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Generating protective immunity against genital herpes

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Abstract

Genital herpes is an incurable, chronic disease that affects millions of people worldwide. Not only does genital herpes cause painful, recurrent symptoms, it is also a significant risk factor for the acquisition of other sexually transmitted infections such as HIV-1. Antiviral drugs are used to treat herpes simplex virus (HSV) infection, but they cannot stop viral shedding and transmission. Thus, developing a vaccine that can prevent or clear infection will be critical in limiting the spread of disease. In this review, we outline recent studies that improve our understanding of host responses against HSV infection, discuss past clinical vaccine trials and highlight new strategies for vaccine design against genital herpes.

Keywords

female genital tract; vaccines; memory T cells; antibodies; protective immunity; cell migration

Introduction

Genital herpes is one of the world's most common sexually transmitted viral infections. Most cases of genital herpes are caused by HSV-2, although the number of cases caused by HSV-1 are on the rise (1). Over 500 million people are infected worldwide, this estimate may underreport the number of cases as many people are unaware they have been infected (1). The most common manifestation is the recurrent outbreaks that result in painful inflammation and lesions in the genital area. Infection in pregnant women may also result in vertical transmission to the fetus, which can cause fatal herpes encephalitis in the newborn child. In the United States, approximately 16% of people ages 14–49 are seropositive for HSV-2, with higher seroprevalence in women (21%) than men (12%) (2). Over 80% of the people who tested seropositive had not yet been diagnosed with genital herpes (2). Cases of HSV-2 are higher in developing regions of the world than in developed, with seroprevalence reaching up to 70% in sub-Saharan Africa (1). Considering that HSV-2 infection is a strong risk factor for HIV-1 acquisition, preventative measures against HSV-2 transmission are of paramount public health importance. While the clinical symptoms of HSV-2 can be controlled with antiviral acyclovir and valacyclovir, even high doses of these drugs are insufficient to stop subclinical shedding and potential for transmission (3). Ideally, a prophylactic vaccine to prevent infection could protect both the recipient of the vaccine as

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well as any potential sexual partners. While several candidate vaccines against genital herpes have been tried in humans, these vaccines have failed and none exists for public use (4, 5). In this review, we outline our current knowledge of HSV-2 pathogenesis, immune responses to infection and both past and current vaccine strategies for the prevention of genital herpes.

Pathogenesis of primary genital HSV-2 infection

Upon sexual transmission, HSV infects epithelial cells in the female genital mucosa. Entry of HSV into a cell occurs primarily by viral fusion and is mediated by engagement of multiple receptors by viral glycoproteins (6). Once inside the host cell, the de-enveloped capsid introduces viral DNA into the host cell nucleus. Epithelial cells support the lytic replication of HSV, which leads to the rapid death of the host cell. During the lytic cycle, viral genes are transcribed in a cascade-like fashion whereby intermediate-early (IE, alpha) genes are transcribed first, followed by early (beta) and late (gamma) genes (7). After the replication cycle is complete, virions are enveloped and are released from the cell. Infectious virions can go on to infect other epithelial cells or can invade nerve endings throughout the tissue.

HSV-2 spreads from the epithelium to the peripheral nervous system (PNS) during the course of infection. Virus spreads from infected epithelial cells to the neurons through the axon termini within the tissue (8). Retrograde transport of the de-enveloped capsid to the neuronal cell body is mediated by dynein traveling along microtubules that line the length of the axon (8). Once in the soma, the virus exudes its genetic material into the nucleus and the HSV DNA circularizes to form episomes (7). HSV-2 does not undergo lytic infection in neurons; rather, it is maintained in a latent state until stress and other unknown factors cause reactivation (9).

