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Unique features of antiviral immune system of the vaginal mucosa

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Abstract

A vast majority of human vaccines rely on neutralizing antibodies for protection. With the exception of vaccines against human papillomavirus, despite a great amount of dedicated effort by the scientific community, development of vaccines against sexually transmitted viruses has generally been unsuccessful. Understanding the immunobiology of the genital tract is key to designing vaccines that prevent spreading of these viruses. Recent studies demonstrate that adaptive immunity in the vaginal mucosa is uniquely regulated compared to other mucosal organs. In particular, development of virus-specific CD4⁺ and CD8⁺ T cells is critically important for antiviral defense in vagina. In this review, we provide an overview of our current understanding of a wide spectrum of immune responses in vagina - from innate viral sensing to memory development.

Introduction

The lower reproductive tract represents a unique site for both pathogen entry and dissemination between individuals. However, despite its importance in sexually transmitted diseases, mechanisms of antiviral immunity in the genital mucosa have attracted relatively little attention compared to those in other mucosal surfaces such as gastrointestinal and respiratory tracts. Compared to the monolayer epithelia in the intestine and in the lung, the vaginal tract is covered with stratified epithelia. In addition, the vaginal mucosa differs from other mucosae with respect to mucus composition, microbiota and innate and adaptive immune mechanisms. Here we discuss recent progress in understanding how antiviral immunity is initiated and maintained in the female genital tract.

Constitutive barrier mechanisms in the female genital mucosa

The female genital tract consists of two different types of mucosal surfaces. The upper genital tract (endocervix and endometrium) surfaces represents the type I mucosal surface, which is covered with a monolayer of columnar epithelial cells with tight junctions and secretory IgA. In contrast, the lower genital tract (vagina and ectocervix) represents the type II mucosal surface, lined by stratified squamous epithelia that lack luminal IgA and mucosa-associated lymphoid tissues [1,2]. The boundary between type I and type II mucosa, known as the cervical transformation zone, is most vulnerable to invasion by pathogens (Figure 1) and populated heavily with T cells and antigen presenting cells (APCs) compared to other

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regions of the female genital tract [3]. In addition, both molecular and cellular antiviral events in the female genital tract are heavily affected by sex hormones, which is discussed extensively elsewhere [4,5].

The epithelial surfaces of the female genital tract are covered with mucus. Mucus consists of mucin proteins that may inhibit viral entry and also contains secretory proteins that have microbicidal and antiviral activity [2]. In addition to mucus, the lower genital tract is populated with endogenous bacteria and fungi, with a predominant *Lactobacillus* species, that keep an acidic environment in the vagina. The acidic pH, epithelial barrier, mucus and innate immune responses triggered by the commensal flora act in concert to prevent virus infections in the female genital tract against pathogens including *Haemophilus ducreyi*, HSV—2 and *Chlamydia trachomatis* [6]. However, the influence of the vaginal microbiota on the adaptive immune responses to sexually transmitted viruses is unknown.

Inducible host immune responses to viral infection in the vagina

As in the other sites of the body, antiviral immune responses in the genital tract consist of four different phases – 1) recognition of the virus by invariant receptors of the innate immune system, leading to the activation of cytokines and antiviral response genes, 2) processing and presentation of the virus antigens by APCs to naïve lymphocytes leading to priming of adaptive immunity, 3) elimination of the virus by various effector mechanisms, and 4) establishing long-term memory (Figure 1). This review will use examples from human immunodeficiency virus 1 (HIV-1), herpes simplex viruses (HSV-1 and HSV-2) and human papilloma viruses (HPVs), which are clinically relevant sexually transmitted viruses in humans. Although kinetics of the immune response and viral elimination differs greatly between viruses, we here discuss general principles of these processes in the vagina.

Innate viral recognition

In general, pathogen recognition by the host innate immune system relies on receptors, known as pattern recognition receptors (PRRs) that recognize molecular patterns shared by different classes of pathogens [7]. In response to viral infection, genital epithelial cells produce pro-inflammatory cytokines, IFN β and antimicrobial peptides such as defensins [5]. The tissue-resident macrophages, DCs and intraepithelial $\gamma\delta$ T cells also recognize the virus immediately after initial infection, and secrete antiviral factors such as type I IFNs and cytokines [2]. DCs have the capacity to link innate detection of viruses to the generation of adaptive immune responses. Nevertheless, virus infection is likely sensed by both the hematopoietic and non-hematopoietic compartments for the full induction of T cell-mediated adaptive immunity against genital HSV-2 infection [8].

