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Immunology of Uterine and Vaginal Mucosae

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Abstract

Along with maintaining symbiotic mutualism with commensal microbes and protection against invasive infections common to all mucosal barrier tissues, female reproductive tissues have additional unique tasks that include dynamic cyclic cellular turnover in menstruation and immunological tolerance to genetically foreign fetal antigens in pregnancy. Herein, we review current knowledge on distinct features of the immune cells in the female reproductive tissue with regards to antimicrobial host defense and adaptations to accommodate the fetus during pregnancy. Outstanding areas for future research to shed new functional insights on this enigmatic mucosal barrier is also highlighted.

The unique female reproductive mucosa

Mucosal barrier tissues harbor specialized features to protect against infection and other noxious environmental insults while simultaneously maintaining symbiotic mutualism with commensal microbes. These **tissue-resident immune cells** (see **Glossary**) and their effector mechanisms have been extensively characterized in the intestine and respiratory tissues. However, female reproductive mucosal tissues are unique in that they also require adaptation to specialized physiological situations such as menstruation, fertilization and parturition. Reproductive mucosal tissues are also crucial for nourishing and maintaining tolerance to the **semi-allogeneic** fetus during pregnancy. The upper female reproductive mucosa, the

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uterus and endocervix, is lined with **columnar epithelial cells**, whereas the lower portion, the ectocervix and vagina, is lined with stratified **squamous epithelial cells** (Figure 1). Epithelial cells in both upper and lower female reproductive tissue sit apical to a bed of stromal cells that interact with the epithelial cells to regulate their differentiation and function. During pregnancy, the inner lining of the uterus, the **endometrium**, transforms into the **decidua**, or physical interface between genetically foreign maternal and fetal-origin tissues. The immunology of the placenta is the topic of another review and not discussed here.

Cervicovaginal mucosa

Along with protecting against injury that preserves fertility, the cervicovaginal mucosa is also an attractive site for vaccines that induce immunity against urogenital pathogens [1, 2] and immune modulatory therapies that promote tolerance to dietary and paternal antigens [3, 4]. To this end, the specialized functions of local immune cells in the cervicovaginal mucosa need to be understood more precisely to fine-tune their activation in specific antigenic or pathogenic contexts.

Antigen-presenting cells in the vaginal mucosa

Within the human vaginal mucosa, 4 unique myeloid-derived antigen-presenting cells (APCs) subsets have been characterized: cervicovaginal Langerhans cells (cvLCs), CD14⁺ dendritic cells (CD14⁺ DCs), CD14⁻ DCs and CD14⁺ macrophages [5] (Table 1). LCs were initially identified in the epidermis (epLCs) as APCs that express the C-type lectin molecule Langerin and can migrate to draining lymph nodes to present antigens and activate or tolerize T cells [6]. The ontogeny and function of cvLCs are much less understood than that of epLCs. cvLCs are located in the human cervicovaginal epithelium under steady state, whereas the other 3 vaginal APC populations are found in the subepithelial **lamina propria** [5] (Figure 1A). When co-cultured with naive CD4⁺ T cells, all 4 APC subsets can induce production of the prototypic T helper type 1 (T_H1) cytokines interferon (IFN)- γ and tumor necrosis factor (TNF), but only cvLCs and CD14⁻ DCs induce production of the T_H2 cytokines interleukin (IL)-5 and IL-13. Transcriptional profiling showed that the CD14⁻ populations (cvLCs and CD14⁻ DCs) displayed T_H2-inducing and regulatory properties, whereas the CD14⁺ APCs (CD14⁺ DCs and macrophages) exhibited signatures of innate defense and pro-inflammatory responses [7]. However, cvLCs can also have important functions in controlling viral pathogens. For examples, the Langerin molecule they express could mediate Human Immunodeficiency Virus (HIV)-1 uptake and degradation, potentially allowing cvLCs to be a barrier against HIV-1 transmission [8]. Suppression of cvLC activation was seen in patients infected with certain genotypes of Human Papilloma Virus (HPVs) [9, 10], suggesting a role for viral interference of cvLC function in evading host immunity.

In mice, although the structure of the vaginal epithelium is different from that of the human vaginal epithelium, similar vaginal mucosal APC populations distinct from those in other mouse mucosal areas are also found, and their functions have primarily been investigated after intravaginal infection with Herpes Simplex Virus-2 (HSV-2). In mice, epLCs arise from

