Published in final edited form as:

Future Microbiol. 2010 January ; 5: 15–22. doi:10.2217/fmb.09.111.

Mucosal treatments for herpes simplex virus: insights on targeted immunoprophylaxis and therapy

Chris L McGowin and

LSU Health Sciences Center, Department of Medicine, Section of Infectious Diseases, 533 Bolivar Street, CSRB 701 New Orleans, LA 70112-2822, USA, Tel.: +1 504 568 7281, Fax: +1 504 568 8825, cmcgow@lsuhsc.edu

Richard B Pyles[†]

Department of Pediatrics, 3.206 Mary Moody Northern Pavilion; L22486, 301 University Blvd, Galveston, TX 77555-0436, USA, Tel.: +1 409 747 8140, Fax: +1 409 747 8150, rbpyles@utmb.edu

Abstract

Herpes simplex virus (HSV) serotypes 1 and 2 establish lifelong infections that can produce reactivated pools of virus at mucosal sites where primary infections were initiated. No approved vaccines are available. To break the transmission cycle, interventions must either prevent infection or reduce infectivity at mucosal sites. This article discusses the recent experimental successes of immunoprophylactic and therapeutic compounds that enhance resistance and/or reduce viral loads at genital and ocular mucosa. Current data indicate Toll-like receptor agonists and selected immunomodulating compounds effectively increase the HSV infection threshold and hold promise for genital prophylaxis. Similarly, immunization at genital and extragenital mucosal sites is discussed. Finally, preclinical success with novel immunotherapies for ocular HSV that address herpetic keratitis and corneal blindness is reviewed.

Keywords

herpes simplex; HSV-1; HSV-2; genital herpes; immunotherapy; keratitis; mucosa; therapy; TLR

Persistent problem of herpes infection

Herpes simplex virus (HSV) serotypes 1 and 2 primarily cause infections of the oral–facial, ocular or genital mucosa with high worldwide prevalence [1–4]. In most cases, HSV-1 causes self-limiting oral–facial infections, which affect an estimated 58% of US adults [5]. Globally, depending upon the age range evaluated, HSV-1 prevalence is as high as 90% [6], and in North America and several European countries, HSV-1 accounts for up to half of new genital herpes cases [7]. Ocular HSV-1 infections most often affect the cornea, resulting in keratitis, and can lead to corneal scarring and blindness [8,9]. HSV-2, affecting approximately 17% of Americans [5], commonly infects the genital tract of men and women, resulting in self-limiting

© 2010 Future Medicine Ltd

[†]Author for correspondence: Department of Pediatrics, 3.206 Mary Moody Northern Pavilion; L22486, 301 University Blvd, Galveston, TX 77555-0436, USA, Tel.: +1 409 747 8140, Fax: +1 409 747 8150, rbpyles@utmb.edu.

Financial & competing interests disclosure

Richard B Pyles and Chris L McGowin received research support from the Gulf South STI/Topical Microbicides Cooperative Research Center (NIAID U19 AI61972). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

lesions that can serve as portals for acquisition of secondary infections. Recurrent genital infections by HSV-2 are of significant medical and psychosocial concern [10]. Indeed, because genital herpes is so common, these infections have significant medical and economic impacts [11,12]. In rare cases, primary HSV infection can also lead to encephalitis, hepatitis, ocular keratitis and be transmitted to newborns with considerable morbidity and mortality [13]. After transmission, HSV establishes lifelong latent infections from which reactivation can produce symptomatic disease that is associated with increased susceptibility to other pathogens, including HIV-1 [14].

After almost 90 years of research, no vaccines to prevent HSV-2 disease have been licensed for use by the US FDA [15]. However, there have been encouraging clinical successes. In recent Phase III clinical trials of a recombinant glycoprotein D vaccine, approximately 74% prevention of genital HSV disease was observed but only in women seronegative for both serotypes of HSV [16]. Clearly, further research is required to address this gender-specific effect and the lack of efficacy in HSV-seropositive males and females, but these results provide solid evidence that vaccine-mediated prevention of HSV disease is attainable.

Reactivation of HSV leads to symptomatic and asymptomatic shedding of virus at effected mucosa, where transmission to the next host occurs [17]. Therefore, novel approaches to reducing transmission and preventing HSV infections are of utmost importance. Successful reductions in both recurrent disease and asymptomatic shedding have been achieved using available antivirals. Several antiviral compounds are approved for therapeutic management of recurrent disease, most notably acyclovir, valacyclovir (Valtrex[®]) and famciclovir [18]. These oral medications are used widely to reduce the duration and severity of genital HSV lesions [18,19]. Long-term use of these antivirals as suppressive therapy (e.g., daily dosing of Valtrex) also reduced viral shedding from the genital tract [20] but relies upon strict compliance to reduce the likelihood of drug-resistant variants [19,20] or recurrent episodes if therapy is discontinued. The recent successes and shortcomings of such efforts provide rationale and optimism for continued investigation of traditional antiviral HSV therapy, but also highlight the need for alternative methods of prevention or suppression.

