Herpes Simplex Virus: Rapidly Cleared Reactivation Episodes, Treatment with Topical Resiquimod, and Incidence and Clinical Management of Newly Diagnosed Symptomatic Disease

Karen E. Mark

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2013

Reading Committee:

Anna Wald, Chair

Matthew R. Golden

Amalia Sophia Magaret

Program Authorized to Offer Degree:

Public Health – Epidemiology

University of Washington

Abstract

Herpes Simplex Virus: Rapidly Cleared Reactivation Episodes, Treatment with Topical Resiquimod, and Incidence and Clinical Management of Newly Diagnosed Symptomatic Disease

Karen E. Mark

Chair of the Supervisory Committee:

Professor Anna Wald

Departments of Medicine, Epidemiology, and Laboratory Medicine

Objectives: We sought to 1) characterize the frequency and duration of herpes simplex virus (HSV) oral and anogenital reactivation in both immunocompetent and HIV-infected adults, 2) determine whether topical resiquimod 0.01% gel, a toll-like receptor 7 and 8 agonist, has post-treatment efficacy in reducing time to first anogenital herpes recurrence, and 3) determine the incidence and clinical management of newly diagnosed symptomatic genital herpes in western Washington.

Methods: 1) Twenty-five HSV-2 seropositive and 18 HSV-1 seropositive healthy adults collected anogenital and oral swabs, respectively, and 20 HSV-2 seropositive, HIV seropositive adults, including 9 (45%) who were also HSV-1 seropositive, collected both anogenital and oral

swabs, 4 times a day for 60 days. Samples were positive for HSV if we detected ≥ 150 copies of HSV DNA/mL of specimen. 2) Three phase III randomized, double-blind, vehicle-controlled trials of topical resiquimod 0.01% gel to reduce anogenital herpes recurrences were conducted in healthy adults with ≥4 recurrences within the prior year. 3) Surveillance data collected in King and Pierce Counties, Washington ware used to identify persons ≥ 18 years of age with newly diagnosed symptomatic genital herpes; patients and their clinicians were interviewed.

Results: 1) Among immunocompetent and HIV-infected participants respectively, 24% and 29% of anogenital and 21% and 35% of oral reactivations lasted ≤ 6 hours, while 49% and 53% of anogenital and 39% and 59% of oral reactivations lasted ≤ 12 hours. The median maximum level of HSV DNA detected in an episode increased with episode duration for both oral and anogenital episodes in both immunocompetent and HIV-infected participants. 2) Median time to first recurrence was similar for resiquimod and vehicle treated participants in all three trials, while median time to healing of initial treated recurrence was longer for resiquimod. In two of the three trials, moderate to severe erythema and erosion/ulceration at the application site were more common in resiquimod recipients. 3) Incidence of newly diagnosed symptomatic genital herpes decreased with age and was almost 3 times higher in women than men and in Blacks than Whites. Patients reported condom use was discussed in 75% of clinical encounters, suppressive therapy in 69%, and suppressive therapy to decrease transmission in 39%; 30% reported taking suppressive therapy. Both discussion of suppressive therapy and discussion of suppressive therapy for transmission prevention were associated with suppressive therapy use ($p<0.001$ for both), as was a lesion culture or PCR positive for HSV-2 as opposed to HSV-1 ($p=0.016$).

Conclusions: 1) Rapidly cleared episodes of oral and anogenital HSV shedding occur in both immunocompetent and HIV-infected hosts, strongly suggesting that the peripheral mucosal

immune system plays a critical role in clearing HSV reactivations and illuminating how frequent anogenital mucosal immune activation caused by HSV-2 could contribute to increased risk of HIV acquisition and transmission. 2) No post-treatment efficacy of resiquimod 0.01% gel was observed, while increased application site reactions and initial recurrence healing time are consistent with resiquimod-induced cytokine effects. 3) Although clinicians usually discuss condoms and suppressive therapy with patients diagnosed with genital herpes, only a minority discuss suppressive therapy to prevent transmission and only 30% of patients take suppressive therapy, suggesting that suppressive therapy may be an underutilized tool for HSV-2 transmission prevention.

.

Table of Contents

Chapter 1: Rapidly Cleared Episodes of Herpes Simplex Virus Reactivation in Immunocompetent Adults

This is a non-final version of an article published in final form in the *Journal of Infectious Diseases* (http://jid.oxfordjournals.org).

Complete journal citation of final published article:

Mark KE, Wald A, Magaret AS, Selke S, Kuntz S, Olin L, Huang ML, Corey L. Rapidly cleared episodes of herpes simplex virus reactivation in immunocompetent adults. *J Infect Dis* 2008;198(8):1141-9.

ABSTRACT

Background: Herpes simplex virus (HSV) remains latent in nerve root ganglia of infected persons and is thought to reactivate several times yearly. Recent *in situ* data show the localization of HSV specific CD8+ T cells at the dermal epidermal junction next to peripheral sensory nerve endings, suggesting that viral reactivation may occur more frequently than previously appreciated.

Methods: Twenty-five HSV-2 seropositive and 18 HSV-1 seropositive healthy adults collected anogenital and oral swabs, respectively, 4 times a day for 60 days. Swabs were assayed for HSV using a quantitative PCR assay.

Results: Twenty-four percent of anogenital and 21% of oral reactivations lasted ≤ 6 hours, and 49% of anogenital and 39% of oral reactivations lasted ≤ 12 hours. Lesions were reported in only 3 (7%) of 44 anogenital and 1 (8%) of 13 oral reactivations lasting ≤ 12 hours. The median copy number of HSV DNA per mL at initial and last detection in an anogenital reactivation was $10^{3.5}$ and $10^{3.3}$, respectively, and $10^{3.7}$ and $10^{3.0}$ in an oral reactivation, respectively.

Conclusions: This high frequency of short subclinical HSV reactivation in immunocompetent hosts strongly suggests the peripheral mucosal immune system plays a critical role in clearing HSV reactivations.

INTRODUCTION

Seventeen percent of the United States adult population is seropositive for herpes simplex virus type 2 (HSV-2) and 58% for HSV-1, indicating chronic infection with the viruses that cause genital and oral herpes [1]. HSV acquired from sexual exposure infects and remains latent in the sacral ganglia [2], and HSV acquired non-sexually usually infects and remains latent in the trigeminal ganglia. Intermittent reactivations can be either clinical, causing typical herpetic genital or oral lesions, or subclinical, causing asymptomatic viral shedding [3]. Studies in the last decade have highlighted the importance of subclinical reactivation in the transmission of genital herpes to sexual partners and newborns [4, 5]. With daily genital mucosa sampling, HSV-2 shedding as detected by PCR is observed on 12–25% of days; ~60% of episodes are subclinical [6-8]. With daily oral mucosal sampling, HSV-1 shedding as detected by PCR is observed on 5-9% of days [9, 10]. Recent studies have raised important questions about the mechanism and frequency of HSV reactivation in humans. Mathematical modeling of daily genital shedding patterns in a cohort of women with genital herpes suggested that shedding episodes of several days duration may be caused by multiple short overlapping rather than single ganglionic HSV reactivations [11, 12]. In addition, recent immunohistologic studies have shown the persistence of HSV-2 specific T cells in genital skin contiguous to sensory neuronal nerve endings, suggesting that peripheral mucosal immune responses may help rapidly clear ganglionic HSV reactivations [13]. The above studies suggest that the frequency of mucosal HSV reactivation may be underestimated and the typical reactivation duration overestimated. We designed a study to determine if frequent short subclinical bursts of mucosal HSV reactivations occur, and, if so, to determine their frequency, duration, and pattern.

METHODS

Study Participants and Procedures

During the years 2004–2007, HSV-2 seropositive participants were asked to collect anogenital and HSV-1 seropositive participants oral swabs for HSV DNA PCR 4 times a day at home for 60 days. All participants were HIV negative healthy men and women aged ≥ 18 years. Participants were recruited by word of mouth and advertising and were enrolled if they met the above serologic criteria and could comply with the intensive study protocol. Three participants who were both HSV-1 and HSV-2 seropositive collected both anogenital and oral swabs. Participants took no antiviral medication for the study duration and collected swabs upon awakening, in the mid-morning, afternoon, and bedtime, as close as possible to every 6 hours, and recorded the exact swab time and any symptoms present in a diary. Anogenital swabs were performed by rubbing a Dacron swab across the surface of the penile and perianal areas (in that order) for men and across the posterior cervical/vaginal, vulvar, and perianal areas (in that order) for women. Oral swabs were performed by rubbing a Dacron swab across the buccal mucosa and tongue. A separate swab was collected from any lesions. These sampling and collection methods were identical to those used in our previous studies of daily sampling, except that sampling frequency was increased [6, 7, 10]. Self-collected swabs are as reliable a measure of detecting HSV reactivations as clinician-collected swabs [3]. Participants were seen in the clinic every 2 weeks for collection of samples and diary review. These studies were approved by the University of Washington Institutional Review Board and all participants gave written informed consent.

Laboratory Methods

HSV serologic testing was performed by Western blot [14]. Swabs were placed into vials containing 1 mL of PCR transport media and refrigerated until laboratory processing. HSV DNA was detected using a quantitative PCR assay and expressed as HSV DNA copies per ml of transport media [15, 16]. The initial PCR assay uses type common primers to the HSV glycoprotein B gene. Positive samples were subsequently analyzed using type specific primers to examine whether DNA detected was HSV-1, HSV-2, or both [15, 17]. An internal control was included in the PCR reaction to ensure negative samples were not due to inhibition. Samples were considered positive if we detected ≥ 3 copies of HSV DNA/20 ul of specimen (≥150 copies of HSV DNA/mL of transport media) [16]. Laboratory personnel were blinded to clinical data.

Statistical Analysis

HSV anogenital shedding was considered to occur if either an anogenital or lesion sample at a given time was positive, and oral shedding if either an oral or lesion sample at a given time was positive for HSV. If both were positive, the higher copy number of the 2 samples was used in further analyses. Shedding rates were calculated as number of swabs with HSV DNA detected divided by total number of swabs collected. A shedding episode of known duration was defined as a series of positive swabs immediately preceded and followed by at least 2 negative swabs [18]. Since not all time periods had swabs available, some subjects shed HSV for an unknown time period. These are referred to as shedding episodes of uncertain duration since they may have been longer than observed. Any shedding episode (of known or uncertain duration) could include 1 missing or 1 negative swab within the episode.