Following the first wave of responses by epithelial cells and tissue-resident leukocytes, more leukocytes such as neutrophils, monocytes, natural killer (NK) cells and pDCs are recruited to the vaginal mucosa, which, at least to some extent, was shown to be protective to the host during genital HSV infection in mice [4,9]. Of these cell types, pDCs are equipped with a robust capacity to respond to a variety of viruses through TLRs and provide a large burst of type I IFNs necessary for restricting virus infection [10]. Interestingly, however, neither pDCs nor type I IFNs are required for the development of adaptive immunity in genital HSV infection in mice [9,10].

Priming adaptive immunity

DCs present antigens most efficiently to naïve T cells and are thereby a key APC type that bridges innate and adaptive immunity. In both humans and mice, there are multiple DC subsets in the vaginal mucosa including intraepithelial Langerhans cells (LCs) and submucosal DC [11]. Unlike the skin, vaginal LCs in the mouse derive from circulating

radiosensitive precursors at steady state [12]. Upon inflammation, other DC subsets are recruited including monocyte-derived DCs and pDCs [10,13]. Recent studies indicate differential roles of various DC subsets in priming, maintenance and execution phases of immune responses in the vaginal mucosa.

Immune priming by non-infected DCs—For non-viral antigens, DCs in the female genital tract are seemingly conditioned to be tolerogenic [14–17]. However, in the case of viral infection, DCs are capable of initiating robust T cell responses. Although genital epithelial cells may directly present antigens to T cells in some settings [4,18], direct viral antigen presentation by the infected epithelial cells is not likely to prime naïve lymphocytes, which preferentially circulate to secondary lymphoid tissues. In addition, directly infected DCs are incapable of presenting antigens as viruses are armed with molecules that inhibit their activation and function [19]. Accordingly, LCs are incapable of cross-presenting HSV antigens [20,21] or physically depleted by HPV E6 protein [22]. Furthermore, rather than priming T cells, LCs spread HIV infection beyond the site of viral entry by transmitting virions to CD4⁺T cells [23,24]. Thus, antigens are likely cross-presented by non-infected DCs.

Upon vaginal viral infection, virus-specific T cells are primed exclusively in vaginal draining lymph nodes (dLNs), which are iliac and inguinal LNs [1]. Once infection takes place in the vaginal mucosa, these dLNs undergo various adaptations to prime optimal immune responses. One such adaptation involves increasing naïve lymphocyte influx by enlargement of the feed arteriole [25,26]. Upon vaginal HSV-2 infection, only the migratory CD11b⁺ DCs, and not the lymph node-resident CD8a⁺ DCs effectively prime CD4⁺ T cells in the dLNs [21]. Another study demonstrated that intravaginal inoculation of ovalbumin mixed with the cholera toxin B subunit adjuvant leads to presentation of MHC class I epitopes by CD11b⁺ DCs, but not CD8⁺ DCs [27]. In contrast, vaginal HSV-1 infection results in CD4⁺ and CD8⁺ T cell priming by migrant CD11b⁺ and CD8a⁺ DCs [28]. How does this compare to antigen handling in the skin? Interestingly, while primary epicutaneous infection with HSV-1 results in antigen presentation by the LN-resident CD8a⁺ DCs [20,28,29], antigen presentation during recrudescence is handled almost exclusively by the CD103⁺ dermal DCs [30]. While similarities do exist between the skin and the vaginal mucosa in regard to inability of LCs and potential role for subepithelial DCs in cross-presenting antigens, further studies are needed to characterize vaginal DC subsets as counterparts for CD103⁺DCs have not been described for the vaginal submucosa [11].

Antiviral effector responses at the site of infection

Recent evidence indicates that effector responses are most efficient in exerting their protective function locally. Both humoral and T cell-mediated immunity against genital HSV infection are most efficiently induced by attenuated HSV when the vaccination is given intravaginally [31,32]. Here, we discuss the advantages of local effector responses and relate this to strategy for vaccine design.

