The 8 known human herpes viruses (HHV) are divided into 3 groups designated as alpha, beta, and gamma. Together with varicella-zoster virus (VZV), herpes simplex virus (HSV) 1 and 2 (HSV-1, HSV-2) make up the alpha-herpes virus group within the family of human herpesviridae. Knowledge of herpes can be traced back to the early Greek civilization, as Hippocrates used the word “herpes” to describe lesions that appeared to “creep” or crawl along the skin. John Astruc, physician for King Louis XIV of France, is credited for initially recognizing and publishing the connection between herpes and the genital organs, at a time when French prostitutes were under medical surveillance.1 First grown in vitro in 1925, it was some 45 years later before it became known that 2 antigenic strains of these enveloped, double-stranded, linear DNA HSVs actually existed.2 Historically, awareness of the contagiousness and ability of HSV to be passed from 1 person to another dates back to the early 20th century, though not until 1970 was genital herpes (GH) acknowledged as being an unintended sexually transmitted disease (STD). The extent and perceived seriousness of genital HSV infections came to the forefront of the public’s attention with Time magazine’s cover story in 1982.3 This was shortly before the period when anti-viral drug therapy first became available for the treatment of HSV.

Genital HSV is the leading cause of genital ulcer disease worldwide, with HSV-2 accounting for 60%-80% of the cases. Although more than 90% of adults have serologic evidence of having been infected with HSV-1 by the fifth decade of life, this simplex virus serves as the etiologic agent in GH disease much less frequently. Nevertheless, HSV-1 is now being reported as an increasingly common cause of primary GH infection, particularly among men who have sex with men (MSM), as well as in younger individuals.4 Herpes simplex virus 2 has a cumulative lifetime incidence, with a prevalence ranging from about 20% in white men to nearly 80% in black women. Currently, approximately 45 million U.S. adults are infected with HSV-2.5

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residents are estimated to be seropositive for antibodies against HSV-2, though less than 10% actually report a history of GH disease. Because almost all HSV-2 infected individuals shed virus genitally (even those with no prior history of genital lesions), this asymptomatic seropositive population serves as a potential major source of infection for their previously unexposed sexual partners.

Not surprisingly, HSV-2 is 1 of the most common infections among those persons infected with the human immunodeficiency virus (HIV). A shared route of sexual transmission is likely the cause for HSV-2 coinfection in 50%-90% of HIV-positive individuals. Interactions between HIV and HSV in the coinfected person may have several potential effects that tend to alter the natural history of HSV-2 infection. Coinfection with HIV facilitates the acquisition and transmission of HSV due to the fact that the frequency, severity, and duration of clinical reactivation of HSV-2 is increased by HIV infection. In addition, in the presence of HIV infection, individuals coinfected with HSV-2 experience more frequent episodes of mucosal shedding, most of which is subclinical. Moreover, the dually infected individual experiences more frequent clinical and subclinical reactivation and therefore, a subsequent increase in asymptomatic viral shedding. Furthermore, the immunosuppression resulting from HIV disease leads to more severe and prolonged symptomatic GH episodes with a greater propensity for dissemination when HSV outbreaks do occur. Accumulating data suggests that a significant biological interaction occurring between these 2 viruses results in an augmented rate of HIV replication during both clinical and subclinical HSV reactivation, allowing for more efficient sexual transmission of HIV to an uninfected partner. Presumably, this heightened infectiousness is a consequence of elevated HIV RNA levels that have been observed in the plasma and genital tract of HIV-infected persons associated with HSV coinfection. The mechanistic explanation for this phenomenon is thought to involve HSV proteins amplifying HIV expression through post-transcriptional stabilization of HIV RNAs. Moreover, HSV stimulates HIV replication through cytokines released from HSV-infected cells.

