



Published in final edited form as:

Expert Rev Anti Infect Ther. 2009 June ; 7(5): 559–568. doi:10.1586/eri.09.34.

Novel approaches in fighting herpes simplex virus infections

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Abstract

The development of novel strategies to eradicate herpes simplex virus (HSV) is a global public health priority. While acyclovir and related nucleoside analogues provide successful modalities for treatment and suppression, HSV remains highly prevalent worldwide and is a major cofactor fueling the HIV epidemic. HSV is the predominant cause of genital ulcerative disease, and neonatal and sporadic infectious encephalitis. Asymptomatic shedding, which occurs more frequently than previously appreciated, contributes to viral transmission. Acyclovir resistance may be problematic for immunocompromised patients and highlights the need for new safe and effective agents. Ideally, vaccines to prevent infection, drugs to inhibit the establishment of or reactivation from latency, or vaginal microbicides to prevent sexual and perinatal transmission are needed to control the epidemic. This review summarizes current therapeutic options and strategies in development.

Keywords

acyclovir; herpes simplex virus; microbicide; vaccine

The discovery of acyclovir (ACV), a nucleoside analogue that is selectively phosphorylated by herpes simplex virus (HSV) and inhibits viral replication by acting as a substrate for viral DNA polymerase, ushered in a new era in antiviral chemo therapy [1,2]. Despite the overwhelming success of ACV and related drugs, HSV remains a major global health problem, and is the leading cause of encephalitis and genital ulcerative disease, and a major cofactor for HIV infection. This reflects, in part, the ability of the virus to uniformly establish latency, reactivate frequently and to be horizontally and vertically transmitted during periods of

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Financial & competing interests disclosure

This work was supported by NIH grants AI065309, AI070202, AI079763 and AI077549. 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.

unrecognized or asymptomatic shedding. Thus novel strategies to treat, suppress, prevent and eradicate HSV infection are needed.

Critical scientific advances over the past decades have enhanced our understanding of the natural history and pathogenesis of HSV, and provide the framework for the development of novel prevention and treatment strategies. The spectrum of disease caused by both HSV-1 and -2 is impacted by the portal of entry, host immune response and whether the infection is the result of primary infection or viral reactivation.

Clinical syndromes & current treatment

Gingivostomatitis

Gingivostomatitis is the most common manifestation of HSV infection in children and is more often associated with HSV-1. Primary infection is characterized by an ulcerative enanthem involving the gingiva and mucous membranes, often with vesicular lesions. Fever and other constitutional symptoms may also occur. In the immunocompetent host, symptoms are self-limited and treatment is not routinely recommended, although antiviral therapy (ACV, valacyclovir [VCV] or famciclovir; see later) initiated within 72 h of the onset of symptoms may shorten the duration of clinical manifestations and viral shedding [3].

Because inflammation may contribute to the symptoms associated with herpes labialis, a topical cream that combines 5% ACV with 1% hydrocortisone (ME-609) has been developed. ME-609 provided a benefit to subjects with experimental UV radiation-induced herpes labialis, reducing healing time, lesion size and lesion tenderness [4]. A Phase III clinical trial comparing the efficacy and safety of ME-609 versus ACV cream for the treatment of recurrent herpes simplex labialis has been completed, but the results have not yet been published.

Treatment of recurrent herpes labialis is not routinely recommended, as minimal therapeutic benefit has been demonstrated in controlled studies with currently licensed drugs. However, patients with frequent recurrences may benefit from suppressive antiviral therapy.

Keratitis

It is estimated that there are 59,000 new and recurrent episodes of HSV eye disease annually in the USA, most commonly manifesting as keratitis [5]. Most ocular infections are associated with HSV-1 and may occur in association with oral lesions. Keratitis typically presents as a unilateral 'red eye', which may be painful and accompanied by photophobia. Topical application with nucleoside analogues including vidarabine, trifluridine, ACV or ganciclovir results in healing, usually within 1 week. Interferon may be effective when combined with another antiviral agent, such as trifluridine [6]. Recurrences are common, and suppressive oral ACV or VCV therapy may be beneficial, and reduce the rate of recurrent HSV epithelial keratitis and stromal keratitis [7].

Encephalitis

Herpes simplex encephalitis (HSE), the most common cause of sporadic viral encephalitis, is associated with 70% mortality in untreated patients and a high incidence of severe and permanent neurological sequelae in treated cases [8]. Most adult cases are caused by HSV-1, whereas neonatal HSE is more often associated with HSV-2. Whether encephalitis is due to viral reactivation or primary infection is unclear, as it occurs only in a small minority of otherwise healthy HSV-1-infected individuals. A recent study identified a potential genetic association in two children with autosomal recessive deficiency in the intracellular protein UNC-93B, resulting in impaired cellular IFN- α/β and - γ antiviral responses [9], and a subsequent study demonstrated an association with autosomal-dominant Toll-like receptor

(TLR)-3 deficiency [10]. These findings provide theoretical support for the treatment of HSE patients with IFN- α in addition to ACV.

Genital HSV infections

HSV-2 is the major cause of genital herpes, a highly prevalent infection among sexually active people worldwide. In the USA, 17% of the population is seropositive for HSV-2 and 58% is seropositive for HSV-1, which is emerging as a major cause of genital herpes infections [11]. The HSV-2 seroprevalence rates are much higher in some populations, reaching 80–90% among HIV-infected individuals and female sex workers [12].

Primary genital herpes may be clinically silent or associated with significant genital lesions. Symptoms usually resolve within 2 weeks without specific antiviral therapy, but treatment with oral ACV, VCV or famciclovir decreases the duration of symptoms and viral shedding. Symptomatic recurrences, which are more common during the first year after primary infection, tend to be milder and of shorter duration than primary disease, but some patients benefit from oral therapy. Suppressive therapy is recommended, especially in patients with frequent and/or severe episodes, and has been demonstrated to decrease the incidence of clinical recurrences and to reduce viral shedding and transmission of HSV-2 in discordant, heterosexual couples by approximately 50% [13]. A recent study found that VCV was somewhat more effective than famciclovir for the suppression of genital herpes and associated viral shedding [14].

