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Immune responses in the human female reproductive tract

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Summary

Mucosal surfaces are key interfaces between the host and its environment, but also constitute ports of entry for numerous pathogens. The gut and lung mucosae act as points of nutrient and gas exchange, respectively, but the physiological purpose of the female reproductive tract (FRT) is to allow implantation and development of the fetus. Our understanding of immune responses in the FRT has traditionally lagged behind our grasp of the situation at other mucosal sites, but recently reproductive immunologists have begun to make rapid progress in this challenging area. Here, we review current knowledge of immune responses in the human FRT and their heterogeneity within and between compartments. In the commensal-rich vagina, the immune system must allow the growth of beneficial microbes, whereas the key challenge in the uterus is allowing the growth of the semi-allogeneic fetus. In both compartments, these objectives must be balanced with the need to eliminate pathogens. Our developing understanding of immune responses in the FRT will help us develop interventions to prevent the spread of sexually transmitted diseases and to improve outcomes of pregnancy for mothers and babies.

Keywords: ILC; macrophages; mucosa; T-cells; uterus.

Introduction

Mucosal barriers are critical interfaces between the host and the environment. Over the past decades, much effort has been dedicated to understanding immune protection in the intestinal and pulmonary mucosae, but our grasp of immune mechanisms in the female reproductive tract (FRT) has lagged behind. Although this partly reflects a climate in which research into women's health has been underfunded,[1] it is also a result of the not inconsiderable difficulties of studying the FRT.

The immune system encounters vastly different challenges in the various compartments of the FRT, and as a result differs between the lower (vagina and ectocervix) and upper (uterus and endocervix) FRT. Like the gut, the vagina is populated by a commensal flora, and here the immune system must allow the growth of beneficial microbes while preventing that of pathogens. The cervix acts as a gatekeeper, preventing the entry of microbes into the uterus, while permitting the passage of sperm. Finally, the immune system in the uterus faces the challenge of allowing the fetus, which is immunologically distinct from its mother, to co-exist with her for 9 months, while simultaneously eliminating any pathogens that may enter. Every site within the FRT must mediate a fine balance between protection from pathogens and maintenance of tissue integrity and function, allowing fertilization, implantation and pregnancy to occur. The two are not necessarily at

Abbreviations: APC, antigen presenting cell; EVT, extravillous trophoblast; FRT, female reproductive tract; HCMV, human cytomegalovirus; HIV, human immunodeficiency virus; HPV, human papilloma virus; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; KIR, killer immunoglobulin-like receptor; LC, Langerhans cells; LP, lamina propria; MAIT, mucosal-associated invariant T cell; NK, natural killer; TGF, transforming growth factor; TNF, tumour necrosis factor; VT, villous trophoblast

odds; however, because infections are among the common causes of infertility[2] and late pregnancy failure.[3]

Another challenge of studying the FRT is the additional complexity of hormone-driven alterations over the course of the menstrual cycle. It has long been appreciated that the uterine mucosa and cervix change over the cycle, allowing sperm to enter the uterus at roughly the time of ovulation, and the conceptus to implant about 9 days later. The uterine immune system, too, changes with the menstrual cycle and pregnancy. It is now coming to be appreciated that cyclical changes also occur in the vagina, and that these could have an impact on susceptibility to disease.

The vagina

Physical and chemical barriers in the lower reproductive tract

In contrast with the upper reproductive tract, which is lined by a monolayer of columnar epithelial cells, the ectocervix and vagina are lined by protective layers of non-keratinized stratified squamous epithelium. In addition to the physical barrier that a stratified epithelium constitutes, chemical and biological barriers form a first line of defence, with mucus and antimicrobial peptides protecting the vagina from pathogens.[4,5] The epithelial layer may also play a role in the success of human immunodeficiency virus (HIV) containment using antiretroviral therapy, as FRT epithelial cells, as well as the underlying fibroblasts, can deliver and store antiretrovirals, promoting sustained protection of vaginal $CD4^+$ T-cells from viral infection.[6]