The mechanism by which latency is initiated within the neuron is still unclear. Latency-associated transcript, or LAT, is the only HSV-associated transcript that is produced during the latent phase of infection, and it has been proposed that LATs actively promote viral latency and host cell survival through several different mechanisms (7). Reactivation from latency leads to the production of lytic genes and the anterograde transport of virus from the cell body back into the epithelial cells at the original site of infection. Upon release of infectious virions, the epithelium becomes reinfected and lytic replication of the virus leads to host cell lysis, which in turn leads to the symptoms that are commonly associated with genital herpes, such as inflammation and ulceration of the genital skin. Generally, the most severe symptoms are associated with primary infection, although many patients who become infected by HSV-2 are unaware of the infection (2, 10). In clinical episodes of disease, genital ulcers form after viral release from nerve endings into the epithelium and increase in size by spread of cell-associated HSV (11). Additional lesions, on the other hand, appear to develop from the spread of cell-free virus released from the initial site of infection, leading to the appearance of closely spaced individual lesions (11). Studies have shown that viral reactivation, and more importantly viral shedding, can occur in the absence of any clinical symptoms (12, 13). While it was originally thought that HSV-2 was largely silent during asymptomatic phases, mathematical modeling suggests that HSV-2 may be reactivating with great frequency in very small numbers of neurons at any given time, leading to the near-continuous release of HSV-2 into the genital epithelium, although this release may not necessarily be detectable (14). Even patients treated with high doses of acyclovir or valacyclovir, two antiviral drugs commonly used to control HSV-2 infection, had frequent episodes of viral shedding despite a lack of any clinical symptoms (3). Thus, the risk of transmission from an infected patient to a partner may be present even though overt signs of viral reactivation are absent.

Immune responses against genital HSV-2 infection

Innate link to adaptive immunity

Invasion of the genital mucosa by HSV-2 generates both innate and adaptive immune responses in the tissue. Upon infection, engagement of pattern recognition receptors (PRRs) by the virus initiates an antiviral program by the host. Response to infection first occurs in the target keratinocytes and tissue-resident hematopoietic cells. Inflammation and cytokines produced by this initial response recruits a variety of innate immune cells to the site of infection, including inflammatory monocytes, natural killer (NK) cells, dendritic cells (DCs) and plasmacytoid dendritic cells (pDCs). Recognition of HSV can occur through a toll-like receptor (TLR) 9, which recognizes viral DNA, and in some cases, TLR2, which reportedly binds viral glycoprotein (15, 16). HSV has also been reported to engage multiple different cytosolic receptors that bind nucleic acids, including IFI16 (17–20), retinoic acid inducible gene I (RIG-I) through an RNA polymerase III-dependent mechanism (21). More recently, cyclic-GMP-AMP synthase (cGAS) has also been shown to bind cytosolic DNA, which can then go on to activate stimulator of interferon genes (STING) through a secondary messenger (22, 23). HSV can also lead to the activation of inflammasomes, although unlike other dsDNA viruses, this activation occurs independently of absent in melanoma 2 (AIM2) (24) and is instead mediated by IFI16 (25). Innate viral recognition may also occur through mechanisms independent of PRRs, such as viral fusion with the host cell (26).

Activation of the innate immune response is critical for the induction of adaptive immunity. Mouse model of genital herpes has taught us that TLR signaling provides a crucial link between innate virus recognition and adaptive immunity to HSV-2. Furthermore, MyD88 signaling downstream of TLRs in both the hematopoietic and stromal cell compartment are required for the induction of optimal T helper 1 (Th1) responses to genital HSV-2 infection (27). Despite the numerous immune evasion mechanisms that HSV employs, a robust adaptive immune response does occur after infection (4). After genital HSV-2 infection, activation of naïve T cells occurs in the iliac lymph nodes, which are the draining lymph nodes (dLNs) for the genital tract (28). Naïve T cells are primed by migratory DCs that carry antigen from the infected tissue into the dLN (29, 30). Once activated, Th1 cells are recruited into the genital tract first, appearing within 3 days post-infection (p.i.) and peaking around day 6 p.i. (31). Activated CD8 T cells enter the vagina with slightly delayed kinetics as compared to CD4 T cells. Entry of effector CD8 T cells into the genital tract is critically dependent on IFN- γ production by Th1 cells, which in turn stimulates production of the inflammatory chemokines CXCL9 and CXCL10 (31). Th1 entry into the genital tract does not appear to depend on these two chemokines, and thus far the signals required to recruit Th1 cells into the genital tract are unknown (31). Upon genital HSV-2 infection, Tregs unexpectedly appear to regulate the proper localization of innate immune cells. Depletion of Tregs after infection alters the chemokine gradient within the lymph node and the site of infection, leading to decreased homing of NK cells, pDCs and CD11b⁺ DCs to the vagina (32).