embryonic fetal liver and yolk sac progenitors [11], self-renew locally in the skin under steady state [12], and can be replenished from blood monocytes following skin injury [13], but whether this is true for mouse cvLCs is unknown. The relationship between mouse cvLCs and Langerin⁺ DCs [14–16] is also unclear. The location of mouse cvLCs changes during the mouse **estrous cycle**, with many in the vaginal epithelium during **diestrus** and late metestrus, but very few in the **estrus** and early **metestrus** [17], implying hormonal regulation of the local cellular populations which is not well understood. Mouse vaginal DCs were shown to comprise at least 3 populations (CD11b⁺F4/80^{hi}, CD11b⁺F4/80^{int} and CD11b⁺F4/80^{lo}) with very low Langerin expression that are mostly derived from bone marrow non-monocytic precursors under steady state but can be replenished by blood Ly-6C^{hi} monocytes after vaginal HSV-2 infection [18]. It is unclear which of these vaginal DC populations migrates to the draining lymph nodes upon vaginal infection, and their relative contribution in immunity versus tolerance is also unknown. However, vaginal submucosal CD11b⁺ DCs, but not cvLCs, were found to be capable of inducing vaginal T_H1 responses to HSV-2 [17]. This echoes the aforementioned human studies [5, 7] and suggests tolerogenic functions of cvLCs.

Vaginal tissue-resident lymphocytes

There has been a resurgence of studies on reproductive mucosal tissue-resident memory CD4⁺ and CD8⁺ T (T_{RM}) cells because of their function in rapidly responding to reproductive pathogens and the activation of innate, humoral and cell-mediated immunity (Table 1). In contrast to circulating central memory (T_{CM}) and effector memory T (T_{EM}) cells that were first defined based on C-C chemokine receptor 7 (CCR7) expression [19], CD69 and α_E integrin (CD103) were originally used to define T_{RM} cells [20, 21], but T_{RM} cells lacking one or both markers were also found in the vaginal mucosa [22]. Parabiosis experiments, which allow functional identification of T_{RM} cells based on their lack of recirculation, showed that most memory CD8⁺ T cells in mouse non-lymphoid tissues (NLTs), including the vagina, were T_{RM} cells [22]. In a mouse model of vaginal HSV infection, CD8⁺ T_{RM} cells offered much more potent and long-lasting protection than circulating memory T cells [23]. When vaginal CD8⁺ T_{RM} cells recognize their antigens and are reactivated, they release IFN- γ to evoke vaginal tissue inflammation and chemokine expression, which rapidly activates local innate, cellular and humoral immunity, including DC maturation, NK and endothelial cell activation [24], and recruits circulating B cells and memory CD8⁺ T cells to the vagina [25]. CD4⁺ T_H cell entry into the vaginal tissue was required early in this process to provide cytokines such as IFN- γ and chemokines to facilitate circulating memory CD8⁺ T cell entry into the vaginal tissue [26], and a preexisting pool of vaginal CD4⁺ T_{RM} cells maintained by chemokines from local macrophages was required for full protection [27]. These findings demonstrate the coordinated actions of CD4⁺ T_{RM} cells primed by initial antigen exposure and CD8⁺ T_{RM} cells in subsequent infections in the protection against microbial infection in the vaginal mucosa (Figure 1A), and highlight the necessity of establishing vaginal T_{RM} cells by vaccines for achieving effective local protective immunity in the lower female genital tissues [28, 29].

Besides T_{RM} cells, multiple other lymphocyte populations known to establish long-term residence in other mucosal areas have been described in the vaginal mucosa. These include innate lymphocytes, such as NK and helper innate lymphoid cells (ILCs) and B cells (Figure 1A and Table 1). Human vaginal NK cells exhibit a phenotype largely resembling that of peripheral blood NK cells [30], implying a function in innate mucosal defense. Plasma cells in the human vaginal mucosa contribute to protection by secreting IgM, IgG and IgA, with IgG being more abundant than IgA, a feature distinct from that of other mucosal areas. Interestingly, in the female reproductive tract of both rodents and humans, IgG and IgA secretion appears to be hormonally regulated [31]. ILC1s in mouse vaginal mucosa were recently found to inhibit cytotoxic CD8⁺ T cells after local vaccination [32], suggesting their unique regulatory functions in this tissue. In order for vaccines to achieve optimal local protection, research efforts should be devoted to understanding the ontogeny of these vaginal immune cells, including whether they undergo renewal, differentiation and activation *in situ* or rely on blood cell input in steady state and during mucosal infection.

The cervical mucosa

The cervix, which connects the vagina and uterus, consists of the upper endocervix and the lower ectocervix (Figure 1B). The endocervix is lined with **columnar epithelial cells**, like the uterus, whereas the ectocervix is lined with stratified **squamous epithelial cells**, similarly to the vagina. In the human ectocervix, nearly one third of all CD45⁺ mononuclear cells were T cells, and they are capable of forming **lymphoid aggregates** that have been frequently observed in the ectocervix [33]. Approximately 40% of the cervical T cells are CD4⁺ T cells, the majority of which exhibited an effector memory or effector phenotype, and the other 60% being CD8⁺ T cells, most of which expressed an effector phenotype, which is consistent with the cervix being an effector site of cell-mediated immunity and the tissue residency of cervical CD4⁺ and CD8⁺ T cells. NK and B cells were also found, albeit much less prevalent than T cells. Both T and B cells were more abundant in the ectocervix than the endocervix, thereby positively correlating with the microbial load, and the numbers of CD8⁺ T, B and NK cells decrease after menopause [33], suggesting hormonal regulation of cervical immune cells in humans. Myeloid APCs are also prevalent in both the human ectocervix and endocervix. Within the APC compartment, CD14⁺ cells are the most abundant, with the largest populations being CD11c[−] macrophages, followed by CD11c⁺ cells which may encompass tissue DCs and/or monocytes that have not yet fully differentiated into macrophages [33]. Conventional DC subsets were also detected within the CD14[−] subset of APCs; however, their abundance, although greater than in the peripheral blood, was much lower than that of the CD14⁺ cells. Despite the phenotypic characterization of these human cervical immune cells, the regulation of their differentiation and functions by systemic and local immune signals remain to be studied.

Uterine mucosa

Uterine mucosa in non-pregnant state

The **endometrium**, or inner lining of the uterus, is unique compared to many other mucosal surfaces in that it is highly responsive to reproductive hormones, and promotes immune defense as well as tissue remodeling associated with cyclic cellular shedding and

regeneration. For example, endometrial cells in non-pregnant mice can produce IFN- ϵ , an IFN unique to the reproductive tract, to confer protection against HSV2 and *Chlamydia muridarum* infection [34]. The expression of IFN- ϵ is significantly reduced during pregnancy, but partially restored in late gestation [34]. Interestingly, endometrial expression of IFN- ϵ is independent of stimulation from microbial pattern recognition receptors (PRRs) [34]. The human endometrium contains lymphoid aggregates composed of a B cell core surrounded by CD8⁺ T cells with an outer circle of macrophages, which are fully formed during the secretory phase of the menstrual cycle [35] (Figure 1C). Although these endometrial lymphoid aggregates share organizational similarity to **isolated lymphoid follicles** (ILFs) and **Peyer's patches** in the intestinal mucosa, their biological function remains unclear and the phenotype of the B cells, CD8⁺ T cells and macrophages in them are poorly characterized. For example, CD8⁺ T cells in uterine lymphoid aggregates are not cytotoxic after *in vitro* activation, in contrast to CD8⁺ T_{RM} cells in the vaginal mucosa [35]. We speculate that these cells confer immune protection by scavenging pathogens and limiting their systemic spread during menstruation when the endometrium is disrupted and the host is susceptible to infection. The lack of cytotoxic function of lymphoid aggregate CD8⁺ T cells in the secretory phase is likely controlled by reproductive hormones and could have a role in avoiding blastocyst rejection as the time coincides with the receptive window of implantation.

In addition to lymphoid aggregates, the human endometrium contains macrophages, neutrophils and NK cells. Each of these leukocyte subsets undergoes dynamic quantitative and qualitative shifts during the menstrual cycle, suggesting specialized functions with regards to endometrium degradation and repair besides uterine immune defense (Figure 1C and Table 1). Tissue macrophages have been broadly categorized into 2 subsets, M1 and M2, based on their functional characteristics. M1 macrophages are seen as being inflammatory and M2 macrophages considered to be less inflammatory and pro-wound healing [36]. Macrophages within the human endometrium produce angiogenic factors as well as pro- and anti-inflammatory mediators, characteristic of a subtype of M2 macrophage [37]. During menstruation, endometrial macrophages upregulate molecules of cell adhesion and alternative activation, such as intercellular adhesion molecule 1 (ICAM-1) and CD71 [38], suggesting a role in endometrium repair after the secretory phase. Consistently, an influx of monocytes and monocyte-derived macrophages into the endometrium to regions of tissue breakdown and repair was found during menstruation [39]. Similar to macrophages, neutrophils that infiltrate into the human endometrium during the proliferative phase may assist in the breakdown of endometrial tissues by releasing proteases [40, 41]; they also express vascular endothelial growth factor (VEGF) that can promote endometrium regeneration [42]. Despite these dynamic and qualitative cellular differences, the exact role uterine macrophages and neutrophils play and the effector molecules they release in the uterine cycle remain largely undefined.

NK cells are a major endometrial leukocyte population in humans, and endometrial NK cells are phenotypically distinct from their blood counterpart [43]. The presence of immature NK cells in the human uterine mucosa, suggests the uterus as a site of NK cell residency and development [44]. NK cells undergo proliferation and granulation during the secretory phase

of the menstrual cycle. If fertilization occurs, they continue to proliferate, or otherwise die prior to menstruation and are shed with the menses [45]. These findings are consistent with uterine NK (uNK) cells being a crucial source of angiogenic and chemotactic factors for tissue remodeling in menstrual cycles, and for trophoblast invasion and placentation in early pregnancy [46] (discussed below). Nonetheless, even with the results of these recent studies, the immunological signals that regulate the maintenance of these uterine immune cells and their crosstalk with uterine epithelial and stromal cells, as well as the hormonal signals controlling their behaviors in the menstrual cycle remain to be delineated.

Uterine mucosa during pregnancy

Most of the studies on pregnant uterine mucosa have been directed towards understanding the function of each uterine immune cell type during implantation, decidualization, placentation, fetal tolerance and parturition. Particularly, fetal tolerance has been investigated for decades, yet the immunological pathways that prevent maternal immune cells from rejecting genetically foreign tissues of fetal origin remain to be fully elucidated. While some of the immune homeostatic mechanisms in other mucosal surfaces are found to be in play at the maternal-fetal interface, many unique mechanisms exist in the pregnant uterine mucosa to mediate fetal tolerance. After ovulation and successful fertilization and implantation, the uterine endometrium undergoes hypertrophic and vascular transformation into the **decidua**, the interface whereby maternal tissues and fetal trophoblasts physically approximate. The human decidua consists of multiple histologically distinct layers that includes the **decidua basalis** (Figure 2A), **decidua capsularis** and **decidua parietalis**. These latter two layers fuse by mid-gestation to form the **choriodecidua** (Figure 2B).

The negative regulation of decidual cells with potential cytotoxic functions, mainly cytotoxic ILCs (decidual NK (dNK) cells) and CD8⁺ T cells, is crucial for preventing fetal rejection [48]. dNK cells are abundant in early human decidua basalis [43], with the vast majority being CD16⁺CD56^{hi}, in contrast to pbNK cells which are predominantly CD16⁺CD56^{lo} [43]. dNK cells contain cytotoxic granules [49, 50], but, unlike pbNK cells, are only weakly cytolytic to cancer cell lines and do not lyse first-trimester trophoblasts *in vitro* [51, 52]. Instead, dNK cells could dampen maternal anti-fetal T_H17 response via IFN- γ and perhaps also IL-10 and IL-1 receptor antagonist (IL-1RA), and their expression of IFN- γ , IL-10 and IL-1RA was reduced in patients with recurrent abortion [53]. The cytotoxic function of dNK cells is controlled by invading extravillous **trophoblasts** (EVTs) uniquely present in the pregnant uterine mucosa which express non-classical MHC-I molecules such as HLA-E, HLA-F and HLA-G. In turn, cell intrinsic stimulation through these receptors is believed to engage inhibitory receptors on dNK cells to promote their inhibition [54, 55]. Decidual DCs may also negatively regulate dNK cell cytotoxicity via HLA-G and IL-10 [56]. The bi-directional crosstalk of dNK cells with decidual DCs and trophoblasts further promotes the pro-angiogenic function of dNK cells via stimulating dNK cell production of chemotactic and angiogenic factors, including VEGF and IFN- γ [46, 54, 57–61]. Consistently, mouse dNK cells accumulate in the decidua in early gestation, and at mid-gestation, are found near the spiral arteries and express VEGF [57]. Therefore, multiple decidual immune interactions redirect dNK cells from a cytotoxic fate to more regulatory or pro-remodeling functions optimized for placentation and fetal tolerance (this scenario is also true for decidual

macrophages as discussed later). Related helper ILCs, including ILC1s and two subtypes of ILC3s, namely lymphoid tissue inducer (LTi)-like ILC3 and NKp44 natural cytotoxicity receptor (NCR)⁺ ILC3, were also found in human first trimester decidua [62]. Decidual ILC1s produced IFN- γ and may promote vascular remodeling similarly as dNK cells, whereas decidual LTi-like ILC3s produced IL-17 and TNF, and NCR⁺ ILC3s produced IL-22 and IL-8, which could contribute to uterine defense [62]. ILC2s in human late gestation choriodecidua have not been reported, and we speculate that ILC2s, if existent in early and/or mid-gestation, might help to maintain fetal tolerance.

Similar to dNK cells, the cytotoxic effector function of maternal CD8⁺ T cells against the semi-allogeneic fetus must also be contained. Mouse studies indicated that fetal alloantigens are mostly presented by maternal APCs in draining lymph nodes, rather than by fetal tissues, to maternal CD8⁺ T cells, a process that commences only in mid-gestation [63]. This both prevents maternal CD8⁺ T cell activation during the critical window of fetal acceptance in early pregnancy and avoids direct recognition of fetal tissues. Fetus-specific maternal CD8⁺ T cells failed to differentiate into cytotoxic effector cells and, instead, largely underwent clonal deletion after antigen presentation [63]. Moreover, decidua-resident DCs are trapped within the decidua due to the downregulation of the lymph node-directing chemokine C-C chemokine ligand 21 (CCL21) [64], and effector CD8⁺ T cells are excluded from the decidua due to epigenetic silencing of effector T_H1/T_C1 cell chemokines C-X-C chemokine ligand 9 (CXCL9), CXCL10 and CCL5 [65], thereby limiting fetus-specific T cell activation at the inductive site and access to the effector site. However, discrepant findings were obtained in human pregnancy, showing fetus-specific CD8⁺ T cells were not deleted and retain cytotoxicity and continue to proliferate throughout pregnancy [66]. Effector memory CD8⁺ T cells were also found in human late gestation decidua, albeit with reduced expression of cytolytic molecules [67]. Activation and decidual trafficking of maternal CD8⁺ T cells with fetal specificity are also actively suppressed by maternal CD4⁺ Tregs. In mice, maternal Tregs are induced by allogeneic pregnancy and expand in mid-gestation [68–71]; they are fetus-specific [69, 70], and differentiate into memory cells that critically protect subsequent pregnancies from immune-mediated fetal rejection [70]. Because maternal Tregs were detected in the decidua [71], it remains to be determined whether they also establish uterine/decidual tissue-residence to mediate more potent immune suppression in the decidua compared with the draining lymph nodes. Other T cell subtypes, including $\gamma\delta$ T cells [72–74], NKT cells [75] and CD4[−]CD8[−] $\alpha\beta$ T cells distinct from V α 24⁺ type-I NKT cells [72], were also found in pregnant human decidua and proposed to have tolerogenic roles, but their regulation and exact function have not been carefully explored.

Decidual macrophages have long been suggested to promote vascular remodeling in early pregnancy by expressing cytokines, chemokines, proteases and angiogenic factors [76]. Human decidual macrophages consist of a CD11c^{hi} subset that promotes antigen presentation and immune regulation and a CD11c^{lo} subset which predominantly assists in building the extracellular matrix and cellular networks [77]. Both subtypes constitutively express the anti-inflammatory cytokines IL-10 and TGF- β as well as pro-inflammatory cytokines such as IL-1 β , IL-6, MIP-1 β , and TNF. This mixed expression pattern of anti- and pro-inflammatory cytokines allows decidual macrophages to promote fetal tolerance as well as facilitate vascular remodeling in the first trimester of pregnancy. During the growth phase

of pregnancy, a more anti-inflammatory state is induced in these cells [78], indicating a functional shift to the maintenance of fetal tolerance during this period. Besides macrophages, decidual neutrophils with an alternative phenotype and function to peripheral blood neutrophils were recently found in human second-trimester decidua. These cells expressed pro-angiogenic and immunoregulatory molecules in the decidua, such as VEGF, CCL2 and arginase-1, [79], and progesterone and estrogen could convert peripheral blood neutrophils into those capable of inducing Tregs and angiogenesis [80]. These findings are reminiscent of the B-helper neutrophils we identified in the marginal zone of the human spleen [81], and suggest additional heterogeneity, regulation and function of neutrophils.

A role of B cells in pregnancy has been suggested by the observations on the changes in the circulating B cell compartment in pregnancy [82–84] and the association of B cell dysfunctions with **preeclampsia** [85, 86]. Many studies concluded that B cells and plasma cells are rare in the pregnant human decidua under physiological conditions [87, 88], and some studies regarded them as absent and not important [89, 90]. However, B cells are also not the most abundant leukocyte in the blood. Our work recently revealed diverse B cell subpopulations, including B-1 cells and plasma cells, in human late-gestation choriodecidua [91]. These choriodecidual B cells could support local immune defense, promote tissue integrity, or might mediate vascular protection by secreting antibodies, including natural IgM, and the dysregulation in these events in the decidua can directly cause pregnancy complications. Consistently, choriodecidual B cells are phenotypically and functionally altered in spontaneous human **preterm labor** [91]. In addition, choriodecidual B cells exhibited a phenotype associated with tissue residence and memory, and are a significant source of progesterone-induced blocking factor 1 (PIBF1), which could protect against inflammation-induced preterm labor in mice by suppressing uterine inflammation. Consistently, a lower serum PIBF1 level in late pregnancy was associated with an increased risk of preterm birth in humans [92]. Choriodecidual B cells induced PIBF1 expression in late gestation in response to IL-33, a mucosal alarmin that can signify tissue stress, and their IL-33 responsiveness and PIBF1 expression were reduced in preterm labor patients [91]. These findings provide insights into the heterogeneity, regulation and new effector functions of mucosal B cells, particularly their role in promoting tissue homeostasis by responding to local tissue stress signals.

Reproductive mucosal microbiota

Like at other mucosal surfaces, the microbiota is an integral part of the female reproductive mucosa and is important to gynecologic and obstetric health. The composition of a “healthy” human vaginal microbiota appears to be dominated by *Lactobacillus*, with moderate amounts of *Atopobium*, *Prevotella*, *Bifidobacterium* and *Firmicutes* [93, 94], some of which can also be found in other mucosal areas, such as the intestinal and oral mucosae [95]. Vaginal microbial **dysbiosis**, often a reduction in *Lactobacillus* spp., has been associated with gynecological and obstetric disorders, such as bacterial vaginosis, vaginal candidiasis, sexually transmitted infection [96] and preterm birth [97, 98]. A plethora of immunological knowledge is available on how host–microbiota mutualism is maintained in the intestinal mucosa, including the induction of effector and regulatory immune cells by specific microbial metabolites [99]. Whether some or all those mechanisms are in place in the

cervicovaginal mucosa is not yet definitively shown, and much further research is required to fully delineate the cross-regulation of the female cervicovaginal microbiome and the local immune system.

Recently studies have suggested the presence of a placental microbiota in normal pregnancy [100, 101] and its alterations in preterm birth [102, 103]. However, such a finding remains controversial as additional studies employing negative control samples have argued against the existence of a normal placental microbiota [104, 105]. Additionally, many genera of bacteria in the non-pregnant human uterus have also been found [106]. A significant fraction of the non-pregnant uterine microbiota consists of *Lactobacillus* spp., which is similar to the vaginal mucosa. The metabolic profiles of the uterine bacteria changed throughout the menstrual cycle. The functions of these microbes in uterine mucosal immune homeostasis in non-pregnant and pregnant states remain to be studied.

Concluding remarks and future directions

Recent advances in our understanding of the complex cellular dynamics in female reproductive mucosal tissues have suggested that precise changes take place to ensure both surveillance against potential invasive pathogens and tolerance to the commensal microbiota and the fetus. A myriad of unique molecular mechanisms underlies the dynamic shift of mucosal behavior between immune defense and tissue remodeling in reproductive physiology, such as menstrual cycles and pregnancy. Nonetheless, establishing the identity and function of leukocytes in female reproductive tissue still lags behind that of other mucosal barrier tissues. This may reflect the more complex and challenging nature of experimentally investigating female reproductive mucosa given the drastic anatomic and physical changes associated with menstruation, and further tissue remodeling during pregnancy that accommodates at least three genetically independent entities, the mother, the fetus and the microbiota. We feel that many insights, tools and approaches can be borrowed from the studies of the intestinal and other mucosal areas, but this entails solid expertise that spans mucosal immunology and reproductive biology. In addition, technological advances, such as single-cell RNA sequencing (scRNA-Seq), mass cytometry (CyTOF), deep sequencing of bacterial 16S-rRNA and internal-transcribed spacer regions of fungal rRNA genes, metagenomics and metabonomics, should allow the major Outstanding Questions listed here that are relevant to immunological and reproductive health to be tackled with unprecedented resolution.

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Glossary

Decidua

the uterine lining resulting from the hypertrophic and vascular transformation of the endometrium during pregnancy.

Decidua basalis

the endometrium between the implanted chorionic sac and the myometrium, which develops into the maternal side of the placenta.

Decidua capsularis

the luminal surface of the embryo that encloses the embryo into the uterine cavity.

Decidua parietalis

the endometrium lining the uterine wall other than at the implantation or placental site

Choriodecidua

the decidua formed after the fusion of decidua capsularis and parietalis after mid-gestation

Dysbiosis

imbalance or maladaptation of microbial flora in mucosal areas.

Endometrium

the mucosa lining the interior surface of the uterus.

Estrous cycle

a reproductive cycle in mice consisting of 4 stages (proestrus, estrus, metestrus and diestrus) and repeating every 4–5 days in non-pregnant state.

Isolated lymphoid follicles (cryptopatches)

clusters of B cells surrounded by DCs and CD4⁺ T cells in the intestinal mucosa capable of functioning as a site for inducing IgA production.

Lamina propria

a loose connective tissue layer beneath the epithelium in mucosal areas.

Menstrual cycle

a reproductive cycle in humans that repeats on average every 28 days, with 2 components (ovarian cycle and uterine cycle) and alternating levels of reproductive hormones.

The ovarian cycle has 3 phases

follicular phase in which the ovarian follicles mature, ovulation phase in which the mature egg is released into the oviduct, and the luteal phase which corresponds to the secretory phase of the uterine cycle.

The uterine phase also has 3 phases

menstruation in which the endometrial lining is shed, proliferative phase in which the uterine lining grows and proliferates, and secretory phase in which the corpus luteum produces progesterone to facilitate blastocyst implantation into the endometrium.

Peyer's patches

lymphoid follicles beneath the follicle-associated epithelium in the small intestine that consist of T cells, B cells and other APCs and function as an inductive site of intestinal antigen sampling and antibody production

Preeclampsia

a complex pregnancy disorder characterized by hypertension and proteinuria.

Preterm labor

labor prior to 37 weeks of gestation in humans.

Semi-allogeneic

difference in major histocompatibility genes that can lead to rejection.

Tissue-resident immune cells

tissue immune cells that do not recirculate and maintain themselves locally in the tissue.

Trophoblast

an epithelial cell type of fetal origin that separates the mother and the conceptus and invades into the decidua to mediate implantation and placentation.

Uterine lymphoid aggregates

uterine immune structures consisting of a B cell core surrounded by CD8⁺ T cells with an outer circle of macrophages.

Trends Box

- The vaginal mucosa is populated by several APC populations with distinct inflammatory or tolerogenic properties.
- Resident memory T cells in the vaginal mucosa mediate rapid protection upon infection of this tissue. CD4⁺ T cell entry into the tissue facilitates the entry of circulating memory CD8⁺ T cells through the production of IFN- γ .
- Uterine immune cells are functionally adapted to the changing environment during the menstrual cycle. For example, endometrial/decidual NK cells and macrophages have mixed inflammatory, regulatory and pro-remodeling functions that are optimized for menstruation, placentation and fetal tolerance.
- The vaginal immune systems must coordinate with the local microbiota to maintain mucosal homeostasis. There is controversy surrounding the existence of a microbiota in the human placenta. Some studies found traces of microbial products in the womb; yet other studies refute these claims in favor of a sterile womb hypothesis.

Outstanding Questions

- How are the trafficking, residence, differentiation and function of female reproductive tract immune cells regulated immunologically, metabolically and hormonally? What are the unique immunological features of the female reproductive mucosa as compared to other mucosal tissues?
- How is the reproductive mucosal immune system involved in the execution of menstrual cycles and parturition? What are the mucosal immunological defects associated with or driving various gynecologic infections and obstetric complications?
- What are the molecular interactions between the vaginal immune cells and the vaginal microbiota?
- Is the human placenta sterile or harboring a microbiome under physiological conditions? What is the characteristic composition of a vaginal microbiota with prognostic value for diseases or therapeutic functions for reproductive health?

Figure 1. The immunology of the cervicovaginal and non-pregnant uterine mucosae
(A) The cervicovaginal mucosa maintains homeostasis with the vaginal microbiome, composed primarily of *Lactobacillus*, while, at the same time, surveying for pathogens. In the human cervicovaginal mucosa, 4 major myeloid APCs were described, with epithelial cvLCs and CD14⁺ LP-DCs showing gene signatures associated with tolerogenic or T_H2-inducing functions. Additionally, plasma cells participate in immune defense by secreting IgG, IgA and IgM. Most CD4⁺ and CD8⁺ T cells in the cervicovaginal mucosa are tissue-resident memory (T_{RM}) cells which can confer rapid immune protection against pathogens by producing IFN- γ and eliciting epithelial production of the chemokines CXCL9 and CXCL10 to activate the mucosal endothelium and recruit additional immune cells. **(B)** The human cervix harbors many effector and effector memory CD4⁺ and effector CD8⁺ T cells, and to a lesser extent, B cells, NK cells, DCs and monocytes/macrophages. **(C)** Many of the innate immune cell types, such as macrophages, neutrophils and NK cells, are involved in facilitating the structural and physiological changes during the menstrual cycle via the production of chemokines, proteases and angiogenic factors. Additionally, macrophages are involved in the formation of lymphoid aggregates and may participate in antigen presentation and assisting CD8⁺ T and B cell activation. The function of the lymphoid aggregates in the human uterus is currently unclear, but may be involved in antigen scavenging and antibody production. Some of the information presented in the figure was extrapolated from mouse studies.

Figure 2. The immunology of the decidua basalis and choriodecidua during pregnancy (A) dNK cells are a major immune cell type in human early decidua basalis. They crosstalk with decidual DCs and EVTs, and are diverted from a cytotoxic fate to an alternative one with the production of angiogenic and immunomodulatory factors that are optimal for placentation and fetal tolerance (a scenario also seen in decidual macrophages). dNK cells can be regulated by invading EVTs expressing MHC-I molecules, such as HLA-G, which can engage the inhibitory NK receptors. Neutrophils that are of an alternative phenotype found in this tissue could promote blood vessel remodeling by producing angiogenic factors. Additionally, Tregs are essential for the establishment and maintenance of fetal tolerance through the inhibition of effector and inflammatory cell types. (B) In addition to T cells, macrophages and neutrophils present in the human middle and late pregnancy choriodecidua, B cells recently found in this tissue can respond to tissue stress signals, such as IL-33, and, in response, secrete PIBF1 to suppress decidual inflammation and offer protection against the ensuing premature onset of labor. Tregs are also present in the choriodecidua and can inhibit inflammation and promote fetal tolerance. Some of the information presented in the figure was extrapolated from mouse studies.

Table 1

Summary of the phenotype and effector functions of the immune cells in the female vaginal and uterine mucosae.