Potential of HSV immunoprophylaxis & therapy

Although collectively unsuccessful to date, HSV vaccine research has shed light on several key elements of mucosal infection and has led to design and testing of novel prophylactic and therapeutic interventions. Preventing primary infection is the most desired approach, thereby alleviating primary disease, establishment of latency and subsequent reactivation, shedding and transmission. Should prophylaxis fail, HSV accesses sensory ganglia after replication in mucosal epithelial cells, ultimately establishing latency [13]. Reactivation of HSV from sensory ganglia apparently occurs frequently, leading to occasional symptomatic and frequent asymptomatic shedding of virus at the mucosa [17,18,20–22]. Thus, the local mucosal immune environment likely plays a key role in susceptibility and regulating magnitude if not frequency of viral shedding. Alternatively, therapies that limit or reduce viral titers at the mucosa could attenuate recurrent disease and decrease the chance of transmission to the next host.

Herpes simplex virus infection at an anatomical site appears to provide some level of protection from reinfection at the same site [23]. Therefore, the immune responses induced by natural infection appear to be protective at the mucosal level and provide compelling rationale for HSV therapies targeted to the mucosa. If a prophylactic approach can mimic natural exposure and the corresponding mucosal immunity, it appears reasonable to predict that susceptibility to primary infection could be reduced. To this end, a more contemporary approach has been to deliver antiviral or immunomodulating compounds directly to the mucosal surface, thereby conferring protection from subsequent exposure.

Prophylactics & therapeutics that target the innate immune response

Primary HSV exposures first elicit innate immune responses and, therefore, immunomodulatory approaches are being explored that boost innate immunity and engender increased resistance to HSV infection. Several highly conserved mechanisms of the innate immune system recognize HSV components for recognition of infection and activation of the innate response. Most notable is the Toll-like receptor (TLR) family that includes 13 proteins identified in mammals to recognize pathogen-associated molecular patterns produced during microbial infection [24]. TLRs are now known to be an important, primary mechanism of innate immune response activation, especially at mucosal sites of infection. Importantly, because of their evolutionary conservation in humans, they have been intensely investigated as targets for intervention of viral and bacterial infections. TLRs are expressed throughout the female reproductive tract at both lower and upper genital tract sites [25–30]. Of the lower tract, epithelial cells of the vagina and cervix express robust levels of TLR2, 3, 5 and 6, and CD14, with low levels of TLR1, 4 and 7–9 [27]. Ocular and oral mucosae, as well as the epithelial cells of the skin, also express TLRs with distinct patterns that appear to have evolved based upon the common pathogens encountered at this surface [31–33].

Activation of TLRs ultimately results in secretion of cytokines and other immunostimulatory molecules via an intracellular signaling pathway using MyD88 or an alternate MyD88-independent pathway involving interferon response factor 3 [24]. Of the TLRs, current data indicate that intact HSV or specific structural or nucleic acid components of HSV bind and activate TLR2, 3, 7 and 9 (reviewed in [34]). TLR3, 7 and 9 are localized to intracellular sites along the secretory and endocytic pathways for recognition of virus during replication (reviewed in [35]). TLR3 and 7 recognize RNA species produced by viruses during their natural replication cycle [35]. With regard to HSV, surface viral glycoproteins activate TLR2, resulting in NF- κ B activation and subsequent cytokine production (reviewed in [34]). Using this information, several groups have utilized synthetic agonists for TLR3 and 7 to provide transient protection from experimental HSV exposure [36–38], as discussed below.

Regarding the TLR expression profile in the female genital tract, immunoprophylactics and therapies targeting TLR2, 3, 7 and 9 of the mucosa have been investigated for utility in preventing or attenuating HSV-2 disease. For prophylaxis, these synthetic agonists were designed to transiently activate the innate immune response to establish a more HSV-resistant environment, thereby increasing the threshold of infection or attenuating recurrent shedding events [39]. Transient immunomodulation is beneficial to prevent long-term inflammation but also limiting in that the window for effective prophylaxis could be shorter than desired. Practically, these compounds would ideally be applied prior to or just after a high-risk sexual encounter to prevent primary mucosal infection. The first such compound, imiquimod (AldaraTM), an imidazoquinoline amine analog to guanosine and a TLR7 agonist [40], was shown to provide significant protection against genital HSV-2 in small animal models [41, 42]. In a randomized, controlled human trial of resiquimod, a derivative of imiquimod, vaginal application of this TLR7 agonist resulted in reduced frequency of genital HSV-2 reactivation events and viral shedding [43], indicating these compounds have therapeutic utility as well.

