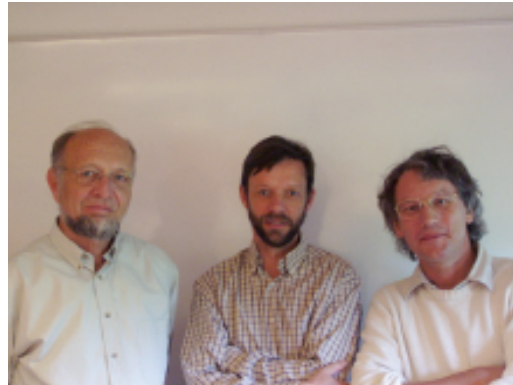


EXTRACELLULAR MATRIX BREAKDOWN

Pierre J. COURTOY, Member (right)
Yves EECKHOUT, Member (left)
Etienne MARBAIX, Member (middle)

Patrick HENRIET, Member
Hervé EMONARD, Guest Investigator
Christine GALANT, Assistant Member
Alix BERTON, ICP Fellow
Patricia CORNET, Graduate Student
Christine PICQUET, Graduate Student
Vassil VASSILEV, Graduate Student
Denis DELVAUX, Technician
Pascale LEMOINE, Technician
Olga MEERT, Technician
Yves MARCHAND, Secretary



The extracellular matrix (ECM) plays a central role in the structural and functional organization of tissues and organs. ECM constituents, in particular fibrillar collagens, are the most abundant proteins of the human body. Physiological and pathological breakdown of ECM is predominantly achieved by a family of neutral metalloproteinases, called matrix metalloproteinases (MMPs). Our group has a long-standing expertise in the biochemistry and molecular biology of collagenase and related MMPs (1,2). We have demonstrated that menstrual and abnormal uterine bleeding in women are due to the expression and activation of some MMPs (3,4). This seminal observation led us to : (i) exploit this system as a human model to study the regulation of MMPs, in particular cellular interactions that integrate overall hormonal impregnation with local environmental changes (5, 6 and ongoing research programme); and (ii) to explore whether this basic knowledge can lead to a rational treatment of abnormal uterine bleeding..

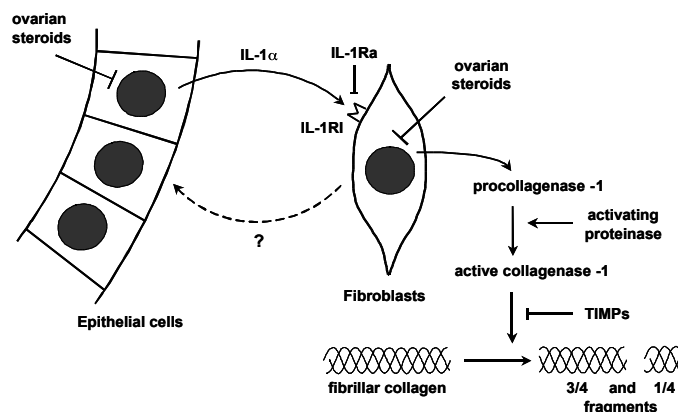


Fig. 1. Model of MMP regulation in the human endometrium : the case of MMP-1. Upon ovarian steroids withdrawal, IL-1_α is released by endometrial epithelial cells and triggers in adjacent fibroblasts the production of proMMPs, including interstitial procollagenase-1. Fibroblast activation involves interleukin-1 receptor (IL-1RI) and is opposed by various soluble factors, such as interleukin-1 receptor antagonist (IL-1Ra). Expression of proMMPs by stimulated fibroblasts is also blocked by ovarian steroids. Secreted proMMPs are then activated by proteinases currently under investigation. Active collagenase-1, if not neutralised by its tissue inhibitors (TIMPs), cleaves fibrillar collagens at position 3/4 of the distance from the amino-terminus.