Another contributor to protection from pathogens in the lower reproductive tract is the population of commensal bacteria. The human vaginal microbiome can be classified into five core microbial communities, of which four are dominated by species belonging to the genus *Lactobacillus*, and one is characterized by higher levels of strict anaerobes, including *Prevotella, Gardnerella, Dialister* and *Atopobium*.[7–9] The abundance of the latter microbial community is higher among Black and Hispanic populations[9] and individuals displaying a microbiome characterized by dominant strict anaerobes other than *Gardnerella* display higher vaginal inflammation and an increased susceptibility to HIV-1 infection.[10,11] Nevertheless, the beneficial effects of specific bacterial species in protecting the FRT are well established. In particular, lactobacilli produce lactic acid, establishing an acidic environment that limits colonization by other microorganisms.[12] Lactobacilli also produce bacteriostatic compounds,[13] compete with opportunistic pathogens for attachment to the vaginal epithelium[14] and secrete antimicrobial peptides.[15] Overall, the vaginal microbiome has, over the past decade, emerged as a critical modulator of inflammation in the reproductive tract, and the full extent of its impact on susceptibility versus protection against infection is an active area of research.

At the cervix, mucus serves as a physical barrier, changing its consistency over the course of the menstrual cycle to allow the passage of sperm at ovulation.[16] Cervical mucus also contains immune mediators, including antibodies, complement and cytokines.[17,18] In addition to these barriers, the lower FRT is populated by immune cells, which participate in its protection and regulation. The barrier and immune mechanisms protecting the reproductive tract are depicted in Fig. 1.

Myeloid cells in the vagina

The lower FRT is populated by a variety of immune cells, which constitute between 6% and 20% of all cells and protect against invading pathogens.[19] Whilst immune cell composition in the upper FRT changes over the menstrual cycle in response to hormonal changes, it does not seem to fluctuate in the lower FRT.[20] However, there is emerging evidence that immune function in this region, and thus disease susceptibility, may vary across the menstrual cycle.[21,22]

Four main subsets of antigen-presenting cells (APCs) are present in the lower FRT: (i) intraepithelial Langerhans cells (LCs); (ii) lamina propria (LP) $CD14^-$ dendritic cells (DCs); (iii) $CD14^+$ DCs; and (iv) macrophages. These comprise between 10% and 50% of leucocytes in the lower FRT. The vaginal mucosa does not contain mucosal-associated lymphoid tissue so priming of adaptive responses takes place in draining lymph nodes. Upon infection, APCs are mobilized to the draining lymph nodes to prime naïve T-cells. Functional and transcriptomic analysis indicates specialization among FRT myeloid populations.[23] LP $CD14^-$ DCs and LCs are skewed towards Th2 cell activation and regulatory functions.[24] In contrast, $CD14^+$ DCs and macrophages resemble classical innate cells, which respond to pathogen-derived molecules via TLRs and contribute to priming of Th1 responses.[24]

Further immune cell populations can populate the reproductive tract during an infectious challenge, and their protective roles in the context of HIV are beginning to emerge. Neutrophils were recently shown to release neutrophil extracellular traps in response to co-culture with HIV viral-like particles, contributing to viral inactivation.[25] While myeloid cells serve critical functions in the surveillance of the FRT, inflammation may also increase susceptibility to HIV infection. LCs and $CD14^+$ DCs support HIV-1 infection,[26] and HIV-1 DNA has been detected in LCs and $CD14^+$ cells isolated from HIV-infected women.[27,28] Critically, HIV-1 was detected in immune cells in individuals starting antiretroviral therapy as early as 10 days after the onset of symptoms of primary infection, and replicative virus was detectable in immune cells despite undetectable blood viremia.[29] Therefore, targeting mucosal immune cells may be key to achieving sterilizing immunity to HIV.

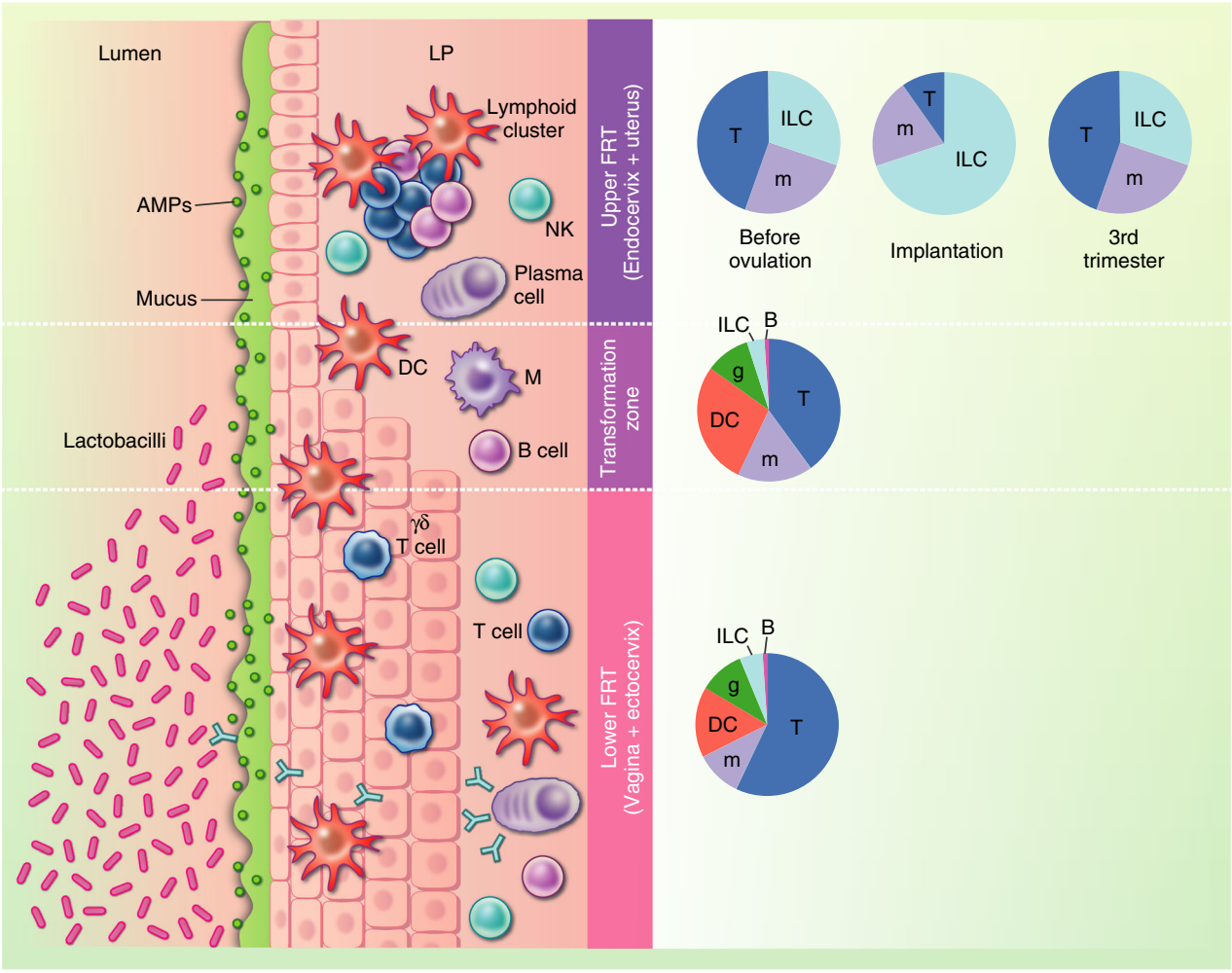

Figure 1. Immune and non-immune barriers in the female reproductive tract (FRT). The FRT can be broadly divided into an upper section, comprising the uterus and the endocervix, and a lower section, which includes the vagina and ectocervix. The upper reproductive tract is lined by a single layer of columnar epithelium, while the lower reproductive tract is lined by stratified squamous epithelium. The zone where the two types of epithelia meet is called the transformation zone. Several non-immune barriers form a first line of defence against pathogen invasion: the presence of tight junctions constitutes a physical barrier, mucus and antimicrobial peptides form a chemical barrier, and the *Lactobacillus*-rich vaginal milieu creates a biological barrier. This multi-layered defence strategy is further reinforced by a variety of immune cells that reside within the epithelium and the lamina propria (LP), patrolling for invading microorganisms. Pie charts indicate the composition of immune cells along the FRT. In the upper FRT, the composition differs by stage of the menstrual cycle or pregnancy where 'Implantation' represents the composition in the secretory phase of the menstrual cycle and early pregnancy. AMPs, antimicrobial peptides; DC, dendritic cell; FRT, female reproductive tract; g, granulocyte; ILC, innate lymphoid cell; LP, lamina propria; m, monocyte/macrophage; NK, natural killer.

Innate lymphoid cells and innate-like T-cells in the vagina

In addition to APCs, several innate and innate-like lymphocyte populations contribute to immune surveillance and protection of the lower FRT. Natural killer (NK) cells, which are members of the innate lymphoid cell (ILC) family,[30] are present in the vagina. In contrast to those present in the upper FRT, NK cells in the lower FRT resemble blood NK cells[31] and play an important role in limiting viral infections. NK-deficient individuals have an increased risk of herpes virus infection[32] and a higher incidence of cervical cancer resulting from human papilloma virus (HPV) infection.[33]

Additional innate-like lymphocyte populations, including γδ and mucosal-associated invariant T-(MAIT) cells, may play important roles in local protection against sexually transmitted infections (STIs). γδ T-cells in the FRT predominantly express a Vδ1 TCR, in contrast with the $V\gamma9V\delta2^{+}$ γδ T-cell subset predominant in peripheral blood.[34] The balance between Vδ1 and Vδ2 cells is altered during bacterial vaginosis, with endocervical Vδ2 cells expressing CD4 and CCR5, which are required for HIV cell entry, increasing in frequency.[35] This points to a

potential link between microbial dysbiosis in the FRT and HIV transmission. Female genital MAIT cells produce interleukin (IL)-17 and IL-22 in response to *Escherichia coli* stimulation, indicating a potential role in protection against bacterial infections.[36] The location of these unconventional T-cell populations at key sites of infection and cellular transformation, together with their acute sensitivity to tissue perturbation, makes them an attractive target for immune intervention. Defining the cues underlying their activation and their contributions to protection will be central to capture the complexity of protective responses within the FRT.

Classical adaptive responses in the vagina

Antigen-specific lymphocytes participate in the containment of numerous infections in the FRT, with T-cells comprising between 35% and 50% of leucocytes, and B-cells representing under 1% of all immune cells. Indeed, orchestration of a Th1 and cytotoxic T-cell response is critical for containment of *Chlamydia trachomatis*, which accounts for a third of all new STI cases worldwide.[37] Chlamydia is often asymptomatic, but 10% of women develop pelvic inflammatory disease with ascending infection often leading to lasting sequelae, including ectopic pregnancy, infertility and chronic pelvic pain.[37] The quality of the immune response elicited by *Chlamydia* correlates with pathology, with elevated levels of cervical type I interferons (IFNs) and decreased IFN-γ associated with severe ascending infection.[38]

Persistent herpes simplex virus (HSV)-2 infection is associated with formation of lymphoid clusters in the vagina and cervix of both humans and mice.[39,40] These aggregates of $CD4^+$ and $CD8^+$ T-cells, B-cells, DCs and macrophages can persist for months to years after viral clearance, potentially conferring lasting protection against reinfection. In mice, $CD8^+$ T-cells are recruited to peripheral nerve endings following HSV-2 reactivation, where they remain after viral containment.[41] Subsequent viral reactivation at sites where $CD8^+$ cells were present did not result in lesion formation, suggesting a role for local $CD8^+$ T-cells in limiting reactivation.

In the FRT, antibodies contribute to pathogen clearance through mechanisms including pathogen neutralization, opsonization and complement-driven lysis. Plasma cells secreting IgG and IgA can be detected in the LP of both the cervix and the vagina,[42] although in contrast with other mucosal sites, IgG, rather than IgA, is the predominant antibody isotype in the lower FRT.

The uterus

Immune interfaces in the uterus

In contrast to the lower FRT, commensal microbes have long been thought to be absent from the upper FRT. Some reports suggest that a small commensal population may be present in the uterus,[43,44] although a recent study that carefully controlled for the effects of contamination could find no evidence of commensals in placenta and uterine lining from healthy pregnancies.[45] However, infections of the uterus with pathogens are widely recognized, occurring both during and outside of pregnancy. This is commonly a result of ascent of bacteria from the vagina, as is the case for infection with sexually transmitted *Neisseria gonorrhoeae* or *C. trachomatis*. The immune system responds to these infections in broadly the same way that it would in any other tissue, recruiting immune cells from the blood to produce an inflammatory response which, in pregnancy, may result in miscarriage or preterm birth.[3,46]

The primary foreign cells with which immune cells in the healthy uterus interact are those of the placenta. The placenta invades through the uterine lining, so that the placental villi, which are the site of nutrient and gas exchange between mother and fetus, are bathed in the mother's blood. This means that significant numbers of circulating immune cells are present. The villi are covered in villous trophoblast (VT) cells, whereas a population of extravillous trophoblast (EVT) cells invade into and transform the spiral arteries of the uterus, allowing blood to flow to the placenta at low pressure. Both trophoblast populations are exposed to circulating maternal immune cells, and have several features to prevent their recognition and elimination by these cells.[47–50] Another interface between the placenta and the maternal immune system occurs within the uterine lining (the endometrium), which in pregnancy is called the decidua. In early pregnancy, the decidua basalis lies beneath the site of placental implantation, the decidua capsularis encloses the fetal membranes, and the decidua parietalis lines the opposite wall of the uterus. By the fourth month of pregnancy, the decidua capsularis and parietalis fuse. Studies that have compared the basalis and the parietalis have found the frequencies of immune cells to differ slightly between them,[51,52] with evidence of greater immune activation in the basalis towards term. In the decidua basalis, interstitial EVT cells encounter specialized immune cells present at this mucosal site. The three interfaces between fetal trophoblast and maternal immune cells are depicted in Fig. 2.

Before pregnancy, the frequency of immune cells in the endometrium varies over the menstrual cycle. ILCs are sparse before ovulation, in the proliferative phase of the cycle. They increase rapidly following ovulation, in the secretory phase, and at the time of implantation represent about 70% of the immune cells present.[53,54] Like ILCs, macrophage numbers increase over the course of the menstrual cycle,[55,56] whereas T-cells remain roughly constant.[53,54] If pregnancy occurs, the high frequency of ILC characteristic of the secretory phase of the menstrual cycle is maintained into the first trimester of pregnancy, with approximately 70% of decidual immune cells varieties of

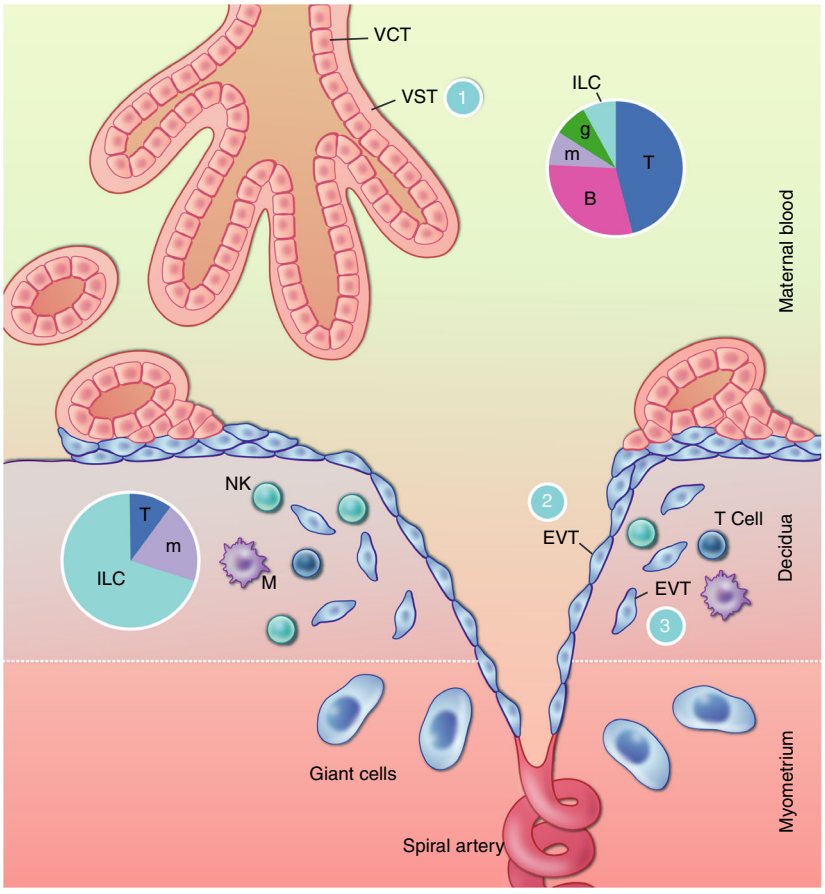

Figure 2. Interfaces between the placenta and the maternal immune system. The placental villi are bathed in the maternal blood. The outer layer of the villi is covered by VST with an underlying layer of mononuclear VCT. Villous trophoblast (VT) is protected from recognition by T-cells, which are frequent in the maternal blood, by their complete lack of MHC expression (interface 1). They are further protected by immune cell recognition by their syncytial nature and thick glycocalyx. The inner layer of VCT grows out of the villi to anchor the placenta to the maternal decidua. VCT differentiates to extravillous trophoblast (EVT), some of which migrates down the spiral arteries, replacing the endothelial cells as far as the inner third of the myometrium. This process aids in the transformation of the spiral arteries, allowing blood to flow to the placenta at low pressure. These cells are in contact with the maternal blood (interface 2). Some EVT cells are also present in the decidua, where they interface with the unique immune cells present in this microenvironment (interface 3). EVT is largely protected from T-cell recognition because they do not express the major TCR ligands HLA-A and -B, but they do express the natural killer (NK) cell ligands HLA-C, -E and G. At interface 2, this may protect EVT from recognition by blood NK cells. At interface 3, the expression of HLA-C is likely to allow recognition by decidual NK cells, which are not cytotoxic but rather seem to have a role in tissue remodelling. Pie charts indicate the composition of maternal immune cells in the blood and decidua. VCT, villous cytotrophoblast; VST, villous syncytiotrophoblast; EVT, extravillous trophoblast; T, T-cells; B, B-cells; m, monocytes/macrophages; g, granulocytes; ILC, innate lymphoid cells.

ILC. Macrophages are the next most frequent immune cell in first-trimester decidua (20%), followed by T-cells (10%).[57–59]

Some studies have been unable to locate DCs in the human decidua,[59] while others have described CD209$^+$ cells that could represent immature DCs occurring at a low frequency,[60] and evidence from mice suggests that the rarity of DCs in the decidua may be among the mechanisms preventing the initiation of classical immune responses to the placenta.[61] B-cells are also largely absent.[59] The number and frequency of ILCs declines over the course of pregnancy, although significant numbers are still detectable at term. Meanwhile, the numbers of macrophages and T-cells remain roughly constant, although the decline in ILCs means that the proportion of decidual immune cells accounted for by these cells increases.[51,60] These changes in the composition of the endometrial/decidual immune system are depicted in Fig. 1.

ILC in the uterus

The ILC family is divided into five groups: NK cells, ILC1, ILC2, ILC3 and lymphoid tissue inducer cells.[30] A large population of cells that resemble NK cells is present in the decidua, with smaller populations of ILC1 and ILC3. ILC2 are rare.[59] Unlike circulating NK cells, decidual NK cells

are not cytotoxic,[62–66] and their presence in large numbers at the time and site of implantation led to the suggestion that they might have a role promoting placentation. In support of this, decidual NK cells produce pro-angiogenic and trophoblast-chemoattractant factors, as well as factors that may promote tissue remodelling by their action on macrophages.[64,67] Most suggestively, women who have genes (KIR2DS1, KIR2DS5 or KIR2DS4) for receptors that activate NK cells when they bind to HLA-C2 expressed by fetal EVT are less likely to be affected by pre-eclampsia, fetal growth restriction and recurrent miscarriage,[72–75] pointing to the importance of decidual NK cell activation in successful pregnancy.

Single-cell RNAseq approaches have recently been used to show that decidual NK cells consist of three subpopulations, which have been called dNK1, -2 and -3.[59] Among these, dNK1 have the highest levels of killer immunoglobulin-like receptors (KIRs) and LILRB1, which recognize HLA molecules expressed on EVT. Therefore, this subset is likely to be the one that responds to EVT. dNK3 are phenotypically similar to ILC1 identified in human lymph nodes,[76] and may represent uterine ILC1. Intriguingly, dNK2 display some features that are intermediate between dNK1 and dNK3 (or ILC1), which could indicate a certain level of plasticity between the NK and ILC1 lineages within the uterus.

