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Steroid Hormone Dependent Inflammation and Regulation in the Endometrium in Women with Dysfunctional Menstrual Cycles: Is There a Role for Toll-Like Receptor Activation via PAMPs and DAMPs?

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Summary Sentence

The role of microbial and immunological menstrual cycle dependent changes within the endometrium may provide insight into the underlying causes of dysfunctional menstrual cycles.

Abstract

The ovarian steroid hormones control cyclic cellular proliferation, differentiation, inflammatory cell recruitment, apoptosis, tissue degradation and regeneration associated with the menstrual cycle as well as the response to pathogen challenge. Women with dysfunctional menstrual cycles (menorrhagia and dysmenorrhea) exhibit altered cytokine and prostaglandin expression in the endometrium implying ongoing recruitment of innate immune mediators. Activation of TLRs by endogenous and/or exogenous ligands caused by cell damage resulting from ongoing inflammation, endogenous microbiota or dysbiosis may contribute to the inflammatory symptoms associated with these conditions. The role of the upper genital tract endogenous microbiota in promoting genital tract homeostasis through possible promotion of re-epithelialization or anti-inflammatory mediators warrants further investigation.

Keywords: Menorrhagia; Dysmenorrhea; Toll-like receptor; Pathogen associated molecular patterns; Damage associate molecular patterns

Dysfunctional Menstrual Cycles – Menorrhagia and Dysmenorrhea

Primary menorrhagia represents dysfunctional menstrual bleeding with a menstrual loss in excess of 80 millilitres per cycle, in the absence of abnormal uterine pathology. This condition affects between 10-30% of reproductive aged women [1]. Similar to primary menorrhagia, no identifiable uterine abnormality is detected in women with primary dysmenorrhea. Primary dysmenorrhea is characterized by painful menstrual cramps as a result of abnormal uterine contractility [2]. Reduced blood flow due to ischemia is correlated with more severe pain. Together, these conditions affect up to 50% of women during reproductive life and result in significant morbidity, incapacitation and lost work days in some 10% of sufferers. Half of all women who have a hysterectomy for menorrhagia before age 60 years have an anatomically normal uterus [3].

Steroid Hormones and the Menstrual Cycle

Cyclic tissue remodelling in the endometrium occurs under the influence of fluctuating levels of the ovarian steroid hormones estradiol and progesterone [4,5] (Figure 1). Steroid receptors for estrogens, progestins and androgens have been reported to fluctuate throughout the menstrual cycle. The ovarian steroid hormones modulate the ability of each to respond to the other and other hormone–dependent factors [6]. Both estrogen and progestin receptors peak during the second half of the proliferative phase of the cycle, and decline during the secretory phase of the cycle in response to progesterone, though to varying degrees for each isoform [7-10]. Androgen receptor expression decreases from the proliferative until the mid-secretory phase and is undetectable by the late secretory phase [11].

Menstrual phase

The menstrual phase of the cycle is characterized by progesterone withdrawal. The endometrial response to progesterone withdrawal preceding menstruation involves mediators of apoptosis, haemostasis and wound healing. An influx of inflammatory cells into the endometrium occurs prior to menstruation.

Prostaglandins are key mediators in menstrual cycle processes [12]. Prostaglandins are regulated by progesterone, and are implicated in menstruation [13]. Prostaglandins promote uterine contraction, and vasoconstriction leading to hypoxia, ischemia and pain [14]. Prostaglandins are eicosanoid cyclooxygenase (COX) metabolites of arachidonic acid, promoting angiogenesis by binding to specific cell receptors. Dysregulation in biosynthesis or signalling of the prostaglandin receptors can result in abnormal vasculature leading to dysfunctional uterine bleeding. COX-2 is up-regulated during the peri-menstrual period [15]. Matrix metalloproteases (MMPs) have been implicated in the irreversible tissue breakdown associated with menstruation in response to progesterone withdrawal during the pre-menstrual period [16].

Proliferative phase

Estrogen dominance results in significant changes in growth factor secretions and endometrial regeneration causing increased endometrial height. Estradiol stimulates the expression of epidermal growth factor

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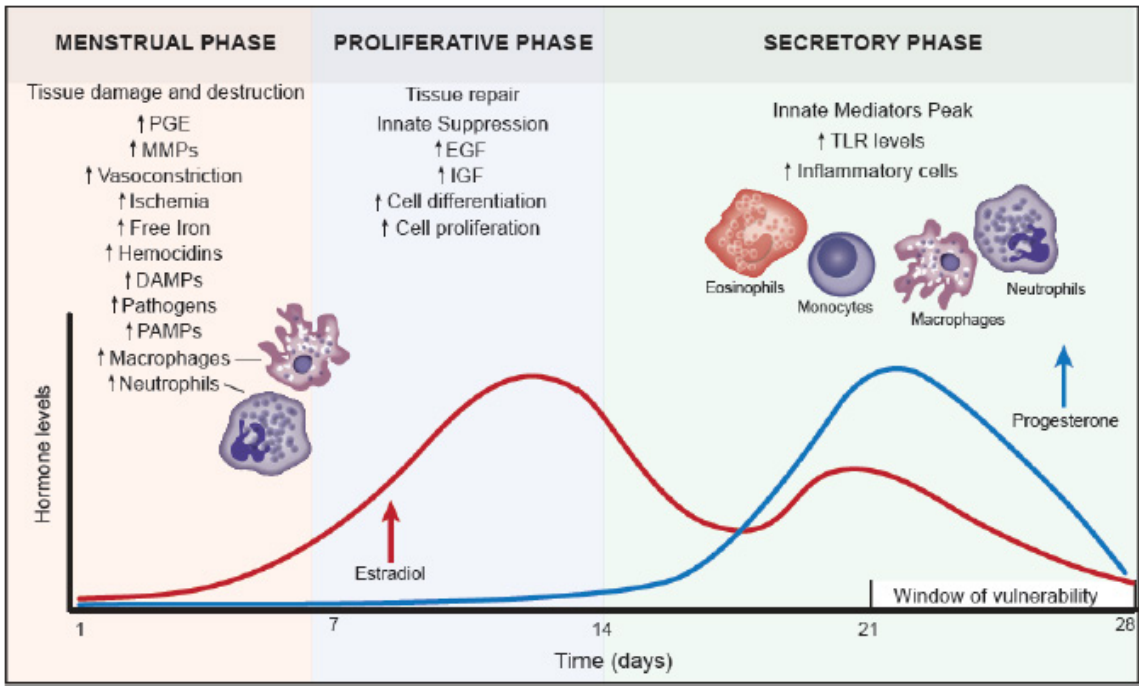

Figure 1: Menstrual cycle phase and immune mediators respond to estradiol and progesterone in a coordinated manner.

(EGF) and insulin-like growth factor (IGF), factors promoting cell division, differentiation and estradiol-induced endometrial growth [17,18].

Secretory phase

The secretory phase of the menstrual cycle is characterized by progesterone dominance. Proliferation ceases and endometrial differentiation occurs, however the endometrial height is fixed due to growth inhibition by progesterone interference with estrogen receptor expression [19,20]. Progesterone levels peak in the estrogen primed endometrium resulting in increased inflammatory cell infiltrates and commencement of decidualization.

Steroid Hormones and Immunity

Mucosal immunity within the female genital tract is also regulated by the steroid hormones. Cyclic changes in estradiol and progesterone concentrations result in controlled recruitment of immune cell populations, antigen presentation and cytokine secretion [6]. The steroid hormones influence both the innate and the adaptive immune responses, contributing to the immediate non-specific response to pathogen associated molecular patterns (PAMPs) and the specific but delayed humoral and cell mediated antigen presentation in adaptive immunity [21]. Estrogens enhance the humoral immune response whereas progesterones suppress the proliferative capacity of lymphocytes within the endometrium; increasing infection risk [22-24] Androgens inhibit both cell-mediated and humoral immunity.

Estradiol attenuates inflammation and modulates the immune response [25]. Previous studies revealed that estradiol causes inhibition of lipopolysaccharide (LPS) mediated interleukin (IL)-6 secretions in uterine epithelial cells [21,26]. Estradiol and progesterone both inhibit LPS-mediated prostaglandin production in endometrial cells, further highlighting the role of the ovarian steroid hormones in infection sequelae [24].

Mediators of the Innate Immune System Respond to Menstrual Cycle Steroid Hormone Fluctuations

The composition of the genital tract epithelium, the secretion of mucus and the localized immune response follow a similar pattern of hormonal dependence [27-30]. Importantly, there appears to be a relationship between innate immune mediators and endogenous stress response proteins in the development of pelvic inflammation [31].

Human uterine epithelial cells express Toll-like receptors (TLR) 1-9 [32,33]. TLRs interact with specific PAMPs, and receptor binding results in the up-regulation of a pro-inflammatory cytokine cascade and cellular activation [34,35]. Cell surface TLRs (TLR 1,2,4,5,6) recognize microbial products and endogenous ligands while intracellular TLRs (TLR3,7,8,9) recognize nucleic acids [36,37] (Table 1). Therefore, inflammation can be initiated under infectious and non-infectious conditions via TLR activation. Pro-inflammatory cytokines and chemokines are expressed in response to TLR ligand binding in quantitatively and temporally specific patterns [38,39]. Many of these chemical mediators are tightly regulated throughout the menstrual cycle, therefore, changes in secretion induced by TLR activation may well impact on endometrial function in susceptible women [32]. Despite varying patterns of expression for individual immune mediators throughout the menstrual cycle, the cycle-specific changes in immune mediator expression capitulates in the endometrium during the late secretory to early proliferative phase of the cycle [40,41].

There are distinct cell and site-specific differences in TLR expression throughout the female reproductive tract [42]. TLR-2 and TLR-4 are predominantly expressed in the female upper reproductive tract, but rarely in the lower reproductive tract [43-45]. In contrast to all other epithelia, TLR-4 is expressed at much higher levels than TLR-3 within the endometrium possibly due to redundancy between TLR-3, TLR-4 and dsRNA binding proteins [38,46].

Toll-like receptor	Endogenous ligands	Exogenous ligands	Cell localization	Genital tract expression
1		Lipopeptides Modulin	Cell surface	Decidual cells Endometrial epithelial cells
2	Biglycan Endoplasmin Hsp60 Hyaluronan Monosodium urate crystals Veriscan	Lipopolysaccharide (LPS) Lipoproteins Lipoproteins Lipotechoic acid (LTA) Mannuronic acid polymers Modulin Peptidoglycan Zymosan	Cell surface	Natural killer cells
3	mRNA	Vrial dsDNA	Intracellular	
4	Biglycan CD138 Endoplasmin Fibrinogen Fibronectin Heparan sulphate HMGB1 HSP22 Hsp60 HSP60 Hsp70 HSP70 HSP72 Hyaluronan Monosodium urate crystals Oxpapc Resistin Surfactant protein A α-crystallin A chain β-defensin 2	LPS Mannuronic acid polymers	Cell surface	
5		Flagellin	Cell surface	
6	Veriscan	Lipoprotein Modulin	Cell surface	
7	RNA Small interfering RNA (siRNA)	Viral ssRNa	Intracellular	
8	Human cardiac myosin siRNA	Viral ssRNA	Intracellular	
9	DNA HMGB1	Unmethylated CpG-DNA	Intracellular	

Table 1: Toll-like receptor ligands.

Recently, TLR-4 activation by commensal Gram-negative genital tract bacteria (*E. coli*, *Veillonella parvula* and *Neisseria mucosa*) was shown to inhibit human immunodeficiency virus (HIV)-1 macrophage infection *in vitro* [47]. In contrast, TLR-2 activation by Gram-positive bacteria including lactobacilli enhanced HIV-1 infection of macrophages. The capacity for commensal bacteria to alter the immune response to a potential pathogen via PAMP recognition by TLRs highlights the potential impact of dysbiosis within the commensal microbiota on reproductive health outcomes.

TLRs can also be activated by endogenous ligands (damage associated molecular patterns, DAMPs), which are released from damaged cells during tissue inflammation and injury [48]. Hyaluronic acid and biglycan bind to, and activate, TLR-2 and/or TLR-4 and heparin sulphate, fibrinogen, fibronectin [48]. Heat sock proteins activate TLR-4 [48,49]. Fibronectin is released by endometrial cells in response to progesterone [50]. The activated TLRs then activate macrophages to produce inflammatory chemokines and cytokines [33]. Activation of TLR-2 and TLR-4 by microbial pathogens activates pathways involved in inflammation, tissue repair and adaptive immunity, whilst endogenous ligands binding TLRs -2 and -4 induce inflammation and tissue repair processes [48]. It is proposed that chronic inflammation, which leads to elevated levels of endogenous TLR ligands causes an accumulation of DAMPs thereby lowering the threshold of cellular responsiveness to PAMPs [51]. The net result of which is likely to be ongoing, acute inflammation.

Some bacteria (*E. coli*, *Streptococcus* sp., *Pasteurella* sp., *Neisseria meningitidis*) avoid the host immune response through molecular mimicry of host glycan structures (heparan sulphate, hyaluronic acid, biglycan) or by altering the cell surface domains releasing charged moieties, which then bind the secreted endogenous antimicrobials such as defensins and lysozyme to neutralize the innate host response. Bacterial species including *Streptococcus agalactiae, Neisseria* sp. *Pseudomonas aeruginosa and Haemophilus* sp. appear to promote the cell surface expression of specific glycans and sialic acids in a commensal role to avoid activating the innate immune response [52-55].

Secretory immunoglobulin levels also fluctuate throughout the menstrual cycle in response to the steroid hormones. Secretion is highest during the secretory phase of the cycle, significantly reduced during the proliferative phase and even further reduced at the time of menstruation [56].

The endogenous antimicrobials in the female upper genital tract are capable of inducing cell proliferation, chemotaxis and cytokine secretion. Secretory antimicrobials from the upper genital tract tissues demonstrate selective toxicity against sexually transmitted pathogens, when tested against lower genital tract pathogens and the commensal

organism *Lactobacillus crispatus* [57]. It has been postulated that the microbial cell wall and cell membrane composition may be the reason for differential activity against pathogens [58]. The ovarian steroid hormones produce differential effects on the expression levels of endogenous antimicrobials between the upper and the lower genital tract sites [26,59]. The role of immune response or surveillance in the presence of an endometrial-specific endogenous microbiota has not yet been explored. The upper and lower genital tract sites function in a coordinated but separate fashion, highlighting the need to further investigate the role of the upper genital tract microbiota in susceptibility to infection [60].

Menstrual phase

Menstruation involves a breach in the epithelial lining, which may elicit an up-regulation of immune mediators to prevent infection [61-63]. Decreases in estradiol and progesterone levels correlate with changes in the genital tract microbiota. Many antimicrobial peptides including human beta defensins (HBD), elafin and secretory leukocyte protease inhibitor (SPLI) are over expressed in the peri-menstrual epithelium [4]. Elafin is also expressed during menstruation [63]. HBD2 levels peak during the menstrual phase of the cycle [59,64].

Proliferative phase

Elevated estradiol levels during the proliferative phase of the menstrual cycle result in suppression of innate immunity and the resultant secretion of endogenous antimicrobials. HBD4 levels peak during the proliferative phase of the cycle indicating that there is some level of innate immune response at this stage [65]. However, whilst HBD4 is chemotactic for monocytes, it has no effect on neutrophils or eosinophils [66]. This may provide some insight into the increased diagnosis of genital tract infection during the proliferative phase of the menstrual cycle.

Secretory phase

Several natural antimicrobials including HBD and SLPI are also maximally expressed during the secretory phase of the cycle [67,68]. HBD1, 2, 3 and 5 levels and SLPI levels peak during the second half of the menstrual cycle [59,64].TLR-2-6, and 9 are expressed at maximum levels during this same phase. TLR-4 expression is increased during the secretory phase, which is consistent with its increased expression in response to progesterone [61,62,69,70].

Several innate immune mediators, known to exhibit steroid hormone dependent expression, peak during the period from ovulation to implantation, but have the capacity to deviate from the traditional antigenic response in order to induce immune tolerance, indicating their role in maintaining an adequate endometrial environment for initiating pregnancy [71]. Wira et al. [60] identified the 'window of vulnerability' during the 7-10 days post ovulation, where there is increased susceptibility to viral (and possibly bacterial) infection as a result of increased steroid hormone expression leading to decreased innate, humoral and cell mediated immune activity [60].

Steroid hormones regulate cytokine and chemokine secretion throughout the menstrual cycle

Chemokine and cytokine production and secretion is also regulated by the ovarian steroid hormones [72]. These inflammatory mediators are constitutively secreted by the epithelial cells within the uterus [42]. Together, the chemokines and cytokines create an environment with a resident immune cell population that contributes to both the inflammation and repair associated with the menstrual cycle and innate immune surveillance [5,73,74]. Seven cytokines and chemokines are constitutively expressed within the genital tract epithelium throughout the menstrual cycle (IL-6, (tumour necrosis factor (TNF)-α, (granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage migration inhibitory factor (MIF), IL-8, monocyte chemo-attractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1 β [75]. The TNF system is known to be regulated by steroid hormones, supporting a key role for the family in uterine function [76]. What remains to be elucidated is whether there are distinct differences between the pro-inflammatory cytokine profile required for endometrial remodelling throughout the menstrual cycle, and pathogen initiated cytokine expression [71,77].

Inflammatory Cell Function During the Menstrual Cycle: Steroid Hormones as Inflammatory Regulators of Myeloid Cells

Inflammatory cells of the immune system express estrogen, progesterone and androgen receptors, therefore the functional role and spatial distribution of these cells also appears to be menstrual cycle dependent [78] (Figure 2).

Neutrophils express estrogen but not progesterone receptors [79,80]. Both stimulatory and inhibitory effects of estrogen and progesterone on neutrophil release of reactive oxygen species (ROS) have been reported [81-84]. Neutrophil numbers are relatively constant throughout the menstrual cycle, but peak during menstruation following steroid hormone withdrawal and IL-8 up-regulation [85]. Neutrophils are the major responders to infection via TLR activation. Neutrophils phagocytoze pathogens, produce oxidation compounds, secrete microbicides and secrete chemokines for cell recruitment in adaptive immunity [6]. Neutrophils also activate prostaglandins and leukotrienes via catalysis of arachidonic acid derived from bacterial cell membranes. Within the tissues, the neutrophils initiate wound healing and repair by promoting angiogenesis and neutrophil extracellular trap formation [86,87].

Progesterone stimulates the production of ROS in monocytes [88]. Progesterone also promotes TNF-α, IL-1β and IL-8 secretion in LPS challenged cells [89,90]. Estradiol, progesterone and androgens reduce the secretion of IL-6 in LPS challenged cells [90]. Estradiol and progesterone also cause reduced secretion of IL-1α and IL-1β [91]. Androgen exposure results in reduced production of prostaglandin E2 [92]. Estradiol reduces IL-8 secretion and inhibits chemotaxis of monocytes to the monocyte chemotactic protein-1 (MCP-1) [93]. The steroid hormones influence the cytokine profile and apoptotic pathways in monocytes.

Macrophages accumulate in the endometrial tissue in the pre-menstrual period when levels of both estradiol and progesterone decrease, and levels of macrophage chemo-attractants are up-regulated [15,94,95]. Estradiol has been shown to suppress the production of TNF- α, IL-1β and MIP-2, while progesterone stimulates these same factors [96]. Estradiol also appears to block LPS induction of TLR-4 [97]. TLR-4 expression is increased during the secretory phase of the menstrual cycle when progesterone levels peak, and estradiol levels are low. The macrophage phenotype (pro-inflammatory or anti-inflammatory) appears to be induced by TLR signalling in response to either exogenous or endogenous ligands [98].

Eosinophils express estrogen receptors. Estrogen treatment enhances eosinophil adhesion and degranulation of cells [99,100].

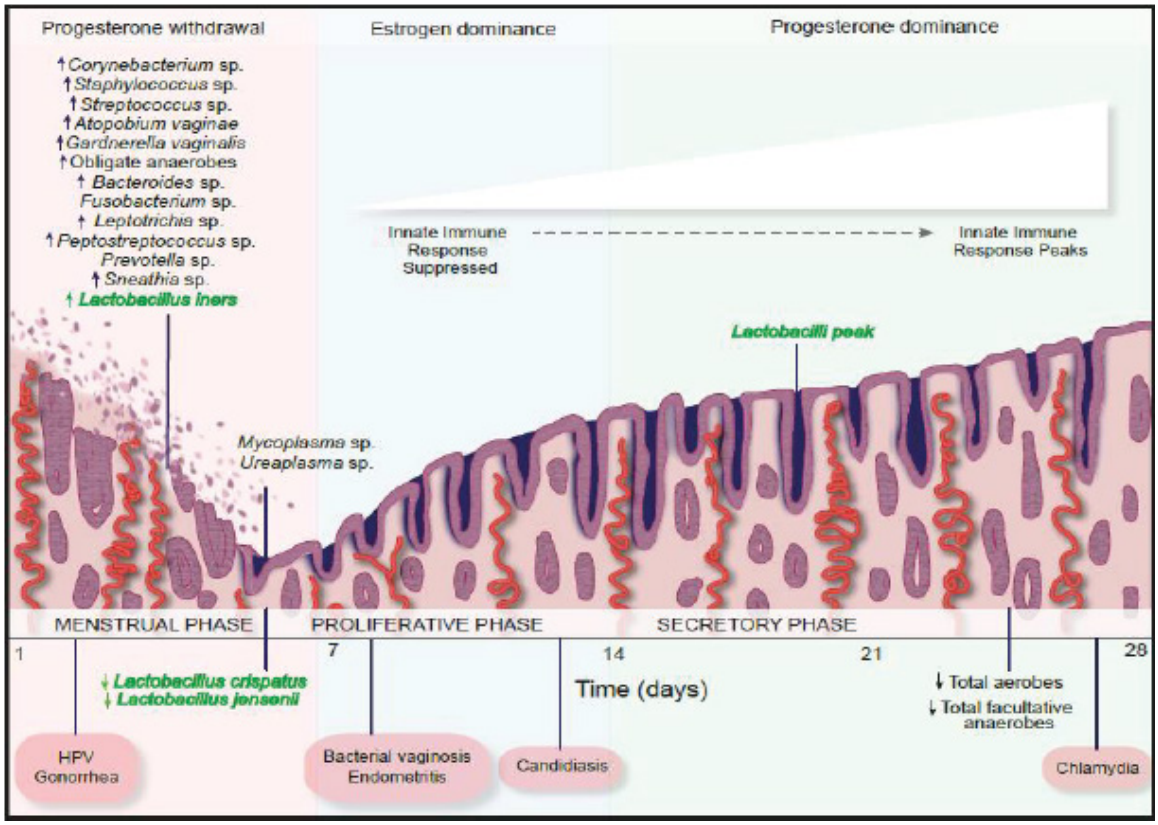

Figure 2: Genital tract commensals and pathogens fluctuate throughout the menstrual cycle.

Eosinophils do not express progesterone receptors [80]. There is little known about the effects of steroid hormones on basophils, however treatment of cells with immunoglobulin (Ig) E results in enhanced histamine secretion [101]. Estradiol stimulates mast cell degranulation and histamine secretion [102,103]. In contrast, progesterone inhibits mast cell proliferation but stimulates histamine secretion and platelet aggregation factor [104-106]. Platelet aggregation is inhibited by both estradiol and progesterone [107].

The uterine natural killer (NK) cells are low in number during the early proliferative phase of the menstrual cycle, increasing in number until the late secretory phase. The uterine NK cells express TLR-2, TLR-3 and TLR-4, however the cellular response to activation is dependent upon interactions between the multiple endometrial cell populations [98].

The steroid hormone dependent expression of myeloid cells throughout the menstrual cycle is responsible for the simultaneous controlled endometrial tissue degradation, remodelling and repair, and the ability of the epithelium to recognize and respond to microbial pathogens. (See Table 2 for a summary of immune mediator expression throughout the menstrual cycle)

Dysregulation of Inflammation in Menorrhagia and Dysmenorrhea

Women with dysfunctional menstrual cycles exhibit alterations in steroid hormone levels, and inflammatory mediators such as prostaglandins, chemokines and cytokines (Table 2). The systemic estradiol levels in women with dysmenorrhea are higher in the late proliferative phase of the cycle when compared to normal cycling women [108].

Genes encoding the pro-inflammatory cytokines IL-1β, IL-6 and TNF were up-regulated during the menstrual and secretory phases of the cycle in dysmenorrheic women whilst members of the TGF-β superfamily were down-regulated in these women when compared to women with pain-free menstrual cycles [109]. The authors concluded that the anti-inflammatory response was insufficient to dampen the pro-inflammatory cascade. There was an increased level of expression of growth factors during the menstrual phase of the cycle, which decreased during the proliferative and secretory phases [109]. The down-regulation of TGF-β super family members and the corresponding up-regulation of pro-inflammatory cytokines results in prostaglandin release, induction of additional inflammatory mediators, hypoxic ischemia within the uterus and pain.

Prostaglandin synthesis and the number of prostaglandin receptors are increased in uterine tissues in women with menorrhagia compared to women with normal menstrual loss, and levels correlate with menstrual loss [12]. Prostaglandin concentrations are also higher in the menstrual blood collected from women with dysmenorrhea compared to women without menstrual pain.

It is possible that the altered expression of pro-inflammatory mediators within the endometrial epithelium of women with dysfunctional menstrual bleeding may lead to alterations in the cellular responses necessary for maintaining homeostasis or responding to pathogen challenge. Constant activation of TLRs during prolonged bleeding, inflammation and repair may reduce the threshold for TLR activation by exogenous ligands leading to hyper-responsiveness to PAMP recognition leading to ongoing inflammation and pathology.

Mediator	Normal menstrual cycle	Menorrhagia	Dysmenorrhea	References
Cytokines/chemokines				
GMCSF	Regulator and activator of granulocytes and macrophages			[183]
Gro α	Neutrophil chemotaxis			[184]
IL-1	Ovulation promotion Induces IL-6 production for angiogenesis Enhances IL-8 production Inhibited by progesterone Inhibited by estradiol Induces NO production			[185,186] [187,188]
IL-1 α	Stimulates T and B lymphocyte proliferation Stimulates prostaglandin production			[189]
IL-1 β	Endometrial inflammation at menses Inhibits apoptosis by NO production Stimulates T and B lymphocyte proliferation Stimulates prostaglandin production			[190]
IL-1 ra	Antagonises IL-1 to prevent tissue damage after ovulation			[191]
IL-2	Progesterone stimulation HCG suppression			[192]
IL-6	Role in steroid hormone production Reduces granulosa cell proliferation Inhibited by estradiol		Elevated at menstruation	[188,193,194]
IL-8	Up-regulated by progesterone withdrawal Chemotaxis and activation of monocytes and neutrophils Proliferation Menstruation Angiogenesis Prevention of infection Prevention of tissue damage after ovulation			[72,94,195]
IL-10	Inhibition of progesterone production Anti-inflammatory antagonist to IL-1, IL-2, IL-6, TNF α			[196]
LIF	Estradiol biosynthesis			[193]
MCP-1	Up-regulated by progesterone withdrawal Chemotaxis and activation of monocytes and neutrophils Proliferation Angiogenesis Menstruation			[72,94,195]
RANTES	Androgen production and induction of LH receptors Inhibited by estradiol			[197]
TGF β			Down regulated at menstruation	[109]
TNF α	Endometrial inflammation at menses Androgen production and induction of LH receptors Inhibited by estradiol	Increased at menstruation	Increased	[109,188,197]
Growth factors				
EGF	Up-regulated with estradiol Endometrial proliferation			[198]
FGF	Endometrial maturation and regeneration Elevated during proliferative and secretory phases	Decreased receptor expression		[199,200]
VEGF	Angiogenesis	Increased proliferative activity		[199]
Prostaglandins				
COX-2	Activated by progesterone withdrawal Prostaglandin activation resulting in leukocyte influx Endometrial breakdown	Elevated during menstruation	Elevated	[201,202]
PGE	Angiogenesis	Elevated at menstruation	Elevated	[199,201,203]
PGF2 α	Up-regulated following progesterone withdrawal Inflammation at menstruation	Elevated in secretory phase	Elevated	[195,203-205]
TXA2	Vasoconstriction of spiral arterioles inducing hypoxia Stimulates smooth muscle contraction for endometrial sloughing Menstrual pain nociception		Elevated at menstruation	
Matrix metalloproteases				
MMP-2	Relaxation of endometrial blood vessels enhancing edema and leukocyte recruitment Menstrual pain nociception	Reduced at menstruation		[206]
MMP-9	Menstrual pain nociception	Reduced at menstruation		[206]

Table 2: Cytokine/chemokine/prostaglandin expression in endometrium in normal and in pathology.

Infection Risk and Commensal Microbes

The menstrual cycling of the endometrium represents a constant tissue remodelling process encompassing cell death, proliferation and migration. The endometrium is one of very few tissues that can undergo such significant remodelling without scar tissue formation, further supporting the tight regulation surrounding inflammation during this process [110]. The commensal microbiota is responsible for maintaining epithelial homeostasis and integrity, and contributes to the tissue damage response via recognition by innate immune cells expressing TLRs [111-113].

There exists a close relationship between regulated microbial growth in the female genital tract and the menstrual cycle. Genital tract infections occur in a menstrual cycle-dependent fashion, which can be attributed to changes in secreted levels of the steroid hormones estradiol and progesterone, which influence the expression of immune mediators [30,61,114]. Many members of the endogenous microbiota use these hormones as growth factors, while for others they are inhibitory [115]. The concentration of opportunistic pathogens increases during menstruation and the early proliferative phase of the cycle at the time of progesterone withdrawal. Levels of the endogenous lactobacilli peak with estradiol levels during the secretory phase of the cycle [116].

Previous studies indicate that there are some changes in *Lactobacillus* sp. community composition, but that *L. crispatus* is associated with long-term stability within the vaginal microbiome [117]. However, absence of *Lactobacillus* sp. has been reported in healthy, asymptomatic women with a lower genital tract microbiota dominated by other lactic acid producing species including *Atopobium* sp., *Megasphaera* sp. or *Leptotrichia* sp. [118,119]. These reports may suggest that maintenance of the host microbiome (expression of microbial genes and subsequent secretion of metabolites) rather than the host endogenous microbiota (individual microbial species) is key in preserving a healthy genital tract environment. The inability to identify a site-specific (vaginal) 'core microbiome' in the healthy asymptomatic women of reproductive age supports the concept of functional redundancy in the microbiome [120-123]. Whilst functional redundancy may imply overall equilibrium in the system, it is reasonable to assume that the relative abundance of some members of the commensal microbial community will activate the innate immune response to a greater extent than for example the non-antigenic endogenous lactobacilli, leading to activation of a pro-inflammatory cascade, dysbiosis and instability within the site-specific microbiome. Functional redundancy has also been reported for the human microbiome in healthy individuals and in the gut microbiome in lean and obese individuals [124,125].

Microbial pathogens have at their disposal diverse mechanisms for interacting with and manipulating host cells for the benefit of their survival [126]. Bacteria are capable of selectively inducing either pro-apoptotic or anti-apoptotic pathways in human cells to mediate their survival within the host environment [127]. Apoptosis plays a key role in microbial pathogenesis and antibacterial immunity [128]. The recognition of apoptotic cells by the immune system leads to phagocytosis and activation of anti-inflammatory mediators [129]. However, for intracellular pathogens, survival is dependent on inhibition of apoptosis of the host cell [130]. The endogenous genital tract *Lactobacillus* sp. are capable of inducing this switching independently of pH and lactate, further supporting a role for the microbiome in reproductive pathology or homeostasis [131-133].

Numbers of anaerobic Gram-positive cocci increase during the menstrual phase of the cycle, and in response to antibiotic administration [134]. An increase in the presence of the Gram-positive cocci including *Streptococcus anginosus*, *Peptostreptococcus anaerobius* and *P. asaccharolyticus* has been linked to disturbances in the lower genital tract microbiota. Interestingly, these species appear to exist in equilibrium with the lactobacilli in healthy women, but can be disrupted in susceptible women during the menstrual phase of the cycle. The complexity of the host-microbe interaction is further highlighted by the inability to achieve resolution of all dysbioses following antibiotic treatment [135]. Treatment resistance of *Gardnerella vaginalis* in confirmed cases of bacterial vaginosis further highlights the need to consider the microbiome in toto, rather than only an individual member of the genital tract microbiota when treating genital tract dysbiosis [136-138]. Further, abnormalities in the lower genital tract microbiota are implicated in endometrial pathology including adverse obstetric outcomes [139-141].

Evidence of transient exposure to microbial commensals or pathogens has been reported in the upper genital tract [142-145]. Microorganisms have been isolated from the endometrial cavity in both the presence and the absence of symptoms of infection and/or inflammation, suggesting that the presence of microorganisms within the upper genital tract may not always reflect a pathological process; rather the endometrial cavity is not sterile, and harbours an endogenous microbiota [146-150]. Cultures of vaginal and endocervical specimens have proven to be poor indicators of the presence of microorganisms within the endometrial cavity and discordant results for microbial species were obtained when sampling both sites in asymptomatic women [151,152]. In susceptible women, the endogenous endometrial microbiota may lead to endometrial pathology or may serve to maintain homeostasis.

Nutritional Requirements of the Host Microbiota

A critical factor controlling the composition and distribution of the host microbiota is the nutritional requirements of individual microbial community members. Members of the commensal microbiota implement an array of direct interaction strategies to compete with opportunistic pathogens present as minority community members. Commensals produce bacteriocins and toxins that inhibit members of the same or closely related species [153]. Members of the endogenous microbiota are also capable of altering the host environment by shifting the pH creating a niche prohibitive to pathogen growth [153-155]. The consumption of nutrients and production of metabolites by endogenous microbiota can also alter pathogen growth and virulence [156,157]. However, some pathogens (including members of the Enterobacteriaceae and obligate anaerobes) have evolved to use alternative nutrients to manage nutritional competition by commensals [158,159]. Alterations of host inflammation in response to pathogens can impede the growth of some community members including obligate anaerobes from the families Bacteroidetes and Firmicutes more rapidly than others (*E. coli*) due to their inability to utilize electron acceptors such as nitrate, which is generated by an inflamed epithelium [160]. There are also opportunistic pathogens capable of using resources more efficiently than commensals. Iron, an essential resource for bacterial growth is utilized by iron-chelating siderophore producing bacteria. Host cells can actively block siderophore production and cellular proliferation in members of the endogenous microbiota making competition with opportunists difficult [161]. Hemoglobin is a precursor of many natural antimicrobial peptides [162]. Erythrocytes from the endometrium may therefore be another source of antimicrobial activity acting alongside epithelial cells and leukocytes [163]. Hemoglobin derived peptides exhibit diverse activities including immunomodulatory action [164]. In

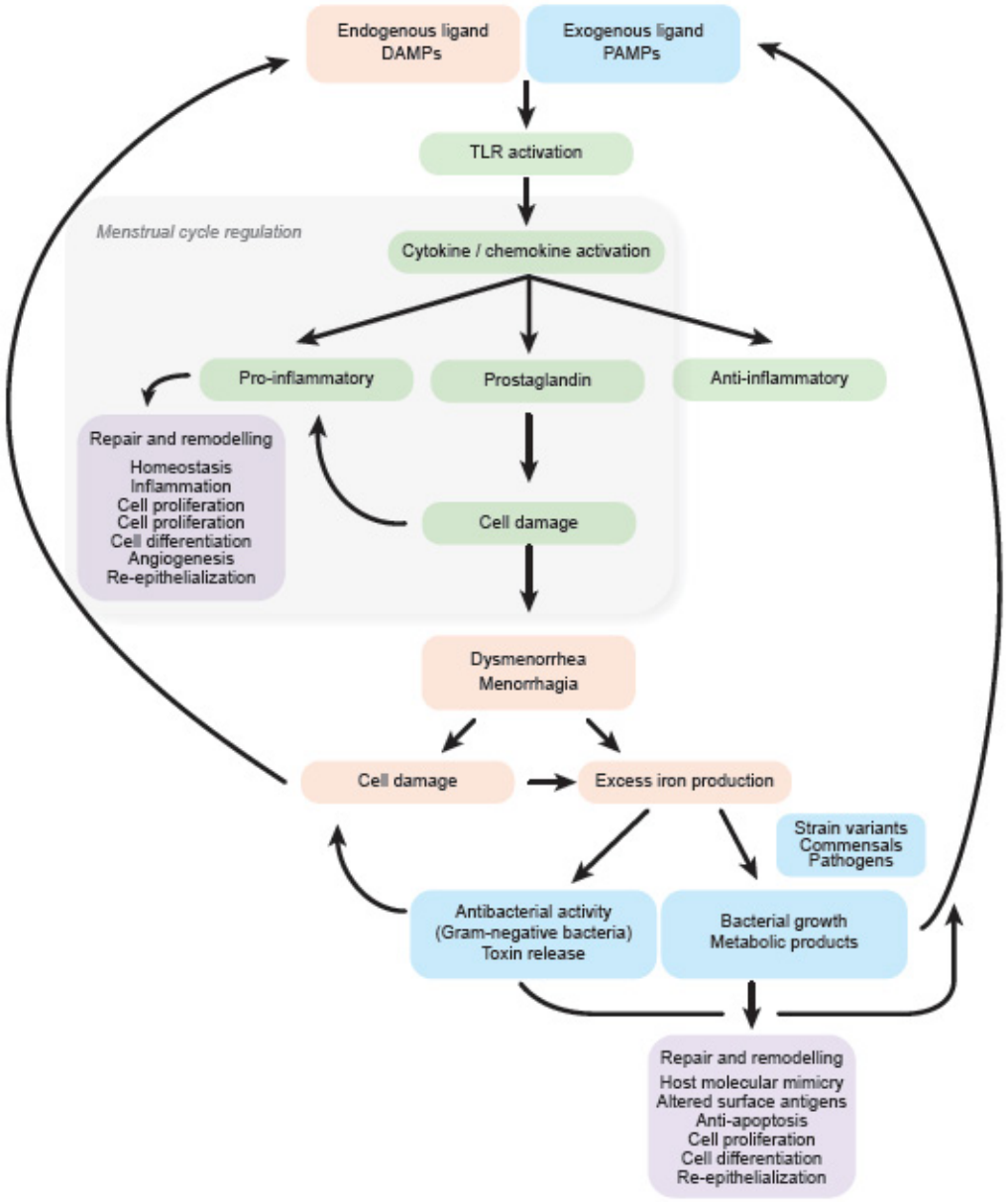

Figure 3: Proposed pathways of DAMP and PAMP-associated TLR activation in normal and dysfunctional menstrual cycling.

contrast, hemoglobin can also promote microbial growth by providing iron or interfering with oxygen metabolism by leukocytes. Hemocidins (short chain alpha-hemoglobins) demonstrate antimicrobial activity against bacteria, particularly Gram-negative species. Hemocidins have been isolated from post cesarean section uterine lochia [162,165].

