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REVIEW

Type I IFNs in the female reproductive tract: The first line of defense in an ever-changing battleground

Helen E. Cumming[1] | **Nollaig M. Bourke**[2]

[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Monash University, Clayton, VIC, Australia

[2]Department of Medical Gerontology, Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland

Correspondence
Helen E. Cumming, Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Monash University, Clayton, VIC, Australia.
Email: helen.cumming@hudson.org.au

Abstract

The primary function of the female reproductive tract (FRT) is to enable successful reproduction, yet the biologic mechanisms required to accomplish this, which include fluctuating sex hormones and tolerance of semen and a semi-allogeneic fetus, can leave this unique mucosal environment susceptible to pathogenic challenge. Consequently, the FRT has evolved specialized innate and adaptive immune responses tailored to protecting itself from infection without compromising reproductive success. A family of innate immune cytokines that has emerged as important regulators of these immune responses is the type I IFNs. Type I IFNs are typically rapidly produced in response to pathogenic stimulation and are capable of sculpting pleotropic biologic effects, including immunomodulation, antiproliferative effects, and inducing antiviral and bactericidal molecules. Here, we review what is currently known about type I IFN-mediated immunity in the FRT in human, primate, and murine models and explore their importance with respect to three highly relevant FRT infections: HIV, Zika, and *Chlamydia*.

KEYWORDS

Chlamydia, female reproductive tract, HIV, hormonal regulation, mucosal immunity, Type I IFNs, Zika

1 | THE FRT: A DYNAMIC IMMUNE ENVIRONMENT

The female reproductive tract (FRT) is a site of unique mucosal immune regulation; it must be capable of detecting and inducing immune responses against pathogenic infections yet maintain tolerance to commensal bacteria, semen, and the semi-allogeneic fetus. The FRT can be broadly divided into two main anatomic sites: the lower reproductive tract, which includes the vagina and ectocervix, and the upper reproductive tract, which includes the uterus, fallopian tubes, and ovaries. The structure of the lining of the FRT varies considerably between these sites and reflects their respective functional characteristics. The lining of the lower FRT comprises multilayered squamous epithelium, which represents a large barrier defense and supports high levels of commensal microbes. In contrast, the upper FRT comprises a single layer of columnar epithelium, which is remodeled in response to sex hormones to enable support of successful implantation and pregnancy.[1] Between these two sites of the FRT is the transformation zone, where the squamous epithelium of the ectocervix meets the columnar epithelium of the endocervix. FRT immunity is predominately mediated by both epithelial cells and the immune cells that underlie the epithelium in both the upper and lower reproductive tract.

Abbreviations: AMPs, antimicrobial peptides; cGAS, cyclic GMP-AMP synthase; E2, estrogen; FRT, female reproductive tract; HAART, highly active antiretroviral therapy; IRF, IFN regulatory factor; IRG, IFN regulated gene; ISG, IFN stimulated gene; P4, progesterone; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; SHIV, simian-HIV; ZIKV, Zika virus

2 | REGULATION OF FRT IMMUNITY: SEX HORMONES

An important aspect of immunoregulation within the FRT is that the FRT immune cells are continuously modulated in response to fluctuating levels of the sex hormones estrogen (E2) and progesterone (P4) during the menstrual cycle. The first line of defense against pathogen infection is usually the epithelial cells that line the upper and lower FRT. Epithelial cells that line the vagina and uterus are important regulators of immunity in the mucosa, acting through specialized antigen presentation function and secretion of antimicrobial peptides (AMPs), chemokines and mucins, which modulate recruitment and activation of the many innate and adaptive immune cells within the FRT.[2] The

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numbers of these epithelial cells in the uterus dramatically increases during the E2 dominated proliferative phase in preparation for ovulation. Unlike other organs, the uterus contains a much higher frequency of CD8+ CTLs than CD4+ T lymphocytes and unique to the uterus, these CTLs form lymphoid aggregates with B lymphocytes.[3] The size of these lymphoid aggregates increases during secretory phase, suggesting that this is a mechanism for maintaining CTL and B cell numbers during secretory phase, yet shielding them from stimuli to establish tolerance.[1] Uterine NK (uNK) cells are an immune cell type exclusively found in the FRT and they differ from their blood NK cell counterparts in that the former are predominantly CD16-CD56^{bright}, whereas the latter are predominantly CD16+CD56^{dim} and more cytotoxic.[4] In humans, uNK cells numbers fluctuate throughout the menstrual cycle, representing over 30% of all endometrial cells by late secretory phase. In mice, which do not undergo cyclic decidualization, uNK cells do not appear until after implantation. Cyclic infiltration of uNK cells into the uterus during decidualization correlates with a hormonal driven increase in expression of the innate immune regulated chemokines (CXCL10 and CXCL11), highlighting the link between hormonal regulation of the FRT innate immunity and changes to immune cell numbers.[5]

