Published in final edited form as:

Nat Rev Immunol. 2010 October ; 10(10): 699–711. doi:10.1038/nri2836.

Antiviral immune responses in the genital tract: clues for vaccines

Akiko Iwasaki

Department of Immunobiology, Yale University School of Medicine, 300 Cedar Street, New Haven CT 06520 USA

Akiko Iwasaki: akiko.iwasaki@yale.edu

Abstract

Mucosal surfaces are often exploited as a portal of entry by a wide variety of microorganisms. An advanced understanding has been gained over the past decade of the immune system of the gastrointestinal and the respiratory mucosae. However, despite the fact that many viruses are transmitted sexually through the genital tract, the immune system of the male and female genital mucosae has received much less attention. Here, I describe and highlight differences of the innate and adaptive immune systems of the genital and intestinal mucosae, and discuss the challenges we face in the development of successful vaccines against sexually transmitted viral pathogens.

Introduction

Pathogens often rely on contact between host organisms for transmission. Many pathogens have adapted their transmission mechanisms to take advantage of behaviours that are essential for the survival of the host species, such as eating (oral), breathing (respiratory) and procreating (genital), whereas others use insect vectors to inoculate themselves into their mammalian hosts. Some pathogens have evolved to exploit sexual contact of the host organism for their transmission. Transmission of this class of pathogen that causes sexually transmitted infections (STIs) depends on the sexual maturity and promiscuity of humans. The World Health Organization (WHO) estimates that one million new cases of STIs occur every day. Yet, for the most part, vaccines that prevent STIs are not available.

Compared to the intestinal mucosa, the female and male genital tracts are covered by distinct epithelial cell layers and mucus types, are inhabited by a unique set of microbial flora, and use distinct innate and adaptive effector mechanisms. Unlike STIs caused by bacterial pathogens, which are treatable by antibiotics, there are no cures for viral STIs. Currently available antiviral agents are unable to eliminate the latent pool of viruses such as HIV-1 or HSV. This review is not intended to be a comprehensive overview of vaccines against STIs. Instead, in this review I use examples of three major human sexually transmitted viral pathogens – HIV-1, human papillomavirus (HPV) and herpes simplex virus type 2 (HSV-2) – to describe our current understanding of modes of viral entry, innate detection, initiation of adaptive immune responses and virus clearance. Further, I discuss how understanding the innate and adaptive immune mechanisms of protection in the genital mucosa could be applied for rational design of vaccines against STIs.

Sexually transmitted virus infections

Human infection with sexually transmitted viruses

Several viruses use the genital mucosa as a portal of entry into human hosts. Clinically relevant sexually transmitted viruses (STVs) include HIV-1 that causes acquired immunodeficiency syndrome (AIDS), HPV that causes cervical cancer and genital warts,

HSV that causes genital herpes, and hepatitis B virus. Epidemiology of these STVs has been covered extensively elsewhere¹. Here, I briefly describe the STVs and their impact on human populations.

HIV-1 is a member of the lentivirus family of retroviruses, and structurally consists of an envelope enclosing a capsid containing two copies of positive single-stranded RNA genome (~9 kb). HIV is transmitted through sexual contact (vaginal, penile and rectal), through blood, or by vertical transmission from infected mother to child. Since its discovery in the early 1980s, AIDS has already claimed the lives of more than 25 million people, and has generated 14 million orphans in Sub-Saharan Africa alone². In endemic countries, death by HIV-1 and the reduced fertility rate of HIV-positive women has dramatically affected life expectancy and population demographics (Figure 1)³.

Genital herpes is caused by infection with HSV-1 and HSV-2. HSV is an enveloped virus containing a double-stranded DNA genome (~150 kb) within the capsid. HSV is transmitted through mucosal contact through vaginal, penile and oral routes. Even though HSV-1 is traditionally associated with oral herpes, adults without immunity to HSV-1 who practice oral sex are at risk for genital HSV-1 infection⁴. Vertical transmission of HSV-2 from infected mother to newborns often leads to lethal neonatal herpes. In the USA, prevalence of genital herpes is rising at an alarming rate (Figure 2a).

HPV is a member of the papillomaviridae family, consisting of a capsid containing a circular dsDNA genome (~8 kb). On sexual contact, HPV establishes productive infections only in the stratified epithelium of the skin or mucous membranes. Although the majority of the nearly 200 known types of HPV cause no symptoms in most people, 'high risk' types of HPV are the causal agent of more than 90% of all cervical cancer, the second leading cause of death among women worldwide. High risk HPV types, such as HPV type 16 (HPV-16) and HPV-18, also cause anal, penile and vulvar cancer, and 'low risk' HPV types cause genital warts⁵. HPV infection is also common in men although it is usually asymptomatic. HPV is the most common sexually transmitted disease in the world, causing 260,000 deaths annually, 80% of which occur in developing countries⁶.

Anatomy of the female and male genital mucosa

Although men can also be infected, the global prevalence of HSV-2 and virus-associated morbidity is significantly higher in women (Figure 2b). HPV causes morbidity and mortality almost exclusively in women. The underlying mechanism for such gender-based differences is undoubtedly complex, including differences in societal rank, behaviour, sexual practices, sex hormone regulation of the immune system and exposure dose and route. For example, in many countries women are less able to negotiate condom use and are more likely to be subjected to non-consensual sex. In the genital tract of premenopausal women, sex hormones control expression of many genes including cytokines and chemokines and dictate cellular composition, immunoglobulin secretion and antigen presentation during menstrual cycle⁷. In addition to these factors, anatomical differences between male and female genitalia have an important role in gender-based differences in acquisition and progression of disease following STV exposure (Figure 3). In addition, circumcision significantly reduces STI rates in men⁸ (Box 1). In both sexes, anal intercourse also has an important role in the transmission of STIs. A comparison of the genital and rectal mucosae reveals important barrier differences in these organs (Figure 3 and Table 1).

Mucosal surfaces can be generally divided into two types – type I mucosal surfaces are covered by a simple columnar epithelium (such as the gut and lungs), whereas type II mucosal surfaces have protective stratified squamous epithelial layer (such as the vagina, eyes and mouth)⁹ (Table 1). The female genital tract consists of type II mucosa (outer

vagina, inner vagina and ectocervix) and type I mucosa (endocervix and the uterus). The male genitalia is composed of the external (penis and scrotum) and the internal genitalia (epididymis, vas deferens and prostate gland). Other important distinctions of the two types of mucosa include the presence (type I) or absence (type II) of IgA transport mechanisms. In addition to the difference in epithelial cells, the submucosa of type I and type II tissues differ with respect to cell composition and the presence (type I) or absence (type II) of mucosa-associated lymphoid tissues (MALT). In general, submucosa underlying type I epithelia is constitutively filled with dendritic cells, macrophages and memory lymphocytes, while the submucosa of the type II tissues contains a sparse network of dendritic cells, macrophages and rare lymphocytes. Uterus contains lymphoid aggregates of unknown function made up of B cell core surrounded by CD8 T cells, which become enlarged during the secretory phase⁷.

The uncircumcised penis glans is covered by a loose fold of skin known as foreskin or prepuce (Figure 3b). During coitus, the foreskin is pulled back exposing the glans. The penis is covered by external skin, except for the inner foreskin that consists of a typical type II mucosal epithelium¹⁰. The foreskin mucosal surface is akin to the inner vaginal canal and may be more susceptible to invasion by STIs. The foreskin fold could also trap materials longer, providing a greater window of opportunity for the STI pathogens to enter the host. Circumcision surgically removes the foreskin just below the glans. The glans penis of circumcised male is covered by the protective cornified external skin (Figure 3c). Accumulating data suggest that male circumcision has long-term beneficial health effects on both males and females by limiting the spread of STIs (Box 1).

In contrast to the genital tract, the rectum is part of the gastrointestinal tract and shares its type I mucosa characteristics (Figure 3d). Large aggregates of submucosal lymphoid follicles are present in the rectal mucosa adjacent to the recto-anal junction¹¹, which defines the area where the columnar epithelia of the rectum end and the squamous epithelia of the anal canal begin. The lower half of the anorectal canal is lined by stratified squamous (type II) epithelium, providing a stronger physical barrier than the upper half.

Invasion mechanisms used by STVs

STVs have evolved to use the biology of the genital mucosae for their transmission among humans. HIV-1 uses the CD4 molecule as its receptor and CC-chemokine receptor 5 (CCR5) or CXCR4 as co-receptors to enter a host target cell. Once the viral genome is reverse transcribed into DNA, it is integrated into the genome of the infected cells. Activation of CD4⁺ T cells induces the transcription of the provirus, leading to viral synthesis and release of new virus particles. Several modes of entry have been suggested including transcytosis through M cells (only present over the MALT in type I mucosal epithelia), uptake by dendritic cells (DCs) that extend dendrites into the mucosal cavity and entry through microabrasion in the epithelial layer¹². Preexisting genital lesions caused by other STIs also contribute significantly to increasing the risk of HIV-1 transmission¹³. Most women who acquire HIV-1 infection do so through heterosexual contact with infected men. Entry of the virus probably occurs near the transformation zone of the endocervix, where the virus first replicates in local CD4⁺ T cells and sets up founder cells¹⁴. Homogeneity of the founder virus indicates that a single virus is responsible for establishing infection in humans^{15, 16}. From there, the virus can disseminate through the lymph to local lymph nodes and further to the systemic circulation. However, the vaginal route of transmission is estimated to be only successful in between 1 in 200 to 1 in 2,000 encounters, likely owing to the protective type II epithelial layer present in this tissue. Risk for HIV-1 acquisition from females to males is even less, particularly in circumcised males (Box 1). By contrast, HIV-1 can enter through the rectal mucosa much more efficiently, with transmission rates estimated to be as high as 1 in 10 encounters (Ref. ¹³). This difference in

transmission rate probably reflects the fact that the rectal mucosa is covered by only a single layer of columnar epithelia, which upon physical trauma during anal intercourse can allow viral access to the systemic circulation. The rectal mucosa also contains many memory CD4⁺ T cells in the lamina propria and in the MALT that could serve as targets for HIV-1 infection (Figure 3d).

