Journal of Antimicrobial Chemotherapy (2000) **45**, *Topic T3*, 1–8

JAC

Herpes simplex virus: the importance of asymptomatic shedding

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Herpes simplex virus (HSV) is frequently shed after infection of the genital or perianal area. HSV shedding, as determined by culture, occurs on about 3% of days for immunocompetent women and men, and more for persons with HIV infection or if measured by polymerase chain reaction (PCR). Most horizontal and vertical transmission of HSV occurs during unrecognized or asymptomatic shedding, and the majority of HSV-2-infected persons are unaware of their infection. Many persons with 'asymptomatic' HSV-2 infection can learn to recognize genital signs and symptoms as recurrences of HSV-2 infection. However, some shedding episodes remain truly asymptomatic even after patient education. Antiviral therapy dramatically reduces asymptomatic shedding, and trials to evaluate its effect on HSV transmission are underway.

Introduction

The prevalence of genital herpes simplex virus (HSV) infection is increasing despite the availability of drugs that inhibit its replication.[1] New strategies for reducing genital HSV transmission and infection are needed.[2] This article reviews recent studies that define the characteristics and predictors of asymptomatic shedding, its role in HSV transmission and the effect of antiviral therapy on asymptomatic shedding.

Pathogenesis of HSV infection

HSV is labile in the environment and is acquired through direct contact with infected tissue or secretions. After binding to and entering cells at the inoculation site through specific receptors,[3,4] HSV replicates with a life cycle of the order of 1 day and a burst size of at least 100–1000 daughter virions per infected cell.[5] During primary infection, progeny viruses infect surrounding cells and traverse the neuroepithelial gap into the distal endings of sensory and autonomic axons. Viral proteins are probably also carried to regional lymphoid aggregates by mobile dendritic-lineage cells, where primary immune responses are initiated.

After HSV has ascended to nerve cell bodies in the relevant ganglia, it is believed to be transmitted to neighbouring neurons and to higher-order neurons in the spinal cord. These events have several consequences: (i) based on animal models, the virus may descend acutely the axons of neighbouring neurons back to the periphery, resulting in the clinical primary infection syndrome;[6] this phenomenon may account for the multiple crops and wide anatomical distribution of lesions during primary genital HSV infection in humans;[7] (ii) ganglionic spread may give rise to an increased number of latently infected neurons—in animal models, the number of such neurons appears to be directly related to the rate of subsequent reactivation;[8] (iii) spread to the CNS may cause meningeal signs and symptoms.[9]

Local peripheral persistence of HSV is detectable in some animal models and in the human cornea, but there is no evidence that the virus persists in the genital epithelium between episodes of shedding.[10]

Asymptomatic and symptomatic recurrences of HSV infection are believed to start in latently infected neurons. During latency, specific and limited viral RNA expression occurs, and very few or no viral proteins are made.[5] During reactivation, the HSV genome switches to a lytic pattern of gene expression, resulting in the delivery of infectious virions from axons to epithelial cells.[11] It is believed that neurons are not killed in the process of HSV reactivation.

Stimuli precipitating HSV reactivation and clinical lesions include both 'ganglionic' triggers, such as nerve section, surgery or fever,[12] and peripheral or 'skin' triggers, such as ultraviolet light or trauma.[13,14] Social or emotional stress is commonly cited by patients as a cause of recurrent HSV episodes, but prospective documentation suggests that stress may often be the effect, rather than the cause, of recurrences.[15]

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The 'skin trigger' theory of recurrent infection states that the presence of the proper local milieu permitting viral replication controls the formation of lesions to a greater extent than does neuronal reactivation and virus delivery to the periphery.[13,16] Irrespective of how peripheral recurrent infection is initiated, HSV then replicates and may potentially be transmitted. After symptomatic recurrent disease, local innate and acquired host immune responses terminate replication.[17] Little, if anything, is known with regard to the local or systemic immune correlates of asymptomatic shedding of HSV.