The local immune system within the FRT is therefore in a continuous, dynamic state of change throughout the menstrual cycle. During the P4-dominated secretory phase, the FRT prepares for fertilization and implantation, immune responses are suppressed and a tolerogenic state is established. However, this privileged environment leaves the FRT susceptible to pathogen invasion. P4 treatment induces susceptibility to *Chlamydia muridarum* and HSV-2 infection in mice.[6] In women, P4, either during secretory phase or in P4-based contraceptive pills, increases susceptibility to STIs such as HIV, *Chlamydia*, HSV-2, and Gonorrhoea.[7,8] This increased susceptibility immediately post ovulation during the early secretory phase of the menstrual cycle has been postulated to therefore be a "window of vulnerability" within the FRT.[1] However, it is still unclear what the mechanisms are in the FRT that link fluctuating levels of these sex hormones directly with these changes in FRT immunity.

3 | REGULATION OF FRT IMMUNITY: SEMEN

Semen and the seminal plasma itself can also interact with the epithelial cells lining the FRT leading to pleiotropic effects on the immune environment. High levels of semen incidence represent an increased chance of fertilization; therefore, the immune environment of the FRT must become tolerant to prepare for implantation of the semi allogeneic fetus. However, exposure to high levels of semen also carries with it an increased likelihood of infectious microbes; therefore, the FRT must also increase immune surveillance to prevent infections. Experiments in mouse and pig models showed that seminal fluid can induce an array of pro-inflammatory cytokines in uterine epithelial cells.[9] Seminal fluid provokes changes to the structure of the epithelial surface, including recruitment of activated macrophages, dendritic cells, and granulocytes.[10] However, semen also contains many factors that can mediate a more tolerogenic profile, including high-levels of IL-10, TGF-β, and Prostaglandin E2.[11,12] These factors have been thought to have an important role in inducing the Treg cells required for fertilization.[13]

One family of cytokines that is emerging as being very important for mediating host immunity in the FRT, and may be regulated by hormones and semen directly, is the type I IFNs.

4 | TYPE I IFNs

The IFNs are a group of cytokines that have a pivotal role in protecting the host against both external threats, such as pathogen infection, and threats from within, , such as in cancer. The IFNs perform this function through several mechanisms: by inducing a potent antiviral response, inhibiting cell proliferation, modulating cell survival, as well as effecting differentiation and migration. Importantly, IFNs also play an essential role in bridging the innate with the adaptive immune response.[14]

IFNs can be separated into three types (I, II, and III) based on their receptor binding, sequence homology, genetic locus, their pattern of expression, and their inducing stimuli. This review will focus on the type I IFNs, which is the largest group of IFNs, containing multiple IFNα's, as well as a single IFNβ, IFNκ, and IFNε. There are also several species-specific IFNs such as IFNτ and IFNζ, neither of which is expressed in humans; however, these examples will not be included in this review. In humans, the type I IFNs are all situated on the short arm of chromosome 9, whereas in mouse, the type I IFN locus is on chromosome 4. Most studies to date have focused on the IFNαs and IFNβ; therefore, these IFNs are called "classical" IFNs.