HSV-1 and HSV-2 enter the human host through a variety of mucosal surfaces including the vagina and the penis and first infect the type II epithelial layer. HSV uses multiple cell surface proteins for entry: heparan sulphate chains on cell surface proteoglycans, a member of the tumour necrosis factor receptor family and two members of the immunoglobulin superfamily related to the poliovirus receptor¹⁷. Primary replication in the keratinocytes results in the subsequent infection of sensory ganglia through nearby nerve endings. The de-enveloped virus migrates to the nerve cell body through retrograde axonal transport. Hiding in the ganglia, HSV establishes life-long latent infection in the host. Immune suppression results in reactivation of the virus, causing lesions in the external genitalia. Notably, HSV virions are shed even in asymptomatic individuals¹⁸, increasing the likelihood of transmission from carriers to their non-infected partners.

HPV infects genital skin - the vagina or the cervix in women, and the penile shaft, glans and foreskin in men¹⁹. To infect, HPV requires disruption of epithelial cell integrity, which allows access to the basement membrane. HPV uses an unconventional mechanism for infection, in that it first binds to the basement membrane for subsequent entry into the basal keratinocytes. Following binding to the basement membrane, the virus undergoes a conformational change that results in cleavage of a capsid protein and transfer of the capsid to the epithelial cell surface²⁰. Once within the basal keratinocyte stem cells, the virus uses the differentiation programme of the epithelial cells to complete its life cycle⁵. HPV virions are shed from the superficial layer of the type II mucosa, and are then transmitted to sex partners. Most subclinical infections with HPV regress spontaneously. Only a very small percentage of HPV-infected women, if untreated, go on to develop cervical cancer over many years.

Innate immunity in the genital tract

Mucus

Mucus covers the internal surface of the vaginal tract, penis and anal canal and functions to trap infectious microorganisms and pollutants. Mucus is made up of mucins, which are complex, high molecular weight glycoproteins that contain at least one and sometimes multiple protein domains that are sites of extensive O-glycan attachment. In the type I mucosa (gut and lungs), goblet cells provide much of the mucus production, whereas in the type II genital mucosal surfaces (vagina, lower half of anal canal and foreskin) mucus is secreted from local mucus-secreting epithelial cells (Table 1). During ovulation, crypts in the cervix secrete mucus that descends and covers the vaginal canal. Cervical mucus is less acidic and more conducive to sperm movement through the cervix. In addition to mucins, mucus contains various other defence molecules including immunoglobulins, complement, antimicrobial peptides, lysozyme and lactoferrin (discussed below).

Antimicrobial factors

Bacteria, fungi, parasites and viruses in the mucus layer are met with a variety of antimicrobial arsenals. Microbicidal molecules, such as complement components and antimicrobial peptides, can directly bind and kill microorganisms before they can reach the host epithelial cell layer. The epithelial cells, glands of the cervix and neutrophils produce most of the antimicrobial peptides present in the vaginal fluid, including calprotectin, lysozyme, lactoferrin, secretory leukoprotease inhibitor (SLPI), human neutrophil peptides

(HNPs) and human β -defensins²¹. In the gut mucosa, a similar array of antimicrobial molecules, including lysozymes, defensins, cathelicidins and secretory phospholipase A2, have an important role in keeping the commensal bacteria at bay and also in defence against pathogenic bacteria²². Paneth cells, which are the main producers of antimicrobial factors in the small intestine, are absent in the rectal mucosa. Instead, rectal epithelial cells are the source of antimicrobial peptides.

Endogenous flora

Commensal bacteria are essential for shaping intestinal immune responses in both health and disease²³. Recent advances in 16S ribosomal RNA sequencing and deep metagenomic sequencing have enabled taxonomic identification of human microbial communities. Human gut-associated communities are dominated by only four phyla - Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria²⁴. More than 1,000 species of bacteria are estimated to live in the gut of the human cohort studied²⁵. In addition to providing key metabolic, trophic and defence functions to the mammalian host, the microbiota shape the gut immune system in various ways (Box 2).

In contrast to the intestinal tract, the normal vaginal flora is predominately composed of *Lactobacillus* species²⁶, which carry out key functions for the female host. Vaginal H₂O₂-producing lactobacilli prevent the outgrowth of harmful bacteria that can cause bacterial vaginosis. Further, lactobacilli keep the vaginal fluid acidic (pH 3.8–4.0) through lactic acid production. The acidic vaginal pH protects against the pathogens that cause STIs, including *Haemophilus ducreyi*, HSV-2 and *Chlamydia trachomatis*²⁷. In addition to *Lactobacillus* (phylum Firmicutes), phyla Proteobacteria and Actinobacteria (mainly the genera *Pseudomonas* and *Gardnerella*, respectively) also constitute significant portions of the vaginal flora of healthy females. Within an individual, vaginal microbiota is not homogenous but differs significantly depending on anatomical location²⁸. However, the influence of the vaginal flora on the adaptive immune responses to STIs is unknown, and will be an important factor to consider for vaccine responsiveness.

Innate immune cells

In the genital mucosa, various innate immune cells provide defence against invading pathogens. At steady state, $\gamma\delta$ T cells, macrophages, Langerhans cells and DCs survey the type II epithelia of vaginal and anal canals (Figure 4). In the uterus, specialized NK cells and Tregs regulate fetal development, and potentially contribute to antiviral defense. Following infection, several cell types are mobilized to the vaginal tissue, including neutrophils, monocytes, plasmacytoid DCs (pDCs) and natural killer (NK) cells. Later on, antigen-specific T and B cells enter the tissue to provide pathogen-specific immune defence. Langerhans cells within the epithelium and DCs in the submucosa at steady state are highly phagocytic and express several pathogen recognition receptors (PRRs) that can recognize a wide array of microorganisms. After recognizing pathogens through PRRs, DCs and Langerhans cells undergo a maturation programme and migrate to the draining lymph nodes to prime naïve T and B cells.

Certain cell types have been identified as having particularly important role in combating known STVs. For HSV-2, a large number of neutrophils are recruited to infected vaginal mucosa and are required for protection during primary and secondary challenge²⁹. NK cells are also important in controlling herpesviruses; people who lack NK cells or have defective NK cell function have increased susceptibility to herpesvirus infections³⁰. Intraepithelial $\gamma\delta$ T cells also contribute to immune protection against vaginal challenge with HSV-2³¹. NK cells have an important role in defence against HIV-1 as indicated by the evasion mechanisms used by HIV-1 to specifically prevent NK cell recognition of infected cells³².

Innate recognition of STVs

Similar to other mucosal surfaces, multiple PRRs expressed by various cell types ensure surveillance of most pathogens that enter the genital mucosa. Research over the past decade has identified several PRR family members including the Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs)³³. Engagement of the PRRs elicits the secretion of type I interferons (IFNs) and other cytokines that are essential for suppressing viral replication and spread through the induction of antiviral effector molecules³⁴. In addition, some PRR-induced responses are essential for the generation of effective adaptive immune responses to pathogens³⁵.

DCs, monocytes and macrophages express distinct sets of TLRs³⁶. Human Langerhans cells express most TLRs but lack TLR7, TLR9 and TLR4³⁷. The type II epithelial cells that line the vaginal canal and ectocervix express TLR2, TLR3, TLR4 and TLR9. RLRs, which include retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), detect the RNA viral genome or replication products³⁸. In addition to RNA viruses, RIG-I is also involved in the recognition of dsDNA following transcription by RNA polymerase III^{39, 40}. RLRs are ubiquitously expressed to ensure that regardless of tropism, viruses can be detected by infected cells. NLRs comprise a large family of intracellular PRRs that regulates innate immunity in response to recognition of various pathogen-associated molecular patterns (PAMPs) and stress signals⁴¹. NLRP1 is widely expressed, whereas NLRP3 expression is restricted to epithelial cells of the oropharynx, oesophagus and ectocervix, and immune cells⁴². The NLR family, pyrin domain-containing proteins (NLRPs) can activate inflammasomes, which in turn activate caspase 1, leading to cleavage of pro-forms of interleukin-1 β (IL-1 β) and IL-18 into mature cytokines. Recent studies show that in addition to NLRPs, absent in melanoma 2 (AIM2) can couple dsDNA recognition to inflammasome activation^{43–46}. NLRs are activated following infection by bacteria, viruses and parasites and are crucial in both innate and adaptive immune responses⁴¹.

