cytokine precursors) and TIMPs exhibit multiple biological functions and, therefore, are likely to play a highly complex and tightly regulated role in endometrial repair. Some intriguing issues remain to be investigated. For example, which MMPs are the key

enzymes in the initiation of menstruation; what are the respective contributions of the various MMPs in the process of endometrial repair; and how does progesterone influence leucocyte migration and activation?

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