Additional experimental success has been reported for other, nucleic acid-based TLR agonists. We first showed that transient activation of TLR9 via vaginal delivery of unmethylated CpG-containing oligonucleotides inhibited replication of HSV-2 in mouse and guinea pig models of genital disease [44]. Subsequent studies with other CpG-containing oligonucleotides suggest they are a promising approach for prophylactic and therapeutic intervention of HSV infections that warrant continued investigation [45–47]. Similarly, topical vaginal application of polyinosine–polycytosine, a stabilized synthetic form of dsRNA that activates TLR3, also increased the amount of virus required to establish an experimental HSV-2 infection of mice

by tenfold [48]. Increased resistance to HSV-2 in mice is difficult to extrapolate to humans and the clinical implications are largely unknown, but this study confirmed a previous observation that polyinosine–polycytosine, but not a TLR4 agonist, increased resistance to HSV-2 [49]. Finally, a role for TLR2 in innate recognition of HSV has been shown *in vitro* for immune and nonimmune cell types [34] and in TLR2-deficient mice [50]. Direct stimulation of TLR2 did not show protection against subsequent HSV-2 challenge [51], but recent studies in our laboratory have shown that stimulation of the TLR2/6 heterodimer, highly expressed in the female genital tract [27], with a vaginally applied synthetic agonist enhanced resistance to HSV-2 challenge in the mouse model [52]. Collectively, these data indicate that immunoprophylaxis and therapies that target TLRs appear to be effective in preventing or reducing primary HSV-2 infection or recurrent disease.

Recent work has also suggested that immunomodulating compounds that do not interact with TLRs hold promise for reducing HSV-2 disease and shedding. In one such study, a synthetic dipeptide termed SCV-07 (γ -D-glutamyl-L-tryptophan) that had shown potent immunomodulatory and antimicrobial activity was evaluated as a therapeutic in the guinea pig model of genital HSV-2 infection [53]. Oral delivery of SCV-07 provided a significant reduction in both incidence and severity of recurrent lesion formation in infected guinea pigs but had no durability of response. In fact, the results were statistically similar to acyclovir dosing completed in parallel [53].

Collectively, immunomodulation appears to be an effective means for increasing the threshold of HSV infection in the genital tract and/or impacting recurrent disease therapeutically. The current data suggest that mucosal epithelial cells are an important target population for stimulation by TLR agonists that can directly reduce viral replication [48,54].

Immunomodulatory approaches also offer the potential of impacting resistance to multiple infections simultaneously because immunomodulation targets the host mucosa and, therefore, is not pathogen specific. Finally, several clinical trials in humans have been completed for evaluation of safety and efficacy of TLR agonists for treatment of anogenital warts and cancers, and have been tested as vaccine adjuvants with a good efficacy and safety track record [55]. In fact, the TLR7 agonist trade named Aldara is used clinically to treat HPV warts and anecdotally for a number of other sexually transmitted infections (STIs), including HSV-2 [38]. Thus, application of this class of compounds for prophylactic and therapeutic use is likely to increase in the future and should continue to be at the forefront of immunotherapy research for HSV infections.

Targeted mucosal interventions for ocular infections by HSV 1

Although there is a clear need for vaccines and alternative therapies to control genital herpes, nongenital infections are of great public health concern and include oral–facial, ocular and neonatal herpes. The vast majority of immune-targeted interventions have been developed and tested for genital infections, probably because of the potentially significant market for an effective therapy. However, ocular HSV-1 infections are the leading cause of unilateral infectious corneal blindness worldwide [2], and novel preventatives and therapies are needed. Ocular HSV-1 infections are, most often, secondary sequelae of latent HSV-1 reactivation from the trigeminal ganglia following primary infection [56]. Virus is transported to the ocular mucosa via antero-grade movement from the ganglia, ultimately causing herpetic keratitis, conjunctivitis and other ocular sequelae [8,9]. Successive reactivation events, including deeper corneal penetration, can increase the probability of subsequent corneal disease and keratitis [56]. Interestingly, current ocular therapies also include chronic administration of antivirals [57] and local steroidal application [58] to minimize inflammatory scarring. The pathology of recurrent disease and the concerns regarding chronic inflammation again support the goal of protecting against primary infection; such protection would prevent latent seeding of the

sensory ganglia and subsequent recurrent corneal disease. As noted for genital infections, this has not yet been achieved with any current vaccine approaches.