The classical type I IFNs are rapidly induced in response to danger signals that are detected by the pattern recognition receptors (PRRs), which are expressed in different cellular compartments and can sense different pathogen-associated molecular patterns (PAMPs). TLRs are PRRs that are expressed on the cell surface and the endosome and can recognize a range of microbial pathogens. The cyclic GMP-AMP synthase (cGAS)-STING pathway and Cytosolic Rig I-like helicases detect viral nucleic acids. Other DNA sensors like the AIM2-like family detect self-DNA.[15] Once a PRR detects a danger signal, a series of signal transduction pathways are triggered, ultimately activating members of the IFN regulatory factor (IRF) family of transcription factors, and /or the NF-κB transcription factor, which induces expression of proinflammatory genes. Expression of the IFNαs and IFNβ is induced by IRFs 1, 3, 5, and 7, whereas IFNβ can also be induced through NF-κB.[16]

All type I IFNs signal through interactions with the type I IFN receptor, which consists of two receptor subunits, IFNAR1 and IFNAR2.[17–19] IFNs bind to the extracellular domain of one IFNAR with high affinity and then the second IFNAR is recruited. This brings the intracellular domains on the receptors into close proximity with their signaling adaptors, and it is the interaction between these that facilitates signaling. The most well characterized signaling cascade is the JAK Stat pathway,[17,20,21] where receptor associated JAK kinases phosphorylate STAT proteins, which then translocate to the nucleus. Within the nucleus, STATs form homo- and heterodimers along with IRF9 to form the ISGF3 complex that binds to DNA to activate transcription of target genes. Other signaling pathways also activated upon IFN-IFNAR binding include PI3/AKT, NF-kB, and MAPK pathways.[22,23] The

contribution of each of these pathways to the IFN response is likely to vary depending on cell type, stimuli, and IFN subtype, but all are required for an appropriate and complete type I IFN response.

The type I IFNs generate their multitude of effects through induction of the IFN regulated genes (IRGs) or IFN stimulates genes (ISGs), which have their expression activated through the ISGF3, STAT, or other transcriptional coactivators. The transcriptional response to IFN stimulation is considerable with hundreds, if not more than one thousand genes induced at a time. There appear to be a core set of ISGs that are induced in all IFN treatments, but there are also larger sets of ISGs that are induced in a temporal and spatial specific manner. The Interferome database (http://www.interferome.org) contains gene expression data from the majority of publicly available microarray experiments from IFN treatments; this database can be interrogated to identify precise ISG responses in many different temporal and cell type-specific conditions.[24]

5 | TYPE I IFNs AND THEIR ANTIPATHOGEN EFFECTS

A primary function of type I IFNs is antiviral immunity and they can impact on virus at multiple points in their lifestyle to limit and prevent their replication and dissemination. Many ISGs have been characterized as encoding direct antiviral effectors and their modes of action give insights into the comprehensive response that IFNs perform. MOV10 prevents viral entry into the cell to block infections, whereas MX1 binds directly to viral components, preventing their function.[25] If the virus does manage to gain entry into cells, ISG20 targets viral RNA synthesis,[26–28] whereas OAS, PKR, and the IFIT family act on the host to inhibit transcription and protein synthesis to prevent viral reproduction.[29–32] Finally, BST2 blocks the egress or budding of nascent virions, so that newly produced virus particles cannot exit the cell and thus prevents the infection spreading.[33–35]

Type I IFNs also play an important role in fighting bacterial infections, where they are required for survival and clearance of many bacterial strains. The type I IFNs impact on bacterial infection through several different mechanisms: locally, the type I IFNs themselves can reduce bacteria's ability to use the hosts intracellular niche for replication by sensitizing these host cells to apoptosis. Several ISGs have been identified as having antibacterial effects, including MYD88 and TRIM1.[36] Furthermore, IFNs can induce a more systemic defense by activating NK and CD8+ T cell cytotoxicity as well as by increasing antigen presentation and recruitment of the adaptive immune system. In addition, type I IFNs can also upregulate the expression of inflammasome components as well as activate the inflammasome itself in response to specific bacterial stimuli.[37]

The type I IFNs act to inhibit pathogenic infections using a range of direct, local, and systemic effects. However, the type I IFN response can also be detrimental to the host, indeed in specific pathogen infections, the actions of IFNs can increase pathogen replication and infection, some examples of these in relation to the FRT will be discussed later.