To compute episode duration, we estimated start and stop times for shedding. Where sampling was consistent, start times were estimated as the chronological midpoint between last

negative and first positive swab, and stop times as the midpoint between last positive and first negative swab. For shedding episodes of uncertain duration, we assumed missing swabs 2 time points before and 2 time points after the positive swab(s) were negative, and we estimated start and stop times as above. We compared duration and median copy number of HSV DNA detected between episodes of known and uncertain length to assess potential bias in our interpolation method.

To compare percent of time shedding if only the first morning versus all 4 daily swabs were collected, we calculated total hours of shedding in each group as above. We then divided this by total hours of follow-up, defined as time from first to last study swab collected. To calculate a reactivation rate per 30 days, we counted all episodes (certain and uncertain length) in the numerator and used follow-up time as defined above in the denominator. Generalized estimating equation models were used to test for significant associations between episode length or mean HSV copy number at onset of shedding and other factors. Median values are reported because they are more robust to outliers. The Wilcoxon rank-sum test was used to compare median shedding rates and numbers of shedding episodes of certain duration between men and women.

RESULTS

Twenty-five genital and 18 oral swabbing participants (Table 1) collected samples for a median of 61 (range 5–73) days, with 38 (88%) participants collecting samples for at least 30 days, 34 (79%) for at least 50 days, and 32 (74%) for at least 60 days. Twenty-one (84%) genital and 15 (83%) oral swabbing participants had at least one sample in which HSV was detected.

Genital Samples

Genital samples were collected for 1287 days and 4706 time periods. HSV DNA was detected on 246 (19%) days and 640 (14%) time periods. HSV typing was available for 572 of these 640 samples. An additional 63 samples were assumed to be HSV-2 because the participant was HSV-2 seropositive only, and the copy number in the remaining 5 (0.8%) samples was too low for typing. HSV-2 alone was found in 598 (93.4%) samples, both HSV-1 and HSV-2 in 25 (3.9%) samples, and HSV-1 alone in 12 (1.9%) samples.

Genital samples were collected at all 4 time periods on 962 (75%) days and at 3 of the 4 time periods on 232 (18%) days, thus 93% of days had at least 3 daily genital samples collected. We initially analyzed data from the 962 days in which all 4 daily samples were collected. Overall, HSV DNA was detected on 197 (20%) of 962 days, including 94 (9.8%) in which HSV DNA was detected during all 4 time periods and 56 (5.8%), 25 (2.6%), and 22 (2.3%) in which HSV DNA was detected during 1, 2, and 3 of the 4 time periods, respectively. The detection of HSV DNA was not influenced by time of collection (Figure 1A).

We identified 109 separate episodes of genital HSV shedding in 21 participants. Complete four times daily sampling allowed calculation of genital shedding duration for 72 (66%) episodes. The median duration of a genital HSV reactivation with complete sampling was

13 hours (range 4 hours–17 days) (Table 2) and the estimated value for the 37 episodes with less frequent sampling was 11 hours (range 2 hours–10 days) (p=0.9). Of the 72 genital shedding episodes of known duration, 35 (49%) lasted ≤ 12 hours (Figure 1B). The median maximum copy number of HSV DNA detected during an episode increased with episode duration (Figure 2A). Seven (28%) participants had at least 1 genital shedding episode which lasted ≤ 6 hours and 14 (56%) had at least 1 which lasted ≤ 18 hours. Of the 72 genital shedding episodes of known duration, 61 were HSV-2 episodes (median duration 14 hours, range 4 hours–17 days), 6 were HSV-1 episodes (median duration 6 hours, range 5–7 hours), 2 included shedding of both HSV-1 and HSV-2, and 3 were untypeable. The participant with the 17 day HSV-2 shedding episode was recently diagnosed with genital herpes (although both HSV-1 and HSV-2 seropositive) and had the highest shedding rate (48% of genital swabs collected had HSV detected) in the study. The 17 day shedding episode consisted of 7 days of pruritic prodrome followed by 5 days of vulvar lesions and 5 days of asymptomatic shedding. In addition to this 17 day episode, she had 3 asymptomatic HSV-2 shedding episodes lasting 42 hours, 48 hours, and 8 days, and one 7 hour asymptomatic genital HSV-1 shedding episode (Figure 3).

Oral Samples

Oral samples were collected for 1045 days and 3651 time periods. HSV DNA was detected on 98 (9%) days and 254 (7%) time periods. HSV-1 alone was found in 253 (99.6%) samples and HSV-2 alone in 1 (0.4%) sample.

Oral samples were collected at all 4 time periods on 691 (66%) days and at 3 of the 4 time periods on 218 (21%) days, thus 87% of days had at least 3 daily oral samples collected. We initially analyzed data from the 691 days in which all 4 daily samples were collected.

Overall, HSV DNA was detected on 80 (12%) of these 691 days. The detection of HSV DNA on oral mucosa was also not influenced by collection time (Figure 1A).

We identified 43 separate episodes of oral HSV shedding, of which 33 were of known duration. Thirty-two of these 33 episodes had HSV-1 detected. The median duration of an oral HSV reactivation episode in which four times daily sampling was obtained was 24 hours (range 4 hours–12 days) (Table 2). Oral shedding episodes of ≤ 12 hours duration occurred in 13 (39%) of 33 episodes (Figure 1C). Six (43%) participants had at least 1 oral episode which lasted ≤ 6 hours and 10 (71%) had at least 1 which lasted ≤ 18 hours. As with genital herpes, the median maximum copy number of HSV-1 DNA detected during an oral-labial episode increased with episode duration (Figure 2B). The single oral HSV-2 episode lasted 7 hours.

Associations Between Shedding and Symptoms

Representative patterns of reactivation are shown in Figure 3. Shorter genital shedding episodes were less likely to be symptomatic than longer ones. Only 3 (7%) genital shedding episodes lasting ≤ 24 hours were associated with reported genital lesions, compared with 8 (29%) genital episodes lasting > 24 hours (p=0.028). Similarly, only 5 (11%) genital episodes lasting ≤ 24 hours were associated with reported genital symptoms, compared with 9 (32%) genital episodes lasting > 24 hours (p=0.027). The median copy number (range) of HSV DNA detected was higher from swabs taken directly from lesions than from swabs obtained at the same time from sampling the entire anogenital area [$10^{5.9}$ (10^{0} – $10^{8.1}$) versus $10^{4.2}$ (10^{0} – $10^{7.3}$), respectively]. Men tended to have more genital shedding episodes lasting < 12 hours than women [median (range) 1.5 (0-7) versus 0 (0-2), p=0.06]. Only 2 oral reactivations, both in the

same person, were accompanied by lesions, both classic labial lesions. An additional two oral reactivations in another person were associated with oral tingling, but no definable ulcerations.

HSV Reactivation Rates as Measured by Four Times Daily Sampling

The median number of HSV reactivations of known duration among those who shed during the 60 day sampling period was 3 (range 1–14) genital and 3 (range 1–4) oral reactivations per person. The median genital HSV reactivation rate was 1.5 reactivations per 30 days (range 0–10.7), or 18 reactivations annually, compared with 0.5 per 30 days and 6 reactivations annually if calculated from once daily morning sampling. Similarly, the median oral HSV reactivation rate was 1.4 reactivations per 30 days (range 0–3.0), or 16.2 reactivations annually, compared with 0.9 per 30 days and 10.8 annually, if calculated from once daily morning sampling. These rates are three times higher for HSV-2 and 1.5 times higher for HSV-1 compared with studies using once daily sampling.

DISCUSSION

Our study demonstrates several new concepts about HSV infection. HSV reactivation has both a more frequent onset and rapid clearance than previously appreciated. Using 4 times daily sampling we showed that approximately half of HSV mucosal reactivations last 12 hours or less, and that these short reactivation episodes are largely asymptomatic, characterized by rapid emergence of 10^3–10^4 copies of HSV DNA in the skin or mucosa, and accompanied by rapid viral clearance by the host. Prolonged genital shedding was associated with higher initial viral copy number and greater likelihood of symptoms and lesions. Median genital HSV reactivation frequency was 18 episodes annually, 81% of genital mucosal HSV reactivations were subclinical, and half lasted < 12 hours. Only 19% of genital shedding episodes were associated with symptoms and 15% with overt genital lesions, illustrating the importance of subclinical HSV reactivation in the biology of HSV reactivation. Similarly, reported oral ulcerations accompanied only 2 of 33 oral HSV reactivations of known duration.

Our data raise several issues pertinent to HSV reactivation pathogenesis. Most samples were collected from a large surface area and placed into 1 mL of viral transport solution. Although this method of collection is consistent and reproducible, HSV reactivations are exquisitely anatomically localized [13] and, hence, the in vivo number of HSV virions released from subclinical ulcerations is undoubtedly markedly higher then the 10^3–10^4 copies we detected. This hypothesis is supported by the higher viral copy number found from swabs of identifiable lesions than samples collected at the same time from the entire anogenital area ($10^{5.9}$ versus $10^{4.2}$ copies per mL). Rapid HSV clearance within 6–12 hours of shedding appearance illustrates prompt and effective immunocompetent host defense mechanisms. Prior work has shown that HSV-2 clearance from genital lesions is associated with HSV-2 specific cytotoxic T

cell activity in the CD8+ fraction of T lymphocytes [19-21]. HSV-specific CD8+ T cells have also been found in ganglia, suggesting that some control occurs in ganglia [22-26]. However, when control at the ganglia fails and virus travels via anterograde transport to genital mucosa, the fact that mucosal replication is effectively eliminated within hours of appearance suggests that host T cells must either move extremely rapidly to the mucosal site of replication or remain in the genital mucosa between recurrences to rapidly control and eliminate HSV mucosal replication when virus first appears from peripheral nerves. Recent immunohistologic studies suggest that HSV-2 specific CD8+ T cells can persist in genital skin for extended time periods and are associated with localized clearance of subclinical HSV-2 reactivation [13]. The rapid host elimination of HSV-2 suggests that much HSV-2 control rests within the peripheral mucosal immune system.

Our study also helps explain the large body of data implicating HSV infections as an important factor in HIV acquisition. The rapid reactivation, release, and resolution of relatively high copy numbers of HSV DNA in genital mucosa may help explain the high rate of sexual transmission of HSV-2 and the increased risk that HSV-2 confers in HIV acquisition [27-29]. Frequent, short, subclinical genital mucosal reactivations place large numbers of activated CD4+ T lymphocytes at risk for HIV infection at the genital mucosa [20]. If subclinical ulcerations are also present during these reactivations, then they would provide portals of entry for HIV, further enhancing the risk due to increased numbers of CD4+ T lymphocytes at the mucosa. Our findings also put into perspective the use of episodic therapy for mucosal HSV infection. Although effective in relieving the discomfort of individual episodes [30-32], such an approach treats only a small fraction of reactivations. Daily suppressive antiviral therapy can reduce genital HSV-2 shedding as detected by once daily sampling by 70-85% of days, but reduces

HSV-2 sexual transmission by only 48% [33]. This disparity between antiviral effects on shedding and transmission may suggest that once daily antivirals do not eliminate short reactivations.

Participants included in these studies were persons well versed in the signs and symptoms of genital and oral herpes. Hence, the ratio of subclinical to clinical ulcerations among HSV-2 infected persons in the general population is probably even higher than the 85% ratio found in our study, as recognition of lesions is likely lower. Frequent sampling is both difficult for participants and results in a large number of samples for the laboratory. We collected and analyzed over 8300 separate HSV PCR samples from 43 participants. The genital shedding rate we found among our 25 HSV-2 infected participants is comparable to historical shedding rates among our previously studied HSV-2-infected patients; among 352 participants (145 men and 207 women) in previous genital HSV-2 shedding studies, 2740 of 11,838 days (23.1%) were PCR positive by once daily sampling, with a median of 57 days per person sampled. Thus data from the present study are likely representative of immunocompetent persons with oral and genital HSV infections in the studied age range. Significant individual variability in HSV shedding frequency is seen and age, gender, immune status, host genetics, and number and density of recurrences in dorsal root ganglia appear to affect the severity of HSV reactivation [10, 34-37]. Whether rapidly cleared episodes of mucosal HSV are seen in immunosuppressed patients remains to be determined. The high combined rate of HSV-1 and HSV-2 reactivation we observed among immunocompetent persons, if subsequently demonstrated among HIV-infected persons, could provide an explanation for how HSV increases HIV plasma viral load [38, 39], as most HIV-infected persons worldwide have HSV-1 and HSV-2 infections.

In summary, our data indicate the frequency and pace of mucosal HSV reactivation and clearance is much faster than previously appreciated. The most common form of HSV reactivation is an asymptomatic reactivation associated with rapid onset and clearance of virus within 12 hours, illustrating a dynamic interaction between virus and host in the peripheral skin and mucosa. These short subclinical reactivations help explain the observations that most HSV-2 transmission events occur during subclinical reactivations and that clinical disease manifestations predict neither mother to child nor sexual transmission. The frequency of these reactivations also provide a possible explanation for how incident and prevalent HSV-2 infection increase both the risk of HIV acquisition and the HIV viral load in co-infected persons.

REFERENCES

1. 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;296:964-973.

2. Croen KD, Ostrove JM, Dragovic L and Straus SE. Characterization of herpes simplex virus type 2 latency-associated transcription in human sacral ganglia and in cell culture. J Infect Dis. 1991;163:23-8.

3. Wald A, Zeh J, Selke S, Ashley RL and Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. N Engl J Med 1995;333:770-5.

4. Mertz GJ, Benedetti J, Ashley R, Selke SA and Corey L. Risk factors for the sexual transmission of genital herpes. Ann Intern Med 1992;116:197-202.

5. Brown ZA, Wald A, Morrow RA, Selke S, Zeh J and Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. JAMA 2003;289:203-209.

6. Wald A, Corey L, Cone R, Hobson A, Davis G and Zeh J. Frequent genital herpes simplex virus 2 shedding in immunocompetent women. Effect of acyclovir treatment. J. Clin. Invest. 1997;99:1092-1097.

7. Wald A, Huang ML, Carrell D, Selke S and Corey L. Polymerase chain reaction for detection of herpes simplex virus (HSV) DNA on mucosal surfaces: comparison with HSV isolation in cell culture. J Infect Dis 2003;188:1345-51.

8. Krone MR, Tabet SR, Paradise M, Wald A, Corey L and Celum CL. Herpes simplex virus shedding among human immunodeficiency virus-negative men who have sex with men: site and frequency of shedding. J Infect Dis. 1998;178:978-82.

9. Tateishi K, Toh Y, Minagawa H and Tashiro H. Detection of herpes simplex virus (HSV) in the saliva from 1,000 oral surgery outpatients by the polymerase chain reaction (PCR) and virus isolation. J Oral Pathol Med 1994;23:80-4.

10. Kim HN, Meier A, Huang ML, et al. Oral herpes simplex virus type 2 reactivation in HIV-positive and -negative men. J Infect Dis. 2006;194:420-7. Epub 2006 Jul 12.

11. Crespi CM, Cumberland WG and Blower S. A queueing model for chronic recurrent conditions under panel observation. Biometrics 2005;61:193-8.

12. Crespi CM, Cumberland WG, Wald A, Corey L and Blower S. Longitudinal study of herpes simplex virus type 2 infection using viral dynamic modeling. Sex Transm Infect 2007;2:2.

13. Zhu J, Koelle DM, Cao J, et al. Virus-specific CD8+ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. J. Exp. Med. 2007;204:595-603.

14. Ashley RL, Militoni J, Lee F, Nahmias A and Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 1988;26:662-7.

15. Jerome KR, Huang ML, Wald A, Selke S and Corey L. Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. J Clin Microbiol 2002;40:2609-11.

16. Magaret A, Wald A, Huang M, Selke S and Corey L. Optimizing PCR positivity criterion for detection of HSV DNA on skin and mucosa. J Clin Microbiol 2007;45:1618-20.

17. Corey L, Huang ML, Selke S and Wald A. Differentiation of herpes simplex virus types 1 and 2 in clinical samples by a real-time taqman PCR assay. J Med Virol 2005;76:350-5.

18. Benedetti J, Corey L and Ashley R. Recurrence rates in genital herpes after symptomatic first-episode infection. Ann Intern Med 1994;121:847-854.
19. Cunningham AL, Turner RR, Miller AC, Para MF and Merigan TC. Evolution of recurrent herpes simplex lesions. An immunohistologic study. J Clin Invest 1985;75:226-33.
20. Koelle DM, Posavad CM, Barnum GR, Johnson ML, Frank JM and Corey L. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. J Clin Invest 1998;101:1500-8.
21. Koelle DM, Liu Z, McClurkan CM, et al. Expression of cutaneous lymphocyte-associated antigen by CD8(+) T cells specific for a skin-tropic virus. J Clin Invest. 2002;110:537-48.
22. Sheridan BS, Khanna KM, Frank GM and Hendricks RL. Latent virus influences the generation and maintenance of CD8+ T cell memory. J Immunol 2006;177:8356-8364.
23. Verjans GM, Hintzen RQ, van Dun JM, et al. Selective retention of herpes simplex virus-specific T cells in latently infected human trigeminal ganglia. Proc Natl Acad Sci U S A 2007;104:3496-501.
24. Liu T, Khanna KM, Chen X, Fink DJ and Hendricks RL. CD8(+) T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons. J Exp Med 2000;191:1459-66.
25. Khanna KM, Bonneau RH, Kinchington PR and Hendricks RL. Herpes simplex virus-specific memory CD8+ T cells are selectively activated and retained in latently infected sensory ganglia. Immunity 2003;18:593-603.
26. van Lint AL, Kleinert L, Clarke SR, Stock A, Heath WR and Carbone FR. Latent infection with herpes simplex virus is associated with ongoing CD8+ T-cell stimulation by parenchymal cells within sensory ganglia. J Virol 2005;79:14843-51.

27. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. J Infect Dis 2002;185:45-52.
28. Corey L, Wald A, Celum CL and Quinn TC. The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. J Acquir Immune Defic Syndr. 2004;35:435-45.
29. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA and Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. Aids. 2006;20:73-83.
30. Leone PA, Trottier S and Miller JM. Valacyclovir for episodic treatment of genital herpes: a shorter 3-day treatment course compared with 5-day treatment. Clin Infect Dis 2002;34:958-62.
31. Wald A, Carrell D, Remington M, Kexel E, Zeh J and Corey L. Two-day regimen of acyclovir for treatment of recurrent genital herpes simplex virus type 2 infection. Clin Infect Dis 2002;34:944-8.
32. Aoki FY, Tyring S, Diaz-Mitoma F, Gross G, Gao J and Hamed K. Single-day, patient-initiated famciclovir therapy for recurrent genital herpes: a randomized, double-blind, placebo-controlled trial. Clin Infect Dis 2006;42:8-13.
33. 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;350:11-20.
34. Seppanen M, Meri S, Notkola IL, et al. Subtly impaired humoral immunity predisposes to frequently recurring genital herpes simplex virus type 2 infection and herpetic neuralgia. J Infect Dis. 2006;194:571-8. Epub 2006 Jul 24.

35. Whitley RJ, Miller RL. Immunologic approach to herpes simplex virus. Viral Immunol. 2001;14:111-8.

36. Bochud P, Magaret A, Koelle DM, Aderem A and Wald A. Polymorphisms in toll-like receptor 2 are associated with increased viral shedding and lesional rate in patients with genital HSV-2 infection. J Infect Dis 2007;196:505-9.

37. Wang K, Mahalingam G, Hoover SE, et al. Diverse herpes simplex virus type 1 thymidine kinase mutants in individual human neurons and Ganglia. J Virol 2007;81:6817-26.

38. Mole L, Ripich S, Margolis D and Holodniy M. The impact of active herpes simplex virus infection on human immunodeficiency virus load. J Infect Dis 1997;176:766-70.

39. Schacker T, Zeh J, Hu H, Shaughnessy M and Corey L. Changes in plasma human immunodeficiency virus type 1 RNA associated with herpes simplex virus reactivation and suppression. J Infect Dis 2002;186:1718-25.

Table 1. Demographic and clinical characteristics of study participants.

Baseline characteristic	Genital swabbing group n = 25[a]	Oral swabbing group n = 18[a]
Mean age (range) in years	44 (24-66)	43 (28-75)
Sex, n (%)		
Male	10 (40)	12 (67)
Female	15 (60)	6 (33)
Race/ethnicity, n (%)		
African American	3 (12)	0
Asian	0	3 (17)
Native American	1 (4)	0
White	21 (84)	12 (67)
Multiracial	0	3 (17)
History of genital herpes, n (%)	19 (76)	8 (44)
Mean (range) no. of genital recurrences in past 6 months[b]	2.3 (0-6)	1.4 (1-3)
History of oral herpes, n (%)	5 (20)	14 (78)
Mean (range) no. of oral recurrences in past 6 months[c]	0	0.75 (0-1)
HSV serostatus, n (%)		
HSV-1 and HSV-2 seropositive	13 (52)	7 (39)
HSV-1 seropositive only	0	11 (61)
HSV-2 seropositive only	12 (48)	0

[a]3 participants participated in both studies

[b]Of persons with a history of genital herpes [c]Of persons with a history of oral herpes

Table 2. Duration of HSV reactivation as defined by 4 times daily sampling, for 72 genital and 33 oral shedding episodes of known duration.

	Genital swabbing group n = 25[a]	Oral swabbing group n = 18[a]
Median duration of HSV reactivation	13 hours (range 4 hours–17 days)	24 hours (range 4 hours–12 days)
No. episodes ≤ 12 hours	35 (49%)	13 (39%)
No. episodes ≤ 6 hours	17 (24%)	7 (21%)
Median copy no. per mL at episode onset	$10^{3.5}$ (range $10^{2.2}$–$10^{7.5}$)	$10^{3.7}$ (range $10^{2.2}$–$10^{6.1}$)
Median copy no. per mL of last positive sample in an episode	$10^{3.3}$ (range $10^{2.2}$–$10^{5.8}$)	$10^{3.0}$ (range $10^{2.4}$–$10^{5.6}$)
Median copy no. per mL at episode onset for episodes > 12 hours vs. ≤ 12 hours	$10^{4.2}$ versus $10^{3.1}$ ($p<0.001$)	$10^{3.9}$ versus $10^{3.1}$ ($p = 0.14$)
Median copy no. per mL at episode onset among women versus men	$10^{4.5}$ versus $10^{3.2}$ ($p<0.0001$)	$10^{3.6}$ versus $10^{3.7}$ ($p = 0.95$)

[a]3 participants participated in both studies

Figure 1. Herpes simplex virus (A) shedding rate by time period (genital shedding in black and oral shedding in grey) and (B) genital and (C) oral shedding episode duration, of 72 genital and 33 oral episodes of known duration.

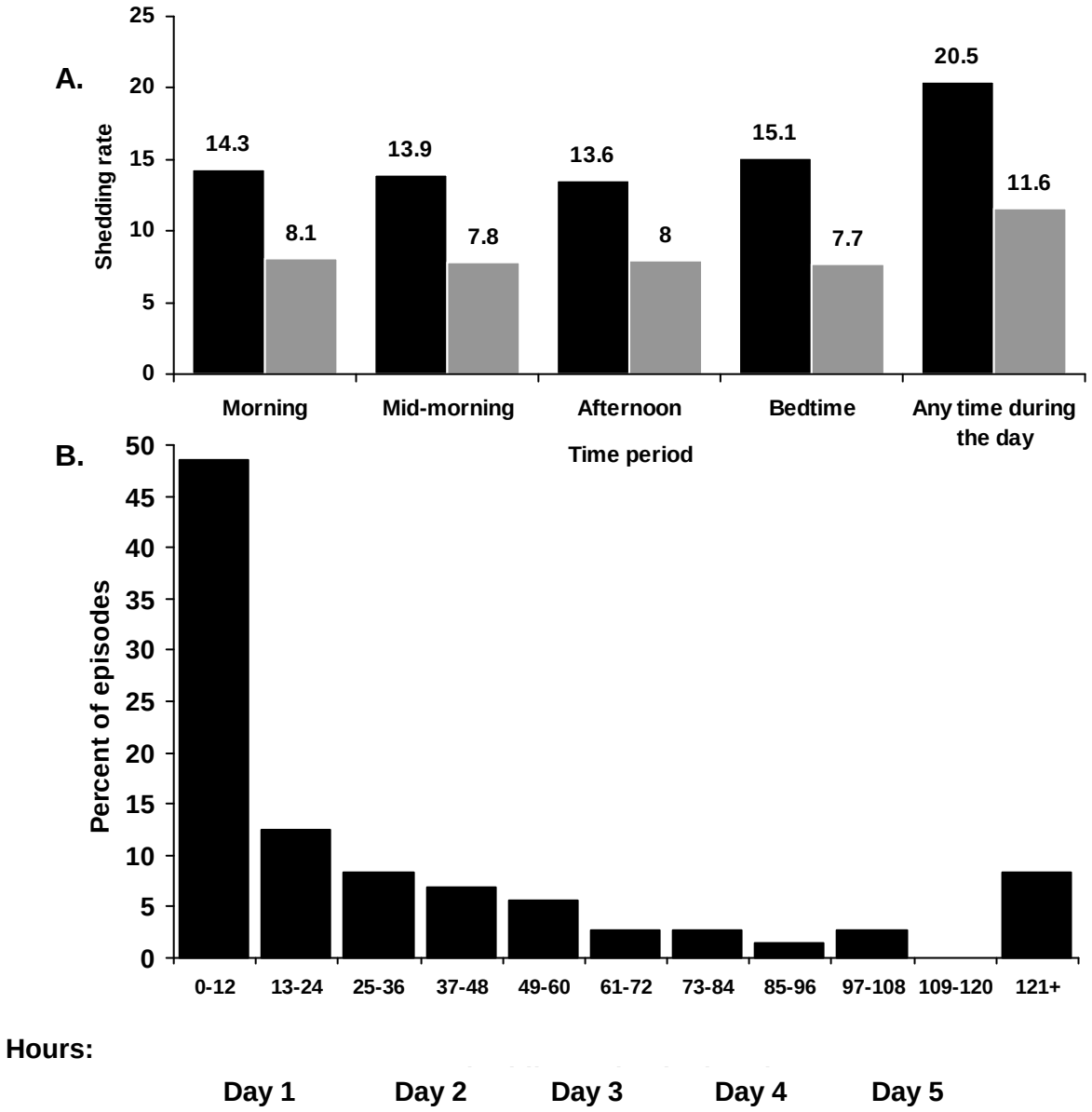
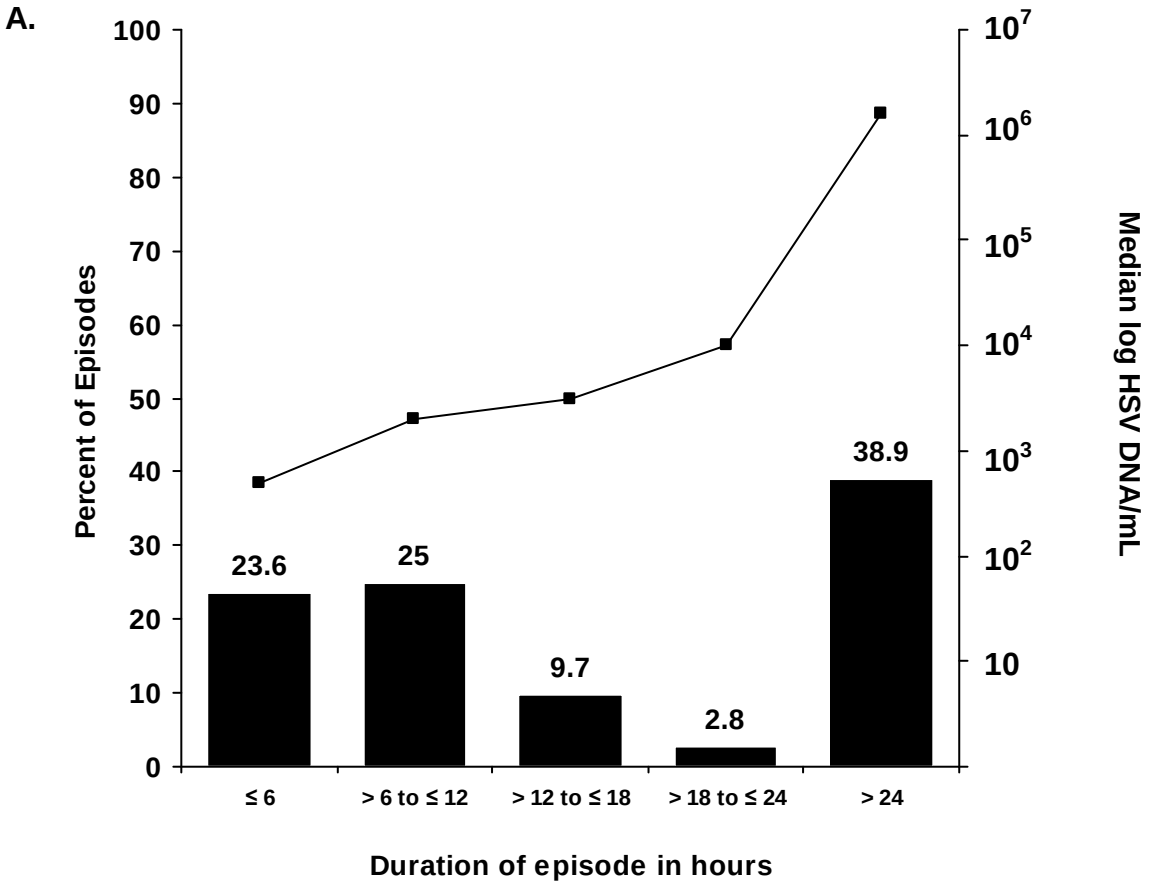

A.
Shedding rate
25
20
15
10
5
0
14.3
8.1
13.9
7.8
13.6
8
15.1
7.7
20.5
11.6
Morning
Mid-morning
Afternoon
Bedtime
Any time during the day
Time period
B.
Percent of episodes
50
45
40
35
30
25
20
15
10
5
0
0-12
13-24
25-36
37-48
49-60
61-72
73-84
85-96
97-108
109-120
121+
Hours:
Day 1
Day 2
Day 3
Day 4
Day 5
C.
Percent of episodes
50
45
40
35
30
25
20
15
10
5
0
0-12
13-24
25-36
37-48
49-60
61-72
73-84
85-96
97-108
109-120
121+
Hours:
Day 1
Day 2
Day 3
Day 4
Day 5
Shedding episode duration

Figure 2. Herpes simplex virus median log copy number by duration of (A) genital and (B) oral shedding episode, of 72 genital and 33 oral episodes of known duration.


A.
Percent of Episodes
Median log HSV DNA/mL
23.6
25
9.7
2.8
38.9
≤ 6
> 6 to ≤ 12
> 12 to ≤ 18
> 18 to ≤ 24
> 24
Duration of episode in hours

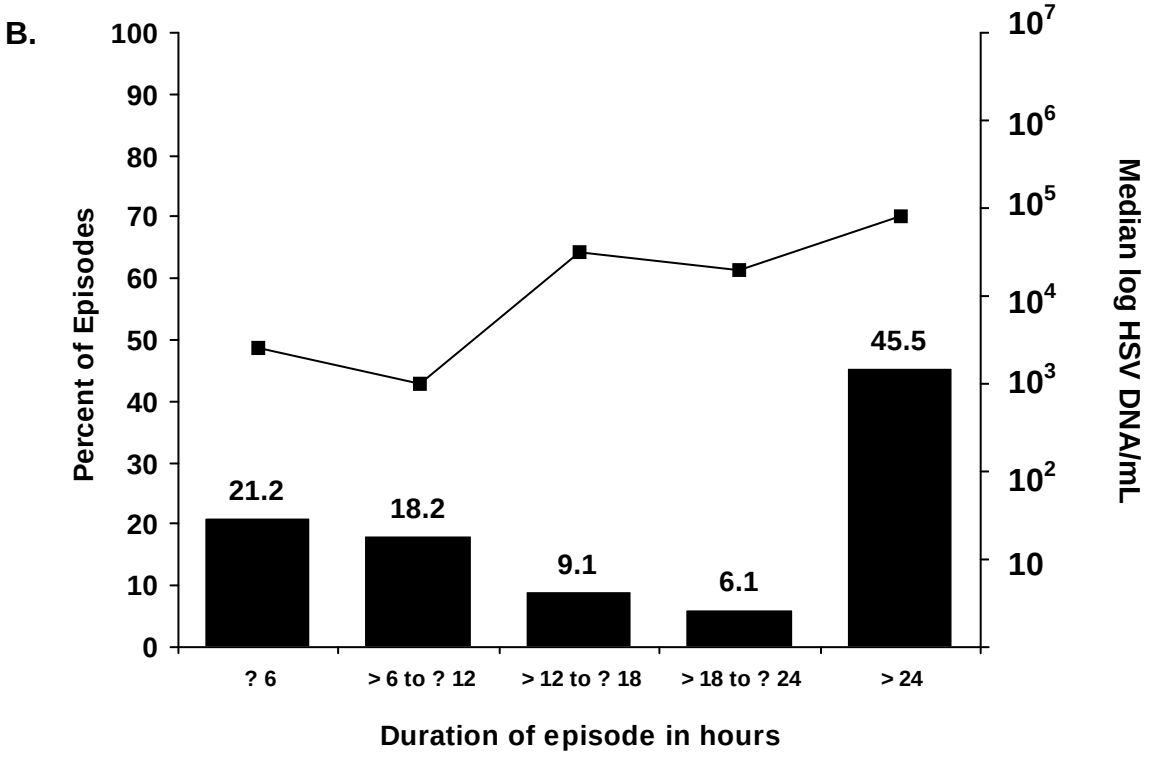
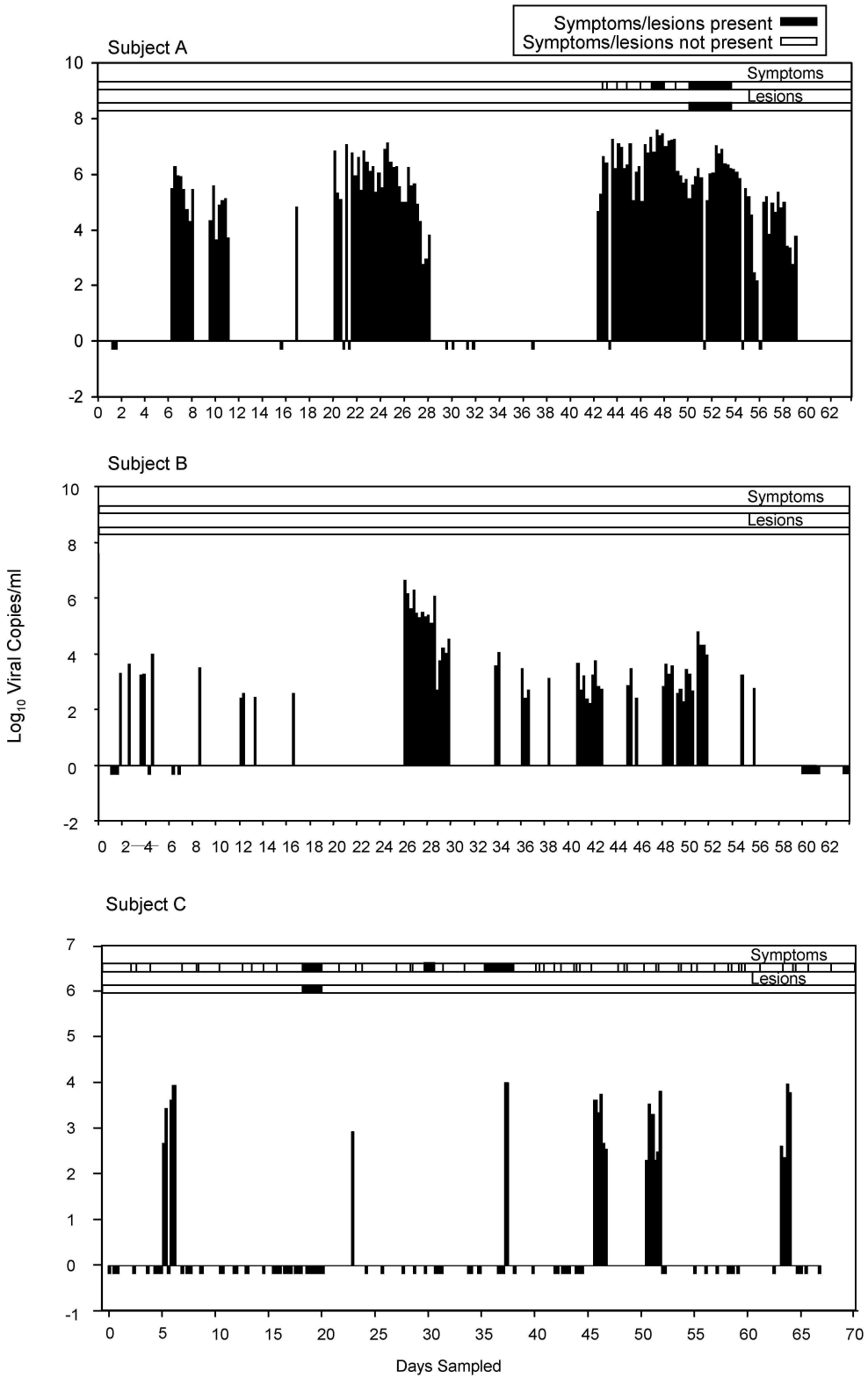

B.
Percent of Episodes
Median log HSV DNA/mL
21.2
18.2
9.1
6.1
45.5
? 6
> 6 to ? 12
> 12 to ? 18
> 18 to ? 24
> 24
Duration of episode in hours

Figure 3. Representative herpes simplex virus shedding patterns of 3 participants. Negative values indicate missing samples. Subject A is a 27 year old woman, HSV-1 and HSV-2 seropositive, with genital herpes diagnosed just prior to study entry. All pictured genital reactivations are HSV-2 except for the 7 hour shedding episode on day 16, which had HSV-1 isolated. Subject B is a 50 year old man, HSV-1 and HSV-2 seropositive, with genital herpes diagnosed 25 years prior to study entry. All pictured genital reactivation episodes are HSV-2 except 1 swab collected at 1 time period on day 41 had both HSV-1 and HSV-2 isolated. Subject C is a 35 year old man, HSV-1 seropositive, with oral herpes diagnosed 5 years prior to study entry.


Symptoms/lesions present
Symptoms/lesions not present
Subject A
Symptoms
Lesions
Subject B
Symptoms
Lesions
Subject C
Symptoms
Lesions
Log10 Viral Copies/ml
Days Sampled

Chapter 2: Rapidly Cleared Episodes of Oral and Anogenital Herpes Simplex Virus Shedding in HIV-infected Adults

This is a non-final version of an article published in final form in the *Journal of Acquired Immunodeficiency Syndromes* (http://journals.lww.com/jaids).

Complete journal citation of final published article:

Mark KE, Wald A, Magaret AS, Selke S, Kuntz S, Huang M-L, Corey L. Rapidly cleared episodes of oral and anogenital herpes simplex virus shedding in HIV-infected adults. *JAIDS* 2010;54(5):482-8.

ABSTRACT

Objective: To determine whether rapidly cleared episodes of herpes simplex virus (HSV) reactivation occur in HIV-infected adults.

Methods: Twenty HSV-2 seropositive, HIV seropositive adults, including 9 (45%) who were also HSV-1 seropositive, collected oral and anogenital swabs for HSV DNA PCR 4 times a day for 60 days. Samples were positive for HSV if we detected ≥ 150 copies of HSV DNA/mL of specimen.

Results: Median HSV shedding episode duration was 7.5 (range 4–253) hours for oral and 11 (range 4–328) hours for anogenital reactivation. Thirty-five percent of oral and 29% of anogenital reactivations lasted ≤ 6 hours, and 59% of oral and 53% of anogenital reactivations lasted ≤ 12 hours. Seven of 9 participants who shed orally and 10 of 15 who shed anogenitally had ≥ 1 reactivation lasting ≤ 6 hours. The median maximum level of HSV DNA detected in an episode increased with episode duration for both oral and anogenital episodes. Concurrent oral and anogenital shedding occurred more frequently than expected: Oral HSV shedding was detected on 17% of time points with anogenital, but 1% of time points without anogenital, shedding ($p < 0.001$).

Conclusions: Rapidly cleared episodes of oral and anogenital HSV shedding occur in HIV-infected persons, supporting the hypothesis that frequent anogenital mucosal immune activation caused by HSV-2 is present in HIV co-infected persons, potentially contributing to HIV infectiousness.

INTRODUCTION

Chronic HSV ulcers were one of the first described manifestations of AIDS,[1] and HIV-infected persons have higher clinical anogenital HSV recurrence rates,[2] higher anogenital HSV-2 shedding rates,[2, 3] and higher oral HSV shedding rates[3] than HIV uninfected persons. Higher rates of both HSV shedding and HSV clinical recurrences among HIV-infected persons compared with HIV uninfected persons are presumed to be caused by decreased host ability to control HSV replication. However, there is considerable variability in HSV-2 reactivation rates in HIV-infected, as in HIV-uninfected, persons, even among those with similar CD4 T cell counts. In general, severe persistent HSV-2 genital ulcer disease has been associated with CD4 T cell counts <50 cells/mm^3.[4] Recent data in immunocompetent adults have shown that both oral and anogenital HSV reactivations occur more frequently and are cleared more rapidly than previously appreciated, with 21% of oral and 24% of anogenital reactivations lasting ≤ 6 hours and 39% of oral and 49% of anogenital reactivations lasting ≤ 12 hours.[5] Because the frequency and duration of short HSV reactivations in HIV-infected persons might differ from immunocompetent adults, and because their presence might affect investigational HSV treatment strategies in HIV-1 co-infected persons designed to decrease HIV-1 transmission,[6] we performed an observational pilot study to examine the frequency of short episodes of oral and anogenital HSV reactivation among HIV-infected persons.

METHODS

Study participants and procedures

HSV-2 seropositive, HIV-1 seropositive participants aged ≥ 18 years were recruited in 2006–2007 from a pool of prior research study participants known to be able to comply with an intensive study protocol and asked to collect oral and anogenital swab specimens for HSV DNA PCR at home 4 times per day for 60 days. Swab specimens were collected at ~6 hour intervals: upon awakening, in the midmorning, in the afternoon, and at bedtime. Participants recorded in a diary exact swabbing times and any symptoms present, and were instructed not to take any herpes antiviral medication during the study. As previously described,[3, 5, 7, 8] anogenital swabs were obtained by rubbing a polyester fiber-tipped swab across first the penile and then the perianal skin for men, or first across the posterior cervical/vaginal, then vulvar, then perianal areas for women. Oral swabs were performed by rubbing one swab across the buccal mucosa and tongue. Separate swabs were collected from any oral or anogenital lesions noted by the participant. Participants were seen in the clinic every 3 weeks for collection of samples and diary review. Blood was drawn for both CD4 T cell count and serum HIV-1 RNA level within one month of starting the swabbing study, and antiretroviral use was obtained by history at the time of study initiation. Highly active antiretroviral therapy (HAART) use was defined as receipt of at least 3 drugs in 2 of the following categories: protease inhibitor, nucleoside analogue, or non-nucleoside analogue, at the time of study initiation. This study was approved by the University of Washington institutional review board, and all participants gave written informed consent.

Laboratory methods

HIV-1 seropositivity was confirmed by standard enzyme-linked immunoassay and Western blot. HSV serologic testing was performed by Western blot.[9] Absolute CD4 T cell counts were determined by flow cytometry. HIV-1 RNA levels were measured using real-time RT PCR technology (detection level, 30 HIV-1 RNA copies per mL). Oral, anogenital, and lesion swabs were placed into separate vials containing 1 mL of PCR transport medium and stored at -20°C until laboratory processing. HSV DNA was detected using a quantitative real-time PCR assay and expressed as copies per mL of transport medium.[10, 11] As previously described, the initial PCR assay uses type-common primers to the HSV glycoprotein B gene, with positive samples subsequently analyzed using type-specific primers to determine whether DNA detected was that of HSV-1, HSV-2, or both.[10, 12] An internal control was included in the PCR reaction to ensure that HSV-negative findings were not due to inhibition. Samples were considered positive for HSV if we detected ≥ 3 copies of HSV DNA per 20 μL of specimen (i.e., ≥ 150 copies of HSV DNA per mL of transport media).[11] Laboratory personnel were blinded to clinical data.

Statistical analysis

Analyses were done using SAS (SAS Institute Inc, Cary, North Carolina). We used identical definitions of HSV shedding, shedding rate, and shedding episode as in our previous work examining shedding in immunocompetent adults.[5] Briefly, HSV shedding was considered to have occurred if an oral or anogenital sample or the corresponding lesion sample was positive for HSV at a given time. If both the oral or anogenital sample and the corresponding lesion sample were positive for HSV, the sample with the higher HSV DNA copy number was used in further analyses. Shedding rates were defined as number of swab specimens with HSV DNA

detected divided by number of swab specimens collected. Number of days with samples collected was defined as total days from first to last day of specimen collection for both oral and anogenital samples, minus any days during which no samples were collected; expected number of days per participant was at least 60. On each day with any genital (or oral) sampling done, 4 genital (or oral) swabs were expected. A shedding episode of known duration was defined as one or a series of HSV-positive swab specimens that were collected immediately before and after at least 2 HSV-negative swab specimens. Episode start time was estimated as the chronological midpoint between the last HSV-negative and first HSV-positive swab specimen, and episode stop time as the midpoint between the last HSV-positive and first HSV-negative swab specimen.

Since not all time points had swab specimens available, some subjects shed HSV for an unknown period. These are referred to as shedding episodes of uncertain duration, because the episodes may have been longer than observed. For these episodes we assumed missing swab specimens 2 time points before and 2 time points after the positive swab specimen(s) were HSV negative and estimated start and stop times as above. Any shedding episode (of known or uncertain duration) could include 1 missing or 1 HSV-negative swab specimen within the episode.

Generalized estimating equations were used to determine whether there was a difference in average episode duration between episodes of known and uncertain duration, whether there was an association between average per-episode maximum HSV DNA copy number and episode duration, and whether concurrent oral and anogenital shedding occurs more frequently than would be expected by chance alone. Wilcoxon rank sum tests were used to test for differences in median CD4 count and HIV viral load among persons taking and not taking HAART and to examine person-level differences in shedding rates and number of reactivations by anatomic site.

Fisher's exact test was used to test for associations between episode length and anogenital symptoms and lesions. Generalized estimating equations were also used to examine associations between measures of HIV disease severity (CD4 count, HIV viral load, and HAART use) and duration of or maximum HSV copy number in shedding episodes. CD4 count was dichotomized at 200 cells/mm^3 and HIV viral load at 10,000 copies/mL based on prior studies indicating this degree of immunosuppression and HIV viral replication were associated with longer HSV shedding duration.[2]

RESULTS

Study population

Twenty participants collected oral and anogenital samples for a median of 62 (range 32-79) days, with 20 (100%) participants collecting samples for at least 30 days, 18 (90%) for at least 50 days, and 15 (75%) for at least 60 days. Participants, who included 19 men and 1 woman, had a median CD4 count of 426 (range 29-1066) cells/mm^3, and a median HIV-1 RNA level of 2280 (range <30 – 150,000) copies/mL (Table 1). Five (25%) had undetectable HIV-1 RNA levels, 12 (60%) were taking antiretroviral therapy [for a median of 4.4 years (range 1.1 – 9.9 years) prior to study entry], and 12 (60%) met the 1993 CDC AIDS surveillance case definition.[13] Of the 12 receiving antiretroviral therapy, 7 (58%) were taking a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen (4 nevirapine and 3 efavirenz), 4 (33%) a protease-inhibitor-based regimen (1 ritonovir-boosted lopinavir, 1 ritonovir-boosted nelfinavir, 1 ritonavir-boosted atazanavir, and 1 unboosted fosamprenavir), and 1 (8%) a triple nucleoside reverse transcriptor inhibitor (NRTI)-based regimen. Three participants had a CD4 count <200 cells/mm^3 at study entry. Median CD4 counts among persons taking and not taking HAART were similar (473 versus 423 copies/mL, p = 0.51) but median HIV viral load among persons taking HAART was lower than among persons not taking HAART (239 versus 32,700 copies/mL, p = 0.01). Seven of 8 (88%) participants not on HAART and 1 of 11 (9%) participants on HAART with viral load data had a HIV viral load above 10,000 copies/mL (p = 0.001), while 1 of 8 (13%) participants not on HAART and 2 of 11 (18%) participants on HAART with CD4 data had CD4 < 200 cells/mm^3 (p = 0.74).

Frequency of mucosal HSV shedding

Oral samples were collected for 1201 days (95% of the expected 1269 days) and 4559 time points (95% of the expected 4804 time points), and anogenital samples for 1199 days (94% of the expected 1269 days) and 4544 time points (95% of the expected 4796 time points). Both oral and anogenital samples were collected at all 4 time points on 87% of days and at 3 of 4 time points on 7% of days, thus 94% of days had at least 3 daily oral and anogenital samples collected. HSV DNA was detected from oral samples on 58 (5%) days and 120 (3%) time points and from anogenital samples on 199 (16%) days and 535 (12%) time points ($p = 0.002$, indicating more frequent anogenital than oral shedding; see Table 2). Nine (45%) participants had at least one oral sample in which HSV was detected and 15 (75%) at least one anogenital sample in which HSV was detected.

HSV typing was available for 109 (91%) of 120 oral samples and 533 (99.6%) of 535 anogenital samples; the copy number in the remaining samples was too low for typing. HSV-1 alone was found in 46 oral samples (42% of all oral samples and 46% of oral samples from HSV-1/HSV-2 seropositive participants) and 4 anogenital samples (1% of all anogenital samples and 5% of anogenital samples from HSV-1/HSV-2 seropositive participants), both HSV-1 and HSV-2 in 11 oral samples (10% of all oral samples and 11% of oral samples from HSV-1/HSV-2 seropositive participants) and 33 anogenital samples (6% of all anogenital samples and 20% of anogenital samples from HSV-1/HSV-2 seropositive participants), and HSV-2 alone in 52 oral samples (48% of all oral samples and 43% of oral samples from HSV-1/HSV-2 seropositive participants) and 496 anogenital samples (93% of all anogenital samples and 75% of anogenital samples from HSV-1/HSV-2 seropositive participants) (Table 2).

Number and duration of shedding episodes

We identified 36 separate episodes of oral HSV shedding in 9 participants and 82 episodes of anogenital HSV shedding in 15 participants. Complete 4 times daily sampling allowed calculation of oral shedding duration for 29 (81%) episodes and anogenital shedding duration for 66 (80%) episodes. The median duration of an oral HSV reactivation with complete sampling was 8 hours (range 4 hours – 11 days) and an anogenital reactivation with complete sampling, 11 hours (range 4 hours–14 days) (Table 2). Of the 29 oral episodes of known duration, 10 (34%) lasted ≤ 6 hours, 17 (58%) lasted ≤ 12 hours and 19 (66%) lasted ≤ 18 hours (Figure 1A). No oral shedding episodes of known duration consisted of sole HSV-1 shedding; 17 (59%) were HSV-2 oral shedding episodes (median duration 12 [range 5-36] hours), 6 (21%) included both HSV-1 and HSV-2 shedding (median duration 34 [range 6-253] hours), and 6 (21%) could not be typed (median duration 6 [range 4-8] hours). Of the 66 anogenital shedding episodes of known duration, 19 (29%) lasted ≤ 6 hours, 35 (53%) lasted ≤ 12 hours and 43 (65%) lasted ≤ 18 hours. Sixty-three (95%) contained HSV-2 only, 2 both HSV-1 and HSV-2, and 1 could not be typed (Figure 1B). The median maximum copy number of HSV DNA detected during both oral and anogenital episodes increased with episode duration (for episodes lasting ≤ 24 hours and > 24 hours $10^{2.9}$ versus $10^{4.7}$ copies orally, $p = 0.009$, and $10^{3.3}$ versus $10^{4.9}$ copies anogenitally, $p < 0.001$, see Figure 1, panels C and D). Seven (35%) participants had at least 1 oral shedding episode which lasted ≤ 6 hours, 10 (50%) at least 1 anogenital episode ≤ 6 hours, 9 (45%) at least 1 oral episode ≤ 12 hours, and 11 (55%) at least 1 anogenital episode ≤ 12 hours. Of 3 people with CD4 < 200 cells/mm^3, one had 1 oral episode and 4 anogenital episodes lasting ≤ 6 hours and 2 had at least 1 oral and at least 1 anogenital episode lasting ≤ 12 hours.

The median number of HSV reactivations of known duration among those who shed during the 60 day sampling period was 3 (range 1–8) oral and 4 (range 1–18) anogenital

reactivations per person. Excluding the 11 participants who were not observed to shed orally, the median oral HSV reactivation rate was 1.4 (range 0.4–9.5) reactivations per 30 days or 16 reactivations annually. Excluding the 5 participants who did not shed anogenitally, the median anogenital HSV reactivation rate was 2.1 (range 0.5–8.6) reactivations per 30 days or 26 reactivations annually ($p = 0.17$ for comparison between oral and anogenital reactivation rates).

Associations Between Shedding and Symptoms

No oral HSV reactivation was accompanied by symptoms or lesions. Three (5%) of 63 anogenital episodes of known duration with symptom and lesion information were associated with lesions and 5 (8%) with symptoms. Shorter anogenital shedding episodes were less likely to be symptomatic than longer ones. None of 45 anogenital shedding episodes lasting ≤ 24 hours had lesions, compared with 3 of 21 (14%) anogenital episodes > 24 hours ($p = 0.029$). Similarly, only 1 of 45 (2%) anogenital episodes lasting ≤ 24 hours was associated with symptoms, compared with 4 of 21 (19%) anogenital episodes > 24 hours ($p = 0.032$).

Concurrent Oral and Anogenital Shedding

Swab samples were collected concurrently from both oral and anogenital sites at 4499 time points, with HSV detected on both oral and anogenital swabs concurrently at 89 time points. Concurrent shedding occurred in 4 participants (shedding data from 2 of these 4 are shown in Figure 2) and occurred more frequently than would be expected by chance alone: oral HSV shedding was detected on 17% (89/532) of time points when HSV was detected anogenitally but on only 1% (30/3967) of time points when anogenital shedding was not occurring ($p < 0.001$). Of the 89 time points with concurrent shedding, most ($n = 37$, 42%) involved HSV-2 at both

sites, 24 (27%) involved oral HSV-1 with both HSV-1 and HSV-2 detected anogenitally, 14 (16%) involved oral HSV-1 and anogenital HSV-2, 6 (7%) involved both HSV-1 and HSV-2 orally and anogenital HSV-2, 6 (7%) involved oral untypeable virus and anogenital HSV-2, 1 (1%) involved both HSV-1 and HSV-2 at both sites, and 1 (1%) involved oral HSV-2 and both HSV-1 and HSV-2 anogenitally.

Effect of immune status on HSV shedding

In our population, CD4 count, HIV viral load, and antiretroviral use did not affect the duration of HSV shedding episodes (the proportion of episodes ≤ 6 hours versus > 6 hours, Table 3). However, univariate analyses showed both antiretroviral use and a plasma HIV RNA <10,000 copies/mL to be associated with a lower average per-episode maximum HSV copy number. In multivariate regression including both HIV viral load and antiretroviral use, HIV viral load ≥ 10,000 copies/mL was found to be associated with a 45% increase in mean per-episode maximum log HSV copy number ($10^{3.1}$ HSV copies/mL among those with HIV viral load < 10,000 copies/mL compared with $10^{4.6}$ HSV copies/mL among those with HIV viral load ≥ 10,000 copies/mL, 95% CI 33% to 59% increase, $p < 0.001$), while the association between maximum HSV copy number and antiretroviral use was no longer significant.

DISCUSSION

Our study indicates that oral and anogenital HSV-1 and HSV-2 reactivation are even more common in HIV-infected persons than previously appreciated. In particular, subclinical oral shedding of HSV-1 and HSV-2 is quite common. Most mucosal HSV reactivations in HIV-infected persons are short and subclinical, with a median anogential HSV reactivation duration of 11 hours. Twenty-nine percent of anogenital HSV reactivations last ≤ 6 hours and over half (53%) last ≤ 12 hours.

We also found that concurrent oral and anogenital HSV shedding, often of the same viral type but sometimes of different viral type, occurred more frequently than would be predicted by chance, supporting the findings of Kim et al.[3] It is of interest that simultaneous reactivation from oral and anogenital mucosa, often with different subtypes, happens more frequently than would be predicted to occur, suggesting common systemic or mucosal host factors influencing shedding. Interestingly, oral HSV-2 shedding was as common as oral HSV-1 shedding; among HSV-1/HSV-2 seropositive participants, 46% of oral samples showed HSV-1 alone and 43% showed HSV-2 alone. We do not know whether oral HSV-2 shedding during episodes of oral-genital contact with a HSV-2 negative partner can lead to partner acquisition of genital HSV-2 infection, but this is certainly biologically plausible.

These data on HSV reactivation duration in HIV-infected persons are similar to what we found in immunocompetent hosts, who had a median anogenital HSV reactivation duration of 13 hours, with 24% of reactivations lasting ≤ 6 hours and 49% lasting ≤ 12 hours.[5] We studied an HIV-infected population that was only moderately immunosuppressed (median CD4 count 426 cells/mm^3) and our results may have been different if we had enrolled only HIV-infected persons with more marked immunosuppression (CD4 < 200 cells/mm^3, for example), but our finding of

some short anogenital HSV reactivations even in our few study participants with CD4 < 200 cells/mm^3 suggests that even quite immunosuppressed HIV-infected persons can continue to have short, rapidly cleared HSV reactivations. The proportion of HSV shedding episodes which were ≤ 6 hours did not differ by CD4 count, plasma HIV RNA, or HAART use, suggesting that level of immunosuppression does not markedly affect HSV episode length, although small numbers of participants limit our power to find subtle differences.

All but one of our participants were men, limiting our ability to draw definitive conclusions about short HSV reactivations in HIV infected women. However, we have previously shown a greater number of short (<12 hour) episodes in immunocompetent men than immunocompetent women and a lower median HSV viral load at genital shedding episode onset in immunocompetent men than immunocompetent women ($10^{3.2}$ copies/mL versus $10^{4.5}$ copies/mL, $p < 0.0001$).[5] Genital HSV shedding rates among immunocompetent women have been shown in some studies to be ~40% higher than among immunocompetent men.[8, 14] Together these data suggest that immunocompetent women may shed genital HSV more frequently and in longer episodes than immunocompetent men; whether the same is true of HIV-infected women is unknown. Our participants were also relatively old (median age 45 years), suggesting many likely acquired HSV-2 many years ago. Whether HSV shedding episode duration differs among HIV infected individuals with more recently acquired HSV-2 requires further study.

Our findings have important implications regarding interactions between HIV-1 and HSV-2 and potential investigational HIV-1 prevention methods which would focus on HSV-2 treatment. Observational data show that HSV-2 co-infection may increase HIV-1 transmission,[15] possibly by HSV stimulating transcription of latent HIV-1.[16] In HIV negative, HSV-2

seropositive persons, HSV-2 specific CD4+ and CD8+ T cells and plasmacytoid and myeloid dendritic cells, including cells expressing the C-type lectin receptor DC-SIGN, persist at the dermal epidermal junction for months after lesion healing, even with daily antiviral therapy.[17, 18] The frequent short bursts of HSV reactivation and the high annual anogenital reactivation rate (median of 26 anogenital reactivations annually) that we demonstrated here in HIV infected persons likely also lead to persistent anogenital mucosal immune activation, potentially contributing to HIV infectiousness. We know that acyclovir 400 mg orally twice daily, the dose used to assess whether HSV suppressive therapy in HIV-1/HSV-2 co-infected persons could help prevent HIV-1 transmission,[19] does not completely suppress HSV reactivation.[7, 20] More potent therapies, or an effective HSV vaccine, are needed to assess whether complete suppression of HSV reactivation in HIV-1/HSV-2 co-infected persons, or prevention of HSV-2 infection, could help prevent HIV-1 transmission.

Our findings also suggest that treatment of HIV-1 in HIV-1/HSV-2 coinfected persons might help prevent HSV-2 transmission. Plasma HIV RNA $\geq$ 10,000 copies/mL and lack of HAART use were associated with higher maximum HSV copy numbers during shedding episodes. These higher copy numbers likely lead to greater HSV-2 infectiousness.