HSV infection is sensed by multiple PRRs. In plasmacytoid DCs (pDCs), HSV is recognized by TLR9 in the endosome^{47, 48}, whereas in other cell types, HSV is recognized in the cytoplasm by the RIG-I pathway following transcription by DNA-dependent RNA polymerase III³⁹. Certain strains of HSV also trigger TLR2 activation on DCs and macrophages⁴⁹. HSV-2 also activates inflammasomes⁵⁰, independent of AIM2⁵¹. Caspase 1 is not required for adaptive immunity to genital HSV-2 infection⁵², however IL-18 is required for innate protection against HSV-1 infection⁵³. pDCs are rapidly recruited to the vagina following HSV-2 infection and provide a local source of type I IFNs for limiting viral replication⁵⁴.

Recently generated HPV vaccines have used HPV-derived virus-like particles (VLPs). In addition to containing viral epitopes, these VLPs stimulate innate immune recognition in DCs through a myeloid differentiation primary-response protein 88 (MyD88)-dependent pathway, and adaptive immunity to VLPs is compromised in the absence of MyD88⁵⁵. It is likely that this activity of stimulating a MyD88-dependent transcription programme contributes to the success of the HPV-based VLP vaccine. Innate recognition system for the intact HPV virions, identification of the PRRs involved in VLP recognition and the nature of the PAMPs being recognized will provide further insights into the basic biology of papillomavirus recognition.

Despite the recent development in our understanding of the cell-intrinsic restriction pathways used to contain retroviruses⁵⁶, it is not clear how HIV-1 or retroviruses in general are recognized by PRRs. Studies in mice indicate that humoral immunity to a retrovirus (Friend murine leukaemia virus) requires MyD88 and DCs⁵⁷. In addition, endocytosed viral RNA is detected by pDCs through a process that probably involves TLR7⁵⁸. However, even

though pDCs can produce IFN- α in response to HIV-1, this appears to be a highly inefficient process requiring as much as 10,000 TCID₅₀ of HIV-1 (compared to 1 infectious HSV or influenza virus per cell being sufficient to trigger comparable level of IFN- α secretion)⁵⁹. In addition, HIV-1 induces a global disruption of innate signalling pathways in infected cells by degrading interferon-regulatory factor 3 (IRF3)⁶⁰, making directly infected cells incapable of producing type I IFNs. Understanding which innate pathways are involved in sensing HIV-1 and which of these pathways link innate recognition to adaptive immune responses will be key to developing a successful HIV-1 vaccine⁶¹.

Adaptive immunity

Initiation of acquired immunity against STVs

The type II mucosa is characterized by the absence of MALT (Table 1). Priming occurs exclusively in the draining lymph nodes. The human vaginal canal is drained by several lymph nodes, including the common iliac, interiliac, external iliac and inguinal femoral lymph nodes (in descending order). The penis is drained by the inguinal lymph nodes. Antigens in the anorectal canal are handled by the local MALT that underlies the type I epithelium (upper rectum) and by the inguinal lymph node draining the lower rectum and anus (Figure 3).

During a natural course of infection, STVs infect specific target cells and are taken up by local antigen-presenting cells. HSV-2 infects the type II epithelium of the vagina and cervix. Virus infection is detected by the infected epithelial cells and by submucosal DCs in a MyD88-dependent manner. Recognition of infection by both haematopoietic and stromal compartments is required for successful induction of protective T helper 1 (T_H1)-type immunity⁵². However, directly infected cells are incapable of priming T cells because HSV-2 blocks MHC class I and class II presentation and is a highly lytic virus⁶². Uninfected submucosal DCs take up viral antigens and migrate to the draining lymph nodes, where they present antigenic peptides to cognate CD8⁺ and CD4⁺ T cells^{63, 64}. Langerhans cells within the vaginal epithelium differentiate from circulating bone marrow-derived precursors, express low level of Langerin⁶⁵ and do not participate in T_H1 cell priming^{63, 64}. In addition, vaginal LCs were shown to induce IL-17-secreting CD8 T cells that suppress CTL responses following intravaginal immunization with ovalbumin and cholera toxin B⁶⁶. Early immune responses against genital HSV infection fail to clear the virus because it can invade the innervating ganglia and establish latent infection prior to the onset of highly effective immunity. Latent virus cannot be cleared by T cells or antibody, although CD8⁺ T cells provide important immune surveillance of the infected neurons through a non-lytic mechanism⁶⁷. Natural immunity against HSV-1 offers some level of protection against the acquisition of genital HSV-2 in women but not in men⁶⁸.

Natural infection with HPV induces poor immunity. HPV-encoded molecules engage multiple mechanisms to prevent the initiation of a robust immune response, including depletion of Langerhans cells by HPV E6 protein⁶⁹, downregulation of MHC class I molecules by HPV E5 protein⁷⁰ and blockade of type I IFN signalling by HPV E7 protein⁷¹. Thus, HPV antigens are likely to be cross-presented only by non-infected DCs the antigen presentation machinery of which is not affected by the virus evasion mechanisms. Presumably such DCs are activated by a cell-extrinsic mechanism through recognition of virus-infected cells by an endosomal PRR³⁵. Although the nature of the DCs that are responsible for this priming is unknown, because HPV infection occurs only within the type II epithelial layer, Langerhans cells in the epithelial layer and possibly submucosal DCs that extend their dendrites towards the epithelial layer are most likely to participate in this process.

During HIV-1 infection, the type of DCs that primes CD4⁺ and CD8⁺ T cell responses is unknown. Rather, much of the focus has been placed on the role of DCs and Langerhans cells in enhancing HIV-1 infection. Langerhans cells can bind to HIV-1 gp120 through mannose C-type lectins⁷². Using an *ex vivo* human organ culture system, Langerhans cells were shown to take up HIV-1 through endocytosis⁷³. As Langerhans cells exit the epithelium at the basal side, they transport intact virions, thereby enabling the infection to spread beyond the site of viral entry¹². Strong immune responses induced by HIV-1 infection occur too late to eliminate the infection⁷⁴. In addition, HIV-1 superinfection can occur in individuals with a strong and broadly reactive virus-specific CD8⁺ T cell response⁷⁵.

These results highlight the need to design vaccines that can induce immune responses that are more potent than those generated by a natural STV infection, as responses elicited by natural infection with viruses such as HPV and HIV-1 provide very poor protective immunity. Vaccines that simply mimic the ‘best’ immune responses generated during natural STV infection will not be effective as a preventative measure. In addition, an “immunogenic” vaccine will not necessarily be protective. Thus, it is important to understand what constitutes a protective immune response to a given pathogen and tailor prophylactic vaccines accordingly.

Effector mechanisms

Once generated, effector and memory lymphocytes specific to a given pathogen can migrate to various sites in the body and provide protection against the infection (Figure 5a). Neutralizing antibodies are the preferred effector mechanism as they can establish sterile immunity and provide complete protection against most viral infections. In addition, as antibodies circulate throughout the body and can access most mucosal tissues, there is no need to generate local antibody-producing cells. Since type II epithelial cells can not transport IgA, antibodies in the vaginal lumen come from two sources, IgA is secreted from the cervix and uterus (type I epithelium) and there is paracellular diffusion of serum-derived and locally produced IgG through the type II epithelium of the vagina⁷⁶. Antibody profiles in male genital tract secretions resemble those that are present in serum rather than those that are characteristic of typical external secretions⁷⁷. This suggests that once circulating levels of antibodies are established, recipients of a vaccine might be protected from challenge through multiple mucosal routes.

In addition to neutralizing antibodies, cell-mediated immunity by CD4⁺ and CD8⁺ T cells have a crucial role in antiviral defence. CD4⁺ T cells provide help for antibody production by B cells. CD4⁺ T cell help is also required for CD8⁺ T cell responses during the priming phase for differentiation into effector T cells^{78–81} and to generate memory T cells^{82–84} that mount a robust secondary response. Furthermore, CD4⁺ T cell help is required for entry of CD8⁺ T cells into the genital mucosa during the effector phase⁸⁵. Finally, CD4⁺ effector T cells themselves have a direct antiviral role. In the mouse genital herpesvirus model, T_H1 cells enter the infected vagina and secrete high levels of IFN γ , which blocks viral replication in infected cells⁸⁶. CD8⁺ T cells (cytotoxic T lymphocytes (CTLs)) are key antiviral effectors that block further dissemination of the virus through direct elimination of infected cells by perforin- and granzyme-mediated cytotoxicity. Immunization protocols that only elicit CTL responses are sufficient to provide protective immunity against genital herpesvirus^{87, 88} challenge in animal models. In addition, CD8 T cells are important in preventing latent HSV from being reactivated in neurons^{89–92}.