The cornea does not actively provide immune protection to the eye but largely relies on other tissues of the ocular mucosal immune system, such as the conjunctiva and the draining preauricular lymph nodes [59]. Extending the immunostimulatory properties of TLR agonists observed at the genital mucosa, the ocular mucosal immune system has also been a target for HSV immunotherapy and immunization. In humans, high levels of TLR1–4 and 6 are expressed in the cornea [60]. Although only a few studies have addressed immunomodulation as an approach to prevent or attenuate disease, topical application of HSV-1 glycoprotein D epitopes with TLR9-stimulating CpG oligonucleotides can induce strong local and systemic immune responses in laboratory rabbits [59], which are aimed to protect against primary infection. Efficacy of these responses depended upon CpG as an adjuvant, indicating that targeted immunostimulation is possible at ocular sites. Although little research has addressed immunomodulation for ocular HSV infections directly, stimulation of the host mucosal immune system has proven to be a viable approach for prophylaxis and treatment of genital HSV-2 infections, and good rationale exists for continued investigation.

Concerns & consequences of immunomodulation

A chief concern with the clinical utility of immunomodulating compounds for genital infections is their impact on HIV-1 infection. An estimated 2.7 million new cases of HIV infection were diagnosed worldwide in 2008 alone [101]. In addition, a well-defined causal relationship exists between HSV-2 status and HIV-1 replication in coinfecting patients [61]. Collectively, the cocirculation of these two pathogens has provided a great challenge for researchers and healthcare providers to develop HSV-2 therapies that either do not increase HIV-1 susceptibility or replication, or simultaneously protect against both infections. Considering the primary target cells for HIV-1 infection are T lymphocytes and macrophages [62], it is not surprising that TLR-dependent stimulation of the innate immune response might also enhance susceptibility to HIV-1 by recruitment of susceptible cells to the mucosa. Although limited studies have addressed this important concept, topical application of TLR agonists has shown to increase susceptibility to HIV-1 in human cells *ex vivo* [63]. In addition, specific stimulation of TLRs results in differential cytokine production, which has been shown to directly enhance HIV-1 replication [64,65]. However, the intricate network of cytokine signaling in response to specific mucosal stimulation is not yet well defined and is a significant impediment to continued understanding of immunomodulatory therapies.

The majority of efforts to study immunomodulation for STI prophylaxis have been in the development and testing of topical vaginal therapies, commonly termed microbicides. Following TLR stimulation, markers of inflammation (e.g., cytokines) have been evaluated for their impact on mucosal integrity and their potential impact on HIV-1 [66], but transient activation of the innate response is the intended mechanism for HSV-2 prophylaxis. Importantly, the difference between inflammation and immunomodulation is not yet clearly defined. For example, TLR2 recognition of HSV is associated with enhanced disease severity [50], but transient stimulation also provides protection from HSV infection. A more thorough understanding of targeted innate stimulation will help define this distinction and will be necessary for effective protection against or therapy of HSV infections. Finally, because of the significant cocirculation of HSV-2 with other STIs, including HIV, immunomodulation at genital sites requires special consideration to tailor responses to those that are specifically able to enhance resistance to HSV-2 exposure rather than broader inflammatory outcomes associated with increased susceptibility to HIV. Such tailoring will require more data from extensive preclinical and clinical investigations prior to widespread use in humans.

Conclusion

Several promising alternative strategies have emerged for control of HSV infections, including immunoprophylaxis, immunotherapy and mucosal vaccination. The ocular, genital tract and oral-facial mucosa are undoubtedly major players in the control of HSV susceptibility and recurrent disease. Thus, more research is required for the continued understanding of the mucosal immune system and for the development of effective prophylactic and therapeutic interventions for HSV, HIV-1 and other STIs. Immunomodulatory approaches as therapy for HSV reactivation also hold great promise as a means to reduce the transmission of this nearly ubiquitous set of human pathogens.

It is evident that the mucosal immune system plays a key role in regulating HSV susceptibility and recurrent HSV disease. Another promising area for increased resistance to HSV infection has been mucosal delivery of vaccines. In general, most systemic vaccines induce protective immune responses but few induce mucosal responses that could prevent initial infection at the mucosal site. Since HSV replicates in mucosal epithelial cells and is periodically shed from mucosal sites, effective vaccines for HSV, regardless of delivery mechanism, depend largely on the induction of long-term mucosal immune responses [67] to prevent primary infection or to reduce shedding.