Given that all type I IFNs signal through the same receptor and can activate a very similar set of genes, an interesting question is why are there so many of them? In humans, there are 14 IFNα subtypes, as well as IFNβ and IFNε. If they all perform the same function, why do we need so many? The answer probably lies in the regulation of the individual IFNs, as the promoter sequences diverge considerably. Therefore, the IFNs response can be very specifically controlled to respond to a wide range of conditions. Further reading into this area can be found in Hertzog and Williams.[38]

6 | TYPE I IFNs IN THE FRT

There is mounting interest in the role of type I IFNs in the FRT and in particular, in more novel, nonclassical IFNs following the discovery and characterization of IFNε. Unlike the classical IFNs, which are rapidly induced in response to danger signals, IFNε is constitutively expressed but tissue specific. At the time of writing, it has been found exclusively in mucosal epithelial layers including in the gut, lung, brain, and in particular the FRT.[39] In fact, in mice, IFNε is not induced at all through PRR signaling, instead it is hormonally regulated, with its expression changing along with the changing hormonal environment of the FRT. The expression of IFNε has been broadly studied in mice where it was defined as changing throughout the estrous cycle, with high levels in the estrous stage and low levels in the diestrus stage. In addition, IFNε is turned off in early pregnancy, theoretically to reduce immune rejection of the invading semi allogeneic fetus. In humans, however, the expression and regulation of IFNε is yet to be comprehensively characterized. However, there are initial data showing that IFNε in humans does change during the menstrual cycle in uterine epithelial cells and that its expression is turned off once menopause in reached.[6] This preliminary data suggest that IFNε is important in either fertility or maintenance of a healthy FRT during the reproductive stage. Like all type I IFNs, IFNε signals through the IFNAR receptors. Interestingly the IFNARs undergo temporal changes in their expression levels through the menstrual cycle, with low expression during the proliferative phase and peak expression during the late secretory phase and menstruation. This suggests that there is a change in the type I IFN system that primes the FRT to be more sensitive and responsive to pathogenic stimulation at particular stages of the reproductive cycle and more tolerant during other stages.

Interestingly, semen may also regulate expression of IFNε in the FRT. Transcriptomic experiments in humans on FRT epithelial cell lines and biopsies taken after coitus show an induction of proinflammatory cytokines including *IL8*, *IL6*, *CSF2*, and *CCL2* as well as IFNε. Supporting this, studies in sex workers with high occurrences of unprotected sex and therefore high seminal fluid exposure had higher levels of IFNε than condom using controls.[40] Therefore, IFNε is induced upon semen exposure, presumably to prime the immune system to defend against microbial attacks.

Confirmation that type I IFNs are indeed important regulators of FRT immunity is evident from studies that have examined type I IFN responses in the context of FRT viral and bacterial infection. In these scenarios, type I IFN induction is rapid following pathogen detection, to enable the FRT immune system to combat the invading microbe. Indeed in the case of IFNε, it can maintain constitutive type I IFN