Prevention or suppression of genital herpes is even more important during pregnancy. The majority of mothers whose infants develop perinatal HSV infection have no history of clinical disease. The risk for transmission to the neonate is highest among women who acquire primary infection near the time of delivery (30–50%), compared with women who have pre-existing immunity (<1%). Pregnant women and their partners should be questioned regarding their HSV histories early in pregnancy, and women with a negative history or who are seronegative should be advised to use condoms if their partners are known or suspected to have genital or orolabial herpes. For women with a known history of genital herpes, daily suppressive ACV (or VCV) during the last 4 weeks of pregnancy decreases the frequency of recurrences and the need for Caesarean delivery, and may also reduce perinatal transmission. However, there is insufficient evidence to determine if antiviral prophylaxis reduces the incidence of neonatal herpes [15]. While the safety of ACV in pregnant women has not been established, extensive experience supports its use.

Impact of genital HSV on HIV

The recognition of genital herpes as a worldwide pandemic, coupled with the consistent epidemiological findings that HSV increases HIV acquisition and transmission, highlights the urgency to develop novel HSV prevention strategies [16]. Prevention is particularly difficult, as transmission often occurs during unrecognized and asymptomatic shedding, which is more common than previously appreciated. In a recent study, HSV-2-seropositive subjects collected genital samples for PCR four-times daily for 60 days. HSV-2 was detected by PCR on 19% of the days, with a median duration of 13 h [17]. The frequency of clinically silent viral shedding may contribute to the observation that being seropositive for HSV-2, even in the absence of clinical disease, accelerates the natural course of HIV, with higher plasma and genital levels of HIV and more rapid progression to AIDS [18]. These epidemiological findings provided the rationale for clinical studies to evaluate whether HSV suppression could impact HIV acquisition, transmission and disease [16,18]. Co-infected patients not on highly active antiretroviral therapy (HAART) for HIV infection were prescribed VCV; results revealed a decrease in the average plasma HIV viral load, suggesting that VCV therapy may delay the initiation of HAART in co-infected patients [12,19,20].

HSV suppression could also be beneficial for prophylaxis against acquisition of HIV in HSV-2-infected patients. However, no significant protection against HIV acquisition was observed in recently completed clinical trials, although the drug-dosing regimen (twice-a-day ACV) and the inability of ACV to fully suppress HSV reactivation may have contributed to the negative results [21]. ACV may not prevent the immune responses associated with HSV reactivation, which include T-cell activation, the induction of inflammatory cytokines and chemokines, and downregulation of antimicrobial peptides, such as secretory leukocyte protease inhibitor, all of which could enhance HIV acquisition and replication [22,23]. These observations highlight the need to develop more potent anti-HSV drugs and ones that prevent viral reactivation.

Notably, recent studies have demonstrated that, in the presence of HSV, phosphorylated ACV directly inhibits HIV by targeting the reverse transcriptase (RT), terminating DNA chain elongation [24]. However, exposure to ACV selects for the RT variant V75I, which is part of the multidrug-resistance pathway that enhances viral resistance to many of the currently licensed RT inhibitors, suggesting that use of ACV monotherapy in co-infected patients may result in the selection of resistant variants [25].

Neonatal HSV

Neonatal HSV infections, which are predominantly associated with HSV-2, present as disseminated disease, CNS disease or disease localized to the skin, eye or mucous membranes. In the pre-antiviral era, 85% of patients with disseminated neonatal HSV disease died by 1 year of age, as did 50% of patients with CNS disease [26]. Multicenter collaborative clinical trials conducted by the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group (CASG) established the efficacy of vidarabine, the first licensed nucleoside analogue, in the treatment of neonatal HSV infections [27]. Vidarabine was subsequently replaced by ACV, which was shown to be as effective with fewer toxicities [27]. A subsequent study demonstrated that a higher dose of ACV (60 mg/kg/day administered intravenously in three divided doses for 21 days) resulted in better outcomes for neonatal HSV infections as compared with historic controls [28].

Cutaneous recurrences following neonatal infection are common, and frequent (more than three in the first 6 months of life), are associated with an increased risk of neurological impairment [29]. The role of suppressive oral ACV (or VCV) is under evaluation in randomized, controlled trials conducted by the NIAID CASG. A small Phase I/II study suggested that almost half of babies on suppressive oral ACV for 6 months developed significant neutropenia. Thus, pending the results of the placebo-controlled Phase III studies, there are insufficient data to recommend routine suppressive therapy following neonatal HSV disease [30].

Licensed systemic antiviral agents for HSV

Currently, there are three classes of drugs licensed for the treatment of HSV infection, all of which target viral DNA replication:

- Guanosine analogues, including ACV, VCV, famciclovir and ganciclovir
- The acyclic nucleotide analogue, cidofovir
- The pyrophosphate analogue, foscarnet

Guanosine analogues

Acyclovir, a guanosine analogue, is selectively converted into acyclo-guanosine monophosphate (acyclo-GMP) by viral thymidine kinase (TK), which is more effective in phosphorylation than cellular kinases. The monophosphate form is further phosphorylated into the active triphosphate form by cellular kinases and is a potent inhibitor of viral DNA

polymerase. It has an approximately 100-times greater affinity for viral than cellular polymerases. As a substrate, acyclo-GMP is incorporated into viral DNA, resulting in chain termination. ACV has a very favorable safety profile. Renal toxicity may result with intravenous therapy because of crystallization of ACV in the kidneys; this is prevented by hydration and slow infusion rates.

The major limitation to ACV is its relatively poor oral bioavailability, which led to the development of VCV, an L-valyl ester prodrug of ACV that is absorbed rapidly and is converted to ACV by the hepatic enzyme VCV hydrolase. Approximately 55% of an orally administered dose of VCV is available as ACV (a three- to fivefold increase in bioavailability). Thus, VCV is preferred in most settings. Thrombotic microangiopathy has been reported in immunocompromised HIV-infected patients with CD4⁺ cell counts of less than 50 cells/ μ l receiving high doses of VCV over long periods of time (median: 54 weeks; range: 8–77 weeks) [31,32], although no episodes of thrombotic micro-angiopathy were observed in a subsequent 6-month study of VCV (500 mg twice daily) for the suppression of recurrent genital HSV [33].

Viral resistance is also a potential limitation of ACV and VCV therapy. The mechanism of resistance is most often a mutation in TK or, less commonly, viral DNA polymerase. Isolation of drug-resistant HSV from immunocompetent patients occurs infrequently (<1%) and has remained stable over the past 20 years, but is more common in the setting of HIV (4–7%) [34]. Notably, a recent study documented a relatively high (6.4%) prevalence of ACV-resistant HSV-1 isolates in immunocompetent patients with herpes keratitis (11 out of 173 patients) and nine out of the 11 patients with resistant isolates were refractory to ACV therapy [35]. Furthermore, in allogeneic bone marrow transplant patients, frequencies as high as 14–30% have been reported [36]. These results emphasize the need to consider ACV resistance in patients with poor clinical response to therapy.