In several studies of HSV-2 and other STIs, mucosal delivery has proven to be the best route to generate specific IgA, IgG and long-term antiviral cytotoxic T lymphocytes in genital-associated lymphoid tissues compared with systemic immunization. A burgeoning area of success is in T-cell epitope-based herpes vaccines that have proven to be a safe, immunogenic and cost-effective means to prevent ocular and genital HSV infections [68]. Collectively, these approaches have provided solid evidence for systemic cellular immunity and show that ocular stimulation is a formidable approach to generating localized and systemic immune responses.

Needle-free mucosal immunization is currently regarded as one of the greatest challenges in modern vaccine development but holds great promise for enhancing resistance to primary infections with HSV [68]. Advanced investigations of HSV immune-based interventions have utilized immunization of alternative mucosal sites that enhance protection from genital HSV challenge [67]. Results from these studies show the importance of continued research into a better understanding of the immune system at all mucosa of the body to fully exploit the avenues for HSV prevention. The field of mucosal vaccination and immunotherapies for HSV prophylaxis and treatment is extraordinarily dynamic and, despite the challenges, has shown considerable progress towards an effective therapy.

Future perspective

Continued investigation of topical immune-based interventions and the mucosal immune responses of the genital tract and the eye are of utmost importance to address HSV infections. Considering the rapid establishment of lifelong latency by HSV, alternative strategies to traditional systemic immunization will be required to prevent primary infection of the mucosa. TLR-mediated immunomodulation and vaccines conjugated to TLR agonists enhance resistance to genital HSV-2 infections but additional work is required to dissect their impact on HIV-1 infections. Collaboration between mucosal immunologists and vaccine experts should result in novel approaches, including mucosal delivery of vaccines and combination therapies, for efficacy testing in well-established animal models of ocular and genital disease. Both HSV serotypes are incredibly well adapted to long-term survival in the human host and, in turn, have provided researchers and healthcare providers a great challenge to prevent or treat these infections. With increasing knowledge of the host immune system, and the continued development of novel immunotherapeutic approaches there is reason for cautious optimism.

regarding the future success of immunoprophylactics and mucosal therapeutics to control the global epidemic of HSV infections.

Executive summary

- The ocular and genital mucosa are key players in regulation of herpes simplex virus (HSV) susceptibility and recurrent disease.
- Prophylactic and therapeutic interventions delivered directly to the target mucosa show promise for HSV infections.
- Mucosal prophylactics that target highly expressed Toll-like receptors of the genital tract have been successful in preventing or attenuating HSV disease in several animal models of infection.
- Effective ocular immunotherapies and vaccines to prevent HSV-1 infection are lacking but the ocular mucosal immune system has shown to be an effective means to generate local and systemic immune responses.
- Mucosal immunizations against HSV can result in long-term antiviral IgA and cytotoxic T-lymphocyte responses in the genital tract.
- Delivery of immunomodulating compounds to the genital mucosa requires considerable investigation into their impact on HIV-1 infections.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Lafferty WE. The changing epidemiology of HSV-1 and HSV-2 and implications for serological testing. *Herpes* 2002;9(2):51–55. [PubMed: 12106513]
2. Liesegang TJ. Herpes simplex virus epidemiology and ocular importance. *Cornea* 2001;20(1):1–13. [PubMed: 11188989]
3. Looker KJ, Garnett GP. A systematic review of the epidemiology and interaction of herpes simplex virus types 1 and 2. *Sex. Transm. Infect* 2005;81(2):103–107. [PubMed: 15800084]
4. Looker KJ, Garnett GP, Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bull. World Health Organ* 2008;86(10):805–812. A. [PubMed: 18949218]
5. Xu F, Sternberg MR, Kottiri BJ, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA* 2006;296(8):964–973. [PubMed: 16926356]
6. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J. Infect. Dis* 2002;186:S3–S28. [PubMed: 12353183]
7. Gupta R, Warren T, Wald A. Genital herpes. *Lancet* 2007;370(9605):2127–2137. [PubMed: 18156035]
8. Labetoulle M, Kucera P, Ugolini G, et al. Neuronal propagation of HSV1 from the oral mucosa to the eye. *Invest. Ophthalmol. Vis. Sci* 2000;41(9):2600–2606. [PubMed: 10937572]
9. Summers BC, Margolis TP, Leib DA. Herpes simplex virus type 1 corneal infection results in periocular disease by zosteriform spread. *J. Virol* 2001;75(11):5069–5075. [PubMed: 11333887]
10. Rosenthal SL, Zimet GD, Leichter JS, et al. The psychosocial impact of serological diagnosis of asymptomatic herpes simplex virus type 2 infection. *Sex. Transm. Infect* 2006;82(2):154–157. discussion 157–158. [PubMed: 16581745]