Establishment of memory foci in the genital mucosa

The vaginal submucosa contains no MALT at steady state. However, notably, once the initial wave of virus is cleared, foci of DCs and T cells form beneath the epithelial layer of the vagina (Figure 5b). In mice, after the clearance of infection with thymidine kinase mutant HSV-2 (TK⁻ HSV-2, which is incapable of reactivation from the sacral ganglia), clusters of cells, consisting largely of memory CD4⁺ T cells, B cells, DCs and macrophages, form along the vaginal submucosa⁸⁶. In humans, similar clusters, also including CD8⁺ T cells, are found in patients with recurrent HSV-2 disease⁹³. Such structures are not found in naïve hosts, but persist for months after HSV-2 infection in mice⁸⁶ and humans⁹³ or after *Chlamydia* infection in mice⁹⁴, long after the pathogens have been cleared. In older humans without any STI symptoms, a distinct band of both CD4⁺ and CD8⁺ T cells with an inner core of B cells is often found in both the cervix and vagina directly under the epithelium⁹⁵. These clusters are probably important for providing an immediate response on secondary infection. Secondary infection of TK⁻HSV-2-primed mice with wild-type HSV-2 results in rapid production of IFN γ by preexisting CD4⁺ T cells, which are presumably stimulated locally by DCs and B cells in the cell clusters⁸⁶. A study that followed HSV-2-specific CD8⁺ T cells in humans revealed that such T cells are recruited rapidly following reactivation and persist for more than two months after reactivation and healing adjacent to peripheral nerve endings at the dermal-epidermal junction⁹⁶. Importantly, subsequent virus reactivation at the site where CD8⁺ T cells are present did not result in lesion formation, indicating that HSV-2-specific CD8⁺ T cells at the site of genital herpesvirus lesions control local viral replication. Therefore, localized mucosal memory T cell populations appear to provide a superior control of viral infection compared with circulating memory T cells, suggesting that vaccines against STIs should generate localized memory T and B cell populations at sites of potential exposure.

Vaccines against sexually transmitted viruses

A success story – HPV vaccines

Two recent vaccines against HPV have resulted in overwhelming success. Gardasil (Merck), which comprises VLPs of the four major mucosal HPVs: HPV-16 and HPV-18 (high risk for primary causes of cervical cancer) and HPV-6 and HPV-11 (the causes of genital warts). Cervarix (GlaxoSmithKline) comprises VLPs of HPV-16 and HPV-18. Both vaccines have been shown in clinical trials to be extremely effective, providing protection from infection in almost 100% of cases⁹⁷. Protection conferred by VLP-based vaccines is thought to be mediated by neutralizing antibodies⁹⁸. The outcomes of these trials highlight that successful vaccines against STVs are possible. However the studies described below show that each STV probably requires a specific type of effector immunity to confer protection in the host.

Challenges ahead

Although the success of HPV vaccines illuminates the way for other STV vaccines, protective immunity based solely on neutralizing antibodies may not be achievable for other STVs. For viruses that undergo rapid mutation, such as HIV-1, a high titre of broadly neutralizing antibodies that target conserved epitopes of HIV gp120 must be generated⁹⁹. However, vaccine candidates tested so far have failed to achieve this goal. Likewise, several clinical trials of antibody-based vaccines for HSV-2 have failed to demonstrate significant protection, despite inducing high titre serum antibody levels in vaccine recipients¹⁰⁰. Therefore, vaccines that simultaneously elicit effective CD4⁺ T cell, CD8⁺ T cell and B cell responses are probably needed to stop the spread of HIV-1 (Ref. ¹⁰¹) and HSV-2 (Ref. ¹⁸). In addition to traditional vaccines, topical microbicides that could prevent entry and infection of STIs are also currently being considered (Box 3).

Although many vaccine approaches have been tested to date, none has proven successful in preventing HIV-1 infection in humans, despite inducing robust levels of antibody or cell-mediated immunity. Although the immune correlate of protection is still unclear, experts agree that establishing a local pool of memory T cells is key to protection against HIV-1 (Ref. ^{14, 101}). Given that a single HIV-1 virion is responsible for establishing infection in humans^{15, 16}, preexisting local memory CTLs might have a significant opportunity to reduce replication and dissemination of the founder virus. Recent data indicate that the establishment of tissue-resident memory T cells provides more robust protection against skin HSV-1 infection than circulating memory T cells¹⁰². Therefore, a key feature to consider for future vaccines against STVs may be the ability of vaccines to recruit and establish resident memory T cells at susceptible exposure sites.

Targeting T cells to the genital mucosa

How can we make vaccines that establish local memory T cells in the genital mucosa? To answer this question, we must turn to the migration behaviour of effector and memory T cells. The current paradigm of cellular migration is that effector memory T cells circulate throughout the peripheral tissues whereas central memory T cells reside in the secondary lymphoid tissues¹⁰³. Indeed, regardless of the site of pathogen or antigen encounter, pathogen-specific memory CD8⁺ T cells¹⁰⁴ and CD4⁺ T cells¹⁰⁵ can be found in various tissues, including the gut, lungs, liver and lymph nodes. However, peripheral tissue distribution of memory T cells occurs mainly following infection with live replicating vectors that cause systemic infection¹⁰⁴ or antigen injected systemically¹⁰⁵ (Box 4). Furthermore, circulating memory CD8⁺ T cells do not migrate efficiently into the brain or intestinal lamina propria even following replicating virus infection¹⁰⁶. Such restricted migration of memory T cells is even more exaggerated in response to localized immunogen or vectors that cause localized infection. In such settings, memory T cells seem to follow two distinct migration patterns – permissive migration (spleen, lungs and liver) and restricted migration (central nervous system¹⁰⁷, skin¹⁰² and vaginal mucosa⁸⁵). Migration into restricted tissues requires a subset of CD4⁺ T cells to pave the way first^{85, 107}. Such pioneering CD4⁺ T cells respond to local chemokines, enter the tissue and induce a subsequent wave of chemokines that enable other CD4⁺ T and CD8⁺ T cells to enter the tissue. These results indicate that pioneering CD4⁺ T cells may be needed to establish tissue-specific populations of both CD4⁺ and CD8⁺ memory T cells. Thus, vaccines based on generating only systemic CD8⁺ T cell immunity are likely to fail because such T cells are not self-sufficient for entry into the genital tissue.

“Prime and pull” approach for STI vaccines

The most effective means to establish a memory T cell response is by intravaginal immunization with live agents that infect the vaginal tissue. However, assuming that this is not a viable option in humans, several other approaches have been tested (Box 4). An ideal HIV-1 vaccine should establish a local memory CD8⁺ T cell population and robust IgG- and IgA-secreting plasma cells in the genital tissues without establishing a large pool of activated CD4⁺ T cells (which can become targets of HIV-1 replication⁹³). However, traditional approaches do not allow for selective recruitment of CD8⁺ T cells and/or plasma cells. Using recent understanding of the recruitment pathways involved for each lymphocyte subset, a new way to approach this issue is to generate robust systemic immunity using conventional immunization that would trigger all arms of the adaptive immune system (prime) and then to reorient selective lymphocyte subsets (CD4⁺ T cells, CD8⁺ T cells or B cells) into the genital mucosae using a second signal (pull). Such a prime and pull strategy alleviates the need to develop a new vaccine formulation and can be applied to any mucosal tissue, as long as the chemokines used to ‘pull’ selective lymphocyte populations into the relevant tissues are identified. For example, humans can be primed with a conventional

vaccine intramuscularly to generate cellular and humoral immune responses. Once the effector cells are generated, CTLs can be pulled into the vaginal tissues⁸⁵ by applying CXCL9 locally. Identification of the molecular signature required for memory and effector T cell entry holds the key to manipulating their migration behaviour.

Concluding remarks

Recent studies have shed light on the biology of genital mucosal immunity against STVs. We now have a better understanding of the invasion mechanisms used by HIV-1, HSV-2 and HPV, the importance of viral replication in target cells in susceptible tissues and the various host factors that restrict viral replication. In parallel, multiple evasion mechanisms used by the viruses to interfere with innate and adaptive recognition, PRR signalling, IFN responses and effector functions of IFN-stimulated genes have been uncovered. A common theme that has emerged is that natural immunity that develops following infection with these viruses provides minimal protection against secondary challenges with a heterologous virus. Thus, a vaccine may need to elicit a different type of immune response altogether to achieve protection in the immunized hosts.

Several basic areas of genital mucosal immunity warrant future investigation. First, we must better understand the rules that govern effector and memory lymphocyte migration into the different areas (epithelium, submucosa, cervix, vagina and penis) of the genital mucosa. Which selectins, integrins and adhesion molecules involved? What chemokines are responsible for the recruitment of each lymphocyte subsets? By what means could such lymphocytes be recruited and maintained in the genital mucosa? To this end, studying the lymphoid clusters that form following exposure to STIs may provide important insights. Second, further understanding of the importance of the innate signals that are required to mobilize DCs and prime appropriate immune responses is crucial. How are the naïve T cells programmed to migrate back to the infected tissue? Is there a unique biology of genital mucosal DCs that enables such programming? What antigen-presenting cells are responsible for the priming and the recall phases of immune responses to STIs? Finally, once an ideal vaccine is made, a key issue is how best to deploy the vaccines economically to the developing countries where they are needed most. The development of do-it-yourself technologies for delivering chemokines and antigens to the relevant mucosa would facilitate various cutting-edge vaccination approaches to establish protective immunity against STIs.