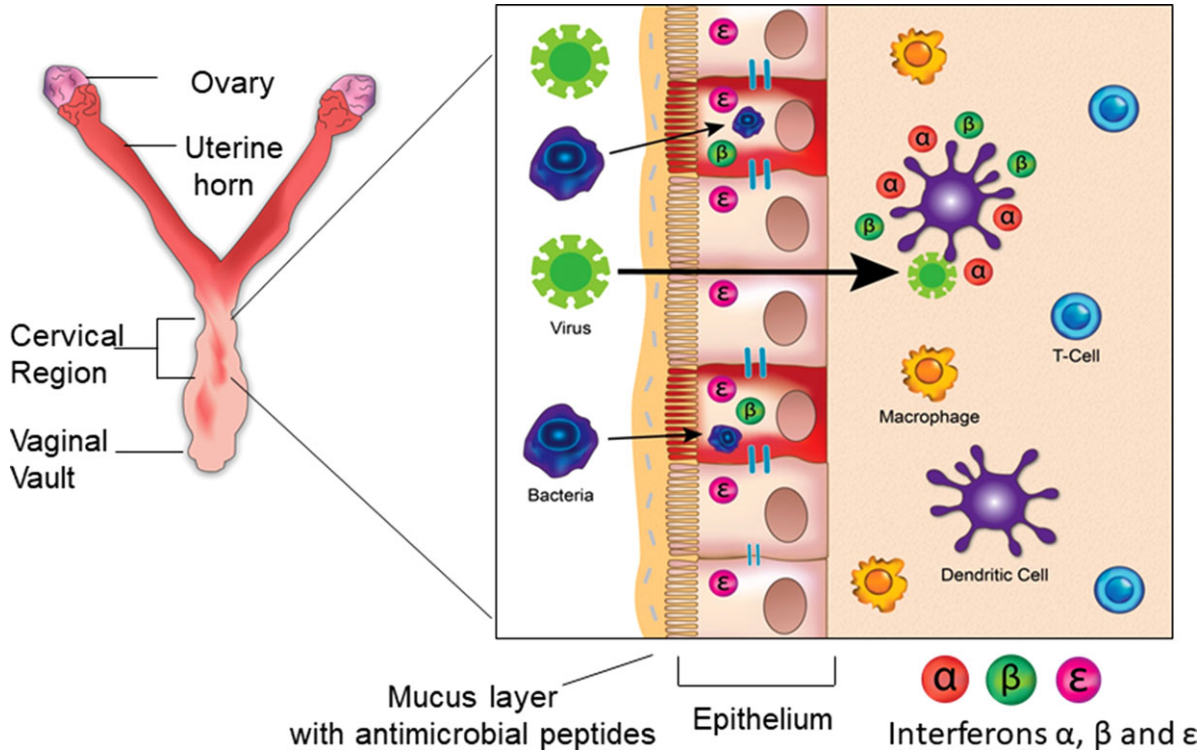

FIGURE 1 **Expression of Type 1 IFNs in the mouse female reproductive tract.** In the epithelial cell layer, IFNε is expressed constitutively and defends against initial infection, when bacteria/virus is detected, IFNβ is rapidly induced and acts by strengthening the tight junctions. In dendritic cells in the surrounding tissue, IFNα and IFNβ are rapidly and strongly induced in response to pathogenic infections

immunity to protect against infection. Here, we review in detail the role of type I IFNs in immunity against three major FRT infections: HIV, a virus that often infects its host via the FRT, Zika virus (ZIKV), where FRT infection can cause devastating side effects on the developing fetus, and *Chlamydia*, the most frequent STI globally.

7 | HIV

HIV-1 virus has been a subject of intense study for the past 40 years due to the devastating epidemic that it has caused. Despite these efforts, there are still currently 37 million people infected worldwide, with the vast majority (69%) residing in Sub-Saharan. Women comprise 51% of infected individuals, with the main route of infection through the mucosal surfaces of the FRT.[41–44] The thick multilayered squamous epithelium of the lower tract appears to provide a considerable barrier to infection, as HIV appears to infect preferentially across the thinner, single layer of the ectocervix.[45–47]

Cervical epithelial cells themselves cannot be infected by HIV *in vivo*,[48] although they can be infected *in vitro*. However, cervical epithelial cells can detect and react to the virus with recent work detecting IFNβ being produced mainly on the basal interface of the epithelium in response to HIV exposure.[49] IFNβ, in this context, protects the epithelial barrier against HIV-mediated damage, which could prevent the HIV from reaching its target cells and thus preventing infection. Once the epithelial barrier is crossed, HIV-1 infects its preferred host cells, CD4+ T cells, dendritic cells and macrophages, where a considerable innate immune response is observed.

7.1 | HIV detection and IFN production

HIV is rapidly detected by innate immune receptors (recently reviewed in Bourke et al.[50]), the PRRs; cGAS and IFI16, which detect viral cDNA. cGAS has been shown to be responsible for sensing of nascent HIV-1 cDNA in infected monocyte-derived dendritic cells and monocytes[51,52] where IFN production is then induced through the STING pathway and IRF3. TLR7 is also capable of sensing HIV genomic DNA in the endosomal compartment and is required for pDCs to respond to HIV. TLR7 then induces type I IFNs through the activation of IRF7 and NF-κB.[50,53–55] IFNα is induced quickly after infection and high amounts of IFNα have been detected in the plasma of individuals that are in the eclipse and exponential viral expansion phases of HIV infection.[56] Indeed before highly active antiretroviral therapy (HAART) was available, IFNα was a candidate for clinical trials and treatment of individuals with asymptomatic HIV was found to decrease the frequency of viral isolation and resulted in fewer patients developing AIDS.[57] IFNα can inhibit HIV infection and replication at multiple stages during its life cycle and this area has been excellently reviewed by Doyle et al.[58] In brief, IFNα can inhibit HIV virus directly or through the activity of ISGs that can inhibit HIV entry, nuclear import, translation, and even release of the budding virions from infected cells. The ISGs responsible for many of these functions are the IFITM family, Trim5a, APOBEC3A, SAMHD1, MxB, and Tetherin.[58]