11. Chesson HW, Blandford JM, Gift TL, Tao G, Irwin KL. The estimated direct medical cost of sexually transmitted diseases among American youth, 2000. *Perspect. Sex. Reprod. Health* 2004;36(1):11–19. [PubMed: 14982672]
12. Szucs TD, Berger K, Fisman DN, Harbarth S. The estimated economic burden of genital herpes in the United States. An analysis using two costing approaches. *BMC Infect. Dis* 2001;1:5. [PubMed: 11472635]
13. Whitley RJ, Roizman B. Herpes simplex virus infections. *Lancet* 2001;357(9267):1513–1518. [PubMed: 11377626]
14. Corey L, Wald A, Celum CL, Quinn TC. The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *J. Acquir. Immune Defic. Syndr* 2004;35(5):435–445. [PubMed: 15021308]
15. Stanberry LR. Clinical trials of prophylactic and therapeutic herpes simplex virus vaccines. *Herpes* 2004;11:161A–169A.
16. Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N. Engl. J. Med* 2002;347(21):1652–1661. [PubMed: 12444179]
17. Magaret AS, Johnston C, Wald A. Use of the designation “shedder” in mucosal detection herpes simplex virus DNA involving repeated sampling. *Sex. Transm. Infect* 2009;85(4):270–275. [PubMed: 19211593] •• Field-adjusting data set showing the frequency of asymptomatic shedding and the differences in the human population.
18. Wald A, Selke S, Warren T, et al. Comparative efficacy of famciclovir and valacyclovir for suppression of recurrent genital herpes and viral shedding. *Sex. Transm. Dis* 2006;33(9):529–533. [PubMed: 16540883]
19. Stanberry L, Cunningham A, Mertz G, et al. New developments in the epidemiology, natural history and management of genital herpes. *Antiviral Res* 1999;42(1):1–14. [PubMed: 10333138]
20. Corey L, Wald A, Patel R, et al. Once-daily valacyclovir to reduce the risk of transmission of genital herpes. *N. Engl. J. Med* 2004;350(1):11–20. [PubMed: 14702423]
21. Crespi CM, Cumberland WG, Wald A, Corey L, Blower S. Longitudinal study of herpes simplex virus type 2 infection using viral dynamic modelling. *Sex. Transm. Infect* 2007;83(5):359–364. [PubMed: 17475687]
22. Wald A, Carrell D, Remington M, et al. Two-day regimen of acyclovir for treatment recurrent genital herpes simplex virus type 2 infection. *Clin. Infect. Dis* 2002;34(7):944–948. [PubMed: 11880960]
23. Lakeman AD, Nahmias AJ, Whitley RJ. Analysis of DNA from recurrent genital herpes simplex virus isolates by restriction endonuclease digestion. *Sex. Transm. Dis* 1986;13(2):61–66. [PubMed: 3012807]
24. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124(4):783–801. [PubMed: 16497588] • Effective overview of the innate immune responses elicited through pathogen recognition.
25. Fazeli A, Bruce C, Anumba DO. Characterization of Toll-like receptors in the female reproductive tract in humans. *Hum. Reprod* 2005;20(5):1372–1378. [PubMed: 15695310]
26. Fichorova RN, Cronin AO, Lien E, Anderson DJ, Ingalls RR. Response to *Neisseria gonorrhoeae* by cervicovaginal epithelial cells occurs in the absence of Toll-like receptor 4-mediated signaling. *J. Immunol* 2002;168(5):2424–2432. [PubMed: 11859134]
27. Herbst-Kralovetz MM, Quayle AJ, Ficarra M, et al. Quantification and comparison of Toll-like receptor expression and responsiveness in primary and immortalized human female lower genital tract epithelia. *Am. J. Reprod. Immunol* 2008;59(3):212–224. [PubMed: 18201283] •• Quantitative analysis of Toll-like receptor (TLR) expression in lower female genital tract reproductive epithelial cells that established the potential for recognition of specific TLR agonists.
28. Pioli PA, Amiel E, Schaefer TM, et al. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. *Infect. Immun* 2004;72(10):5799–5806. [PubMed: 15385480]
29. Pivarsci A, Nagy I, Koreck A, et al. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human β -defensin-2 in vaginal epithelial cells. *Microbes Infect* 2005;7(9–10):1117–1127. [PubMed: 15893496]