Glossary

Virus-like particles	(VLPs). Virion-like structures formed from the self assembly of viral envelope or capsid proteins <i>in vitro</i> . VLPs are not infectious because they do not contain a viral genome.
Targeted lymph node immunization	A subcutaneous immunization technique that aims to administer the vaccine in the proximity of the internal and external iliac lymph nodes. This technique has been proposed as a means to harvest the naturally existing imprinting mechanism for educating effector lymphocytes to migrate to the tissue being drained by the given lymph node (i.e., genital and rectal mucosae).
$\gamma\delta$ T cells	T cells that express a T cell receptor consisting of a γ -chain and a δ -chain. These T cells are present in the skin, vagina and intestinal epithelium as intraepithelial lymphocytes (IELs). Although the exact function of $\gamma\delta$ T cells is unknown, it has been suggested that mucosal $\gamma\delta$ T cells are involved in innate immune responses.

Langerhans cells	A type of dendritic cell that is resident in the epidermal layer of the skin.
Pattern recognition receptors	(PRRs). A host receptor (such as Toll-like receptors or NOD-like receptors) that can sense pathogen-associated molecular patterns and initiate signalling cascades that lead to an innate immune response. These can be membrane bound (e.g. TLRs) or soluble cytoplasmic receptors (e.g. RIG-I, MDA5 and NLRs).
Cross-presentation	The mechanism by which certain antigen-presenting cells take up, process and present extracellular antigens on MHC class I molecules to stimulate CD8 ⁺ T cells
M cells	(Microfold cells). Specialized epithelial cells that deliver antigens by transepithelial vesicular transport from the gut lumen directly to intraepithelial lymphocytes and to subepithelial lymphoid tissues.
Goblet cells	Mucus-producing cells found in the epithelial-cell lining of the intestine and lungs.
Paneth cells	Present at the base of the crypts in the intestinal epithelium, Paneth cells produce antimicrobial proteins and peptides, including phospholipase A2 and defensins.
Founder virus	A transmitted virus or a virus that gives rise to all virus quasispecies in an infected individual.
TCID₅₀	50% Tissue Culture Infective Dose. This is a typical virus infectivity assay that quantifies the amount of virus required to produce a cytopathic effect in 50% of inoculated tissue culture cells.
Transformation zone	Transformation zone is the area around the border between the endocervix and ectocervix. This is where the columnar epithelial cells of the endocervix meets the stratified squamous epithelial cells of the ectocervix. It is the most common area for cervical cancer to occur.

References

1. Starnbach MN, Roan NR. Conquering sexually transmitted diseases. *Nat Rev Immunol.* 2008; 8:313–317. [PubMed: 18309315]
2. UNAIDS/WHO. AIDS epidemic update 2007. Geneva, Switzerland: 2007.
3. WHO. Report on the global AIDS epidemic. 2008.
4. Lafferty WE. The changing epidemiology of HSV-1 and HSV-2 and implications for serological testing. *Herpes.* 2002; 9:51–55. [PubMed: 12106513]
5. Frazer IH. Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol.* 2004; 4:46–54. [PubMed: 14704767]
6. WHO. Comprehensive Cervical Cancer Control: A Guide to Essential Practice. 2006.
7. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev.* 2005; 206:306–335. [PubMed: 16048557]
8. Tobian AA, Gray RH, Quinn TC. Male circumcision for the prevention of acquisition and transmission of sexually transmitted infections: the case for neonatal circumcision. *Arch Pediatr Adolesc Med.* 2010; 164:78–84. [PubMed: 20048246]
9. Iwasaki A. Mucosal dendritic cells. *Annu Rev Immunol.* 2007; 25:381–418. [PubMed: 17378762]

10. Fussell EN, Kaack MB, Cherry R, Roberts JA. Adherence of bacteria to human foreskins. *J Urol.* 1988; 140:997–1001. [PubMed: 2902235]
11. Naylor SW, et al. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect Immun.* 2003; 71:1505–1512. [PubMed: 12595469]
12. Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. *Nat Rev Immunol.* 2008; 8:447–457. [PubMed: 18469831]
13. Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. *Nat Rev Microbiol.* 2003; 1:25–34. [PubMed: 15040177]
14. Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature.* 2010; 464:217–223. [PubMed: 20220840]
15. Keele BF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A.* 2008; 105:7552–7557. [PubMed: 18490657] This study and Ref. # 16 demonstrated that a single HIV-1 virus is responsible for establishing the majority of clinical infection in humans.
16. Abrahams MR, et al. Quantitating the multiplicity of infection with human immunodeficiency virus type 1 subtype C reveals a non-poisson distribution of transmitted variants. *J Virol.* 2009; 83:3556–3567. [PubMed: 19193811]
17. Spear PG, Eisenberg RJ, Cohen GH. Three classes of cell surface receptors for alphaherpesvirus entry. *Virology.* 2000; 275:1–8. [PubMed: 11017782]
18. Koelle DM, Corey L. Herpes simplex: insights on pathogenesis and possible vaccines. *Annu Rev Med.* 2008; 59:381–395. [PubMed: 18186706]
19. Hernandez BY, et al. Circumcision and human papillomavirus infection in men: a site-specific comparison. *J Infect Dis.* 2008; 197:787–794. [PubMed: 18284369]
20. Kines RC, Thompson CD, Lowy DR, Schiller JT, Day PM. The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. *Proc Natl Acad Sci U S A.* 2009; 106:20458–20463. [PubMed: 19920181] An intriguing report demonstrating that HPV evolved a two-phase mechanism of entry - initial steps take place on the basement membrane followed by capsid transfer to basal epithelial cells.
21. Valore EV, Park CH, Igteti SL, Ganz T. Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol.* 2002; 187:561–568. [PubMed: 12237628]
22. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol.* 2010; 10:159–169. [PubMed: 20182457]
23. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. *Science.* 2001; 292:1115–1118. [PubMed: 11352068]
24. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature.* 2007; 449:811–818. [PubMed: 17943117]
25. Qin J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2012; 479:51–56. [PubMed: 22107141]
26. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med.* 2005; 353:1899–1911. [PubMed: 16267321]
27. Cutler B, Justman J. Vaginal microbicides and the prevention of HIV transmission. *Lancet Infect Dis.* 2008; 8:685–697. [PubMed: 18992405]
28. Kim TK, et al. Heterogeneity of vaginal microbial communities within individuals. *J Clin Microbiol.* 2009; 47:1181–1189. [PubMed: 19158255]
29. Milligan GN. Neutrophils aid in protection of the vaginal mucosae of immune mice against challenge with herpes simplex virus type 2. *J Virol.* 1999; 73:6380–6386. [PubMed: 10400730]
30. Orange JS. Human natural killer cell deficiencies. *Curr Opin Allergy Clin Immunol.* 2006; 6:399–409. [PubMed: 17088643]
31. Nishimura H, et al. Intraepithelial gammadelta T cells may bridge a gap between innate immunity and acquired immunity to herpes simplex virus type 2. *J Virol.* 2004; 78:4927–4930. [PubMed: 15078977]

32. Cohen GB, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity*. 1999; 10:661–671. [PubMed: 10403641]
33. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 140:805–820. [PubMed: 20303872]
34. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. *Nat Rev Immunol*. 2008; 8:559–568. [PubMed: 18575461]
35. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science*. 2010; 327:291–295. [PubMed: 20075244]
36. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol*. 2004; 5:987–995. [PubMed: 15454922]
37. Flacher V, et al. Human Langerhans cells express a specific TLR profile and differentially respond to viruses and Gram-positive bacteria. *J Immunol*. 2006; 177:7959–7967. [PubMed: 17114468]
38. Pichlmair A, Reis e Sousa C. Innate Recognition of Viruses. *Immunity*. 2007; 27:370–383. [PubMed: 17892846]
39. Chiu YH, Macmillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell*. 2009; 138:576–591. [PubMed: 19631370] This study and Ref. # 40 demonstrated that certain classes of dsDNA in the cytosol are transcribed by RNA polymerase III, generating ligands for RIG-I for innate recognition.
40. Ablasser A, et al. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol*. 2009; 10:1065–1072. [PubMed: 19609254]
41. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol*. 2009; 27:229–265. [PubMed: 19302040]
42. Kummer JA, et al. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. *J Histochem Cytochem*. 2007; 55:443–452. [PubMed: 17164409]
43. Burckstummer T, et al. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat Immunol*. 2009
44. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature*. 2009
45. Hornung V, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature*. 2009
46. Roberts TL, et al. HIN-200 Proteins Regulate Caspase Activation in Response to Foreign Cytoplasmic DNA. *Science*. 2009; 1169841.
47. Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med*. 2003; 198:513–520. [PubMed: 12900525]
48. Krug A, et al. Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. *Blood*. 2004; 103:1433–1437. [PubMed: 14563635]
49. Kurt-Jones EA, et al. Herpes simplex virus 1 interaction with Toll-like receptor 2 contributes to lethal encephalitis. *Proc Natl Acad Sci U S A*. 2004; 101:1315–1320. [PubMed: 14739339]
50. Muruve DA, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature*. 2008; 452:103–107. [PubMed: 18288107]
51. Rathinam VA, et al. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat Immunol*. 2010; 11:395–402. [PubMed: 20351692]
52. Sato A, Iwasaki A. Induction of antiviral immunity requires Toll-like receptor signaling in both stromal and dendritic cell compartments. *Proc Natl Acad Sci U S A*. 2004; 101:16274–16279. [PubMed: 15534227]
53. Fujioka N, et al. Interleukin-18 protects mice against acute herpes simplex virus type 1 infection. *J Virol*. 1999; 73:2401–2409. [PubMed: 9971824]