Above having an increased frequency of HSV-2 recurrences, those coinfected with HIV are more likely to have atypical GH presentations that at times are not easily recognized. These individuals often have persistent, extensive, deeply ulcerated, and necrotic lesions with increased severity and duration and can be particularly pronounced in the background of low CD4 T-cell lymphocyte counts. Consequently, the diagnosis and prescribing of appropriate anti-herpes therapy for symptomatic genital HSV disease may be delayed in the context of HIV infection. To complicate matters even further, resistance to anti-herpes medications, although uncommon, is influenced by the presence and degree of immunosuppression. What is more, the greater size and extent of the lesions in HIV/AIDS patients may help to facilitate the development of resistant HSV. This mucosal persistence of the virus in this population likely plays an important role in this development.

Recently, we treated a patient who presented with a complex ulcerative genital condition. During his hospitalization, he was discovered to have AIDS. A diagnosis of GH disease confirmed to involve HSV-1 was delayed owing to his atypical presentation void of the classic vesicles on erythematous bases. His lack of clinical response to treatment with acyclovir prompted laboratory anti-viral resistance testing identifying a high-level resistance to this nucleoside analogue, thereby necessitating an alternative treatment option. Hence, we discuss the clinical and therapeutic issues for HSV and HIV coinfection emphasizing the epidemiology of anti-viral resistance in HSV, explain the biologic mechanisms for this decreased susceptibility, and highlight the laboratory’s role in detecting this phenomenon. Finally, we review the available treatment alternatives.

Case Report

A 48-year-old man of Haitian descent presented to the hospital complaining of non-healing soft-tissue lesions in the perineal, scrotal, and penile areas of 1 year’s duration. He previously was prescribed oral antibiotics for a presumed soft-tissue infection without clear benefit. He related an unremarkable review of systems and denied any prior significant medical or surgical maladies. He was unaware of any exposure to STDs and stated that although prolonged, this was the first of such genital lesions. His physical examination was significant only for extensive, deep ulcerations with erythematous bases partially covered with exudate, located alongside his scrotum with extension to the perineum, perianal, and penile areas (Image 1A and Image 1B). There was an absence of bullous formation, and no palpable lymph nodes were appreciated.

The differential diagnosis encompassed genital ulcerative conditions including the following: HSV, chancroid, syphilis, lymphogranuloma venereum, and granuloma inguinale. Pyoderma gangrenosum as well as neoplastic entities were also given consideration. He was empirically treated with oral acyclovir and levofloxacin pending serologic studies.

The rapid plasma reagin (RPR) assay was negative. Antibodies immunoglobulin G (IgG) directed against HSV-1 but not HSV-2 were detectable. An HIV enzyme-linked immunosorbent assay (ELISA) proved positive and was confirmed by Western Blot testing. Based on the measurement of a greatly depressed CD4 T-cell lymphocyte count of 8 cells/mcL (1%) (normal reference range, 544-1212 cells/mcL, 40.4%-57.4%), he was classified as having AIDS. The acyclovir was changed to an intravenous (IV) route of administration. Failing a clinical response, a surgical biopsy was performed with the tissue sample submitted for histologic and microbiologic analysis. Typical viral cytopathic changes were indentified on the hematoxylin and eosin (H&E) stained tissue sections (Image 2A) and confirmed with the aid of immunoperoxidase stains for HSV, which showed strongly positive nuclear immunoreaction corresponding to the intranuclear herpetic viral inclusions (Image 2B) selectively present in the subset of infected cells. Herpes simplex virus (HSV) 1 was isolated from the herpes viral cultures of the tissue and was submitted for antiviral susceptibility testing. Using the dye uptake testing method, the laboratory determined this HSV-1 to be highly resistant to acyclovir, in that the minimal inhibitory concentration (MIC) was >48 µg/mL. Consequently, the acyclovir was discontinued and substituted with IV foscarnet sodium, resulting in a prompt and daily improvement of the patient’s genital ulcers. At the end of 2 weeks of treatment with foscarnet, his genital lesions were clinically resolved and he was discharged with plans for a follow-up visit at the local county HIV medical clinic.
Discussion

This case illustrates many of the clinical issues associated with HSV and HIV coinfection. The atypical, extensive, and somewhat unusual anatomic location of his genital ulcerations, not responsive to standard HSV antiviral medication, led to the need for further diagnostic testing and an alternative treatment regimen. It could not be determined whether this was a primary (previously seronegative) or non-primary (previously seropositive) initial HSV infection episode. In general, non-primary initial HSV presentations tend to be less severe clinically and quite often are asymptomatic. Following primary HSV infection, 70%-90% of individuals will have symptomatic recurrent GH episodes, with an average of 4 events within the first year. The frequency and severity of recurrent GH is known to be even greater in the HIV coinfected person and can be attributed to the degree of immunosuppression.