Like IFNα, IFNε can also protect cells from HIV infection, but its mode of action is somewhat different. In separate experiments, IFNε has been shown to prevent infection of epithelial and T cells.[59] Another direct comparison with IFNα showed that while IFNα could prevent

infection of both T cells and macrophages, IFNε was only able to protect macrophages.[60] The ISGs induced by IFNα can inhibit multiple points in the HIV life cycle, whereas IFNε appears to act primarily on early stages of the HIV life cycle, including viral entry, reverse transcription, and nuclear import. Transcriptomic data from macrophages treated with IFNε and IFNα show that although IFNε activated many classical ISGs, it did not activate several key anti-HIV genes including APOBEC3A and SAMHD1. Furthermore, IFNε induced distinct pathways, including phagocytosis and ROS, which contributed to blocking HIV replication.

Unfortunately, the IFN response against HIV is a double-edged sword. In Abel et al.,[61] the cytokine response was studied in rhesus macaques and it was found that although there is a strong cytokine response in the mucosal tissues after vaginal inoculation of Simian-HIV (SHIV), the response was dominated by proinflammatory cytokines, therefore, recruiting potential SHIV target cells. While the induction of IFN itself occurred later, it was too late to prevent virus dissemination. However, the transmission efficiency of HIV is extremely low, with an infection rate of only 0.0005–0.004 per coital contact. This means that the cervicovaginal mucosal epithelial cell lining provides a robust barrier to HIV-1 infections and is capable of mounting a significant defense against the virus. Recently, there has been a body of work that suggests that type I IFN may be integral to this primary defense (see Table 1 for a summary of experimental data). In 2014, Sandler et al.[62] determined that pretreating rhesus macaques with IFNα prevented SHIV infection, while blocking the IFNAR receptor accelerated SHIV progression. Interestingly, the protective effect of IFNα was only seen for the initial infection. Indeed, in the infrequent cases when SHIV infection was successful in the IFNα pretreated animals, viral loads were in fact increased. This is consistent with the fact that inducing inflammation also recruits target cells for SHIV to infect. A protective role for type I IFNs in HIV was further supported when topical vaginal pretreatment of IFNβ in macaques halved infection rates by SHIV.[63]

7.2 | HIV susceptibility changes during the menstrual cycle

In 2008, Wira and Fahey[64] postulated that women were more likely to be susceptible to HIV during the secretory phase, specifically during the window of vulnerability in the 7–10 day interval following ovulation. This was expected due to the suppression of the humoral, cell-mediated, and innate immune systems by the increasing levels of progesterone.

This theory of a window of vulnerability was supported by work with (SHIV, when it was found that macaque primates were almost only susceptible to SHIV infection near the end of their menstrual cycle.[65] Following this, a more detailed study of macaque susceptibility found that in macaques, there is a window of vulnerability, but preceding and during the menstrual phase,[66] which is later in the phase than the time period suggested by Wira and Fahey. A further study was conducted in the same macaques, using a systems biology approach in which they compared protein secretions and transcriptomic data from biopsies from the vagina during the follicular and luteal phase.[67] They found that innate immune proteins were expressed at higher levels during the follicular phase compared with the luteal phase. In the vaginal biopsies during the follicular stage they identified several enriched gene sets that represented IFNα signaling, TLR signaling, and viral response. Interestingly they did not identify any of the IFNs as differentially expressed between the two phases. Unfortunately, however, the Affymetrix GeneChip rhesus macaque genome array that was used in this experiment does not include a probe for IFNε, which is expressed in macaques and is known to change expression in humans and mice across the menstrual/estrous cycle. It is possible IFNε could be responsible for the changes in immune proteins and genes seen in this study, as well as playing a part in the changing susceptibility of macaques to SHIV.

Studies of HIV infection have of course been limited to *in vitro* work and simian models, but populations of individuals that appear to be resistant to HIV has given more evidence for the role of type I IFNs in defense against HIV. Amongst sex workers that remained HIV negative, one group was found to have increased levels of IFNα in biopsies taken from their ectocervix.[68] Type I IFNs in the FRT in HIV appear to act as an immediate defense, relying not on PRR detection of virus to induce IFNs but rather on constitutive IFNs that are already present, priming the immune system for attack. Therefore, the presence of type I IFN in the reproductive tract (whether topical administration or natural IFNε expression) may protect against HIV infection to prevent viral infection before it crosses the mucosal epithelial barrier. Although these studies point to a potentially important role for FRT type I IFNs in protection against HIV, there are still significant gaps in our knowledge and in particular, little work has been done to investigate the effects of HAART, type I IFNs and HIV infection in the FRT.

8 | ZIKV

In recent years, infection with ZIKV has emerged as a major public health concern due to large outbreaks of the virus in several continents and its association with devastating side effects. ZIKV infection during pregnancy can cause congenital brain abnormalities, in particular microcephaly,[69] and can trigger development of the neurologic disorder Guillain-Barré syndrome, an autoimmune condition where immune cells cause destruction of the myelin sheaths that protect peripheral nerves.[70] ZIKV infection can be transmitted via two major routes: *Aedes* mosquitos and sexual transmission.[71,72] An important role of type I IFNs in controlling ZIKV infection was confirmed in 2016 when the first mouse models were established to examine ZIKV infection; it was found that subcutaneous inoculation relied on inhibition of the type I IFN system, either by using IFNAR$^{-/-}$ mice or antibodies against IFNAR.[73–75]

It has since emerged that type I IFNs in the FRT are also important for limiting ZIKV infection in murine models (see Table 1). Similar to other murine FRT viral infection models, treatment with the hormonal contraceptive medroxyprogesterone acetate (Depo-Provera) 5 days prior to viral challenge permits successful establishment of ZIKV infection in the vagina follow intravaginal ZIKV challenge.[76] Interestingly, previous findings in mouse models of HSV-2 and *Chlamydia* showed that pretreatment with medroxyprogesterone acetate lowered levels

TABLE 1 Summary of Type 1 IFN experimental results in animal models

Infection	Species	IFN subtype	Pathogenic effect
HIV	Human	IFNα	IFNα can inhibit HIV virus directly or through the activity of ISGs that can inhibit HIV entry, nuclear import, translation, and even release.[58]
			IFNα decreased the frequency of viral isolates from HIV infected individuals.[57]
			HIV negative sex workers have higher levels of IFNα.[68]
		IFNβ	HIV induces IFNβ in uterine epithelial cells to protect tight junctions.[49]
		IFNε	IFNε prevents HIV infection in epithelial, T-cells, and macrophages.[59,60]
	Rhesus macaques	IFNα/IFNβ	Simian-HIV induces a primary response includes proinflammatory cytokines, whereas IFNα/IFNβ were induced in a secondary response.[61]
			Pretreating with vaginal or intramuscular IFN prevents SHIV infection.[62,63]
			Blocking the IFN receptor accelerates SHIV progression.[62]
Zika	Mouse	All	IFN receptor knockout mouse are susceptible to sexually transmitted infection. Therefore, a functional type IFN system protects mice against Zika infection.[76]
			Functional type I IFN signaling in the fetus causes spontaneous abortion and fetal growth restriction during Zika virus infection.[78]
Chlamydia	Human	IFNα/IFNβ	IFNα or IFNβ can limit replication of *Chlamydia* bacteria.[85]
	Mouse	IFNα/IFNβ	Type I IFNs are rapidly produced in the FRT upon infection with *Chlamydia*.[79–81]
			IFNα or IFNβ can limit *in vitro* replication of *Chlamydia* bacteria.[85]
			IFNAR−/− mice have decreased susceptibility to lung *Chlamydia muridarum* infection.[88]
		IFNε	Mice lacking IFNε are more susceptible to *Chlamydia muridarum* vaginal infection.[6]
		All	However, mice lacking IFNβ or IFN receptor clear *Chlamydia* infection more rapidly.[86,87]