Famciclovir is rapidly metabolized to the active drug penciclovir, which is a synthetic acyclic guanine derivative with a spectrum of activity and a mechanism of action similar to that of ACV. It has a higher affinity for viral TK, is phosphorylated more rapidly in cells infected with HSV and persists intracellularly for longer than ACV does. However, the overall antiviral effects of the two drugs (ACV and famciclovir) is comparable because penciclovir, which has two hydroxyl groups, is not an obligate DNA chain terminator and is therefore 100-fold less potent in inhibiting the replication of viral DNA than ACV [37]. Both famciclovir and VCV are dosed less frequently than ACV.

Ganciclovir and its prodrug, valganciclovir, differ from ACV and famciclovir because they are efficiently phosphorylated not only by TK, but also by CMV UL97. This contributes to the broader spectrum of antiviral activity of ganciclovir. The drug is as active as ACV against HSV-1 and HSV-2, but is associated with a greater risk of neutropenia and renal toxicity. Thus, it is only recommended if treatment or suppression of both HSV and CMV is required.

Cidofovir (HPMPC, GS 504)

This is an acyclic nucleoside phosphonate that is converted to active cidofovir diphosphate by cellular kinases; thus, it retains activity in the setting of TK mutations. The active form is a competitive inhibitor of viral DNA polymerases and it may act as a chain terminator. It is active against all human herpes viruses; however, its use in HSV treatment should be reserved for confirmed cases of ACV resistance. Cidofovir is nephrotoxic, is routinely administered with probenecid and requires intravenous hydration.

Foscarnet

This is a pyrophosphate that inhibits viral DNA polymerase and does not require phosphorylation by viral kinases. Thus, it is active against HSV or CMV viruses with mutations in TK or UL97, respectively. Resistance to foscarnet is rare but can occur as a result of mutations in viral DNA polymerase. The oral bioavailability of foscarnet is poor and thus it is only available in intravenous formulations. Foscarnet is also nephrotoxic.

Novel therapeutic strategies

The currently licensed drugs are highly effective, but there is a need for drugs that would result in better clinical outcomes following neonatal disease and encephalitis. There is also a paucity of safer drugs for the treatment or suppression of resistant viruses, therapies to prevent, rather than suppress, viral reactivation and novel strategies to prevent infection. The following sections summarize some of the strategies currently in development (Table 1).

New drug targets

Helicase/primase inhibitors

A new class of anti-HSV drugs that recognizes the viral helicase–primase complex has been extensively studied *in vitro* and in small animal models [38]. One candidate drug, BAY 57–1293, was shown to be superior to ACV in murine and rat lethal challenge models of disseminated HSV [39,40], the guinea-pig model of genital herpes [41], and in rabbit and murine models of ocular herpes infection [42]. These findings are promising; however, drug-resistant viruses can be readily selected for by serial passage in cell culture or by plaque selection in the presence of the compounds, and these variants have failed to revert to wild-type in the absence of the drug [43,44]. There is no published clinical experience with this class of drugs.

TLR agonists

Animal model studies have demonstrated that TLR agonists stimulate the immune response and modify HSV disease. For example, BALB/c mice treated with a single intraperitoneal or intranasal injection of polyinosinic:polycytidylic acid (poly I:C), a TLR-3 agonist, prior to challenge with HSV-1 in a model of HSE, demonstrated significantly greater survival than mice pretreated with lipopolysaccharide, a TLR-4 agonist, oligodeoxynucleotide, a TLR-9 agonist or control vehicle, indicating that TLR-3 stimulation may augment the innate immune mechanism of neuro-protection against HSV-1 [45]. These findings are consistent with the recently described genetic studies [9,10]. Additional studies demonstrated that mucosal, but not systemic, delivery of TLR-3 (polyI:C) agonists induced innate immune-mediated protection against genital HSV-2 infection in a murine model [46].

Topical TLR7/8 agonists, including imiquimod and resiquimod, have been evaluated for efficacy against genital HSV in clinical studies with mixed results. In one controlled study, adults with genital HSV-2 applied resiquimod or control vehicle topically to herpes lesions twice weekly for 3 weeks during the initial and recurrent clinical episodes. Swabs were collected for 60 days during the initial study period and again after the 7-month study period for detection of HSV by PCR. The median lesion and shedding rates were significantly reduced for resiquimod compared with vehicle recipients during both sampling periods [47]. Further studies and drug development are needed to determine if TLR agonists could be exploited in the treatment, suppression and/or prevention of HSV infections.

Therapeutic vaccines

An alternative strategy to augment immune responses and suppress or modulate HSV recurrences is the development of a therapeutic vaccine. Although no randomized clinical trial to date has demonstrated a clear therapeutic benefit, they have provided important insights for future studies. A reduction in recurrences was suggested in response to the Lupidon H and Lupidon G vaccines, which are comprised of whole heat-killed virus from HSV-1 and HSV-2, respectively, although the results were inconclusive due to a lack of appropriate controls and the number of other interventions during the study [48]. A reduction in the number and severity of recurrences was also suggested following immunization with the Skinner vaccine, an inactivated subunit vaccine containing mixed HSV-2 glyco proteins; however, the results were not statistically significant [49]. A disabled infectious single-cycle viral vaccine carrying a deletion of glycoprotein H demonstrated initial promise in animal models [50], but failed to demonstrate protection against recurrences in a Phase II trial [51]. The recombinant glycoprotein vaccine developed by Chiron (MA, USA), gD2gB2-MF59, also did not reduce rates of recurrences or viral shedding, but did reduce symptom and lesion duration [52]. Promising results were more recently obtained in Phase I/II clinical trials with a live-attenuated ICPO deletion virus, ICP10DPK, which completely prevented recurrences in 37.5 and 43.5% of the vaccinated patients in two trials [53]. Further development and identification of therapeutic vaccine candidates are needed, but mathematical modeling supports the development of a therapeutic HSV vaccine, which could provide both a therapeutic and epidemic-level benefit [54].