Image 1. Genital infection by drug-resistant herpes simplex virus (HSV) in a HIV-infected patient. (1A) Confluent shallow ulcerations focally covered by purulent exudates. (1B) Marked penile swelling, ulceration, and inflammation without obvious vesicle formation.

Image 2. Histopathologic sampling of the patient's genital lesions. (2A) Typical HSV cytopathic changes in the squamous epithelial cells of the genital mucosa, characterized by focal multinucleation and numerous intranuclear “ground glass” viral inclusions (arrows) (H&E, original magnification ×400). (2B) Immunoperoxidase stain using antibodies against HSV, demonstrating strong nuclear reaction (brown staining) in the cells infected with the virus (avidin-biotin-peroxidase stain methodology, original magnification ×400).
Though HSV-2 is the principal etiology of GH infection, HSV-1 is becoming increasingly identified, particularly in MSM and sexually active young persons, 2 groups at greater risk for acquiring HIV. Interestingly, HSV-1 infection leads to much less reactivated GH disease and less measurable asymptomatic viral shedding in comparison with HSV-2. Both types of HSV are known to disseminate in the immune-compromised HIV patient and may also cause esophagitis, hepatitis, pneumonitis, meningoencephalitis, and retinal necrosis.

As in our case, the diagnosis of HSV infection in the HIV patient may prove difficult and, as a result, is delayed. The varied presentation often renders the clinical diagnosis nonspecific and insensitive. Isolation of HSV grown in tissue culture, with type-specific antigen detection by immunooassay or fluorescent antibody, is highest in the vesicle stage but only has a sensitivity of about 70%\(^\text{15}\) and even lower for recurrent lesions. Polymerase chain reaction (PCR) detection has a 2- to 4-fold increased sensitivity over virus culture,\(^\text{14}\) but it has not been cleared for the testing of genital specimens by the U.S. Federal Drug Administration (FDA). While the use of cytologic detection of HSV infection cytopathic changes may be valuable, it is considered to be an insensitive and non-specific method for genital lesions and should not be relied on. By employing antibodies to HSV glycoprotein G (gG-1 and gG-2 for HSV-1 and HSV-2, respectively), serological assays specific for HSV-1 or HSV-2 have been developed (HerpeSelect-1 ELISA IgG or HerpeSelect-2 ELISA IgG and HerpeSelect 1 and 2 Immunoblot IgG [Focus Diagnostics, Cypress, CA] and HSV-2 ELISA IgG [Trinity Biotech USA, Jamestown, NY]). These type-specific serologic tests have a sensitivity and specificity of >95% in testing for HSV in the HIV/HSV coinfected individual but cannot distinguish between latent and active disease.\(^\text{15}\) Newer tests (eg, HerpeSelect Express, Focus Diagnostics), referred to as point-of-care tests (POCT), can be used at clinic visits. By sampling capillary blood or serum, the identification of HSV-2 antibodies can be achieved in 15 minutes with a sensitivity and specificity of 97% and 98%, respectively.\(^\text{16}\)

Antiviral drug treatment of HSV infections, using analogues of the natural nucleoside deoxyguanosine, began almost 3 decades ago following the initial report detailing the selective activity of acyclovir (Zovirax) against herpesviruses.\(^\text{17}\) Following the approval of acyclovir in 1981, a second structurally related compound, penciclovir, was identified and marketed in the form of its pro-drug, famciclovir (Famvir). Valacyclovir (Valtrex), the valine ester of acyclovir, functions as a pro-drug and is rapidly converted to acyclovir by host acetylases in the intestinal wall and in the liver. It was developed for the purposes of improving oral bioavailability. Currently, these medications are prescribed either for symptomatic outbreaks of GH, termed episodic therapy, or as suppressive therapy to prevent or decrease the number of future outbreaks.\(^\text{18}\) Chronic, daily treatment has also been proven to significantly decrease asymptomatic viral shedding, resulting in reduced transmission of HSV to uninfected partners.\(^\text{19}\)

Having similar mechanisms of antiviral action against HSV, acyclovir and penciclovir are selectively monophosphorylated only within virus-infected cells by viral thymidine kinase (TK). Thereafter, cellular kinases process the compounds to acyclovir or penciclovir triphosphate, which are the active form of the drugs. By competing with the natural nucleotide, deoxyguanosine triphosphate (dGTP), these triphosphorylated drugs inhibit HSV replication by selective inhibition of viral DNA polymerase and by termination of the growing viral DNA chain (Figure 1). Foscarnet (trisodium phosphonoformate; Foscavir) is an inorganic pyrophosphate analogue that is inhibitory for HSV, as well as other viruses (eg, cytomegalovirus [CMV], VZV). Unlike acyclovir and penciclovir, foscarnet does not undergo significant intracellular metabolism and does not depend on viral TK for its activity. It directly inhibits herpesvirus DNA polymerase by reversibly blocking the pyrophosphate binding site of the viral polymerase in a non-competitive manner with respect to deoxynucleotide triphosphates. Although having demonstrated good clinical success for the treatment of HSV infections,\(^\text{20}\) it is considered a second-line therapy due to its required IV route of administration and related renal toxicities. Cidofovir (Vistide) is another IV agent with broad antiviral activity. This monophosphate, acyclic nucleoside analogue is a TK-independent inhibitor of HSV, which acts as a competitive inhibitor and as an alternative substrate for DNA viral polymerase. Though approved for the treatment of CMV retinitis, cidofovir has also been used successfully for difficult-to-treat mucocutaneous HSV infections.\(^\text{21}\)

Beginning with their introduction into clinical practice more than 25 years ago, resistance to the nucleoside analogues acyclovir and penciclovir has been uncommon (less than 1%) and continues to remain infrequent in immunocompetent patients.\(^\text{22}\) With rare exceptions, resistant HSV is cleared normally with no adverse clinical outcomes in those with an adequately functioning immune system. The low prevalence of drug-resistant HSV-2 strains as well as a lack of documented transmission in humans may, in part, be explained by information from animal model studies in which these strains demonstrated reduced fitness.\(^\text{6}\) However, the isolation of resistant HSV from immunocompromised individuals is encountered with greater frequency, generally reported to be between 3.6% and 10.9%.\(^\text{23}\) Moreover, infection with resistant HSV is much more likely to be clinically significant in this population.\(^\text{7}\) The incidence of antiviral resistance appears to vary according to the degree and type of immunosuppression. In 1 study, resistance rates of 4.2% and 18.4% among HSV isolates from HIV-infected persons and from allogeneic bone
marrow transplant recipients, respectively, were reported. Interestingly, all of the isolates in the latter group consisted of HSV-1 strains.

Several factors may influence the emergence and possible spread of resistant HSV and can be divided into HSV, host- and drug-related issues. Resistant HSV spontaneously arises from the natural variability of the HSV population, with resistant mutants comprising a very low percentage of a clinical HSV isolate \(10^{-4} - 10^{-5}\). Amplification of these mutants in infection sites during treatment provides the source for essentially all resistant infections. Nonetheless, in immunocompetent patients, these viral mutants rarely evolve into a significant proportion of HSV, and their presence is transient. On the contrary, cases of persistent infection by resistant HSV occur almost exclusively in immunocompromised persons, and serial recurrence of lesions shedding acyclovir-resistant HSV has been reported. Loss of HSV-specific immune function allows for replication of wild-type and less fit resistant mutants and, in the face of antiviral therapy, will promote the selection of naturally occurring mutations.

Therefore, in the absence of antiviral therapy or when the medication is completely effective, selection for resistant virus does not occur. Intuitively, it would seem obvious that isolation of resistant HSV would correlate with drug use. However, little published evidence demonstrating a clear relationship or correlation between isolation of acyclovir-resistant virus and antiviral therapy exists. Nonetheless, in 1 study, acyclovir resistance was found to be significantly associated with previous episodes of recurrent GH and previous oral acyclovir use in the past year, suggesting that most resistance develops because of the selective pressure created by acyclovir use rather than acquisition of resistant virus from infected sex partners. Curiously, in that same study, a strong association of acyclovir resistance with use of topical acyclovir was also observed.

In 95% of cases, resistance to acyclovir and penciclovir is mediated via mutations in the TK gene leading to a loss of TK activity (the viral-encoded enzyme required to initiate the phosphorylation steps and thus activate the drugs) or due to an alteration of substrate specificity. Three distinct classes of acyclovir-resistant TK mutants have been identified: TK-negative (TK\(^{-}\)), which lacks activity; TK-partial (TK\(^{\ast}\)), which expresses reduced levels of TK activity; and TK-altered (TK\(^{\ast\ast}\)), also referred to as substrate specificity mutants, as it can phosphorylate thymidine but not acyclovir and/or penciclovir. The vast majority of TK mutants are of the TK\(^{-}\) or TK\(^{\ast}\) varieties. In 5% of resistant cases, absence of drug activity is conferred by mutations leading to alteration in the DNA polymerase.

In vitro tests to detect resistance or evaluate HSV susceptibility to antiviral drugs are based on the determination of viral replication inhibition in the presence of increasing concentrations of antiviral drugs. The phenotypic methods used most often include: plaque-reduction, dye-uptake, and viral DNA-inhibition assays. These 3 techniques allow the determination of the antiviral drug concentration leading to viral replication inhibition by 50% (inhibition concentration 50% or IC50). Consequently, all require previous isolation of viral strains in cell cultures and, therefore, often are not available for 7-10 days. An IC50 of >2 µg/mL is generally used as a break point for in vitro assays. The plaque-reduction assay using viral isolates is the most commonly used test of antiviral susceptibility because results most closely correlate with clinical response. However, the latter 2 techniques are less time consuming, as the reading of cytopathic effect is automatable. A rapid assay using a genetically engineered CV19 cell line, which expresses β-galactosidase only after infection with HSV, has been developed. To screen for resistant HSV, viruses are incubated with and without 2 µg/mL of acyclovir and thereafter are subjected to titration. Plaques are stained histochemically for β-galactosidase 2 days later. Genotypic tests, employing amplification by PCR performed directly on biological samples, have been developed, but they pose difficulties since numerous nucleotide substitutions may be found, and these must then be identified as mutations responsible or not for resistance. It is for this reason genotypic testing is not currently available for clinical use.

**Conclusion**

As in our case, unsuccessful treatment of HSV, indicating the possibility of resistant virus, should be suspected when lesions fail to improve after 4-5 days of anti viral therapy and prompt the submission of isolates from the lesion to the laboratory for susceptibility testing. Increasing the dose of acyclovir or changing its administration to an IV formulation would not be anticipated to be beneficial because most resistance results from the TK\(^{-}\) mutation, which lacks the enzymatic activity necessary to monophosphorylate the drug. In a similar fashion, substituting another nucleoside analogue drug (ie, penciclovir), which requires activation by TK, is not recommended, as cross-resistance is most likely present. Foscarnet becomes the alternative treatment of choice since it exerts its antiviral effect by directly acting on the HSV DNA polymerase without the need for activation by any viral or host kinase. Cidofovir is reserved for the rare case not responding to foscarnet.

Fortunately, though a theoretical possibility, there has been no unequivocal evidence of transmission of an acyclovir-resistant HSV strain from person to person. In addition, although cases of recurrent acyclovir-resistant HSV infection due to mucosal persistence of the virus have been reported, subsequent recurrences most often are due to acyclovir-susceptible isolates. Once the infection episode with a laboratory-documented drug-resistant HSV strain resolves, on recurrence, usually the acyclovir-sensitive wild-type virus reactivates.

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