

*Proteinase trigger of menstruation?* In this issue of *The Journal*, Irwin et al. (1) report that a specific member of the matrix metalloproteinase enzyme family may be a likely effector of endometrial menstrual breakdown. The authors cultured human endometrial stromal cells in the presence of progesterone and found an augmentation of proteinase production after withdrawal of progesterone; the same results were achieved by the addition of RU486. Characterization of the enzyme by Western blotting revealed it to be matrix metalloproteinase-2 (MMP-2; 72-kD type IV collagenase, gelatinase A). Northern analysis indicated the differential expression of MMP-2 mRNA in the late secretory phase endometrium. The authors propose that stromal cell-derived MMP-2, acting on components of the extracellular matrix, including epithelial and vascular basement membranes, may play a role in breaching the integrity of the endometrial blood vessels and epithelial lining, thereby causing interstitial bleeding and the entry of blood into the uterine cavity. This hypothesis constitutes a new answer to the classic question of what triggers the initiation of menstruation.

*What is MMP-2?* Matrix metalloproteinases are a growing family of zinc atom-dependent endopeptidases (2). Three characteristics define these proteinases: (a) a conserved catalytic domain containing three histidine residues, (b) a proenzyme domain containing an unpaired cysteine residue that maintains the latent enzyme state, and (c) specific susceptibility to inhibition by the class of natural inhibitors referred to as tissue inhibitors of metalloproteinases (TIMPs). The family of MMP enzymes can be classified into four subgroups based on domain structure and substrate specificity. These are the interstitial collagenases, the type IV collagenases or gelatinases, the stromelysins, and the membrane anchored MT-MMPs. Most of the MMPs are generally secreted in a soluble latent form and activated extracellularly. However, some of the member enzymes in the last two subgroups contain a furin-type protease recognition sequence, RXKR, and are activated before secretion.

MMP-2 or gelatinase A, the enzyme elaborated by the endometrial stroma in the study of Irwin et al. (1), is a member of the second subgroup of MMPs. This group is characterized by the existence of a fibronectin-like gelatin-binding domain adjacent to the catalytic domain (2). MMP-2 was originally discovered because of its ability to degrade basement membrane type IV collagen (3) and thereby to promote tumor cell invasion and angiogenesis. Based on the first substrate, the original name for the enzyme was type IV collagenase. Subsequently, MMP-2 was found to degrade type V collagen, fibronectin, gelatin, and other matrix substrates. MMP-2 is secreted in a soluble latent state requiring activation. Such activation may take place at the cell surface only at the location where it is needed. Latent MMP-2 binds to a cell surface site and is activated by an MT-MMP (4). In addition to localized activation, MMP-2 activity can be regulated by the endogenous inhibitors

TIMPs. There are three members of the TIMP family: TIMP-1, TIMP-2, and TIMP-3. All three TIMPs can inhibit activated MMP-2, but only TIMP-2 can bind to latent MMP-2 and retard its activation (5). Thus, the known substrates, modes of tight regulation, and described functions of MMP-2 are in keeping with a potential role in the breakdown of both the extracellular matrix and the vascular and epithelial basement membranes of the endometrium.

The hypothesis of Irwin et al. (1) can induce a paradigm shift in our concept of the mechanism of menstruation. According to the original model of Markee in 1940 (6), the initiating event of menstruation is the intense vasospasm of the spiral arteries, producing ischemic damage to the vessel wall and surrounding tissue. The ischemic damage causes bleeding and sloughing. Irwin et al. provide support for a different chain of events. Under the new view, decidualized endometrial stromal cells respond directly to progesterone withdrawal by the production of MMP-2, which can directly cleave substrates that maintain the integrity of the endometrium. This proteinase mechanism must be independent of the vasoactive changes, since these investigators found direct progesterone withdrawal-induced tissue breakdown in cultures of isolated endometrial cells.

*Moving from correlation to causality.* Irwin et al. forge new directions but leave two questions unanswered. The first is how and where latent MMP-2 is activated in the endometrium. The second is whether a functional role for MMPs can be proven directly for the onset of menstruation in vivo. Fortunately, new tools exist to answer these questions directly: microdissection analysis and synthetic metalloproteinase inhibitors. Emmert-Buck et al. have described a method to analyze activated MMPs in microdissected cellular samples smaller than one high-power field (7). Using this method, it should be highly feasible to microdissect cycling endometrium and to visualize the cellular location and timing of MMP activation directly. A host of potent synthetic metalloproteinase inhibitors is now being used in animal models and phase I clinical trials (8, 9). MMP inhibitors have validated a mechanistic role for MMPs in tumor invasion and angiogenesis. These same inhibitors can now be turned to the role of MMPs in the endometrial cycle. Inhibitors can be administered systemically or implanted via slow-release pellet form. If such treatment modifies the timing, duration, or magnitude of the menstrual process, it would generate direct proof for the MMP hypothesis, complete the conceptual paradigm shift, and provide new strategies for therapeutic intervention.

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