It is not yet clear how decidual NK cells fit into our current scheme for understanding ILC: are they NK cells, ILC1 or something else? Their low cytotoxicity suggests that they should perhaps be rebranded innate helper cells of pregnancy, rather than killers. Nonetheless, our understanding of circulating NK cells has influenced the kinds of questions that we ask about decidual NK cells. Circulating NK cells have a role in the control of HIV infection and decidual NK cells may act similarly, as they can inhibit HIV infection of decidual macrophages *in vitro*.[77–79] Circulating NK cells are also a major defence against human cytomegalovirus (HCMV), which can cross the placenta. Although they are usually not cytotoxic, decidual NK cells can kill HCMV-infected fibroblasts.[66] Circulating NK cells produce a memory-like response to HCMV,[80] and there is some evidence for a memory-like phenomenon in decidual NK cells during second pregnancies.[81] This could perhaps account for the longstanding observation that second and subsequent pregnancies are more likely to be successful.[82] However, it is not yet clear if the reported phenotype in fact arises in response to HCMV infection.[83]

In contrast to decidual NK cells, ILC3 are present at a relatively low frequency in the uterine mucosa.[59,84] They produce IL-22, which maintains homeostasis at mucosal sites,[87] and evidence from mice suggests that it may help to maintain pregnancy in the face of infection.[88] Decidual ILC3 also produce neutrophil-attractive chemokines and their ability to do this may be associated with better outcomes in early pregnancy.[71]

Macrophages in the uterus

Macrophages are the second most prominent group of immune cells in the uterine mucosa and are present in non-pregnant endometrium throughout the menstrual cycle, with their numbers climbing as menstruation begins.[55,56] They play a role in breakdown of the endometrium in early menstruation, in part by the release of matrix metalloproteinases, as well as repair of the tissue and clearance of debris during the final phase of menstruation.[55,89]

If pregnancy occurs, macrophage numbers stabilize and remain about 20% of the leucocyte population throughout pregnancy.[59] Implantation has been proposed to be an inflammatory process, shaped by macrophage cytokine production.[90] In support of this, inflammatory genes are upregulated in the endometrium during the window of implantation, although some anti-inflammatory genes, such as transforming growth factor (TGF)-β, are also overexpressed.[91] In the first trimester, this continues as macrophages produce IL-6 and IL-8, which can promote placental invasion, in response to EVT.[92,93]

Although macrophages have historically been classified as M1 and M2, with M1 displaying a pro-inflammatory and M2 a pro-repair phenotype,[94] they are now known to be highly heterogeneous and capable of specialization in different tissues,[95] throwing into question whether this is an appropriate way of classifying decidual macrophages. Indeed, in the uterus, an alternative macrophage grouping based on CD11c expression has been suggested.[96] Unbiased RNAseq approaches have also defined two populations of decidual macrophages, dM1 and dM2, whose gene expression profiles match the $CD11c^{hi}$ and $CD11c^{lo}$ profiles, respectively.[59]

Decidual macrophages may also play an important role in the initiation of childbirth, a process in which increased expression of inflammatory mediators promotes uterine contraction, delivery and placental detachment.[97] Macrophages increase in the decidua of rats prior to labour and, in humans, decidual samples from labouring women have greater numbers of macrophages compared with samples from non-labouring women.[98] Macrophages are also recruited to the cervix during ripening, a tissue remodelling process that occurs prior to birth.[99]

T-cells in the uterus

T-cells are present in non-pregnant endometrium with $CD8^+$ T-cells the major population throughout the menstrual cycle.[54] This contrasts with peripheral blood, in which the proportion of $CD4^+$ T-cells is greater than that of $CD8^+$ T-cells.[100] In early pregnancy, T-cells account for a minority of decidual immune cells, but by term just over half of the leucocytes are T-cells,[101] with most of the increase accounted for by $CD4^+$ T-cells.[60]