Regulation of pro-MMP activation in the human endometrium

V. Rigot, A. Berton, E. Marbaix, P.J. Courtoy and Y. Eeckhout

Most matrix metalloproteinases (MMPs) are secreted as inactive pro-enzymes. Their expression is well documented in several human tissues, but their activators *in vivo* are still unknown. To address this question, the activation of progelatinase B (proMMP-9) in the human endometrium was selected as an example. ProMMP-9 was detected by gelatin-zymography in homogenates of fresh endometrial tissue sampled during all phases of the menstrual cycle, whereas its active form was observed only during the late secretory and menstrual phases. Furthermore, proMMP-9 was expressed and activated in endometrial explants sampled outside the perimenstrual phase when cultured in the absence of both progesterone and oestradiol, i.e. mimicking *in vitro* the menstrual condition. Analysis of such tissue cultures by gelatin zymography and Western blotting showed that activation of proMMP-9 depended on a secreted factor and was selectively inhibited by either a synthetic inhibitor of stromelysin 1 (MMP-3) or a monoclonal antibody that specifically blocks MMP-3, thus providing strong evidence for the activation of proMMP-9 *in vivo* by MMP-3. The activation of proMMP-3 was itself inhibited by a broad range MMP inhibitor in most cultures, but seemed to involve multiple pathways, implying both serine proteinases and metalloproteinases, which could operate in parallel or sequentially. Altogether, these data point to a highly-regulated MMP activation cascade (7).

Regulation of the expression of MMPs, TIMPs and endometrial cytokines

P. Henriot, P. Cornet, C. Picquet, V. Vassilev, P.J. Courtoy, Y. Eeckhout and E. Marbaix

Both endocrine (3,8) and paracrine factors (5,6) participate in controlling the expression and activity of the MMPs involved in the menstrual breakdown of the human endometrium. During this year, our research has been focused on potential regulatory cytokines of the TGF- β family: TGF- β 1 and 2, and the recently discovered LEFTY-A, also called endometrial bleeding associated factor (EBAF), the human orthologue of the murine lefty 1 gene. By competitive RT-PCR and, when possible, immunoassay, we have quantified the endometrial expression of these cytokines along the normal menstrual cycle *in vivo* and in explant cultures. Despite important variations between different endometria, our observations point to a down-

regulation of TGF- β 2 by ovarian steroids. We have also found that LEFTY-A and TGF- β 2 mRNA concentrations were strikingly (100-fold) increased *in vivo* in endometria showing signs of menstrual breakdown. A similar increase was observed in proliferative endometria when cultured for 24 h in the absence of ovarian steroids and was prevented by the addition of progesterone, indicating an hormonal control of the expression of LEFTY-A in the human endometrium (9).

Regulation of gelatinases A and B by long-chain unsaturated fatty acids

H. Emonard, A. Berton, V. Rigot and Y. Eeckhout, in collaboration with CNRS, Reims, France

The matrix metalloproteinases gelatinase A (MMP-2) and gelatinase B (MMP-9) are implicated in the physiological and pathological breakdown of several extracellular matrix proteins. We found that long-chain fatty acids selectively inhibit gelatinases A and B. The inhibition of gelatinases increased with fatty acid chain length and unsaturation. *Ex vivo* experiments on human skin tissue sections have shown that micromolar concentrations of a long-chain unsaturated fatty acid (elaidic acid) protect collagen and elastin fibers against degradation by gelatinases A and B, respectively. The role of the fibronectin-like domain, unique to gelatinases, in long-chain fatty acids binding and protease inhibition was demonstrated using a recombinant fibronectin-like domain of gelatinase A and a mutant of gelatinase A deleted of this domain. Moreover, surface plasmon resonance studies with the three individual type II modules of the fibronectin-like domain of gelatinase A identified the first type II module as primarily responsible for long-chain fatty acids binding.

Role of matrix metalloproteinases in abnormal endometrial bleeding

C. Galant, Y. Eeckhout, P.J. Courtoy and E. Marbaix, in collaboration with J.L. Brun, Bordeaux, France

Since matrix metalloproteinases (MMPs) play a key role in initiating normal menstrual breakdown, we looked for their contribution in triggering excessive, prolonged or irregular bleeding, those functional menstrual disorders that lead to one fourth of hysterectomies. Patients upon progestin-only contraception, a characteristic condition in which irregular bleeding is common, were selected for this investigation and paired biopsies compared at the time of bleeding and during non-bleeding intervals. Irregular bleeding was clearly associated with focal menstrual-like stromal breakdown and increased

expression and activation of several MMPs, together with decreased production of TIMP-1 (10). This association was recently confirmed in all major cases of irregular bleeding, irrespectively of hormonal treatment. An ongoing collaboration with Dr. J.L. Brun addresses whether the same molecular mechanisms account for recurrence of bleeding after selective endometrial resection and thermoablation. To clarify the reason for these local disorders, we currently analyse cytokines that control the expression and activation of MMPs and could be abnormally expressed and/or secreted at the time of irregular bleeding.

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