Local humoral immunity—Generally, humoral immunity is thought to provide systemic coverage of various mucosal and visceral tissues through antibodies delivered via the circulation. However, recent studies highlight the importance of generating genital mucosa-resident B cell memory and plasmablast responses. In monkeys immunized against simian-HIV vaginally, virus-specific vaginal IgA and IgG, but not plasma IgG correlated with protection [33]. Another study on genital HSV infection in mice also demonstrated the presence of vagina-resident plasma cells that produce protective virus-specific IgG [32]. How are locally produced immunoglobulins transported to the vaginal lumen? Unlike in type I mucosa such as intestinal or uterine epithelia where IgA is abundant, antigen-specific

antibodies in the vagina are dominated by IgG [2]. Recent studies showed that neonatal Fc receptor (FcRn) is responsible for transcytosis of IgG in the circulation to the apical surface of the vagina [34]. In contrast to basolateral-to-apical IgA transcytosis, FcRn-mediated IgG transcytosis is bidirectional and dependent on acidic pH [35]. Indeed, mucosal application of viral peptide fused with Fc γ leads to protective immunity against genital viral infection in an FcRn-dependent antigen transport [36,37].

Local effector T cell immunity—Although neutralizing antibodies are protective against infections with many viruses such as HPV [38], induction of T cell-mediated immunity, particularly antigen-specific CD4⁺ T cells, is critical for full protection in infections such as HIV and HSV. In murine genital HSV infection models, while B cells and CD8⁺ T cells may contribute to viral clearance, CD4⁺ T cells appear to be more critical in host protection [9]. If the CD8⁺ T cells are the executioner killer T cells, CD4⁺ T cells are the master regulator of innate and adaptive immune responses that go far beyond helping B cells and CD8⁺ T cells, and are themselves potent antiviral effectors during HSV infection [39]. In addition, CD4⁺ T cells are critical for effector CD8⁺ T cell mobilization into otherwise restricted tissues such as the vagina [40–42]. Requirement of CD4⁺ T cells for effector CD8⁺ T cell mobilization is organ-specific, as lung and intestine have been shown to be more permissive to effector CD8⁺ T cell infiltration [43]. CXCR3 expressing virus-specific effector CD8⁺ T cells enter HSV-infected vaginal mucosa via its ligands CXCL9 and CXCL10, which are produced locally in response to CD4⁺ T cell-derived but not NK cell-derived IFN γ [41]. Notably, such local production of IFN γ by CD4⁺ T cells requires infiltration of inflammatory monocyte-derived DCs in the vaginal mucosa [13]. Thus, CD4⁺ T cells provide many facets of local protective immunity by being a potent antiviral inducer and effector and by regulating traffic of other important cell types to the vagina. Nevertheless, cooperation between CD4⁺ and CD8⁺ T cells are particularly important, as suggested by the fact that the crucial role for CD8⁺ T cell in controlling chronically-infected viruses including HIV relies on functionality of CD4⁺ T cells [39].

Establishing local memory responses

In genital HSV infection, once established, both CD4⁺ and CD8⁺ effector T cells persist for a long period and form cluster-like structures in the vaginal tissue [44–46]. Evidence shows that tissue-resident HSV-specific memory CD8⁺ T cells are maintained locally, form a distinct subset from the circulating memory population and continuously monitor the neural endings to suppress reactivation of the virus from neuronal latency [47–50]. Indeed, the density of tissue-resident CD8⁺ T cells critically affects rates of reactivation in both mice and humans [51,52]. Stimulation of these tissue-resident memory CD8⁺ T cells is dependent on DCs and require CD4⁺ T cells in the elicitation phase [53]. In contrast, activation of tissue-resident memory CD4⁺ T cells require either DCs or B cells and is protective to the host against genital HSV-2 infection in an IFN γ -dependent manner [44] (Figure 1). Upon secondary infection, tissue resident memory T cells rapidly produce IFN γ , which serves as a predominant antiviral mechanism in recurrent herpetic lesions in humans [54].