Prevention: vaccines & microbicides

The best way to control the HSV epidemic is prevention. Currently available modalities to prevent genital herpes are condoms and circumcision, but consistent use of condoms and implementation of circumcision programs is difficult and complex [55,56]. Historically, the prevention and ultimate eradication of infections have been achieved through the development of vaccines that elicit sterilizing immunity to completely prevent the establishment of infection. The two major strategies in development are subunit vaccines and live-attenuated viral vaccines. One live-attenuated vaccine candidate was R7020, which was constructed on an HSV-1 background genetically deleted in some of the loci responsible for neurovirulence, inserted with HSV-2 sequences to broaden the immune response and with the TK gene added to retain susceptibility to ACV for safety [57]. However, results of clinical trials were disappointing and two doses of the vaccine failed to deliver enough antigen to induce a sufficient immune response [58]. More promising results have been obtained with subunit vaccines. While the gD2gB2-MF59 vaccine developed by Chiron was found to be ineffective in randomized, double-blind, placebo controlled studies [59], the recently completed studies with a gD-2 vaccine from Glaxo SmithKline show greater potential [60]. The differences between these vaccine candidates may relate to the adjuvant; the adjuvant for the Chiron vaccine was MF-59, a potent inducer of Th2 responses, whereas the adjuvant for the gD-2 vaccine contains alum plus monophosphoryl lipid A, a potent inducer of Th1 responses. Two double-blind randomized trials of the gD-2–alum monophosphoryl lipid vaccine demonstrated efficacy against genital herpes in women who are seronegative for both HSV-1 and HSV-2 at baseline, but failed to demonstrate efficacy in men, regardless of their HSV serologic status [60]. Further studies are ongoing.

Most of the vaccines described have been administered systemically and may not generate sufficient local mucosal immunity at the site of infection. A more effective approach may be mucosal vaccination. However, there are substantial challenges confronting the development of vaginal mucosal vaccines, which include an incomplete understanding of genital tract vaginal mucosal immunity and the need to optimize formulations and delivery systems that will prove safe and immunogenic [61]. Recent successes with intranasally delivered vaccines

suggests that this approach could prove successful, but the genital tract is distinct from other mucosal environments and lacks inductive mucosal sites analogous to nasal-associated lymphoid tissue [62] or intestinal Peyer'S patches [63].

Microbicides

A novel strategy for HSV prevention is the development of mucosally delivered microbicides. While the primary impetus for their development is HIV prevention, several of the candidate drugs in development also have the potential to prevent HSV. The first candidate to be advanced to clinical trials was nonoxynol (N)-9, a spermicidal detergent used since the 1960s that demonstrated *in vitro* activity against HIV and HSV. However, the clinical trials were halted when findings demonstrated an increased risk for HIV acquisition in the treatment compared with the placebo arm, particularly among women who used the product frequently [64]. Subsequent work has demonstrated that the cytotoxic and inflammatory responses to N-9 outweigh any potential benefit [65-67].

The next series of candidate microbicides developed were sulfated or sulfonated polymers including PRO 2000, cellulose sulfate and Carraguard™. These drugs mimic the heparan sulfate receptor for HSV binding and thus competitively block viral entry [68]. One potential concern with this class of compounds is the observation that several of the candidate drugs lose anti-HSV activity *in vitro* and in a murine model if HSV is introduced in seminal plasma. Proteins in seminal plasma competitively interfere with the sulfonated polymer'S ability to bind to the HSV viral envelope [69]. Whether this contributed to the lack of efficacy against HSV observed with PRO 2000 in the recently completed Phase IIb clinical trials requires further study [70]. No significant protection against HIV or other sexually transmitted infections was observed in the Carraguard efficacy trial, although HSV acquisition or recurrences were not reported [71]. The cellulose sulfate efficacy trials were also prematurely halted when the interim analysis indicated a significant increase risk for HIV acquisition in women applying cellulose sulfate compared with placebo gel in one of the two studies. In the final analysis, there were 25 newly acquired HIV infections in the cellulose sulfate group and 16 in the placebo group ($p = 0.13$); HSV acquisition or recurrences were not evaluated in the trial [72]. Other compounds with similar mechanisms of anti-HSV activity are in earlier stages of development and include:

- VivaGel™, a sulfonated dendrimer, which is in early clinical trials [73,74];
- PPCM, a negatively charged nonsulfated compound, which is formed from the reaction of D,L-mandelic acid with sulfuric acid [75];
- A molecular umbrella compound, Spm8CHAS [76];
- Amphipathic DNA polymers including a 40-nucleotide degenerate polymer (REP 9) and a 40-nucleotide polycytidine amphipathic DNA polymer (REP 9C) [77];
- Sodium rutin sulfate [78].

In addition to interference with the antiviral activity of this class of drugs by seminal proteins, another potential problem with microbicides that may have contributed to the lack of efficacy in clinical trials relates to delivery. Current delivery systems are designed to deliver the drug deep into the vagina and cervix, which may be the primary site for HIV acquisition, whereas for the prevention of HSV, the drug may need to be well-distributed at the vaginal orifice and labial surfaces.

Two acid-buffering products, BufferGel® (developed by ReProtect, MD, USA) and ACIDFORM (developed by the Program for Topical Prevention of Conception and Disease [TOPCAD], Rush University and licensed by Instead, CA, USA) have also been advanced to

clinical trials. The goal of these products is to overcome the alkalinizing effects of semen, which neutralizes the normally acidic vaginal pH (3.5–4.5) to pH of approximately 7.0 for hours. Exposure of HSV to pH 4.5 or lower *in vitro* irreversibly inactivates HSV by triggering proteolysis and disrupting the viral particle, but pH 5.0 has little or no effect [79]. How this will translate into the clinical setting will depend on the extent and duration of buffering activity. BufferGel, which is formulated at a pH of 3.9–4.0, has a relatively short duration of action and only buffers to a pH of 5.3–5.5 when mixed 1:3 with semen, a pH that would not inactivate HSV. This may have contributed to the failure of BufferGel to provide any protection against HSV (or HIV) in the recently completed clinical trial [70]. ACIDFORM (pH ~3.5) has greater buffering capacity and durability, but has not yet been evaluated for its anti-HSV activity in clinical trials [80].

Natural antimicrobial proteins or peptides as microbicides

Genital tract secretions obtained by lavage from healthy women inhibit HSV infection *in vitro* and protect mice from genital herpes infection [81]. This antiviral activity is mediated, in part, by defensins. Subsequent studies have demonstrated that the human neutrophil peptides (HNP)-1–3 and the epithelial cell defensins, HBD-5, HBD-6 and HBD-3 inhibit HSV infection *in vitro* through multiple mechanisms [82]. Defensins also contribute to endogenous defense against bacteria [83] and HIV [84]. These observations suggest that defensins or synthetic analogues could be exploited as microbicides. However, defensins have complex effects on the mucosal environment that require further study before being advanced as microbicides. In addition, the cost of synthesis is a major limitation. One approach being pursued is the development of retrocyclins, which are synthetic θ -defensins with antiviral activity. Humans do not express θ -defensins because of the inheritance of genes that contain a premature stop codon that aborts translation [85]. Retrocyclins protect cervical epithelial cells from infection by both HSV serotypes by binding to carbohydrate epitopes on the viral envelope glycoproteins [86]. Retrocyclin-2 protected mice from herpes keratitis when applied prophylactically, but not when added postinfection [87] and also protects mice from genital herpes infection (HEROLD BC, UNPUBLISHED DATA). Retrocyclins and other small molecules are currently being explored as candidate microbicides. One additional strategy in early preclinical development is the delivery of siRNA targeting viral and/or host cellular proteins to prevent HSV infection [88-90]. Optimization of delivery systems, however, is a major hurdle that will need to be addressed before this strategy can be advanced to the clinical arena.

Expert commentary & five-year view

Despite the continued global epidemic of HSV and extensive research, there have been few major breakthroughs in the treatment or prevention of the virus since the introduction of ACV in the 1980s. Major advancements have been the recognition of the frequency with which HSV reactivates and the optimization of the use of ACV and related drugs for suppression, which has the potential to greatly reduce sexual and perinatal transmission. However, options for patients who develop drug-resistant variants are limited, and there are no drugs to eradicate infection or to prevent the establishment of viral latency or its reactivation. Vaccines have had some success and it appears likely that a vaccine for seronegative women may soon be available. However, the discrepancies in the immune responses to vaccines between males and females highlight the challenges in understanding the mucosal immune response to HSV. Microbicides offer a novel approach to prevent herpes infection but have not yet proven successful, and issues with formulation, delivery and adherence must be overcome.

Key issues

- Herpes simplex virus (HSV) poses a great threat to global health as the leading cause of genital ulcerative disease and encephalitis, and as a major cofactor for HIV infection.
- Acyclovir and its derivatives are the drugs of choice for the management of HSV-related disease; however, alternative drugs with different targets of action are needed.
- None of the available formulations can cure HSV and therefore there is an urgent need to develop preventative strategies.
- Vaccines have demonstrated ‘proof of concept’ and present a viable option for future therapeutic and prevention strategies.
- Topical microbicides may provide an alternative prevention strategy but further study is needed.

References

Papers of special note have been highlighted as:

- of interest

1. Elion GB, Furman PA, Fyfe JA, de Miranda P, Beauchamp L, Schaeffer HJ. Selectivity of action of an antitherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. *Proc. Natl Acad. Sci. USA* 1977;74(12): 5716–5720. [PubMed: 202961]
2. Fyfe JA, Keller PM, Furman PA, Miller RL, Elion GB. Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl)guanine. *J. Biol. Chem* 1978;253(24):8721–8727. [PubMed: 214430]
3. Amir J, Harel L, Smetana Z, Varsano I. Treatment of herpes simplex gingivostomatitis with aciclovir in children: a randomised double blind placebo controlled study. *BMJ* 1997;314(7097):1800–1803. [PubMed: 9224082]
4. Evans TG, Bernstein DI, Raborn GW, Harmenberg J, Kowalski J, Spruance SL. Double-blind, randomized, placebo-controlled study of topical 5% acyclovir–1% hydrocortisone cream (ME-609) for treatment of UV radiation-induced herpes labialis. *Antimicrob. Agents Chemother* 2002;46(6): 1870–1874. [PubMed: 12019102]
5. Lairson DR, Begley CE, Reynolds TF, Wilhelmus KR. Prevention of herpes simplex virus eye disease: a cost–effectiveness analysis. *Arch. Ophthalmol* 2003;121(1):108–112. [PubMed: 12523894]
6. Wilhelmus KR. Therapeutic interventions for herpes simplex virus epithelial keratitis. *Cochrane Database Syst. Rev* 2008;1:CD002898. [PubMed: 18254009]
7. Oral acyclovir for herpes simplex virus eye disease: effect on prevention of epithelial keratitis and stromal keratitis. Herpetic Eye Disease Study Group. *Arch. Ophthalmol* 2000;118(8):1030–1036. [PubMed: 10922194]
8. Whitley RJ. Herpes simplex encephalitis: adolescents and adults. *Antiviral Res* 2006;71(2–3):141–148. [PubMed: 16675036]
9. Casrouge A, Zhang SY, Eidschinken C, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* 2006;314(5797):308–312. [PubMed: 16973841]
10. Zhang SY, Jouanguy E, Ugolini S, et al. TLR3 deficiency in patients with herpes simplex encephalitis. *Science* 2007;317(5844):1522–1527. [PubMed: 17872438]
11. 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]
12. Nagot N, Ouedraogo A, Foulongne V, et al. Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus. *N. Engl. J. Med* 2007;356(8):790–799. [PubMed: 17314338]

- 13 • 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] • of interest Demonstrates that once-daily suppressive therapy with valacyclovir significantly reduces the risk of transmission of genital herpes among heterosexual, herpes simplex virus (HSV)-2-discordant couples.
14. 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]
15. Hollier LM, Wendel GD. Third trimester antiviral prophylaxis for preventing maternal genital herpes simplex virus (HSV) recurrences and neonatal infection. *Cochrane Database Syst. Rev* 2008;1:CD004946. [PubMed: 18254066]
16. Abu-Raddad LJ, Magaret AS, Celum C, et al. Genital herpes has played a more important role than any other sexually transmitted infection in driving HIV prevalence in Africa. *PLoS ONE* 2008;3(5):e2230. [PubMed: 18493617]
17. Biswas S, Tiley LS, Zimmermann H, Birkmann A, Field HJ. Mutations close to functional motif IV in HSV-1 UL5 helicase that confer resistance to HSV helicase–primase inhibitors, variously affect virus growth rate and pathogenicity. *Antiviral Res* 2008;80(1):81–85. [PubMed: 18539344]
18. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* 2006;20(1):73–83. [PubMed: 16327322]
19. Zuckerman RA, Lucchetti A, Whittington WL, et al. Herpes simplex virus (HSV) suppression with valacyclovir reduces rectal and blood plasma HIV-1 levels in HIV-1/HSV-2-seropositive men: a randomized, double-blind, placebo-controlled crossover trial. *J. Infect. Dis* 2007;196(10):1500–1508. [PubMed: 18008230]
20. Zuckerman RA, Lucchetti A, Whittington WL, et al. HSV suppression reduces seminal HIV-1 levels in HIV-1/HSV-2 co-infected men who have sex with men. *AIDS* 2009;23(4):479–483. [PubMed: 19169140]
- 21 • Watson-Jones D, Weiss HA, Rusizoka M, et al. Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. *N. Engl. J. Med* 2008;358(15):1560–1571. [PubMed: 18337596] • of interest Representative of clinical trials demonstrating that suppression of HSV with acyclovir may reduce HIV infection.
22. Fakioglu E, Wilson SS, Mesquita PM, et al. Herpes simplex virus down-regulates secretory leukocyte protease inhibitor: a novel immune evasion mechanism. *J. Virol* 2008;82(19):9337–9344. [PubMed: 18667508]
23. 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]
- 24 • Lisco A, Vanpouille C, Tchesnokov EP, et al. Acyclovir is activated into a HIV-1 reverse transcriptase inhibitor in herpesvirus-infected human tissues. *Cell Host Microbe* 2008;4(3):260–270. [PubMed: 18779052] • of interest Demonstrates that phosphorylated acyclovir inhibits HIV and suggests that acyclovir may have efficacy in the setting of dual infection with both pathogens.
25. McMahon MA, Siliciano JD, Lai J, et al. The antiherpetic drug acyclovir inhibits HIV replication and selects the V75I reverse transcriptase multidrug resistance mutation. *J. Biol. Chem* 2008;283(46):31289–31293. [PubMed: 18818198]
26. Kimberlin DW, Lin CY, Jacobs RF, et al. Natural history of neonatal herpes simplex virus infections in the acyclovir era. *Pediatrics* 2001;108(2):223–229. [PubMed: 11483781]
27. Whitley R, Arvin A, Prober C, et al. A controlled trial comparing vidarabine with acyclovir in neonatal herpes simplex virus infection. *Infectious Diseases Collaborative Antiviral Study Group. N. Engl. J. Med* 1991;324(7):444–449. [PubMed: 1988829]
28. Kimberlin DW, Lin CY, Jacobs RF, et al. Safety and efficacy of high-dose intravenous acyclovir in the management of neonatal herpes simplex virus infections. *Pediatrics* 2001;108(2):230–238. [PubMed: 11483782]
29. Whitley R, Arvin A, Prober C, et al. Predictors of morbidity and mortality in neonates with herpes simplex virus infections. *The National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. N. Engl. J. Med* 1991;324(7):450–454. [PubMed: 1988830]

30. Kimberlin D, Powell D, Gruber W, et al. Administration of oral acyclovir suppressive therapy after neonatal herpes simplex virus disease limited to the skin, eyes and mouth: results of a Phase I/II trial. *Pediatr. Infect. Dis. J* 1996;15(3):247–254. [PubMed: 8852914]
31. Bell WR, Chulay JD, Feinberg JE. Manifestations resembling thrombotic microangiopathy in patients with advanced human immunodeficiency virus (HIV) disease in a cytomegalovirus prophylaxis trial (ACTG 204). *Medicine* 1997;76(5):369–380. [PubMed: 9352739]
32. Feinberg JE, Hurwitz S, Cooper D, et al. A randomized, double-blind trial of valaciclovir prophylaxis for cytomegalovirus disease in patients with advanced human immunodeficiency virus infection. AIDS Clinical Trials Group Protocol 204/Glaxo Wellcome 123–014 International CMV Prophylaxis Study Group. *J. Infect. Dis* 1998;177(1):48–56. [PubMed: 9419169]
33. DeJesus E, Wald A, Warren T, et al. Valacyclovir for the suppression of recurrent genital herpes in human immunodeficiency virus-infected subjects. *J. Infect. Dis* 2003;188(7):1009–1016. [PubMed: 14513421]
34. Reyes M, Shaik NS, Graber JM, et al. Acyclovir-resistant genital herpes among persons attending sexually transmitted disease and human immunodeficiency virus clinics. *Arch. Intern. Med* 2003;163(1):76–80. [PubMed: 12523920]
35. Duan R, de Vries RD, Osterhaus AD, Remeijer L, Verjans GM. Acyclovir-resistant corneal HSV-1 isolates from patients with herpetic keratitis. *J. Infect. Dis* 2008;198(5):659–663. [PubMed: 18627246]
36. Frobert E, Cortay JC, Ooka T, et al. Genotypic detection of acyclovir-resistant HSV-1: characterization of 67 ACV-sensitive and 14 ACV-resistant viruses. *Antiviral Res* 2008;79(1):28–36. [PubMed: 18336925]
37. Balzarini J, Naesens L, De Clercq E. New antivirals – mechanism of action and resistance development. *Curr. Opin. Microbiol* 1998;1(5):535–546. [PubMed: 10066527]
38. Kleymann G, Fischer R, Betz UA, et al. New helicase–primase inhibitors as drug candidates for the treatment of herpes simplex disease. *Nat. Med* 2002;8(4):392–398. [PubMed: 11927946]
39. Betz UA, Fischer R, Kleymann G, Hendrix M, Rubsamen-Waigmann H. Potent *In vivo* antiviral activity of the herpes simplex virus primase–helicase inhibitor BAY 57–1293. *Antimicrob. Agents Chemother* 2002;46(6):1766–1772. [PubMed: 12019088]
40. Biswas S, Jennens L, Field HJ. The helicase primase inhibitor, BAY 57–1293 shows potent therapeutic antiviral activity superior to famciclovir in BALB/c mice infected with herpes simplex virus type 1. *Antiviral Res* 2007;75(1):30–35. [PubMed: 17175035]
41. Baumeister J, Fischer R, Eckenberg P, Henninger K, Ruebsamen-Waigmann H, Kleymann G. Superior efficacy of helicase–primase inhibitor BAY 57–1293 for herpes infection and latency in the guinea pig model of human genital herpes disease. *Antivir. Chem. Chemother* 2007;18(1):35–48. [PubMed: 17354650]
42. Kaufman HE, Varnell ED, Gebhardt BM, et al. Efficacy of a helicase–primase inhibitor in animal models of ocular herpes simplex virus type 1 infection. *J. Ocul. Pharmacol. Ther* 2008;24(1):34–42. [PubMed: 18201137]
43. Biswas S, Field HJ. Herpes simplex virus helicase-primase inhibitors: recent findings from the study of drug resistance mutations. *Antivir. Chem. Chemother* 2008;19(1):1–6. [PubMed: 18610552]
44. Biswas S, Smith C, Field HJ. Detection of HSV-1 variants highly resistant to the helicase–primase inhibitor BAY 57–1293 at high frequency in 2 of 10 recent clinical isolates of HSV-1. *J. Antimicrob. Chemother* 2007;60(2):274–279. [PubMed: 17550887]
45. Boivin N, Sergerie Y, Rivest S, Boivin G. Effect of pretreatment with Toll-like receptor agonists in a mouse model of herpes simplex virus type 1 encephalitis. *J. Infect. Dis* 2008;198(5):664–672. [PubMed: 18662130]
46. Ashkar AA, Yao XD, Gill N, Sajic D, Patrick AJ, Rosenthal KL. 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]
47. 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]

48. Weitgasser H. [Controlled clinical study of the herpes antigens LUPIDON H and LUPIDON G]. *Z. Hautkr* 1977;52(11):625–628. [PubMed: 196421]
49. Skinner GR, Turyk ME, Benson CA, et al. The efficacy and safety of Skinner herpes simplex vaccine towards modulation of herpes genitalis; report of a prospective double-blind placebo-controlled trial. *Med. Microbiol. Immunol* 1997;186(1):31–36. [PubMed: 9255764]
50. Bournsnel ME, Entwisle C, Blakeley D, et al. A genetically inactivated herpes simplex virus type 2 (HSV-2) vaccine provides effective protection against primary and recurrent HSV-2 disease. *J. Infect. Dis* 1997;175(1):16–25. [PubMed: 8985191]
51. Koelle DM. Vaccines for herpes simplex virus infections. *Curr. Opin. Investig. Drugs* 2006;7(2):136–141.
52. Straus SE, Wald A, Kost RG, et al. Immunotherapy of recurrent genital herpes with recombinant herpes simplex virus type 2 glycoproteins D and B: results of a placebo-controlled vaccine trial. *J. Infect. Dis* 1997;176(5):1129–1134. [PubMed: 9359709]
53. Casanova G, Cancela R, Alonzo L, et al. A double-blind study of the efficacy and safety of the ICP100PK vaccine against recurrent genital HSV-2 infections. *Cutis* 2002;70(4):235–239. [PubMed: 12403316]
54. Schwartz EJ, Bodine EN, Blower S. Effectiveness and efficiency of imperfect therapeutic HSV-2 vaccines. *Hum. Vaccin* 2007;3(6):231–238. [PubMed: 17881889]
55. Tobian AA, Serwadda D, Quinn TC, et al. Male circumcision for the prevention of HSV-2 and HPV infections and syphilis. *N. Engl. J. Med* 2009;360(13):1298–1309. [PubMed: 19321868]
56. Wald A, Langenberg AG, Krantz E, et al. The relationship between condom use and herpes simplex virus acquisition. *Ann. Intern. Med* 2005;143(10):707–713. [PubMed: 16287791]
57. Meignier B, Longnecker R, Roizman B. *In vivo* behavior of genetically engineered herpes simplex viruses R7017 and R7020: construction and evaluation in rodents. *J. Infect. Dis* 1988;158(3):602–614. [PubMed: 2842408]
58. Cadoz, M.; Micoud, M.; Seigneurin, JM., et al. Phase I trial of R7020: a live attenuated recombinant HSV candidate vaccine.. Presented at: 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy.; Anaheim, CA, USA. 11–14 October 1992;
59. Corey L, Langenberg AG, Ashley R, et al. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection: two randomized controlled trials. Chiron HSV Vaccine Study Group. *JAMA* 1999;282(4):331–340. [PubMed: 10432030]
60. Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-dadjuvant vaccine to prevent genital herpes. *N. Engl. J. Med* 2002;347(21):1652–1661. [PubMed: 12444179]
- 61 • Zhang X, Chentoufi AA, Dasgupta G, et al. A genital tract peptide epitope vaccine targeting TLR-2 efficiently induces local and systemic CD8⁺ T cells and protects against herpes simplex virus type 2 challenge. *Mucosal Immunol* 2009;2(2):129–143. [PubMed: 19129756] • of interest Demonstrates that phosphorylated acyclovir inhibits HIV and suggests that acyclovir may have efficacy in the setting of dual infection with both pathogens.
62. Kozlowski PA, Williams SB, Lynch RM, 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(1):566–574. [PubMed: 12077289]
63. Belyakov IM, Derby MA, Ahlers JD, et al. Mucosal immunization with HIV-1 peptide vaccine induces mucosal and systemic cytotoxic T lymphocytes and protective immunity in mice against intrarectal recombinant HIV-vaccinia challenge. *Proc. Natl Acad. Sci. USA* 1998;95(4):1709–1714. [PubMed: 9465081]
64. Van Damme L, Ramjee G, Alary M, 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(9338):971–977. [PubMed: 12383665]
65. Fichorova RN, Tucker LD, Anderson DJ. The molecular basis of nonoxynol-9-induced vaginal inflammation and its possible relevance to human immunodeficiency virus type 1 transmission. *J. Infect. Dis* 2001;184(4):418–428. [PubMed: 11471099]
66. Galen B, Martin AP, Hazrati E, et al. A comprehensive murine model to evaluate topical vaginal microbicides: mucosal inflammation and susceptibility to genital herpes as surrogate markers of safety. *J. Infect. Dis* 2007;195(9):1332–1339. [PubMed: 17397004]

67. Hillier SL, Moench T, Shattock R, Black R, Reichelderfer P, Veronese F. In vitro and in vivo: the story of nonoxynol 9. *J. Acquir. Immune Defic. Syndr* 2005;39(1):1–8. [PubMed: 15851907]
68. Cheshenko N, Keller MJ, MasCasullo V, et al. Candidate topical microbicides bind herpes simplex virus glycoprotein B and prevent viral entry and cell-to-cell spread. *Antimicrob. Agents Chemother* 2004;48(6):2025–2036. [PubMed: 15155195]
69. Patel S, Hazrati E, Cheshenko N, et al. Seminal plasma reduces the effectiveness of topical polyanionic microbicides. *J. Infect. Dis* 2007;196(9):1394–1402. [PubMed: 17922405]
70. Karim, SA.; Coletti, A.; Richardson, B., et al. Safety and effectiveness of vaginal microbicides BufferGel and 0.5% PRO 2000/5 gel for the prevention of HIV infection in women: results of the HPTN 035 trial.. Presented at: 16th Conference on Retroviruses and Opportunistic Infections.; Montreal, Canada. 8–11 February 2009;
71. Skoler-Karppoff S, Ramjee G, Ahmed K, et al. Efficacy of Carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;372(9654):1977–1987. [PubMed: 19059048]
72. Van Damme L, Govinden R, Mirembe FM, et al. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *N. Engl. J. Med* 2008;359(5):463–472. [PubMed: 18669425]
73. Bourne N, Stanberry LR, Kern ER, Holan G, Matthews B, Bernstein DI. Dendrimers, a new class of candidate topical microbicides with activity against herpes simplex virus infection. *Antimicrob. Agents Chemother* 2000;44(9):2471–2474. [PubMed: 10952597]
74. Gong E, Matthews B, McCarthy T, et al. Evaluation of dendrimer SPL7013, a lead microbicide candidate against herpes simplex viruses. *Antiviral Res* 2005;68(3):139–146. [PubMed: 16219368]
75. Mesquita PM, Wilson SS, Manlow P, et al. Candidate microbicide PPCM blocks human immunodeficiency virus type 1 infection in cell and tissue cultures and prevents genital herpes in a murine model. *J. Virol* 2008;82(13):6576–6584. [PubMed: 18434407]
76. Madan RP, Mesquita PM, Cheshenko N, et al. Molecular umbrellas: a novel class of candidate topical microbicides to prevent human immunodeficiency virus and herpes simplex virus infections. *J. Virol* 2007;81(14):7636–7646. [PubMed: 17494078]
77. Guzman EM, Cheshenko N, Shende V, et al. Amphipathic DNA polymers are candidate vaginal microbicides and block herpes simplex virus binding, entry and viral gene expression. *Antivir. Ther. (Lond.)* 2007;12(8):1147–1156. [PubMed: 18240855]
78. Tao J, Hu Q, Yang J, et al. In vitro anti-HIV and -HSV activity and safety of sodium rutin sulfate as a microbicide candidate. *Antiviral Res* 2007;75(3):227–233. [PubMed: 17459492]
79. • Tuyama AC, Cheshenko N, Carlucci MJ, et al. ACIDFORM inactivates herpes simplex virus and prevents genital herpes in a mouse model: optimal candidate for microbicide combinations. *J. Infect. Dis* 2006;194(6):795–803. [PubMed: 16941346]• of interestRepresentative of the preclinical in vitro and murine model evaluation of the safety and efficacy of a candidate microbicide.
80. Garg S, Anderson RA, Chany CJ 2nd, et al. Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM). *Contraception* 2001;64(1):67–75. [PubMed: 11535216]
81. John M, Keller MJ, Fam EH, et al. Cervicovaginal secretions contribute to innate resistance to herpes simplex virus infection. *J. Infect. Dis* 2005;192(10):1731–1740. [PubMed: 16235171]
82. Hazrati E, Galen B, Lu W, et al. Human α - and β -defensins block multiple steps in herpes simplex virus infection. *J. Immunol* 2006;177(12):8658–8666. [PubMed: 17142766]
83. Valore EV, Park CH, Igrati SL, Ganz T. Antimicrobial components of vaginal fluid. *Am. J. Obstet. Gynecol* 2002;187(3):561–568. [PubMed: 12237628]
84. Venkataraman N, Cole AL, Svoboda P, Pohl J, Cole AM. Cationic polypeptides are required for anti-HIV-1 activity of human vaginal fluid. *J. Immunol* 2005;175(11):7560–7567. [PubMed: 16301665]
85. Lehrer RI. Primate defensins. *Nat. Rev* 2004;2(9):727–738.
86. Yasin B, Wang W, Pang M, et al. θ defensins protect cells from infection by herpes simplex virus by inhibiting viral adhesion and entry. *J. Virol* 2004;78(10):5147–5156. [PubMed: 15113897]
87. Brandt CR, Akkarawongsa R, Altmann S, et al. Evaluation of a θ -defensin in a murine model of herpes simplex virus type 1 keratitis. *Invest. Ophthalmol. Vis. Sci* 2007;48(11):5118–5124. [PubMed: 17962464]

88. Galen B, Cheshenko N, Tuyama A, Ramratnam B, Herold BC. Access to nectin favors herpes simplex virus infection at the apical surface of polarized human epithelial cells. *J. Virol* 2006;80(24):12209–12218. [PubMed: 17005657]
89. Wu Y, Navarro F, Lal A, et al. Durable protection from herpes simplex virus-2 transmission following intravaginal application of siRNAs targeting both a viral and host gene. *Cell Host Microbe* 2009;5(1):84–94. [PubMed: 19154990]
90. Palliser D, Chowdhury D, Wang QY, et al. An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. *Nature* 2006;439(7072):89–94. [PubMed: 16306938]

Table 1

Drugs and vaccines for treatment or prevention of herpes simplex virus.

Class	Specific agents	Current status
Helicase/primase inhibitors	BAY 57-1293	Preclinical
Toll-like receptor agonists	Poly I:C (Toll-like receptor-3)	Preclinical
	Resiquimod (Toll-like receptor-7/8)	Clinical trials
	Imiquimod (Toll-like receptor-7/8)	Clinical trials
Vaccines	Lupidon H and lupidon G (whole heat-inactivated)	Clinical trials completed; discontinued
	Skinner (inactivated subunit)	Clinical trials completed; discontinued
	Disabled infectious single-cycle (gH-deletion)	Clinical trials; ongoing development
	gD2gB2-MF59 (recombinant glycoprotein)	Clinical trials completed; discontinued
	R7020 (live-attenuated virus)	Clinical trials completed; discontinued
	ICP10DPK (deletion virus)	Clinical trials; ongoing development
	gD-2-Alum MPL (recombinant glycoprotein)	Clinical trials ongoing
Microbicides	Nonoxynol-9 (detergent)	Clinical trials failed; discontinued
	Carraguard (sulfated polymer)	Clinical trials completed; ongoing development in combinations
	Cellulose sulfate (sulfated polymer)	Clinical trials completed; discontinued
	PRO 2000 (sulfated polymer)	Clinical trials ongoing
	VivaGel (sulfated dendrimer)	Clinical trials ongoing
	ACIDFORM (acid buffering)	Clinical trials ongoing
	BufferGel (acid buffering)	Clinical trials completed; no efficacy observed
	Retroyclins (defensins)	Preclinical formulation
	Small interfering RNA	Preclinical development

MPL: Monophosphoryl lipid; Poly I:C: Polyinosinic:polycytidylic acid.