Clinical definitions

Asymptomatic shedding is generally defined as the detection of HSV in the absence of genital lesions. Symptoms may be present, but they are not recognized by the patient or clinician as related to herpes. Subclinical reactivation and subclinical shedding are synonymous with asymptomatic shedding,[9,18] the former emphasizing the concepts of ganglionic latency and a discrete neuronal reactivation event. In unrecognized genital herpes, lesions are temporally and spatially correlated with detection of HSV, but although they are within the spectrum of known HSV syndromes,[9] they are not recognized as such by the patient and clinician.

The relationship between unrecognized, symptomatic and asymptomatic infection

Underdiagnosis of genital herpes

Most HSV-2-seropositive persons deny a history of genital herpes.[9] Knowledge of the signs and symptoms of genital herpes is variable, among both clinicians and patients. Clinicians may diagnose correctly a greater proportion of people with genital herpes by having a low threshold for performing culture or other virus detection tests for genital or perianal symptoms, or from subtle changes noted on physical examination. Adjuncts to physical examination may affect the symptomatic status of an HSV shedding event. For example, colposcopy may reveal tiny cervical ulcerations in culture-positive asymptomatic women, which would not be visible during routine examination.[19] Overall, performance of type-specific serology for HSV-2 infection is the most cost-effective way of documenting genital herpes, recognizing that in some geographical areas, up to 50% of genital herpes is due to HSV-1.[9]

Patient education

To address the role of patient education in the recognition of recurrent genital herpes infection, three studies have investigated the frequency of symptomatic, but unrecognized, genital herpes among HSV-2-seropositive persons without a documented history of genital herpes. Langenberg *et al.*[20] studied 62 HSV-2-seropositive women who denied a history of genital herpes. The subjects received a description of typical genital HSV signs and symptoms, a review of transmission and reactivation, and viewed pictures of HSV lesions. Forty-eight (77%) of the women subsequently presented with recurrent HSV lesions. Subjects in a second study[21] of HSV-2-seropositive women without a history of genital herpes were recruited predominantly from a family medicine clinic. Subjects received an educational counselling session, including teaching illustrations of typical and atypical lesions, performed daily home cultures and kept a symptom diary. Overall, 61% of the subjects reported a clinical history consistent with genital HSV infection, and 74% had documented viral shedding. Similar findings were reported by Frenkel *et al.*[22] among pregnant women. Taken together, these studies show that most HSV-2-seropositive persons have signs and symptoms associated with some HSV shedding events.

Even after education, some episodes of HSV shedding remain truly asymptomatic. This was documented, for example, in a study performed by Wald *et al.*[18] Subjects with a history of recurrent genital herpes received intensive education regarding the signs and symptoms of genital herpes. Most cultures were performed at home, but some were performed in the clinic, where an experienced research clinician took a detailed history and performed a complete examination. Several episodes of asymptomatic shedding were detected at study visits. It is important to recognize that essentially all HSV-2-infected people periodically shed virus. Individuals can be categorized as: (i) having symptomatic shedding events that they recognize as herpetic (a minority); (ii) having unrecognized symptomatic disease (a majority); or (iii) having neither class of symptomatic disease (probably a small minority). Most persons with unrecognized disease can be 'converted' to symptomatic disease after education about lesions and symptoms. Importantly, individuals in all three groups have periods of true asymptomatic shedding events, and the data available suggest that the frequency of asymptomatic shedding is similar between these groups.[23] Patient counselling regarding therapy should, therefore, be similar for most HSV-2-infected persons, regardless of symptoms.