Given the importance of local memory T cells in providing protection against subsequent viral challenge, targeting and maintaining cellular immunity to genital mucosa is likely key for efficient vaccination. It has been shown that T cells that migrate to restrictive tissues such as the gut or skin express selective homing markers such as CCR9 and α 4 β 7 or CCR10, respectively. Studies in vaginal non-viral infection models have shown that endothelial cells in infected genital mucosa upregulate ICAM-1, VCAM-1, MAdCAM-1 and E-selectin [55–58], while vagina-recruited T cells preferentially express LFA-1 (ICAM-1 ligand), α 4 β 7 (MAdCAM-1 and VCAM-1 ligand) and α E β 7 integrins as well as cutaneous lymphocyte antigen (E-selectin ligand) [56,58–60]. Accordingly, naïve α E

integrin-deficient mice have reduced numbers of vaginal intraepithelial leukocytes [61], and mice that lack E-selectin and T cells that lack $\beta 7$ integrin were shown to have reduced vaginal recruitment and impaired bacterial clearance in genital *Chlamydia* infection [55]. In addition, similar to HSV-specific CD8⁺ T cells [41], entry of effector CD4⁺ T cells into genital mucosa in murine *Chlamydia* infection requires T cell-intrinsic expression of CXCR3 along with CCR5 [62]. To induce the expression of these homing markers, DCs responding to environmental vitamins imprint tissue tropism of effector T cells by altering expression levels of chemokine receptors and cell adhesion molecules. In the gut, DCs metabolize retinol (vitamin A) into retinoic acid that upregulates gut-tropic receptors CCR9 and $\alpha 4\beta 7$ integrin in T cells [63], whereas in the skin DCs produce 1,25-dihydroxyvitamin D3 from inactive vitamin D3 to suppress CCR9 and $\alpha 4\beta 7$ integrin and upregulate skin-tropic chemokine receptor CCR10 [64]. Interestingly, a recent study demonstrated that retinoic acid used as an adjuvant during immunization with adenovirus-encoded peptide upregulates mucosal homing receptors CCR9 and $\alpha 4\beta 7$ and $\alpha E\beta 7$ integrins in antigen-specific CD8⁺ T cells and successfully induces effector CD8⁺ T cells in mucosal organs including vagina [65]. However, a key question in this regard is what are the cellular and molecular requirements, such as endogenous environmental cues, that enable memory T cell recruitment and residency in the vagina? How do local DCs, if any, imprint vagina-tropic T cells? How is the formation of effector T cell clusters in the vagina controlled and what is the relevance of these clusters in antiviral protection? The answers to these questions will hold the key to designing effective vaccines against sexually transmitted viruses in the future.

Conclusion

Despite a great success in prophylactic HPV vaccine [38], no therapeutic vaccine has been made against any sexually transmitted viruses, nor is there an efficacious preventive vaccine against HIV-1 and HSV infection. While much has been learned from infection models in other mucosal tissues and skin, for a better vaccination strategy against sexually transmitted pathogens, it is critically important to understand cellular and molecular mechanisms of immune protection in the genital mucosa, and translate our basic understandings to clinically relevant outcome. Given the advances made in generating circulating virus-specific T cells and antibodies in humans, we must now focus our attention to developing long-term tissue-specific memory within the genital mucosa. We have recently proposed “prime-and-pull” vaccination strategy, in which antigen-specific lymphocytes are targeted to the genital mucosa by artificially-recruiting memory cells to the vagina [2]. Future studies are needed to test these and other ideas in preclinical and clinical settings.

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Highlights

- Vagina is lined with stratified squamous epithelia with mucus and unique flora.
- Vaginal epithelial cells and innate leukocytes provide the first line of cellular defense.
- Local antibodies and T cells provide long-term protection in the vagina.
- Vagina entry and residency by effector T cells require unique homing properties.
- Long-term tissue-resident memory T cells are key for efficient vaccine.

Figure 1. Antiviral adaptive immune responses in the cervical and vaginal mucosa

Viral exposure is often thought to occur through the transformation zone or through microabrasion. At steady state, vaginal epithelial layer and the submucosa are surveyed by innate leukocytes and lymphocytes, but the recruitment of antigen-specific T and B cells to the vagina is restricted. Once infected, both epithelial cells and innate leukocytes produce type I IFNs, inflammatory cytokines and induce chemokines that recruit NK cells, monocytes, pDCs and neutrophils. Virions and viral antigens are taken up and processed by migrant submucosal DCs or by LN-resident DCs and presented to T cells. Activated effector T cells are recruited to the vagina and can persist for a long period. Vaginal epithelial cells lack polymeric Ig receptor (pIgR) for transport of sIgA. Instead, virus-specific IgG is transcytosed by FcRn into the vaginal lumen, and provides protection.