Human immune responses to recurrent HSV-2 infection

In recurring episodes of genital herpes, the severity of symptoms correlates with the extent of viral spread and the speed with which the virus is controlled. Different immune cell populations, including virus-specific CD4 T cells, pDCs and other DC populations infiltrate and persist at the site of lesion formation (33). Tissue surveillance by the immune system, particularly CD8⁺ T cells in humans that reside at the dermal-epidermal junction (DEJ), helps to keep the severity of disease under control (34). As described above, while shedding occurs more frequently than originally suspected, genital biopsies and mathematical modeling show that the density of CD8 T cells at the site of virus release from the axonal

termini of neuron to the epithelium predicts whether reactivation will lead to a genital ulcer or asymptomatic shedding (35). Containment of viral spread by CD8 T cells appears to occur very rapidly, as infected cells are killed within hours (11). Once lesions have resolved, virus-specific CD8 T cells at the DEJ cluster around the axonal terminal of neurons from which virus is released and may provide viral control upon subsequent release of virus from the neurons into the genital epithelium (35, 36). Collectively, these studies show that while natural immune responses cannot clear genital HSV-2 infection, these responses are critical for controlling the severity of recurrent disease.

Immune responses within the nervous system

While immune responses at the epithelium have been well studied, self-defense mechanisms within the neuron are less well known. Sensory neurons in mice express low levels of TLR1 and 2, moderate levels of TLR3 through 6 and do not express TLR7, 8 or 9 (37). Defects in TLR3 and the downstream signaling molecules UNC-93B and TRIF have been reported in patients suffering from herpes encephalitis, but does not affect susceptibility to other infections, suggesting that this receptor is important for neuronal defense (38). In mice, TLR3 deficiency leads to increased susceptibility to CNS infection after genital infection with HSV-2, and alters the tropism of the virus within the CNS (39). In human iPSC-derived neurons and oligodendrocytes, TLR3 and UNC-93B deficiency leads to an increased susceptibility to HSV-1 infection (40). It is less clear whether TLR3 plays a role in viral recognition in the peripheral nervous system (PNS). Viral titers in the medulla spinalis are similar early after genital HSV-2 infection and increase over time, suggesting that the role of TLR3 is mostly limited to the CNS (39). Thus, other PRRs, such as cytoplasmic RNA and DNA sensors, may be important for mediating cell-intrinsic immunity in sensory neurons after genital infection with HSV-2.

Multicellular organisms rely on apoptosis as a potent antiviral mechanism to halt the spread of viruses. This is a sensible mechanism for renewable cell types, but not for non-renewable cell types such as neurons. This raises the possibility that specialized intrinsic mechanisms may mediate antiviral defense in neurons. Recent reports have shown that autophagy, a cellular homeostatic process that recycles cytoplasmic components, plays an important role in controlling viral infection in neurons. In neurons, absence of Atg5, a protein that is required for the formation of autophagosomes, leads to delayed clearance of viral antigen after CNS infection with Sindbis infection (41). Furthermore, deletion of Atg5 from neurons led to increased viral titers within peripheral nervous tissue after intravaginal (ivag) infection with HSV-1 (42). This study showed that type I IFNs and autophagy work together to provide optimal protection against HSV-1 infection in neurons (42). Whether autophagy plays a similarly important role in controlling neuronal infection with HSV-2 is unknown.