of the type I IFN, IFNε in the FRT.[6] Furthermore, knock out of the type I IFN system receptor (IFNAR1$^{-/-}$) in vaginal models of high dose ZIKV infection is lethal, with high levels of ZIKV noted throughout the FRT and systemically in the brain and spleen.[76] These data therefore strongly implicates type I IFN responses in the FRT in inhibiting local viral replication in the FRT and limiting systemic spread. Sexual transmission of ZIKV is also impacted by type I IFNs in the FRT. In a study by Duggal et al.,[77] sexual transmission from infected males to noninfected females was demonstrated in AG129 mice, which lack IFN α/β and -γ receptors, with virus detected postmating in vaginal wash and uterine tissue. Interestingly, uterine viral titer was higher in pregnant females than nonpregnant females and there was overall increased susceptibility to viral infection in pregnant females, highlighting that pregnancy status, and its associated changes in sex hormone levels in the uterus, may affect susceptibility to ZIKV infection. Although it is clear from these models that type I IFNs are important for limiting early stages of Zika infection in the FRT, a recent study implicated type I IFNs as mediators of spontaneous abortion and fetal growth restriction during ZIKV infection.[78] Therefore, a protective role for FRT type I IFNs during ZIKV infection is complicated and it is yet to be determined how these findings translate into human studies.

9 | CHLAMYDIA

Type I IFNs have been implicated as being both protective and detrimental during infection with the bacteria *Chlamydia*. *Chlamydia* is the most common sexually transmitted bacteria globally and unresolved FRT infection can cause ectopic pregnancy, infertility and pelvic inflammatory disease. Studies from mice and humans (see Table 1) have revealed that type I IFNs are rapidly produced in the FRT upon infection with *Chlamydia*, a process dependent on STING following activation of the DNA sensor cGAS pathway,[79–81] and dependent on activation of the transcription factor IRF3 during early stages of infection.[82] More recently, it has been found that microbial metabolites produced during *Chlamydia* infection not only induce expression of type I IFN via STING, they also activates inflammasome responses.[83] Interestingly, preinfection of mice with *Chlamydia* protects against subsequent infection with HSV-2,[84] which may be due to the initial *Chlamydia* infection driving expression of type I IFN in the FRT and the associated antiviral responses. *In vitro*, stimulation of infected cells with IFNα or IFNβ can limit replication of *Chlamydia* bacteria through depletion of intracellular iron and induction of bactericidal IDO, nitric oxide synthase and IFN-γ, reviewed in Ref. 85. In addition, mice that lack expression of IFNε have increased susceptibility to *C. muridarum* vaginal infection.[6]

Collectively, these data suggest a protective role for type I IFNs in the FRT during *Chlamydia* infection, yet IFNAR$^{-/-}$ mice, or mice treated with neutralizing antibody against IFNβ, clear *Chlamydia* infection more rapidly *in vivo* and display less pathology within their oviducts.[86,87] This clearance is associated with increased levels of the chemokine CXCL9 and enhanced recruitment of CD4+ T cells into cervical tissue. Indeed IFNAR$^{-/-}$ mice also have decreased susceptibility to lung *C. muridarum* infection, which was attributed to a decrease in type I IFN driven macrophage apoptosis.[88] Therefore, these murine models suggest that the protective or deleterious effects of type I IFNs in *Chlamydia* infection, particularly IFNβ, are likely to be temporal where early type I IFN production may limit infection and late production may exacerbate pathology due to mechanisms involving apoptosis

of immune cells. It is also yet to be elucidated what role type I IFNs in the human FRT play during *Chlamydia* infection.

10 | CONCLUDING REMARKS

The unique setting of the FRT demands specialized immune responses that can be modulated by sex hormones to ensure successful reproduction whilst maintaining immunity at this mucosal site. Murine models have clearly established that type I IFN responses are key regulators of FRT immunity, either through maintaining baseline FRT immunity, established by constitutive expression of the novel type I IFN, IFNε, or through inducible immunity following infection, where IFNα and IFNβ can be rapidly induced following detection of pathogenic threat summarized in Figure 1. However, there is a great deal still unknown about the role these type I IFNs play in the human FRT. There are significant gaps in our knowledge concerning the effect that co-infection of multiple sexually transmitted diseases has on type I IFNs in the FRT and their ability to react. The reverse is also of interest, what function do type I IFNs in the FRT perform when assaulted by more than one pathogen at the same time? Another important absence is that the role of the novel IFNε has yet to be elucidated in humans and whether it could emerge as a new tool to combat infections of the FRT.

AUTHORSHIP

Dr. Helen Cumming conceived of the topic for the review. Assistant Prof. Nollaig Bourke provided guidance for the overall structure. Both authors contributed to writing the final manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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