54. Lund JM, Linehan MM, Iijima N, Iwasaki A. Cutting Edge: Plasmacytoid dendritic cells provide innate immune protection against mucosal viral infection in situ. *J Immunol.* 2006; 177:7510–7514. [PubMed: 17114418]
55. Yang R, et al. Papillomavirus-like particles stimulate murine bone marrow-derived dendritic cells to produce alpha interferon and Th1 immune responses via MyD88. *J Virol.* 2004; 78:11152–11160. [PubMed: 15452235]
56. Wolf D, Goff SP. Host restriction factors blocking retroviral replication. *Annu Rev Genet.* 2008; 42:143–163. [PubMed: 18624631]
57. Browne EP, Littman DR. Myd88 is required for an antibody response to retroviral infection. *PLoS Pathog.* 2009; 5:e1000298. [PubMed: 19214214] This report showed that DCs and MyD88 are required to mount antibody responses and antiviral defence against a mouse retrovirus *in vivo*.
58. Beignon AS, et al. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. *J Clin Invest.* 2005
59. Fitzgerald-Bocarsly P, Jacobs ES. Plasmacytoid dendritic cells in HIV infection: striking a delicate balance. *J Leukoc Biol.* 2010; 87:609–620. [PubMed: 20145197]
60. Doehle BP, Hladik F, McNevin JP, McElrath MJ, Gale M Jr. Human immunodeficiency virus type 1 mediates global disruption of innate antiviral signaling and immune defenses within infected cells. *J Virol.* 2009; 83:10395–10405. [PubMed: 19706707]
61. Medzhitov R, Littman D. HIV immunology needs a new direction. *Nature.* 2008; 455:591. [PubMed: 18833256]
62. Leib DA. Counteraction of interferon-induced antiviral responses by herpes simplex viruses. *Curr Top Microbiol Immunol.* 2002; 269:171–185. [PubMed: 12224508]
63. Lee HK, et al. Differential roles of migratory and resident DCs in T cell priming after mucosal or skin HSV-1 infection. *J Exp Med.* 2009; 206:359–370. [PubMed: 19153243]
64. Zhao X, et al. Vaginal submucosal dendritic cells, but not Langerhans cells, induce protective Th1 responses to herpes simplex virus-2. *J Exp Med.* 2003; 197:153–162. [PubMed: 12538655]
65. Iijima N, Linehan MM, Saeland S, Iwasaki A. Vaginal epithelial dendritic cells renew from bone marrow precursors. *Proc Natl Acad Sci U S A.* 2007; 104:19061–19066. [PubMed: 18006657]
66. Hervouet C, et al. Langerhans cells prime IL-17-producing T cells and dampen genital cytotoxic responses following mucosal immunization. *J Immunol.* 2010; 184:4842–4851. [PubMed: 20351191]
67. Divito S, Cherpes TL, Hendricks RL. A triple entente: virus, neurons, and CD8+ T cells maintain HSV-1 latency. *Immunol Res.* 2006; 36:119–126. [PubMed: 17337772]
68. Stanberry LR, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med.* 2002; 347:1652–1661. [PubMed: 12444179] Two double-blind, randomized trials of a HSV-2 glycoprotein-D-subunit vaccine revealed that the vaccine had efficacy against genital herpes only in women who are seronegative for both HSV-1 and HSV-2 but had no efficacy in men, regardless of their HSV serologic status.
69. Matthews K, et al. Depletion of Langerhans cells in human papillomavirus type 16-infected skin is associated with E6-mediated down regulation of E-cadherin. *J Virol.* 2003; 77:8378–8385. [PubMed: 12857907]
70. Ashrafi GH, Haghshenas M, Marchetti B, Campo MS. E5 protein of human papillomavirus 16 downregulates HLA class I and interacts with the heavy chain via its first hydrophobic domain. *Int J Cancer.* 2006; 119:2105–2112. [PubMed: 16823848]
71. Antonsson A, Payne E, Hengst K, McMillan NA. The human papillomavirus type 16 E7 protein binds human interferon regulatory factor-9 via a novel PEST domain required for transformation. *J Interferon Cytokine Res.* 2006; 26:455–461. [PubMed: 16800784]
72. Turville SG, et al. Diversity of receptors binding HIV on dendritic cell subsets. *Nat Immunol.* 2002; 3:975–983. [PubMed: 12352970]
73. Hladik F, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity.* 2007; 26:257–270. [PubMed: 17306567] An excellent analysis of the first events that take place following HIV-1 entry using ex vivo vaginal organ culture demonstrating direct viral infection of CD4 T cells within the epithelial layer.

74. McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. The immune response during acute HIV-1 infection: clues for vaccine development. *Nat Rev Immunol.* 2010; 10:11–23. [PubMed: 20010788]
75. Altfeld M, et al. HIV-1 superinfection despite broad CD8+ T-cell responses containing replication of the primary virus. *Nature.* 2002; 420:434–439. [PubMed: 12459786]
76. Brandtzaeg P. Mucosal immunity in the female genital tract. *J Reprod Immunol.* 1997; 36:23–50. [PubMed: 9430737]
77. Moldoveanu Z, Huang WQ, Kulhavy R, Pate MS, Mestecky J. Human male genital tract secretions: both mucosal and systemic immune compartments contribute to the humoral immunity. *J Immunol.* 2005; 175:4127–4136. [PubMed: 16148163]
78. Bennett SR, et al. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature.* 1998; 393:478–480. [PubMed: 9624004]
79. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature.* 1998; 393:474–478. [PubMed: 9624003]
80. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature.* 1998; 393:480–483. [PubMed: 9624005]
81. Jennings SR, Bonneau RH, Smith PM, Wolcott RM, Chervenak R. CD4-positive T lymphocytes are required for the generation of the primary but not the secondary CD8-positive cytolytic T lymphocyte response to herpes simplex virus in C57BL/6 mice. *Cell Immunol.* 1991; 133:234–252. [PubMed: 1671342]
82. Janssen EM, et al. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nature.* 2003; 421:852–856. [PubMed: 12594515]
83. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science.* 2003; 300:337–339. [PubMed: 12690201]
84. Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science.* 2003; 300:339–342. [PubMed: 12690202]
85. Nakanishi Y, Lu B, Gerard C, Iwasaki A. CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help. *Nature.* 2009; 462:510–513. [PubMed: 19898495]
86. Iijima N, et al. Dendritic cells and B cells maximize mucosal Th1 memory response to herpes simplex virus. *J Exp Med.* 2008; 205:3041–3052. [PubMed: 19047439]
87. Blaney JE Jr, et al. Immunization with a single major histocompatibility complex class I-restricted cytotoxic T-lymphocyte recognition epitope of herpes simplex virus type 2 confers protective immunity. *J Virol.* 1998; 72:9567–9574. [PubMed: 9811690]
88. Orr MT, Orgun NN, Wilson CB, Way SS. Cutting edge: recombinant *Listeria monocytogenes* expressing a single immune-dominant peptide confers protective immunity to herpes simplex virus-1 infection. *J Immunol.* 2007; 178:4731–4735. [PubMed: 17404252]
89. Knickelbein JE, et al. Noncytotoxic lytic granule-mediated CD8+ T cell inhibition of HSV-1 reactivation from neuronal latency. *Science.* 2008; 322:268–271. [PubMed: 18845757] This study showed that CTLs block HSV-1 reactivation by release of lytic granules into neurons and degrading ICP4 by granzyme B.
90. Liu T, Khanna KM, Carriere BN, Hendricks RL. Gamma interferon can prevent herpes simplex virus type 1 reactivation from latency in sensory neurons. *J Virol.* 2001; 75:11178–11184. [PubMed: 11602757]
91. Liu T, Khanna KM, Chen X, Fink DJ, Hendricks RL. CD8(+) T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons. *J Exp Med.* 2000; 191:1459–1466. [PubMed: 10790421]
92. Prabhakaran K, et al. Sensory neurons regulate the effector functions of CD8+ T cells in controlling HSV-1 latency ex vivo. *Immunity.* 2005; 23:515–525. [PubMed: 16286019]
93. Zhu J, et al. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med.* 2009; 15:886–892. [PubMed: 19648930] This study showed that HSV-2 infection results in the formation of persistent localized CD4 and CD8 T cell foci in the dermis below the healed lesion, and that such foci can be readily infected by HIV-1.

94. Johansson M, Lycke N. Immunological memory in B-cell-deficient mice conveys long-lasting protection against genital tract infection with *Chlamydia trachomatis* by rapid recruitment of T cells. *Immunology*. 2001; 102:199–208. [PubMed: 11260325]
95. Johansson EL, Rudin A, Wassen L, Holmgren J. Distribution of lymphocytes and adhesion molecules in human cervix and vagina. *Immunology*. 1999; 96:272–277. [PubMed: 10233705]
96. Zhu J, et al. Virus-specific CD8+ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. *J Exp Med*. 2007; 204:595–603. [PubMed: 17325200] This study followed CD8+ T cell surveillance of reactivating HSV-2 in humans and showed that persistent CD8+ T cells and sensory nerve endings could quickly respond to and eliminate reactivated virus before it undergoes extensive replication.
97. Schiller JT, Castellsague X, Villa LL, Hildesheim A. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. *Vaccine*. 2008; 26(Suppl 10):K53–K61. [PubMed: 18847557]
98. Campo MS, Roden RB. Papillomavirus prophylactic vaccines: established successes, new approaches. *J Virol*. 2010; 84:1214–1220. [PubMed: 19906917]
99. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. *Annu Rev Immunol*. 28:413–444. [PubMed: 20192810]
100. Stanberry LR. Clinical trials of prophylactic and therapeutic herpes simplex virus vaccines. *Herpes*. 2004; 11(Suppl 3):161A–169A.
101. Virgin HW, Walker BD. Immunology and the elusive AIDS vaccine. *Nature*. 2010; 464:224–231. [PubMed: 20220841]
102. Gebhardt T, et al. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol*. 2009 This study demonstrated that memory CD8+ T cells that persist within the skin of primary HSV-1 infection provide enhanced clearance of virus upon secondary challenge.
103. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999; 401:708–712. [PubMed: 10537110]
104. Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science*. 2001; 291:2413–2417. [PubMed: 11264538]
105. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. *Nature*. 2001; 410:101–105. [PubMed: 11242050]
106. Klonowski KD, et al. Dynamics of blood-borne CD8 memory T cell migration in vivo. *Immunity*. 2004; 20:551–562. [PubMed: 15142524] This study analyzed the migratory behaviour of CD8+ T memory cells and found controlled gating for entry into certain tissues including brain, peritoneum and intestinal lamina propria.
107. Reboldi A, et al. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol*. 2009; 10:514–523. [PubMed: 19305396] his study showed that T cell entry into a restricted tissue occurs in two steps a small number of pioneering CCR6+ T cells triggered the entry of a second wave of T cells that migrated into the CNS and caused EAE.
108. Brady MT. Newborn circumcision: routine or not routine, that is the question. *Arch Pediatr Adolesc Med*. 2010; 164:94–96. [PubMed: 20048249]
109. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009; 9:313–323. [PubMed: 19343057]
110. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004; 118:229–241. [PubMed: 15260992]
111. Tezuka H, et al. Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. *Nature*. 2007; 448:929–933. [PubMed: 17713535]
112. Atarashi K, et al. ATP drives lamina propria T(H)17 cell differentiation. *Nature*. 2008; 455:808–812. [PubMed: 18716618]
113. Gaboriau-Routhiau V, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*. 2009; 31:677–689. [PubMed: 19833089]

114. Ivanov II, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009; 139:485–498. [PubMed: 19836068]
115. Caramalho I, et al. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med*. 2003; 197:403–411. [PubMed: 12591899]
116. O'Mahony C, et al. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation. *PLoS Pathog*. 2008; 4:e1000112. [PubMed: 18670628]
117. Hall JA, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity*. 2008; 29:637–649. [PubMed: 18835196]
118. Van Damme L, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. *Lancet*. 2002; 360:971–977. [PubMed: 12383665]
119. Karim QA, et al. Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women. *Science*. 2010
120. VanCott TC, et al. HIV-1 neutralizing antibodies in the genital and respiratory tracts of mice intranasally immunized with oligomeric gp160. *J Immunol*. 1998; 160:2000–2012. [PubMed: 9469464]
121. Di Tommaso A, et al. Induction of antigen-specific antibodies in vaginal secretions by using a nontoxic mutant of heat-labile enterotoxin as a mucosal adjuvant. *Infect Immun*. 1996; 64:974–979. [PubMed: 8641809]
122. Livingston JB, Lu S, Robinson H, Anderson DJ. Immunization of the female genital tract with a DNA-based vaccine. *Infect Immun*. 1998; 66:322–329. [PubMed: 9423874]
123. Klavinskis LS, Barnfield C, Gao L, Parker S. Intranasal immunization with plasmid DNA-lipid complexes elicits mucosal immunity in the female genital and rectal tracts. *J Immunol*. 1999; 162:254–262. [PubMed: 9886393]
124. Parr EL, Parr MB. Immune responses and protection against vaginal infection after nasal or vaginal immunization with attenuated herpes simplex virus type-2. *Immunology*. 1999; 98:639–645. [PubMed: 10594699]
125. Morrison LA, Da Costa XJ, Knipe DM. Influence of mucosal and parenteral immunization with a replication-defective mutant of HSV-2 on immune responses and protection from genital challenge. *Virology*. 1998; 243:178–187. [PubMed: 9527927]
126. Gallichan WS, et al. Intranasal immunization with CpG oligodeoxynucleotides as an adjuvant dramatically increases IgA and protection against herpes simplex virus-2 in the genital tract. *J Immunol*. 2001; 166:3451–3457. [PubMed: 11207303]
127. Gallichan WS, Rosenthal KL. Specific secretory immune responses in the female genital tract following intranasal immunization with a recombinant adenovirus expressing glycoprotein B of herpes simplex virus. *Vaccine*. 1995; 13:1589–1595. [PubMed: 8578847]
128. Kool M, et al. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J Exp Med*. 2008; 205:869–882. [PubMed: 18362170]
129. Mora JR, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science*. 2006; 314:1157–1160. [PubMed: 17110582]
130. Li Z, et al. Novel vaccination protocol with two live mucosal vectors elicits strong cell-mediated immunity in the vagina and protects against vaginal virus challenge. *J Immunol*. 2008; 180:2504–2513. [PubMed: 18250460]
131. Suvas PK, Dech HM, Sambira F, Zeng J, Onami TM. Systemic and mucosal infection program protective memory CD8 T cells in the vaginal mucosa. *J Immunol*. 2007; 179:8122–8127. [PubMed: 18056354]
132. Cuburu N, et al. Sublingual immunization with nonreplicating antigens induces antibody-forming cells and cytotoxic T cells in the female genital tract mucosa and protects against genital papillomavirus infection. *J Immunol*. 2009; 183:7851–7859. [PubMed: 19933861]
133. Lehner T, et al. Protective mucosal immunity elicited by targeted iliac lymph node immunization with a subunit SIV envelope and core vaccine in macaques. *Nat Med*. 1996; 2:767–775. [PubMed: 8673922]

134. Kozlowski PA, et al. Differential induction of mucosal and systemic antibody responses in women after nasal, rectal, or vaginal immunization: influence of the menstrual cycle. *J Immunol.* 2002; 169:566–574. [PubMed: 12077289]
135. Johansson EL, Wassen L, Holmgren J, Jertborn M, Rudin A. Nasal and vaginal vaccinations have differential effects on antibody responses in vaginal and cervical secretions in humans. *Infect Immun.* 2001; 69:7481–7486. [PubMed: 11705923]
136. Rudin A, Riise GC, Holmgren J. Antibody responses in the lower respiratory tract and male urogenital tract in humans after nasal and oral vaccination with cholera toxin B subunit. *Infect Immun.* 1999; 67:2884–2890. [PubMed: 10338495]

Biography

Akiko Iwasaki received her Ph.D. from the University of Toronto (Canada), and her postdoctoral training from the National Institutes of Health (USA). She joined Yale University (USA) as a faculty in 2000, and currently is an associate professor of Department of Immunobiology and Department of Molecular Cellular and Developmental Biology. Akiko Iwasaki's research focuses on the mechanisms of immune defense against viruses at the mucosal surfaces. Her laboratory is interested in how viruses are recognized by the innate immune system and how such information is translated into the generation of adaptive immunity.

Box 1 | Male circumcision and transmission of sexually transmitted infections (STIs)

Three randomized clinical trials have evaluated male circumcision for prevention of STIs in Africa⁸. The trials found that circumcision decreases HIV-1 acquisition by 53–60%, HSV-2 acquisition by 28–34%, and HPV prevalence by 32–35% in men. Among female partners of circumcised men, bacterial vaginosis was reduced by 40%, and *Trichomonas vaginalis* infection was reduced by 48%. Genital ulcer disease was also reduced among males and their female partners. In addition, male circumcision reduces urinary tract infection, reduces penile cancer and reduces hygiene-related conditions (phimosis, paraphimosis and balanitis)¹⁰⁸. The underlying mechanism for such enhanced protection in circumcised men is unknown, but could be related to the absence of the type II mucosal surface. The only mucosal non-keratinized epithelium of the circumcised penis is the urethral opening, stratified squamous cells near the external meatus.

Box 2 | Role of commensal flora in gut immune homeostasis

The commensal flora helps to shape the immune system of the intestinal mucosa. Germ-free mice have underdeveloped gut-associated lymphoid tissues including Peyer's patches, isolated lymphoid follicles and mesenteric lymph nodes¹⁰⁹. Commensal bacteria are sensed by innate pattern recognition receptors and maintain the homeostasis of intestinal epithelial cell turn-over and integrity¹¹⁰. Lamina propria dendritic cells (DCs) are stimulated by commensal bacteria products through Toll-like receptor (TLR) engagement, which leads to the induction of inducible nitric oxide synthase (iNOS) expression and IgA secretion by B cells¹¹¹. Extracellular ATP secreted by commensal bacteria activates lamina propria DCs to induce the generation of T helper 17 (T_H17) cells¹¹². Commensal bacteria, particularly the segmented filamentous bacteria, promote T_H17 cell development in the intestine^{113, 114}. Commensal bacteria can directly activate TLRs expressed by regulatory T (T_{Reg}) cells and promote their proliferation and survival¹¹⁵. Introduction of a single commensal microorganism (*Bifidobacterium infantis*) is sufficient to increase T_{Reg} cell numbers in the intestine and spleen¹¹⁶. By contrast, DNA from commensal bacteria activates TLR9 on lamina propria DCs, which supports the differentiation of effector T cells by limiting the conversion of naïve T cells into T_{Reg} cells in the gut mucosa¹¹⁷.

Box 3 | Vaginal microbicides

Unlike vaccines, microbicides are topical agents that act to block transmission of sexually transmitted infections (STIs) without eliciting specific immune responses. Microbicides must be applied prior to every sexual intercourse to interfere with the incoming pathogen. Over 45 clinical trials have been conducted to test the efficacy of topical microbicides for the prevention of HIV-1 transmission²⁷. As discussed, infection by HIV-1 depends on its ability to enter the vaginal tissue through microabrasion, infect CD4⁺ T cells and macrophages within or beneath the epithelial cell layer and establish a local founder virus population. Microbicides have been developed to interfere with each of these steps. Non-specific microbicides include: surfactants, which disrupt cellular and microbial membranes; protective gel for minimizing mucosal breaks; vaginal milieu protectors to maintain acidic pH (either by buffering mechanism or by introduction of live *Lactobacilli* bacteria); and anionic polymers that block attachment, fusion and entry of viruses. These classes of microbicides have a broad range of targets, and can be effective in preventing entry by viral, bacterial and protozoan pathogens. Virus-specific microbicides against HIV-1 include inhibitors of CC-chemokine receptor 5 (CCR5) and reverse transcriptase. Unfortunately, a Phase III efficacy trial using the surfactant nonoxinol 9 showed increased HIV-1 seroincidence when used more than three times per day¹¹⁸. This is probably owing to the ability of nonoxinol 9 to disrupt the epithelial cell membrane, enabling viral invasion. However, a recent trial of vaginal microbicide containing tenofovir, a nucleotide reverse transcriptase inhibitor, was shown to be efficacious in preventing HIV-1 transmission¹¹⁹.

Box 4 | Intranasal vaccines to induce genital immunity?

It has been known for a long time that intranasal delivery of immunogens results in effector T and B cell responses in the female genital mucosae. Several studies have documented that the intranasal route of antigen delivery, but not intraperitoneal or subcutaneous, induces IgA in vaginal secretion^{120–127}, presumably by recruiting IgA-secreting plasma cells to the cervical mucosa. In addition, CD8⁺ T cells specific for the immunogen have been detected in the vaginal tissue following immunization with adenovirus vector or when antigen is delivered with CpG-containing DNA¹²⁶. What is special about the intranasal delivery route in this regard is unknown. It is possible that since the lung is a highly vascularized tissue, intranasal inoculation often results in delivery of vaccines not only to the respiratory mucosa but also to the gut mucosa and spleen¹²³, enabling dendritic cells (DCs) in various tissues to prime robust T cell responses. By contrast, targeting cutaneous lymph nodes through subcutaneous or intraperitoneal¹²⁸ inoculation of antigens tends to induce only serum IgG and not cervical IgA responses, suggesting that DCs in the skin system are not sufficient to induce IgA responses¹²⁹. However, when a replicating vector is used, other routes of infection can also establish vaginal T cell responses following *Listeria monocytogenes* (intramuscular)¹³⁰ or lymphocytic choriomeningitis virus (intraperitoneal)¹³¹ infection, owing to the ability of these vectors to disseminate and establish systemic infection. In addition to intranasal vaccines, sublingual vaccine delivery¹³² and targeted lymph node immunization¹³³ are being tested for their efficacy in establishing vaginal mucosal immunity. Results from studies in humans indicate that the intranasal route of immunization might hold some promise for establishing effective immunoglobulin responses in the female and male genital mucosae^{134–136}. Whether this and other approaches can be used to establish local T cell immunity in the genital mucosa in humans await further studies.

Online Summary

- Sexually transmitted viruses (STVs) cause major morbidity and mortality in humans worldwide. Yet, for the most part, vaccines that prevent transmission of STVs are not available.
- Certain STVs, such as herpes simplex virus type 2 (HSV-2) and human papillomavirus (HPV), cause much more severe disease in women compared to men. One of the underlying mechanisms is the difference in the anatomy of the genital mucosa, in that female genital tract has a much larger area of viral invasion and contains many more target cell types.
- Innate immunity in the genital mucosa is provided by physical barrier (mucus, epithelial cells), antimicrobial factors (antimicrobial peptides, lactoferrin, complement) and endogenous microflora. In addition, innate immune cells survey the environment for pathogen invasion through expression of a wide array of pattern recognition receptors (PRRs).
- Adaptive immunity in the genital mucosa is mediated by antibodies (IgG in vaginal transudate and IgA from cervix), CD4⁺ T cells and CD8⁺ T cells. After viral clearance, foci of memory lymphocytes form near the vaginal epithelium, which provides an immediate source of virus-specific T cells and B cells for secondary encounter.
- Immune correlate of protection for each STV must be understood before a successful vaccine can be developed. Vaccines against HIV-1 must balance establishing robust local memory CD8⁺ T cells without recruiting memory CD4⁺ T cells that become target of viral replication.

Nature Reviews | Immunology

Figure 1. Effect of HIV-1 infection on human demographics

In countries with high HIV prevalence, life expectancy at birth has fallen dramatically. In southern Africa, the average life expectancy at birth is now below 50 years on average in the subregion and below 40 years in Zimbabwe³. By contrast, the comparatively smaller HIV epidemics in Western Europe and Asia have scarcely affected life expectancy trends in those regions, which continue to rise steadily.

Nature Reviews | Immunology

Figure 2. Prevalence of genital herpes

a | The number of first-time visits to physicians' offices for genital herpes in the USA, as measured by the IMS Health, Integrated Promotional Services, IMS Health Report, 1966–2008. The graph depicts the proportion of persons aged 20 to 29 years with a positive laboratory test result for herpes simplex virus type 2 (HSV-2) from 1966–2008. **b** | An estimate of the global prevalence and incidence of HSV-2 infection plotted by gender in different age groups.

Nature Reviews | Immunology

Figure 3. Anatomy of the genital mucosae and anorectal canal

a | Female genital tract consisting of the upper reproductive tract (uterus, cervix) and the lower reproductive tract (vagina). Lymph drainage is indicated. **b** | Male genital tract – uncircumcised penis. **c** | Male genital tract – circumcised penis. Notice penis glans is covered by skin epithelium. **d** | Anorectal canal consisting of upper half by simple columnar type I epithelia and lower half covered by type II epithelia. Above the anorectal junction, numerous lymphoid follicles are present.

Nature Reviews | Immunology

Figure 4. Innate defense system of vaginal mucosa

Vagina and ectocervix are covered by type II epithelium. Mucus secreted by cervical glands and local epithelial cells provides a barrier to invading pathogens. Lactobacilli constitute the main species of endogenous flora and provides low pH and reactive oxygen species that are toxic to pathogens. **a** | Within and beneath the epithelial layer, there are many innate immune cells, including Langerhans cells, $\gamma\delta$ T cells, dendritic cells (DCs) and macrophages, that survey the environment for invading microorganisms. **b** | After virus infection, inflammatory responses elicit the recruitment of neutrophils, natural killer (NK) cells, monocytes and plasmacytoid DCs. These cell types express various pattern recognition receptors (PRRs) to ensure innate detection and initiation of type I interferon (IFN) responses to block further viral spread.

Figure 5. Adaptive immune system of the vaginal mucosa

a | Following primary virus infection, virus-specific T and B cells generated in the lymph nodes migrate to the site of infection in response to chemokine signals. CD4⁺ T cells migrate into the infected vaginal tissue, secrete interferon- γ (IFN γ), and block virus replication. IFN γ also induces expression of chemokines necessary for CD8⁺ T cell migration. Once in the tissue, CD8⁺ T cells migrate to the infected epithelial cells and induce apoptosis to block viral synthesis. B cells secrete IgG and block the spread of virus. **b** | After the clearance of primary virus infection, clusters of memory lymphocytes and antigen-presenting cells form near or within the epithelial cell layer. Such clusters represent a local and rapid responder unit, capable of secreting antiviral cytokines and releasing killer cells following secondary exposure to the same virus.

Table 1

Comparison of genital and rectal mucosae

	Type I	Type II
Tissues	Endocervix Upper anorectal canal	Vaginal canal Ectocervix Foreskin Lower anorectal canal
Epithelia	Simple columnar	Stratified squamous (non-keratinized)
Polymeric IgR	+	–
Major Ig isotype	sIgA	IgG
Presence of MALT	+	–
Presence of M cells	+	–
Langerhans cells	–	+
Mucus source	Goblet cell (rectum) Glands in crypt (cervix)	Epithelial cells