Once HSV-2 has established latency, control of reactivating virus within the ganglia appears to be heavily dependent on infiltrating virus-specific CD8 T cells. Despite a very low level of MHC class I expression in neurons, contact areas between memory CD8 T cells and the latently infected neuron appear to involve TCR polarization, suggesting antigen presentation may be occurring (43). As latent virus only transcribes LATs, and no viral proteins, memory CD8 T cells in the ganglia are likely responding to antigens produced from the very low level of frequent reactivation that is predicted to occur in neurons (14). While both CD8 and CD4 T cells infiltrate the ganglia after HSV-1 infection, only depletion of CD8 T cells leads to rapid reactivation of virus in the ganglia (44, 45). CD8 T cells control reactivating HSV-1 in neurons using two non-cytolytic mechanisms, 1) by release of granzymes into the neuron cleaves the intermediate-early gene product ICP4 rather than caspases, and 2) through IFN- γ production (45, 46). Unlike the functionally exhausted state of CD8 T cells during chronic infections such as HIV and hepatitis C (47), memory CD8 T cells within the latently infected ganglia appear to remain functional and capable of mounting recall responses (44,

48). However, LAT may play a role in modifying CD8 T cell effector activity, as mice ocularly infected with LAT(+) and LAT(-) HSV-1 appear to have different levels of T cell functionality (49). Thus, cellular immunity plays an important role at both the lytic and latent stages of HSV infection.

Protective immunity against HSV-2 infection

Natural host immune responses are rarely sufficient to control and clear HSV-2 infection. While secondary responses against recurring episodes of genital herpes are potent, once HSV-2 has established a latent reservoir within the peripheral nervous system, it remains there for life. Thus, it has been a challenge to determine what factors are required for sterile immunity against HSV-2 infection. Nevertheless, animal models have provided many clues to the correlates for protection against HSV-2 infection. Multiple studies have emphasized a role for memory T cells in protecting the genital mucosa against infection. After ivag immunization of mice with an attenuated HSV-2, which provides complete protection against death and development of any clinical symptoms after lethal genital challenge with wild type (WT) HSV-2, CD4 T cells form lymphoid clusters in the submucosa of the vaginal tissue that are retained long-term (50). This is in contrast to skin infection with HSV, in which CD4 T cells leave the dermis and enter circulation to mediate immunosurveillance (51). Depletion of CD4 T cells from ivag immunized mice leads to a loss of protective immunity, whereas depletion of CD8 T cells or B cells has minimal effect (50, 52). Immediate IFN- γ production in the tissue was shown to be the main effector mechanism by which Th1 cells protect against genital HSV-2 infection (50, 53). Although the role of memory CD8 T cells in protective immunity may not be obvious when the Th1 response is intact, this population is also capable of mediating protection against HSV infection. More specifically, tissue-resident memory CD8 T cells (CD8 T_{RM}) can provide superior immunity against skin and genital challenges with HSV compared to circulating memory CD8 T cells (54, 55). Thus, both CD4 and CD8 T cells are capable of mediating protection against genital HSV-2 infection, provided that the responses occur rapidly at the site of infection.

While B cell and antibody responses appear to play a less dominant role in the protection of mice immunized ivag with attenuated HSV-2, they can augment T cell responses during both primary and secondary infections. B cell depletion from immunized animals does not appear to affect the outcome of disease after WT HSV-2 genital challenge, but ivag immunized B cell deficient mice do not fare as well as their WT counterparts, suggesting that B cells may play a role in initiating a primary response against genital HSV-2 (50, 56). Several studies have also shown that anti-HSV-2 antibody can protect against HSV-2 infection if concentrations are sufficient. Passive transfer of serum immunoglobulin (Ig) from immune animals to naïve or B-cell deficient animals can help control infection after vaginal challenge with WT HSV-2 (33). Protection conferred by serum Ig against genital HSV-2 challenge appears to occur through the Fc neonatal receptor (FcRn), which is expressed by the epithelial lining of mucosal surfaces, including the genital tract, and mediates transcytosis of IgG to the lumen (57). Thus, while B cells and antibody are not required to mediate protection against genital HSV-2 infection in mice immunized genitally with attenuated HSV vaccines, humoral responses work in concert with cellular responses to enhance overall immunity. Further, antibodies are sufficient to protect the host from primary genital HSV infection if they exist in sufficient quantity at the site of infection. However, as described below, attempts to generate vaccines based on antibody responses in humans have not yet been successful.