The role of asymptomatic shedding in the transmission of HSV infection

In prospective studies of partners discordant for HSV-2 infection, most transmission events were not associated with a clinically recognized HSV recurrence (or prodrome) in the source partner.[23] Earlier cross-sectional studies produced similar results.[24] Many anecdotes address this scenario, including acquisition of genital herpes from partners unaware of their infection status.[25] Even when advice is given to abstain from sex during recurrences and when barrier methods of contraception are used, horizontal trans-

mission may still occur, although compliance with such advice has not been carefully monitored.[23] Most vertical transmission of HSV to the neonate also occurs during asymptomatic shedding. This is rare and most often occurs when genital herpes is newly acquired by the mother in late pregnancy. Transmission may occur before primary infection becomes symptomatic; often, the primary infection in the mother is asymptomatic.[26,27]

Rates of asymptomatic shedding of HSV

Our research group and others have undertaken detailed studies of the rates, anatomical sites and predictors of asymptomatic shedding and its role in the transmission of HSV. Prospective studies have used both intermittent and daily sampling, more recently at multiple anatomical sites and using both culture and highly sensitive quantitative PCR to detect HSV.

Almost all women with symptomatic recurrent genital HSV-2 infection also experience asymptomatic shedding of the virus. When PCR and culture were compared in 20 women studied daily at multiple anatomical sites, shedding was detected in 19/20 women (95%) by PCR compared with 15/20 (75%) by culture.[28] The detection of asymptomatic shedding is dependent upon the system used, the number of anatomical sites and the duration of sampling.

Duration of sampling

Among women who had cultures of the genital and perianal areas performed for >100 days, 100% had at least 1 day of asymptomatic shedding, while only 5.6% of women demonstrated asymptomatic shedding when studied for <25 days.[29]

Anatomical site

Asymptomatic HSV-2 shedding among women with symptomatic recurrent disease from whom cervical/vaginal, vulvar and perianal/rectal specimens are analysed by culture, has been shown to occur during 2–8% of days in several studies.[18,28,30,31] This range is a reasonable estimate to use in counselling immunocompetent subjects with established, symptomatic disease. Larger studies have shown that asymptomatic shedding occurs more often from the perianal area than from the vulva or cervix,[31] although some studies have documented roughly equal rates from each area.[18] Asymptomatic shedding is typically of shorter duration than shedding associated with lesions. Three-quarters of asymptomatic shedding events lasted for only a single day, compared with 57% of symptomatic shedding events in a daily culture study.[31]

Method of detection

PCR is generally a more sensitive tool for HSV detection than culture. Substitution of PCR for culture in shedding studies results in similar findings and trends in asymptomatic shedding, although the absolute magnitude of shedding tends to be higher. In a study that used daily specimen collection, genital or perianal shedding was detected on 27.9% of days (range, 0–77.3% of days) among 20 women with recent onset, symptomatic genital HSV-2 infection. Specimens for the detection of HSV by PCR can be divided into culture-positive and culture-negative specimens. Among the total group of specimens collected on days when genital lesions were and were not detectable, the culture-positive specimens had approximately 100-fold higher mean levels of HSV DNA than did culture-negative specimens. However, some specimens that were highly positive by PCR were negative by culture, and some specimens with very low amounts of HSV DNA (10–100 copies) were positive by culture. Considering the days showing positive HSV cultures only, it is interesting to note that the amount of HSV DNA recovered was approximately the same regardless of the presence of lesions.[28] The presence of culturable virus and relatively high amounts (*c.* 10^4 copies) of HSV DNA in the absence of lesions during asymptomatic shedding is consistent with the possibility that the host inflammatory response may contribute to lesion formation during symptomatic HSV disease.

Impact of patient education

As mentioned above, most women with HSV-2 infection are not aware of their infection, although some can be educated to recognize recurrent symptomatic infection. Daily cultures from 30 women unaware of their HSV-2 infection showed an overall HSV-2 shedding rate of 3.1% and an asymptomatic shedding rate of approximately 2.1% of days, with the majority having had at least one asymptomatic shedding event. While symptomatic shedding rates were lower in this group of women than in women with clinically diagnosed recurrent genital HSV-2 infection, even after intensive education, there was little overall difference in asymptomatic shedding rates between the groups.[21]

HSV-2 seroconversion

Primary genital HSV-2 infection is typically more severe and more prolonged than recurrent infection.[9] However, primary genital HSV-2 infection can also be asymptomatic or unrecognized. Cross-sectional studies found that only 9–25% of HSV-2-seropositive persons had any history of genital herpes, either as a single, initial episode or as recurrent disease.[1,9,32] Prospective studies have documented asymptomatic seroconversion to HSV-2 even in extensively counselled patients.[23,33,34]

To address the frequency of symptoms associated with HSV seroconversion, a large group of HSV-2-seronegative patients was recently studied prospectively for >1 year. Subjects were educated regarding typical signs and symptoms of genital HSV infection at the start of the study, and

asked to report to the clinic if any such events occurred. HSV infection was documented by culture and repeat serology at the end of the study period. Overall, 37% of the HSV-2 acquisition events were symptomatic. Men showed more asymptomatic seroconversion than did women, while women were at a higher overall risk for HSV-2 acquisition. Most of the people with symptomatic seroconversion reported to the clinic at the time of their initial infection. While approximately 75% had typical lesions, some did not have genital lesions and had genital pain or urethral discharge only. Other syndromes included aseptic meningitis and fever with radicular pain. Cases of asymptomatic seroconversion followed by typical symptomatic recurrent disease were also observed.[33]

Predictors of asymptomatic shedding in immunocompetent women

Virus type

The lower symptomatic recurrence rate for anogenital HSV-1 infection in comparison with HSV-2 is also applicable to asymptomatic shedding,[35] reflecting a type-specific difference in pathogenesis. The overall rate of cervical or external genital shedding is 1.2% for HSV-1 and 4.3% for HSV-2 during the first year after primary infection.[30] In patients with established recurrent disease (median 462 days since the first clinical episode), the rate of shedding for HSV-1 was 0.7%, compared with 2.0% for HSV-2.[31]

Duration of infection

The rate of recurrence and the rate of shedding of HSV-2 from genital and perianal areas both decrease over time.[36] It is not known if this is related to the acquisition of immunological control or to a neuronal factor. Among 227 women with primary genital HSV-2 infection, the rate of shedding from the cervix or vulva decreased from 3.1% of days during the first year, to 2.3% during the second year and 2.1% during the third year ($P < 0.005$).[30] In a multivariate analysis of predictors of asymptomatic shedding, women studied <1 year after the acquisition of genital herpes had an adjusted odds ratio of 1.9 (95% confidence interval, 1.1–3.1) for asymptomatic shedding compared with women who had had genital herpes for >1 year.[31] However, asymptomatic shedding persists even in patients with long-standing disease.[28]

Frequency of recurrences

In a multivariate analysis,[31] the rate of asymptomatic shedding of HSV from the anogenital area was also shown to be related to the frequency of recurrences of genital herpes. The relationship persisted regardless of whether recurrences were symptomatic or culture-confirmed and was most dramatic for women with >12 recurrences per year. The host or virus factors that lead to an increased total burden of HSV shedding in apparently immunocompetent persons are unknown.

Asymptomatic shedding in immunocompetent men

Anecdotal case reports have recently been supplemented with prospective observational studies.[25] In homosexual men, the most common site of shedding was the perianal area followed by the penile skin and, rarely, the urethra and semen.[9] In heterosexual men, the penile skin was the most common site of asymptomatic shedding. The subclinical shedding rate for men with a history of genital herpes was 2.0% of days using daily home cultures. As in women, lesions and symptoms were absent on one-third of the days with positive cultures. In a small group of HSV-2-seropositive men without a history of genital herpes, subclinical shedding was detected on 3.9% of days.[37] Similar results were noted in a smaller study of HIV-negative homosexual men.[38] HSV infection of men and women appears to be similar with regard to the overall rate of asymptomatic shedding and the presence of shedding in persons without a clinical history of genital herpes.

Host immune status and asymptomatic shedding

The viral or host factors controlling the development of lesions and symptoms when HSV reactivates are unknown. Immunity to HSV-1 does not confer protection against asymptomatic shedding of HSV-2. Two studies of women have failed to show an association between HSV-1 serostatus and the rate of asymptomatic recurrent anogenital shedding of HSV-2.[30,31] Recent data showing that previous HSV-1 infection does not prevent HSV-2 acquisition has led to a reassessment of the overall concept of protective type-common immunity to HSV.[26,34] In a large prospective study, the presence of previous HSV-1 infection did not protect against HSV-2 seroconversion. An immunological influence on the recognition of disease during initial HSV-2 infection could be discerned, as asymptomatic seroconversion to HSV-2 was more common in people with pre-existing HSV-1 infection.[33]

Men infected with HIV-1 form the largest group of immunodeficient subjects studied to date. The overall rates of asymptomatic shedding of HSV-2 from the anogenital tract in HIV-infected men in two recent daily home culture studies were 7.3%[39] and 5.1%.[40] Approximately two-thirds of overall shedding was asymptomatic, and both total and asymptomatic shedding in this group was higher than in a group of HIV-negative homosexual men who were studied in parallel. The most common area of shedding was the perianal area, where some proportion of shedding may be

unrecognized rather than being truly asymptomatic. However, the majority of shedding from penile skin was also asymptomatic. In a multivariate analysis, there was more asymptomatic and total anogenital HSV shedding in subjects with a $CD4^+$ count of <200 cells/μL of blood than in those with >500 $CD4^+$ cells/μL.[39]

Data from women are limited to a cross-sectional study, although a prospective daily home sample study is nearing completion. Both total and asymptomatic shedding of HSV-2 from the cervicovaginal area was higher in women with HIV-1 and HSV-2 infection than in demographically similar women without HIV-1 infection. Most HSV-2 shedding in HIV/HSV-2-co-infected people was asymptomatic, and total and asymptomatic shedding was greater in those with low peripheral $CD4^+$ T-cell counts.[41]

Acquired cellular immune responses are best correlated with the severity of recurrent HSV infection.[42] The number of circulating precursors of HSV-specific cytotoxic $CD8^+$ T cells was correlated with the severity of symptomatic HSV-2 shedding in a cross-sectional study,[43] but the immune changes associated with increased asymptomatic shedding are unknown. No data are available about the effect of highly active antiretroviral therapy (HAART) and possible immune reconstitution on HSV-specific immune responses, HSV shedding or the need for continuation of suppressive therapy for HSV during effective HAART.

Asymptomatic shedding of HSV from the oropharynx and its role in genital HSV disease

The proportion of newly acquired genital HSV infections caused by HSV-1 is increasing and is >50% in some parts of the world.[9] As mentioned previously, the total and asymptomatic shedding rates for HSV-1 from the genital tract of women appear to be lower than those for HSV-2. The rate of asymptomatic shedding of HSV-1 from the orolabial area may be of relevance in transmission to the anogenital region during oral sex. While oral–genital transmission of HSV-1 has been documented[44] (small studies suggest an asymptomatic oral shedding rate of up to 7–10%),[45,46] few large studies have addressed asymptomatic shedding of HSV from the mouth. For example, most HSV-1-infected individuals are asymptomatic, and little is known about the potential for shedding of infectious virus by such people.

Effect of antiviral therapy on asymptomatic shedding

Antiviral therapy is known to reduce epithelial HSV replication and the symptoms of initial and recurrent genital HSV infection. Clinical trials have not shown an association between antiviral treatment of primary genital infection and symptomatic recurrence rates.[47] As mentioned previously, the number of latently infected neurons in the guinea-pig model of genital HSV infection may influence the frequency of reactivation.[8] However, treatment of primary infection with acyclovir did not alter the pattern of recurrent disease or overall ganglionic latency.[48] The potential effects of aggressive antiviral therapy for initial HSV on subsequent asymptomatic shedding in humans have not been studied.

The nucleoside analogues acyclovir, famciclovir and valaciclovir all suppress asymptomatic genital and perianal shedding of HSV-2. Acyclovir has been studied in a group of young, non-pregnant, predominantly Caucasian, HSV-2-only seropositive women. Women were selected who had a clinical history of genital herpes of <2 years, as the rate of asymptomatic shedding is high at this stage. Patients were randomized to receive oral acyclovir 400 mg bd or placebo for 70 days, followed by a 14 day washout period and 70 days of placebo or active drug. Measurements included daily cultures of the cervical, vulvar and perianal areas. Asymptomatic shedding accounted for nearly half of the total viral shedding noted during the trial. In an intent-to-treat analysis, asymptomatic shedding was detected on 6.9% of days for placebo and on 0.3% of days for acyclovir—a 94% reduction. Reduction of asymptomatic shedding was noted at all anatomical sites. When analysed by PCR, the shedding rates for individual women were reduced by a median of 80% (range, 34–91%). Among specimens that contained HSV DNA, treatment with acyclovir reduced the amount of DNA detected by 90%.[28] While the genital/perianal HSV asymptomatic shedding rate in HSV-2-seropositive women without a clinical history of genital herpes is similar to that in women with recognized recurrent disease (unpublished observations), antiviral therapy has not been studied separately in this population.

Studies of newer antiviral agents for the treatment of herpes are currently in progress. A crossover study using oral acyclovir 400 mg bid, oral valaciclovir 500 mg bid and placebo examined asymptomatic shedding in both men and women. Both acyclovir and valaciclovir were >90% effective at reducing asymptomatic shedding as assessed by culture, while the shedding rate by PCR decreased by approximately 80%.[49] Oral famciclovir at 125 or 250 mg tid or 250 mg bid in a small group of immunocompetent individuals reduced asymptomatic HSV shedding, compared with placebo, as assessed by culture (unpublished observations).[50,51] Famciclovir 500 mg bid reduced asymptomatic HSV shedding by 76% compared with placebo ($P < 0.02$) when assessed by culture in a group of moderately immunocompromised HIV-1-infected men before HAART was available.[40]

Transmission is of great concern to most HSV-infected people. A study is under way of the effect of oral valaciclovir 500 mg od on transmission of genital herpes when given to persons with genital HSV-2 in relationships with an HSV-2-seronegative partner. In this international, randomized, controlled study of HSV-2-discordant couples, the primary endpoint is clinical acquisition of genital

herpes in the susceptible partner. Enrolment is currently under way and the results are eagerly anticipated.

Conclusions and clinical recommendations

As noted in a recent Centers for Disease Control and Prevention recommendation for the treatment of HSV infection,[52] the development of guidelines for the management of asymptomatic shedding is a challenge, since suppressive antiviral therapy reduces, but does not eliminate, shedding, and there are, as yet, no data showing reduction of transmission with therapy. Our current approach, therefore, emphasizes counselling. Time should be committed to explaining the concept of asymptomatic shedding. It is best to discuss this at a follow-up visit, rather than at the initial diagnostic visit, once the acute symptoms and psychological distress have abated and the patient and physician are looking towards the future. An exchange of information about atypical signs and symptoms the patient may be experiencing, and the spectrum of HSV disease, may allow unrecognized recurrent disease to be diagnosed as genital herpes. Sexual abstinence during clinical recurrences is recommended given the high titre of virus present at these times.[28]

Condom use should be encouraged because of its proven efficacy for other sexually transmitted diseases. Limited observational data indicate that HSV transmission tends to be reduced when barrier methods are used,[23] but their effectiveness has not been prospectively evaluated for this infection.[9] Wide anatomical distribution of HSV shedding argues for limited protection at best. Nonoxynol-9 has anti-HSV activity in animals, but there are no human data supporting its use in the prevention of HSV transmission.[53]

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