30. Schaefer TM, Desouza K, Fahey JV, Beagley KW, Wira CR. Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. *Immunology* 2004;112(3):428–436. [PubMed: 15196211]
31. Diamond G, Beckloff N, Ryan LK. Host defense peptides in the oral cavity and the lung: similarities and differences. *J. Dent. Res* 2008;87(10):915–927. [PubMed: 18809744]
32. Kumar A, Yu FS. Toll-like receptors and corneal innate immunity. *Curr. Mol. Med* 2006;6(3):327–337. [PubMed: 16712478]
33. Pearlman E, Johnson A, Adhikary G, et al. Toll-like receptors at the ocular surface. *Ocul. Surf* 2008;6(3):108–116. [PubMed: 18781257]
34. Morrison LA. The Toll of herpes simplex virus infection. *Trends Microbiol* 2004;12(8):353–356. [PubMed: 15276609]
35. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin. Microbiol. Rev* 2009;22(2):240–273. [PubMed: 19366914]
36. Gill N, Davies EJ, Ashkar AA. The role of Toll-like receptor ligands/agonists in protection against genital HSV-2 infection. *Am. J. Reprod. Immunol* 2008;59(1):35–43. [PubMed: 18154594]
37. Herbst-Kralovetz M, Pyles R. Toll-like receptors, innate immunity and HSV pathogenesis. *Herpes* 2006;13(2):37–41. [PubMed: 16895653]
38. Miller RL, Meng TC, Tomai MA. The antiviral activity of Toll-like receptor 7 and 7/8 agonists. *Drug News Perspect* 2008;21(2):69–87. [PubMed: 18389099]
39. Miller RL, Tomai MA, Harrison CJ, Bernstein DI. Immunomodulation as a treatment strategy for genital herpes: review of the evidence. *Int. Immunopharmacol* 2002;2(4):443–451. [PubMed: 11962724] • Review of the first reports of immunomodulators applied mucosally to alter the course of herpes simplex virus (HSV)-2 disease.
40. Hemmi H, Kaisho T, Takeuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat. Immunol* 2002;3(2):196–200. [PubMed: 11812998]
41. Bernstein DI, Harrison CJ, Tepe ER, Shahwan A, Miller RL. Effect of imiquimod as an adjuvant for immunotherapy of genital HSV in guinea-pigs. *Vaccine* 1995;13(1):72–76. [PubMed: 7762282]
42. Bernstein DI, Spruance SL, Arora SS, Schroeder JL, Meng TC. Evaluation of imiquimod 5% cream to modify the natural history of herpes labialis: a pilot study. *Clin. Infect. Dis* 2005;41(6):808–814. [PubMed: 16107978]
43. Mark KE, Corey L, Meng TC, et al. Topical resiquimod 0.01% gel decreases herpes simplex virus type 2 genital shedding: a randomized, controlled trial. *J. Infect. Dis* 2007;195(9):1324–1331. [PubMed: 17397003] • First randomized trial of TLR agonist showing reduced genital shedding.
44. Pyles RB, Higgins D, Chalk C, et al. Use of immunostimulatory sequence-containing oligonucleotides as topical therapy for genital herpes simplex virus type 2 infection. *J. Virol* 2002;76(22):11387–11396. [PubMed: 12388699] • First report of synthetic CpG-based TLR agonists to increase resistance to HSV-2 challenge in an animal model.
45. Harandi AM. The potential of immunostimulatory CpG DNA for inducing immunity against genital herpes: opportunities and challenges. *J. Clin. Virol* 2004;30(3):207–210. [PubMed: 15135735]
46. Herbst MM, Pyles RB. Immunostimulatory CpG treatment for genital HSV-2 infections. *J. Antimicrob. Chemother* 2003;52(6):887–889. [PubMed: 14585849]
47. Sajic D, Ashkar AA, Patrick AJ, et al. Parameters of CpG oligodeoxynucleotide-induced protection against intravaginal HSV-2 challenge. *J. Med. Virol* 2003;71(4):561–568. [PubMed: 14556270]
48. Herbst-Kralovetz MM, Pyles RB. Quantification of poly(I:C)-mediated protection against genital herpes simplex virus type 2 infection. *J. Virol* 2006;80(20):9988–9997. [PubMed: 17005677]
49. Ashkar AA, Yao XD, Gill N, et al. Toll-like receptor (TLR)-3, but not TLR4, agonist protects against genital herpes infection in the absence of inflammation seen with CpG DNA. *J. Infect. Dis* 2004;190(10):1841–1849. [PubMed: 15499542]
50. Kurt-Jones EA, Chan M, Zhou S, et al. Herpes simplex virus 1 interaction with Toll-like receptor 2 contributes to lethal encephalitis. *Proc. Natl Acad. Sci. USA* 2004;101(5):1315–1320. [PubMed: 14739339] • Demonstrates the impact of TLR2 responses upon exacerbated HSV disease in experimentally infected knockout mice.