Menorrhagia is characterized by excessive menstrual blood loss, whilst dysmenorrhea is associated with increased inflammation and cell damage. Therefore, in these women, the haemoglobin concentration is likely to exceed that of other endogenous antimicrobial peptides within the uterine cavity. Notably, despite producing an excessive amount of bacterial nutrition (in the form of menstrual blood), menorrhagia has not been associated with an increased incidence of pelvic infection. The endogenous *Lactobacillus* sp. do not require iron for their growth, suggesting that their numbers are not likely to increase significantly during menstruation in contrast to many opportunistic members of the endogenous microbiota (including *G. vaginalis*) who have developed multiple systems to exploit both low level and excess free iron [166,167]. It is therefore possible that for women with dysfunctional menstrual bleeding there exists a delicate balance between increased numbers of iron-dependent pathogens, and increased levels of antimicrobial hemocidins. A robust endogenous microbiota, such as that linked with localized administration of exogenous progesterone is more likely to tolerate fluctuations in individual species numbers and nutrient supply. It seems reasonable to assume that in these women, the immune system must choose between maintaining homeostasis and mounting an immune response to pathogen challenge.

Microbes and their Interaction with Immune Regulators

Staphylococcus aureus and microbial products signalling via TLR-

2 and TLR-5 induce epithelial repair, survivial and growth [168]. This repair occurs via an independent non-inflammatory pathway linking TLR activation and epidermal growth factor receptors associated with epithelial cells [168]. Peptidoglycan has been associated with wound closure and repair via epithelial cell proliferation and migration. TLR-2 and TLR-5 ligands (peptidoglycan, lipotechoic acid and flagellin) all appear capable of promoting wound repair. It is well accepted that TLR activation causes pro-inflammatory cytokine (IL-1β, TNF-α, IL-6 and IL-8) release by epithelial cells, however TLR mediated epithelial repair appears independent of pro-inflammatory cytokine induced cellular migration and proliferation at the wound site. This data suggests that the endogenous microbiota is capable of enhancing tissue remodelling and repair.

Whilst TLRs are powerful and somewhat specific immune regulators, recent evidence suggests that activation of both TRL-2 and TLR-6 is required for an adequate innate response to *Mycoplasma* sp. infection [169]. Distinct TLRs have the potential to mediate epithelial homeostasis in favour of ongoing pathology or regeneration and repair. Many bacteria, particularly anaerobic species, which are common inhabitants of the endogenous genital tract microbiota produce large quantities of short chain fatty acids (acetic, butyric and propionic acid). Short chain fatty acids are capable of modulating the immune response by inhibiting the production of pro-inflammatory cytokines, chemotaxis and phagocytosis [170-172]. The metabolites also cause apoptosis in neutrophils [173,174]. Commensal bacteria reported to be opportunistic pathogens demonstrate the capacity to alter the immune response as a trade-off for virulence. Highly invasive strains of *Prevotella bivia* produced the weakest pro-inflammatory (IL-6 and IL-8) response during *in vitro* culture when compared to non-invasive strains [175].

Lactobacillus iners, a common genital tract commensal is capable of up-regulating constitutive SLPI secretion, however, increased levels of *L. iners* results in the reverse effect [176]. Interestingly, *L. iners* is the *Lactobacillus* sp. frequently associated with intermediate or bacterial vaginosis vaginal flora and is the dominant vaginal taxon reported after treatment for bacterial vaginosis [177-181]. *L. crispatus* causes a strong reduction in SLPI secretion, and presence of this *Lactobacillus* sp. is associated with an absence of bacterial vaginosis [181]. Current evidence suggests that the dominant genital tract lactobacilli can contribute to the mucosal immune response in diverse ways. The regulatory effect of SLPI secretion is inversely associated with the capacity of the invading or commensal bacteria to evoke a pro-inflammatory response. It is therefore possible that microbes present in the genital tract are capable of causing down-regulation of the immune response in exchange for reduced virulence [182].

Microbial pathogens and their products appear to have two possible roles in epithelia: Cell death, injury and inflammation, or tissue repair, cell migration and proliferation, and limited inflammation. Therefore, under physiological conditions, the presence of certain members of the endometrial microbiota may control epithelial barrier integrity without causing inflammation (Figure 3).

Conclusion

Subacute inflammation associated with dysbiosis in the endogenous endometrial microbiota in women with primary menorrhagia and/or dysmenorrhea may cause TLR binding, and subsequent activation of the pro-inflammatory cascade leading to increased secretion of mediators involved in apoptosis, haemostasis, inflammation and repair. Alterations in prostaglandin and cytokine expression in the endometrium of these women, combined with an altered microbiome may well contribute to ongoing activation of an innate immune response leading to dysregulation of the normal menstrual cycle associated with tissue remodelling. The menstrual phase of the cycle is frequently associated with an increased notification of genital tract infection possibly due to heightened immune surveillance, or a reduced response threshold due to persistent TLR activation by DAMPS. Subsequent increases in cellular damage and the release of free iron and hemocidins within this niche may lead to dysbiosis of the endometrial microbiome and may explain why some women do not respond to treatment. Characterization of the endometrial microbiome in women with normal menstrual cycles and in those with dysfunctional menstrual bleeding may elucidate redundancy within the microbiome and identify targets for restoring eubiosis.

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