$CD8^+$ T-cells in early pregnancy express reduced levels of cytotoxic molecules,[102,103] a phenomenon that may occur under hormonal control.[104] They do not degranulate in response to EVT, but are nevertheless capable of activation and killing.[103,105] This may suggest that they stand ready to act as a defence should a viral infection occur, a hypothesis supported by the observation that HCMV-specific decidual $CD8^+$ T-cells expand and express increased granzyme B.[103] On the other hand, it has also been proposed that, like decidual NK cells and macrophages, decidual $CD8^+$ T-cells produce cytokines, such as IL-8 and IFN-γ, that may promote EVT invasion.[105] $CD4^+$ T-cells in the uterine lining have an effector memory phenotype and are better able to produce cytokines than their counterparts in the peripheral blood.[106,107] Their ability to produce IFN-γ decreases as pregnancy progresses, while their ability to produce IL-4 goes up.[108]

Tregs are present in the lining of the uterus at a higher frequency than in peripheral blood both before and throughout pregnancy.[109] This is likely to be a consequence of high levels of TGFβ in the decidua,[59,110] and they may also be induced by the immunomodulatory enzyme IDO produced by decidual macrophages and/or by interactions with EVT cells.[111,112] The regulatory environment is also supported by decidual γδ T-cells, which are enriched in first-trimester decidua and produce high levels of IL-10 and TGFβ.[113,114] In addition to classical $FoxP3^+$ Treg, the decidua contains two FoxP3-negative populations of $CD4^+$ T-cells, which express the regulatory molecules PD-1 or TIGIT.[112] All three of these can suppress T-cell proliferation, but they differ in their ability to impact cytokine production, with $FoxP3^+$ Tregs most effective at inhibiting IFN-γ and tumour necrosis factor (TNF-α) production, while PD-1+ $CD4^+$ T-cells promote the production of IL-10. It has been proposed that decidual Tregs may promote tolerance to the placenta, as a subset of $FoxP3^+$ Treg is reduced in decidua from miscarriages compared with healthy pregnancies.[115] However, it is difficult to determine if this represents a cause or an effect of the miscarriage. Another possibility is that Tregs in the decidua have a role in tissue repair and regeneration, as is seen in other organs.[116] This is in line with current ideas about the roles of the two other major immune cell populations in the decidua, decidual NK cells and macrophages.

A key question is whether decidual T-cells can recognize allogeneic proteins expressed by trophoblast. Such T-cells have been described in mice,[49] but in humans this has not been an easy question to address. The approaches that have most often been used to investigate decidual T-cell reactivity have looked at their responses to umbilical cord blood cells or HY antigens being presented on HLA-A or -B, but neither of these is representative of the molecules expressed by trophoblast. However, an expansion of trophoblast-specific Tregs is indirectly suggested by the finding that particular Treg clones are expanded in the decidua compared with the blood.[117] In the future, better defining the reactivity of these T-cells will be a key challenge in properly understanding the decidual immune response to trophoblast.

Perspectives and opportunities

Although our understanding of immune responses in the FRT has lagged behind our understanding of those at other mucosal surfaces, we are beginning to make progress in this area. It is an exciting time to be a reproductive immunologist.

The discovery that immune responses in the lower FRT fluctuate over the menstrual cycle has led to the proposal of a 'window of susceptibility' for sexually-transmitted diseases such as HIV[21] and chlamydia.[22] This may have an impact on public health recommendations and vaccination strategies for these diseases. Likewise, our emerging understanding that vaginal dysbiosis and inflammation may be harmful in the context of HIV transmission[35] will help shape therapeutic and preventative approaches. A better understanding of local protective immune responses will also be important in the design of interventions against emerging sexually transmitted diseases, such as multidrug-resistant *N. gonorrhoea* and Zika virus.

In the upper FRT, the development of novel multiparametric approaches means that we now have a better understanding of the diverse populations of immune cells that are present, including which are mucosal and which come from the blood.[59] This means that several questions that had been considered resolved will have to be reopened, but we have the opportunity to make rapid progress. The recent development of trophoblast[118] and endometrial[119] organoid cultures means that we will be able use *in vitro* approaches to understand how immune cells interact with these cells. This will have a significant impact on our understanding of the immunology of pregnancy, and will help in the design of interventions to improve outcomes for mothers and babies.

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Disclosure

The authors declare no competing interests.

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