Vaccines against genital herpes

Past prophylactic vaccine trials against HSV-2

In the past decade or so, two prophylactic vaccines for the prevention of genital herpes have been tested for efficacy and safety in large phase III clinical trials. Both vaccines were subunit vaccines targeted against either HSV-2 glycoprotein B and D (Chiron) or glycoprotein D (HerpeVac, GSK) alone. Both vaccines induced high serum titers of HSV neutralizing antibody, and HerpeVac also elicited Th1 responses (58, 59). Despite this, the Chiron vaccine showed no efficacy in prevention of either disease or infection with HSV-2 in enrollees (58). Furthermore, no difference was observed between the control and vaccinated groups in the duration of the first episode of clinical symptoms or in the recurrence of disease (58). The first HerpeVac study found that while men were unprotected by the vaccine, 74% vaccine efficacy was observed in women from discordant couples who were seronegative for both HSV-1 and HSV-2 (59). However, a follow-up study with the same vaccine in a generalized group of women seronegative for both HSV-1 and -2 found no protection against HSV-2 infection or disease, although a higher rate of efficacy was observed against HSV-1 (60). Currently, no vaccines are available for the prevention of genital herpes caused by HSV-2.

It is still unclear why these two vaccines failed in clinical trial. Neutralizing antibody levels waned in the months after boosting with HerpeVac, but no correlation was found between antibody levels and increased risk for HSV-2 contraction (58, 60). Furthermore, there was no difference in cellular immune responses between patients who contracted HSV and those that remained uninfected during the trial (60). However, all responses during these trials were measured in the blood, which is representative only of systemic immunity. Genital herpes is a local infection that is contracted through a mucosal surface, which suggests that optimal protection against HSV-2 requires robust mucosal responses. The female genital tract is a tissue that is resistant against immunosurveillance by circulating immune cells in the absence of infection or inflammation (31, 61). Compared to circulating memory T cells, T_{RM} can provide enhanced immunity against local viral infections in peripheral sites such as the skin, vagina and lung (54, 55, 62–64). Furthermore, immunization of monkeys with HIV-1 gp41 through the mucosal intranasal (i.n.) route, but not an intramuscular (i.m) route, gives rise to IgA in the vagina that is capable of blocking viral transcytosis (65). When i.n. or i.m. immunized monkeys are challenged vaginally with SHIV, i.n. immunized monkeys are better protected (65). This protection directly correlates with an increase in vaginal antibodies, but not serum IgG, suggesting that antibodies may also need to be concentrated within the target tissue in order to provide effective protection (65). Thus, it may be important to consider both the generation of robust immune responses as well as the localization of virus-specific memory lymphocytes when designing future vaccines.

Ongoing strategies for prophylactic HSV vaccines

In addition to subunit vaccines, other types of vaccines such as peptide vaccines and DNA vaccines have gone to clinical trial, and have been reviewed extensively elsewhere (4, 5). Of these, a phase I study using a DNA vaccine encoding HSV glycoprotein D was most recently completed. The vaccine was found to be safe in humans, but cellular responses occurred only in a small fraction of subjects immunized with the highest dose of DNA and elicited no antibody response (66). Live attenuated vaccines, while an attractive option for stimulating both cellular and humoral immune responses to a variety of epitopes, present safety issues that may complicate their use. However, viral mutants such as dl5–29, which is a replication-deficient HSV-2 strain lacking the UL5 and UL29 genes, have been shown to be efficacious and non-pathogenic in mouse and guinea pig models, and are being prepared

for testing in humans (67, 68). Of note, most of these vaccines are administered systemically or locally, which may not generate sufficient mucosal immunity against genital HSV-2.

Therapeutic vaccine trials against HSV-2

Therapeutic vaccines are designed to treat or eliminate recurrent genital herpes in previously infected patients by boosting the natural immune response that has already developed against the virus. Like prophylactic vaccines, multiple platforms have been tested in humans, but no effective therapeutic vaccine yet exists (4, 5). The most recent therapeutic vaccine to complete phase III clinical trial was the disabled infectious single cycle (DISC) vaccine, which was a replication deficient gH deletion HSV mutant (69). Although safe to use, the vaccine failed to meet its clinical endpoints. Currently, two therapeutic peptidebased vaccines are at phase I and phase II clinical trials, respectively. One is a subunit vaccine composed of glycoprotein D and a peptide derived from infected cell protein 4 (ICP4) (Genocea) (70). Immunization of mice induces both CD4 and CD8 T cell responses, while immunization of both mice and guinea pigs elicits serum antibody. Vaccination of guinea pigs genitally infected with HSV-2 leads to a decrease in mean lesion score, recurrence and viral shedding (70). The other vaccine candidate is composed of 32 synthetic peptides derived from HSV conjugated to human heat shock protein 70 (hsp70) (Agenus) (71). This vaccine elicits IFN- γ -producing cells in mice. When tested for therapeutic activity in guinea pigs, while there was no significant decrease in lesion score or mean duration, there was a significant decrease in the frequency of recurrences (71). Testing in humans during a phase I trial shows that the vaccine is safe and welltolerated, and can induce T cell responses (72). The availability of either an effective therapeutic or prophylactic vaccine could help control the spread of HSV infection.

Future strategies for herpes vaccines – eliciting local protection

Intravaginal immunization of mice with an attenuated HSV-2 prevents disease and death caused by a lethal challenge with WT HSV-2 (73). This route of immunization establishes tissue-resident cellular responses and increases virus-specific IgG in the vagina, which together can confer robust immunity (73). Although ivag immunization is a useful tool for studying immune responses against HSV-2 in animal models, it has not translated into a vaccine option in humans. Immunization through other mucosal routes, such as intranasal, has been explored as possible options for eliciting genital immunity. Accordingly, immunizations that take advantage of the purported “common mucosal system” have been tested in animal models as potential strategies for future vaccines. Intranasal immunization can lead to higher titers of HSV-specific IgA and IgG in the genital tract, increases the number of cytolytic lymphocytes in the tissue and improved protection against genital challenge compared to controls immunized through other routes (74, 75). More recently, it has been shown that i.n. immunization of mice with an IgG Fc fragment conjugated to gD, which is transported across the mucosal barrier of the lung by FcRn, leads to systemic cellular immunity, high levels of gD-specific IgG in the genital tract, and robust protection against a genital challenge (76). Thus, modifying the route by which vaccines are administered may have an impact on the level of mucosal immunity, which in turn may affect the efficacy of the vaccine.

Recently, a study from our lab offered an entirely different approach for inducing immunity at the genital tract (55). Our goal was to design a potential vaccine that would establish a pool of tissue-resident memory T cells in the vagina without direct immunization of the genital tract and that could be used in conjunction with standard parenteral immunization routes. Our strategy, which we call “prime and pull”, first generates robust systemic cellular immune responses and then redirects those responses to the genital tract with a topical application of chemoattractants. Mice are immunized subcutaneously with attenuated

HSV-2, which induces a systemic HSV-specific T cell response. During the effector phase, mice are treated ivag with a solution of the chemokines CXCL9 and CXCL10 (55). This chemokine treatment, or “pull”, recruits a large number of circulating HSV-specific CD8 T cells, and a proportion of these cells are retained in the vagina up to 12 weeks post-pull. When challenged genitally with a lethal dose of WT HSV-2, the prime and pull mice are fully protected from death, lose significantly less weight and develop significantly less severe clinical symptoms than the prime only controls (55). Immunity is not sterilizing, however, as there is no significant difference in mucosal viral titers compared to prime only controls, likely due to a lack of antibody and/or tissue-resident CD4 T cells. Mucosal control within the prime and pull system could be improved with different pull signals or alternative priming agents or routes, as other studies have suggested that local T cell populations are capable of reducing viral titers within the epithelium. However, very little virus is detected in the ganglia, suggesting that prime and pull may prevent establishment of latency in neurons. Preventing HSV-2 latency by prime and pull could in turn prevent reactivation, viral shedding and transmission to others, making it a potentially attractive option for reducing primary infection as well as recurrent disease. While other systemic vaccine strategies may provide similar protection against vaginal HSV-2 challenge in animal models, clinical trials of vaccines that rely solely on circulating immunity have proven ineffective in the past. Specifically, mouse models of HSV-2 vaccines do not predict protection in humans. This difference in protection in vaccinated mice and humans can be explained by many factors, likely including vaccine access to lymphoid tissues, antigen handling by DCs, and memory lymphocyte circulation pattern. We predict that prime and pull approach may bypass possible difference in the circulation pattern of memory T cells in humans vs. mice, and facilitate translation of existing preclinical vaccine approaches to humans. Needless to say, this hypothesis must be rigorously tested in humans.

Future challenges

Sexually transmitted viral infections such as HSV-2 represent a significant burden on global health. Despite several past and ongoing attempts to develop an efficacious vaccine to control genital herpes, none have been successful. Understanding how to design a vaccine that will control either infection or disease has been difficult because humans rarely control HSV-2 infection naturally. However, the use of animal models has increased our understanding of the infection and how different branches of the immune system contribute to controlling it. Recent reports have highlighted the importance of establishing local immunity in protecting against infections in peripheral tissues, and these observations may be key to designing a prophylactic vaccine that can rapidly control HSV-2 at the site of infection.

The vast majority of existing successful vaccines for infectious agents rely on antibodies for protection (77). An obvious question in this regard is why antibody-based vaccines against genital HSV-2 have not been successful. One possibility is that the magnitude and the quality of antibody responses generated by vaccines are not sufficient to neutralize the virus. Even naturally induced immune responses to HSV-2 may not always be protective against exogenous reinfection by another HSV-2 strain (4). This means that a vaccine must generate a completely different type of immune response in order to protect the host against HSV-2 challenge. As described above, it is also possible that antibody-mediated protection may require a local B cell response. How we might design a vaccine that establishes resident antibody-secreting cells in the genital mucosa is a worthy question to address in the future.

Moving forward, it will be important to keep in mind that obvious differences exist between animal genital herpes models and humans, as many vaccines that offer robust protection in animal models often fail during clinical trial. However, the holes in our understanding of

how to build the “right” type of immunity against HSV-2 infection in humans are slowly closing. A future challenge is to understand the determinant of protective immunity in humans if we were to have any chances of creating an efficacious vaccine against genital herpes.

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Highlights

- HSV shedding occurs with great frequency and cannot be entirely controlled.
- Tissue-resident T cells mediate control of HSV-2 infection.
- Antibody may need to be localized to the vagina to be effective against HSV.
- Generation of mucosal immunity may be critical for the design of an efficacious vaccine.

Figure 1. Different vaccination techniques lead to distinct types of immunity in the genital tract

A) Intravaginal immunization with attenuated HSV can establish lymphoid clusters containing CD4 T cells in the lamina propria, CD8 T_{RM} in the epithelium and may retain B cells/antibody secreting cells (ASC). HSV-specific IgG titers also increase, and may be concentrated by FcRn in the lumen. Ivag immunization leads to complete protection against vagina HSV-2 infection. B) Parenteral immunization, on the other hand, only leads to circulating immunity, which is suboptimal in protecting against contraction of genital herpes in humans. C) Immunization through mucosal routes such as intranasal can lead to a high titer of HSV-2 antibody in the genital tract. T cells may also be recruited to the vagina after i.n. immunization. This is sufficient to protect against vaginal challenges with HSV-2, although protection may decrease over time. D) Prime and pull can establish CD8 T_{RM}, but not CD4 T cell containing lymphoid clusters, in the vagina. This pool of CD8 T_{RM} can protect against disease through a neuroprotective mechanism, rather than through mucosal viral control.