51. Gill N, Deacon PM, Lichty B, Mossman KL, Ashkar AA. Induction of innate immunity against herpes simplex virus type 2 infection via local delivery of Toll-like receptor ligands correlates with β interferon production. *J. Virol* 2006;80(20):9943–9950. [PubMed: 17005672]
52. Rose WA 2nd, McGowin CL, Pyles RB. FSL-1, a bacterial-derived Toll-like receptor 2/6 agonist, enhances resistance to experimental HSV-2 infection. *Viol. J* 2009;6:195. [PubMed: 19903337]
53. Rose WA 2nd, Tuthill C, Pyles RB. An immunomodulating dipeptide, SCV-07, is a potential therapeutic for recurrent genital herpes simplex virus type 2 (HSV-2). *Int. J. Antimicrob. Agents* 2008;32(3):262–266. [PubMed: 18619817]
54. Nazli A, Yao XD, Smieja M, et al. Differential induction of innate anti-viral responses by TLR ligands against herpes simplex virus, type 2, infection in primary genital epithelium of women. *Antiviral Res* 2009;81(2):103–112. [PubMed: 19013198]
55. Meyer T, Stockfleth E. Clinical investigations of Toll-like receptor agonists. *Expert Opin. Invest. Drugs* 2008;17(7):1051–1065.
56. Pepose JS, Keadle TL, Morrison LA. Ocular herpes simplex: changing epidemiology, emerging disease patterns, and the potential of vaccine prevention and therapy. *Am. J. Ophthalmol* 2006;141(3):547–557. [PubMed: 16490506]
57. Kumar M, Hill JM, Clement C, et al. A double-blind placebo-controlled study to evaluate valacyclovir alone and with aspirin for asymptomatic HSV-1 DNA shedding in human tears and saliva. *Invest. Ophthalmol. Vis. Sci* 2009;50(12):5601–5608. [PubMed: 19608530]
58. Toma HS, Murina AT, Areaux RG Jr, et al. Ocular HSV-1 latency, reactivation and recurrent disease. *Semin. Ophthalmol* 2008;23(4):249–273. [PubMed: 18584563] ■ Includes results of ocular sensitivity to CpG-based immunomodulation.
59. Nesburn AB, Bettahi I, Zhang X, et al. Topical/mucosal delivery of sub-unit vaccines that stimulate the ocular mucosal immune system. *Ocul. Surf* 2006;4(4):178–187. [PubMed: 17146573]
60. Jin X, Qin Q, Chen W, Qu J. Expression of Toll-like receptors in the healthy and herpes simplex virus-infected cornea. *Cornea* 2007;26(7):847–852. [PubMed: 17667620]
61. Van de Perre P, Segondy M, Foulongne V, et al. Herpes simplex virus and HIV-1: deciphering viral synergy. *Lancet Infect. Dis* 2008;8(8):490–497. [PubMed: 18652995]
62. Coleman CM, Wu L. HIV interactions with monocytes and dendritic cells: viral latency and reservoirs. *Retrovirology* 2009;6:51. [PubMed: 19486514]
63. de Jong MA, de Witte L, Oudhoff MJ, et al. TNF- α and TLR agonists increase susceptibility to HIV-1 transmission by human Langerhans cells *ex vivo*. *J. Clin. Invest* 2008;118(10):3440–3452. [PubMed: 18776939]
64. Bafica A, Scanga CA, Schito ML, Hieny S, Sher A. Cutting edge: *in vivo* induction of integrated HIV-1 expression by mycobacteria is critically dependent on Toll-like receptor 2. *J. Immunol* 2003;171(3):1123–1127. [PubMed: 12874196]
65. Equils O, Schito ML, Karahashi H, et al. Toll-like receptor 2 (TLR2) and TLR9 signaling results in HIV-long terminal repeat *trans*-activation and HIV replication in HIV-1 transgenic mouse spleen cells: implications of simultaneous activation of TLRs on HIV replication. *J. Immunol* 2003;170(10):5159–5164. [PubMed: 12734363]
66. Fichorova RN. Guiding the vaginal microbicide trials with biomarkers of inflammation. *J. Acquir. Immune Defic. Syndr* 2004;37:S184–S193. [PubMed: 16419271]
67. Rosenthal KL, Gallichan WS. Challenges for vaccination against sexually-transmitted diseases: induction and long-term maintenance of mucosal immune responses in the female genital tract. *Semin. Immunol* 1997;9(5):303–314. [PubMed: 9327525] ■ Discusses T-cell epitope-based vaccines for genital and ocular herpes.
68. Dasgupta G, Chentoufi AA, Nesburn AB, Wechsler SL, BenMohamed L. New concepts in herpes simplex virus vaccine development: notes from the battlefield. *Expert Rev. Vaccines* 2009;8(8):1023–1035. [PubMed: 19627185]

Website

101. Joint United Nations Programme on HIV/AIDS: 2008 Report on the Global AIDS Epidemic. www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp