Immunostimulatory CpG treatment for genital HSV-2 infections

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Keywords: herpes simplex virus, genital herpes, immunostimulatory sequences, CpG motif, topical microbicides, vaginal microbicides, sexually transmitted diseases

Genital herpes simplex virus type 2 (HSV-2) infection is one of the most common sexually transmitted diseases, infecting approximately 20–30% of US adults. Despite efforts to reduce HSV-2 transmission rates through behavioral modifications and chemical interventions, a 30% increase in seroprevalence was reported recently indicating the ongoing nature of this epidemic and the inadequacies of current therapeutic approaches. Primary HSV-2 infections produce vesicular-ulcerative lesions and the establishment of a life-long latent infection in innervating sensory neurons. Recurrent ulcerative lesions produced by viral reactivation predispose to human immunodeficiency virus type 1 (HIV-1) infection and colonization by other pathogens. Viral reactivation also results in asymptomatic viral shedding episodes that are a major factor in viral transmission. The majority of HSV-2-seropositive people are unaware that they are infected and can transmit the virus in the absence of clinically apparent lesions further complicating control of infection. Such asymptomatic viral shedding events occur as frequently as 40% of sampled days as measured by PCR. Effective therapeutic approaches ideally would not only minimize the severity and duration of primary and recurrent disease but also would reduce the shedding of virus from genital mucosa to lower transmission rates.

Nobel Laureate Gertrude Elion and her colleagues introduced one of the most successful approaches for chemical intervention against HSV infections based upon the promiscuity of the viral thymidine kinase (TK) and its phosphorylation of nucleoside analogues. Incorporation of the activated analogue terminates synthesis of the viral DNA genome. Aciclovir, the lead compound from Elion’s work, is used widely; however, poor bioavailability requires high doses and inconvenient dosing schedules leading to poor compliance and treatment failures. Derivatives of aciclovir and other nucleoside analogues have been identified with better profiles, but also require multiple dosing regimens. More recently, chronic suppressive drug therapy (e.g. valaciclovir, 3–5 doses/day for life) has been reported to reduce inapparent viral shedding and possibly transmission. When dosed appropriately, the current interventions modestly reduce lesion duration and viral shedding is reduced only with chronic suppressive therapy, indicating the need for improved or synergistic intervention strategies. Multiple high-dose regimens and the specific targeting of a viral function like TK can be expected to select progeny with adaptive mutations resulting in antiviral resistance. As predicted, HSV-2 antiviral-resistant viruses are becoming more common especially in immunocompromised patients.

There is increasing interest in work evaluating methods for preventing transmission of HSV-2 and other genital pathogens through the use of topical microbicides. Such compounds act to block attachment or prevent entry of the virus to the host cell. The ideal microbicide would be safe and efficacious, undetectable, inexpensive and have broad range efficacy against a variety of pathogens. Preclinical studies have indicated that these compounds are effective if given immediately before or just after pathogen exposure limiting convenient and covert application. A number of microbicide candidates are in clinical evaluation; however, at present, none have shown the level of efficacy against HSV-2 observed in animal models. Additionally, many of the microbicides under evaluation are contraceptive and therefore not useful for discordant couples attempting to conceive.

An obvious alternative to chemical interventions is to develop vaccines that prevent, or, perhaps more realistically, reduce viral infection and disease. Several attempts to develop HSV-2 vaccines have generated candidates that provide protection in animal models but have not proven efficacious in clinical trials. Recently, clinical evaluation of an HSV-2 subunit vaccine has provided intriguing data indicating efficacy in HSV-1/HSV-2 double-seronegative women. Although promising, at present this single successful vaccine appears to have limited usefulness suggesting a continued need for vaccine development and other interventions. Alternative to vaccines, immunotherapeutic approaches have shown promise in animal models of herpetic disease. Evaluation of immune response modifying compounds from the imidazoquinoline family has shown efficacy against HSV in animals, although they have not performed well in clinical evaluations. The pre-clinical results do demonstrate the potential of this type of intervention and support continued evaluation of other immunomodulators.

Experiences with the current antiviral approaches collectively indicate that the ideal HSV-2 intervention would be safe, non-conceptive, effective prophylactically and therapeutically, require minimal dosing, have efficacy before or after exposure and have a reduced chance of selecting drug-resistant mutants. Recently an...
exciting approach that may satisfy many of these expectations has been evaluated pre-clinically utilizing oligodeoxynucleotides (ODN) that contain a sequence motif with a C-p-G (CpG ODN) at the core now known to stimulate innate and acquired immune responses through toll-like receptor 9 (TLR9). Studies in both mouse and guinea pig models of vaginal HSV-2 infection by our laboratory have shown significant efficacy utilizing an immunostimulatory CpG ODN designated ISS1018 (Dynavax Technologies, Inc., Berkeley, CA, USA). Specifically, a single 100 µg dose of ISS1018 in PBS applied topically to the genital mucosa protected 50% of outbred mice from a lethal HSV-2 challenge significantly reducing disease incidence relative to animals treated with an ODN that lacked CpGs but was identical in the remaining sequence (non-ISS1040). Therapeutically, ISS1018, delivered as a single intravaginal dose, also significantly impacted recurrent herpetic disease in latently infected guinea pigs when administered 21 days post-inoculation. Compared with non-ISS1040, ISS1018 significantly reduced recurrent herpetic lesions and limited the magnitude but not the frequency of shedding in treated guinea pigs. Finally, CpG ODN do not appear to directly inhibit viral infection of cells but rather act through stimulation of innate immune responses.

The reduction in magnitude but not frequency of viral shedding in our guinea pig studies suggests that ISS1018 application produced a local response rather than altering reactivation from the latent neuronal site. Consistent with a local effect, our results and those of others indicate CpGs are most effective when delivered locally. Minimal activity was provided by systemic administration in both mouse and guinea pig models (R. Pyles, D. Higgins & G. VanNest, unpublished data). When delivered to guinea pigs at 2 h after viral challenge, ISS1018 significantly reduced primary and recurrent disease relative to non-ISS treated animals. The ISS1018 animals also had significantly less latent viral DNA in sacral ganglia harvested 70 days post-inoculation (M. Herbst & R. Pyles, unpublished data). Reductions in latent HSV-2 DNA burden have been correlated with reduced recurrence in other model systems and support the hypothesis that effective CpG therapy during the primary exposure may reduce the establishment of latency ultimately reducing the potential for viral transmission. Importantly, reductions in herpetic lesions should also decrease susceptibility to secondary pathogen infections including HIV.

The potential of this herpetic intervention has been confirmed and extended by other groups that have evaluated other CpG ODN in inbred lines of mice. Collectively the findings indicate that several CpG motifs provide protection to multiple strains of mice against several tested HSV-2 isolates. To begin to address mechanisms, Harandi and colleagues tested the efficacy of a CpG ODN in a variety of immunocompromised mice reporting that animals lacking T cells were less protected from lethal HSV-2 challenge than those lacking B cells. This report also indicated that CpG, delivered 2 days before exposure in the absence of viral components, established an HSV-2-resistant environment relative to controls. Our evaluations in the guinea pig model provided an interesting anecdotal observation that supports the hypothesis that CpG treatment primes a response that must be further triggered by viral components within ~2 days of administration. A single animal failed to respond to CpG therapy administered 21 days post-inoculation. PCR analysis of daily vaginal swab samples from this animal indicated an absence of HSV-2 DNA for the 4 days before and the 3 days after ISS application. It is clear that dosing and challenge studies must be carried out to test the hypothesis that viral components must be present and define the window of time provided by CpG priming before viral product exposure. The current findings that establish an effective therapy window extending from 2 days before through to 6 h after viral challenge, suggest CpG therapy may provide a larger intervention window than most of the microbicides under evaluation.

Mechanistically, CpGs appear to provide non-specific priming through TLR9 as indicated by the lack of activity afforded by CpG ODN in TLR9 knock-out mice. A careful examination of TLR9/ CpG motif interactions has indicated that mouse TLR9 recognizes an optimal motif that is less well recognized by human TLR9 potentially explaining some of the failures to translate successful CpG animal studies to efficacy in human cells. ISS1018 was chosen for evaluation because it contains both a murine- and human-optimized CpG and has been shown to have potent activity in a variety of animal species. ISS1018 has been evaluated in unrelated clinical trials and has proven to be safe and has provided efficacy in limited evaluations. It is reasonable to predict that future human applications would make use of a mixture of CpG motifs contained within a single or multiple ODN. ISS1018 has been evaluated in unrelated clinical trials and has proven to be safe and has provided efficacy in limited evaluations.

Although the cellular mechanism has yet to be elucidated, CpG motifs appear to act by stimulating an innate resistance to HSV-2 probably through induction of Th1-type cytokines and chemokines, as well as activating and/or recruiting dendritic cells (DCs). DCs express high levels of TLR9 and are important effector cells bridging the innate and acquired immune responses during HSV-2 vaginal clearance. DCs reside beneath the vaginal epithelial cell layer requiring that CpG pass through 1–12 layers of cells depending upon the oestrus phase. Because the epithelial cell is probably the predominant cell type contacted by the CpG, we have hypothesized that TLR9 is expressed in this cell layer and leads to a direct responsiveness to the CpG stimulus resulting in production or transduction of pro-inflammatory signals. Recently, CpG treatment of mouse vaginal mucosa has been reported to induce a thickening of the epithelial layer that may increase resistance to HSV-2 infection; however, this cell type is the target of the initial infection and may increase vaginal viral titres. During dioestrus phases when vaginal epithelial layers are thinnest, the opportunity for HSV-2 to infect the host is greatest due to lack of protective cornified epithelium and reduced thickness of stratum spinosum. Consistent with this observation, mice are found to be susceptible to HSV-2 only at these stages. It is possible then that CpG therapy will be less effective during specific phases of oestrus and therefore should be evaluated for activity and toxicity at representative stages.

CpG therapy offers an alternative or supplement to the chronic suppressive therapies currently in use today to reduce the likelihood of transmission. Currently all of our studies have examined a single intravaginal administration of CpG. The effect of multiple dosing, as often as daily, needs to be completed to examine potential tolerance or toxicity associated with the promising therapeutic approach. Delivering a mix of CpG ODNs may be necessary due to differences in TLR9 responsiveness indicated in the human population. A single administration, essentially unformulated in PBS, has proven to be well tolerated and effective in multiple mouse strains and in our outbred guinea pig studies. Evaluations of other CpG sequence motifs alone and in combination will help to establish an optimal formulation for use in human therapies. The current findings indicate the exciting potential of this novel therapy that satisfies most of the criteria for an ideal HSV-2 antiviral.
References