Cells	Vaginal mucosa	Uterine mucosa		Ref.
		Non-Pregnant (endometrium)	Pregnant (decidua)	
Macrophage	CD11c ^{lo} CD86 ⁺ CD163 ⁺ → Antigen Presentation, T _H 1 polarization	M1: CD14 ⁺ CD68 ⁺ CD80 ⁺ CD86 ⁺ MHC-II ⁺ → Inflammation, antigen clearance (TNF, IL-1β, IL-6, IL-12) M2: CD163 ⁺ CD68 ⁺ → Potential role in endometrium angiogenesis repair post menstruation (TNF, IL-6, IFN-γ, IL-1RA, IL-17, IL-1β, IL-12p70, IL-10, VEGF, PDGF, FGF2, G-CSF, GM-CSF, CXCL8, MIP-1α, MIP-1β upon LPS stimulation)	CD11c ^{hi} : CD14 ⁺ CD209 ^{+/-} → Gene signature for antigen presentation, inflammation in early pregnancy (MIP-1β, IL-10, IL-6, TNF, TGF-β) CD11c ^{lo} : CD14 ⁺ CD209 ⁺ → Gene signature for tissue remodeling in early pregnancy (IL-6, TNF, TGF-β) M2: CD14 ⁺ CD209 ⁺ CCL8 ⁺ → Protects fetus from inflammatory attack (IL-10, TGF-β)	[5, 37]
DC	CD11c ⁺ HLA-DR ^{hi} (two subsets) CD14 ⁺ LP-DC → Antigen presentation, T _H 1 polarization (<i>in vitro</i>) CD14 ⁻ LP-DC → Antigen presentation, T _H 1 and T _H 2 polarization (<i>in vitro</i>)	MHC-II ⁺ CD11c ⁺ CD103 ^{+/-} (mouse) → antigen presentation	CD14 ⁺ CD16 ⁺ CD83 ⁺ (tolerogenic DC ₁₀ also express HLA-G) → Antigen presentation, NK cell regulation (IL-15, IL-10 (DC ₁₀))	[5, 64, 107]
LC	Langerin ⁺ CD1a ⁺ CD11c ⁺ CD86 ⁺ CD83 ⁺ CD324 ⁺ C CR4 ⁺ CCR6 ⁺ CX3CR1 ⁺ → Antigen presentation, T _H 2 polarization, immune tolerance, viral clearance via CD8 ⁺ T cell activation	N/A	N/A	[5]
NK cell	?	Stage 3 immature NK cell (NK committed): CD56 ⁺ CD34 ⁻ CD117 ⁺ CD94 ⁻ CD161 ⁺ → Migrate from blood and mature into uNK cells in uterus (IL-22) Stage 4 mature NK cell: CD56 ⁺ CD34 ⁻ CD117 ^{+/-} CD94 ⁺ CD161 ⁺ → Proliferate and granulate after ovulation; shed during menstruation if no fertilization	CD56 ⁺ CD16 ⁻ → Vascular remodeling, T _H 17 inhibition (VEGF, PlGF, NKG5, IL-8, IP-10, CCL5, IFN-γ, IL-10, TNF, Perforin, Granzyme A, TIA-1)	[44, 46, 49, 57–59]
Plasma cell	CD19 ⁺ CD38 ^{hi} CD27 ^{hi} → antibody secretion (IgG > IgA > IgM)	?	CD19 ⁺ CD20 ⁻ CD38 ^{hi} CD27 ^{hi} → antibody production	[31]
CD8 T cell	CD3 ⁺ CD8 ⁺ (mouse) → Pathogen control via inflammation and cytotoxicity. Also exhibit a tissue-resident memory phenotype to protect from	CD3 ⁺ CD8 ⁺ → Participation in lymphoid aggregates	CD3 ⁺ CD8 ⁺ CCR7 ⁻ CD45RA ⁻ (mouse) → majority in the decidua are T _{RM} cells; promote rapid immune defense (Perforin, Granzyme, IFN-γ)	[5, 25, 28, 29]

Cells	Vaginal mucosa	Uterine mucosa		Ref.
		Non-Pregnant (endometrium)	Pregnant (decidua)	
	subsequent infections (IFN- γ , TNF)			
CD4 ⁺ T cell	CD3 ⁺ CD4 ⁺ T _H 1: T-bet ⁺ → Inflammation, macrophage activation (IFN- γ , TNF) T _H 2: GATA3 ⁺ → Humoral immunity (IL-4, IL-5, IL-13) T _H 17: ROR γ t ⁺ → Inflammation, fungal/microbiota control (IL-17, IL-22) Treg: Foxp3 ⁺ → Immune suppression/tolerance (IL-10, TGF- β , IL-35)	CD3 ⁺ CD4 ⁺	Treg: Foxp3 ⁺ → promote fetal tolerance and suppression of effector T cells (IL-10, TGF- β) T _H 2: GATA3 ⁺ → Anti-inflammatory in contrast to Th1 (IL-4, IL-5, IL-13)	[27, 69, 70, 108]
CD4 ⁺ CD8 ⁻ T cell	?	?	CD3 ⁺ CD4 ⁺ CD8 ⁻ → ?	[72]
B Cell	IgG, IgA, IgM → Antibody production	CD19 ⁺ CD20 ⁺ → Participation in lymphoid aggregates; antibody production?	CD19 ⁺ CD20 ⁺ → Protection against preterm labor; response to uterine stress (PIBF1)	[91]
Lymphoid aggregate	?	B cell core surrounded by CD8 ⁺ T cells and macrophages → Unknown function, possibly antibody production and pathogen scavenging in menstrual cycles	?	[35]
EVT	N/A	N/A	CD45 ⁺ HLA-G ⁺ HLA-C ⁺ → Invade into the decidua for artery remodeling and dNK cell regulation	[54, 61]

Shaded boxes contain data from mouse studies.