In summary, we found that frequent short episodes of oral and anogenital HSV reactivation occur in HIV-infected persons, even those with relatively advanced immunosuppression, and that the median oral and anogenital HSV reactivation duration in HIV-infected hosts is surprisingly similar to that in HIV-uninfected hosts. Further study of the pathogenesis of HSV-induced chronic mucosal immune activation and its effect on HIV-1 transmission is warranted.

Table 1. Demographic and clinical characteristics of study participants.

Baseline characteristic	n = 20
Median age (range) in years	45 (36 – 58)
Men, n (%)	19 (95)
Race/ethnicity, n (%)	
White	13 (65)
Black	5 (25)
Other	2 (10)
Symptomatic anogenital herpes, n (%)	14 (70)
Symptomatic oral herpes, n (%)	6 (30)
HSV serostatus, n (%)	
HSV-1 and HSV-2 seropositive	9 (45)
HSV-2 seropositive only	11 (55)
HIV positive, n (%)	20 (100)
Antiretroviral use during study, n (%)	12 (60)
Median (range) CD4 count[a], cells/mm^3	426 (29 – 1066)
Median (range) HIV RNA[a], copies/mL	2280 (<30 – 150,000)

[a]One subject did not have CD4 or HIV RNA data within 1 month of study initiation.

Table 2. Proportion of days, time points, and participants with HSV detected in at least 1 sample, and HSV typing. Duration of HSV reactivation as defined by 4 times daily sampling is shown for 29 oral and 66 anogenital shedding episodes of known duration.

	Oral	Anogenital
Days sample(s) collected, n	1201	1199
Days HSV detected, n (%)	58 (5%)	199 (16%)
Time points samples collected, n	4559	4544
Time points HSV detected, n (%)	120 (3%)	535 (12%)
Participants with HSV detected at any time point, n (%)	9 (45%)	15 (75%)
HSV type*		
HSV-1, n (%)	46 (42%)	4 (1%)
HSV-2, n (%)	52 (48%)	496 (93%)
HSV-1 and HSV-2, n (%)	11 (10%)	33 (6%)
HSV reactivation duration		
Median episode duration	8 hours	11 hours
Range episode duration	4 hours – 11 days	4 hours – 14 days
No. (%) episodes ≤ 6 hours	10 (34%)	19 (29%)
No. (%) episodes ≤ 12 hours	17 (58%)	35 (53%)
Median (range) maximum copy number per episode	$10^{2.2}$ ($10^{3.2}$ – $10^{7.0}$)	$10^{3.4}$ ($10^{2.1}$ – $10^{8.4}$)

*Type-specific data available for 109 (91%) of 120 oral samples and 533 (99%) of 535 anogenital samples.

Table 3. Effect of immunological, viral, and treatment status on oral and anogenital HSV shedding.

Measure of systemic immune function	Oral episodes				Anogenital episodes			
	Proportion of episodes ≤ 6 hours		Maximum HSV copy number per episode		Proportion of episodes ≤ 6 hours		Maximum HSV copy number per episode	
	n (%)	p	Median (range)	p	n (%)	p	Median (range)	p
CD4 < 200	1/7 (14%)		$10^{3.4}$ ($10^{3.2}$-$10^{3.8}$)		4/12 (33%)		$10^{3.7}$ ($10^{2.2}$-$10^{4.2}$)	
CD4 ≥ 200	9/22 (41%)	0.39	$10^{2.8}$ ($10^{2.2}$-$10^{7.0}$)	0.89	14/53 (27%)	0.64	$10^{3.2}$ ($10^{2.3}$-$10^{8.4}$)	0.72
HIV VL < 10,000	4/10 (40%)		$10^{2.9}$ ($10^{2.3}$-$10^{7.0}$)		14/46 (30%)		$10^{3.0}$ ($10^{2.2}$-$10^{8.4}$)	
HIV VL ≥ 10,000	6/13 (32%)	0.67	$10^{3.3}$ ($10^{2.2}$-$10^{5.8}$)	0.35	5/20 (25%)	0.60	$10^{4.2}$ ($10^{3.2}$-$10^{7.2}$)	**0.005**
HAART	4/10 (40%)		$10^{2.9}$ ($10^{2.3}$-$10^{7.0}$)		13/45 (29%)		$10^{3.0}$ ($10^{2.2}$-$10^{8.4}$)	
No HAART	6/19 (32%)	0.67	$10^{3.3}$ ($10^{2.2}$-$10^{5.8}$)	0.35	6/21 (29%)	0.97	$10^{4.2}$ ($10^{3.2}$-$10^{7.2}$)	**0.009**

Figure 1. Herpes simplex virus (HSV) shedding episode duration and viral copy number, of 29 oral and 66 anogenital shedding episodes of known duration. (A) Oral and (B) anogenital episode duration and (C) oral and (D) anogenital HSV copy number by episode duration.

Figure 2. Concurrent oral and anogenital herpes simplex virus shedding in two HSV-1/HSV-2 seropositive participants. Participant 1, a 37 year old man diagnosed with genital herpes 17 years earlier, had been off antiretrovirals for ~4 months prior to study entry and had a CD4 of 160 cells/mm^3 and an HIV viral load of 148,000 copies/mL. Participant 2, a 49 year old man diagnosed with asymptomatic HSV-2 infection at least 4 years earlier but subsequently noted to have typical genital, perianal, and perineal recurrences of which he was previously unaware, was on antiretrovirals with a CD4 of 706 cells/mm^3 and an undetectable HIV viral load. The asterix in Figure 2B shows the one day that genital symptoms and lesions were present; all other shedding was asymptomatic.

Figure 1.

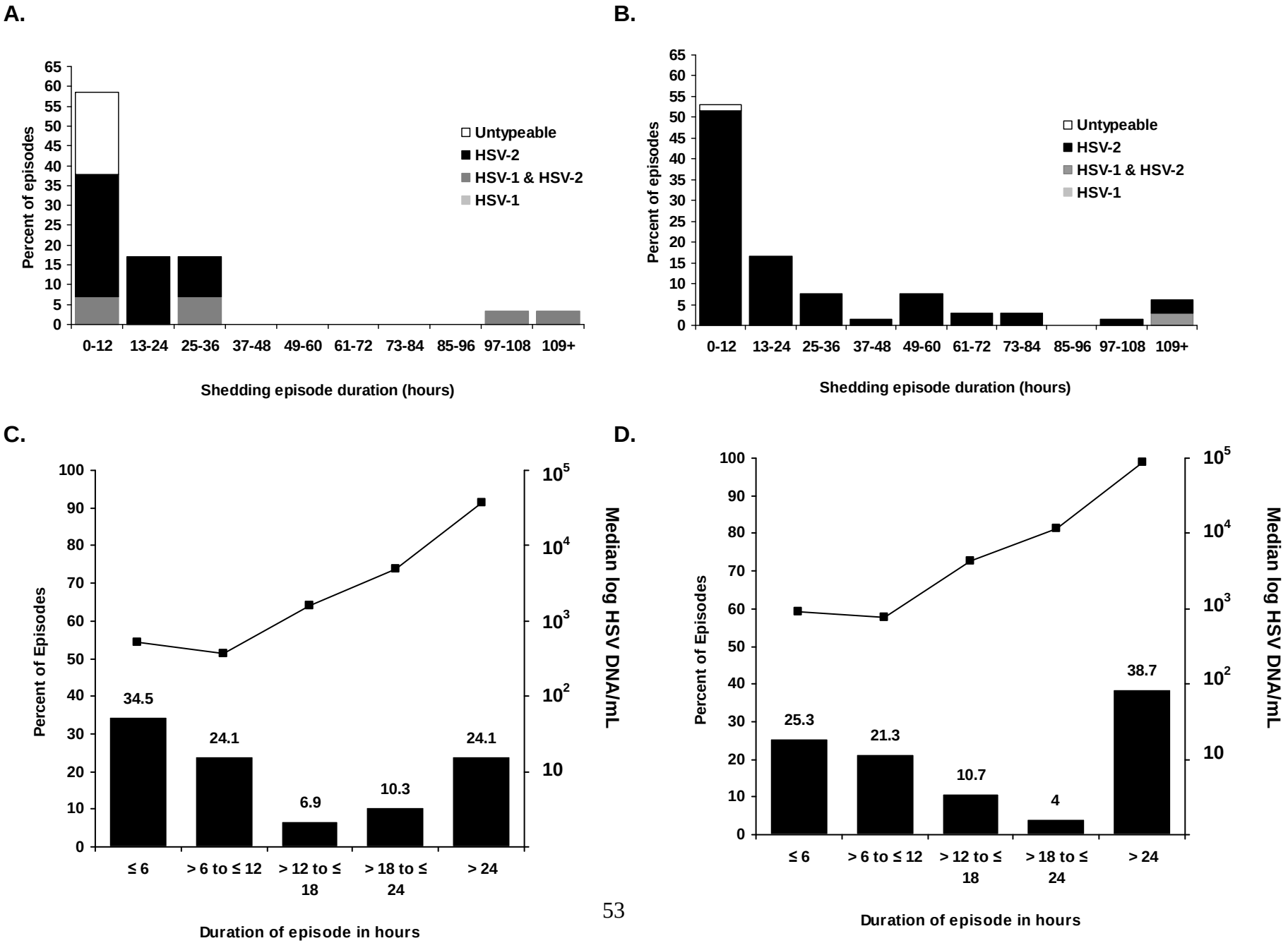

A.
Percent of episodes
Untypeable
HSV-2
HSV-1 & HSV-2
HSV-1
0-12
13-24
25-36
37-48
49-60
61-72
73-84
85-96
97-108
109+
Shedding episode duration (hours)
B.
Percent of episodes
Untypeable
HSV-2
HSV-1 & HSV-2
HSV-1
Shedding episode duration (hours)
C.
Percent of Episodes
Median log HSV DNA/mL
34.5
24.1
6.9
10.3
24.1
≤ 6
> 6 to ≤ 12
> 12 to ≤ 18
> 18 to ≤ 24
> 24
Duration of episode in hours
D.
Percent of Episodes
Median log HSV DNA/mL
25.3
21.3
10.7
4
38.7
Duration of episode in hours

Figure 2A.

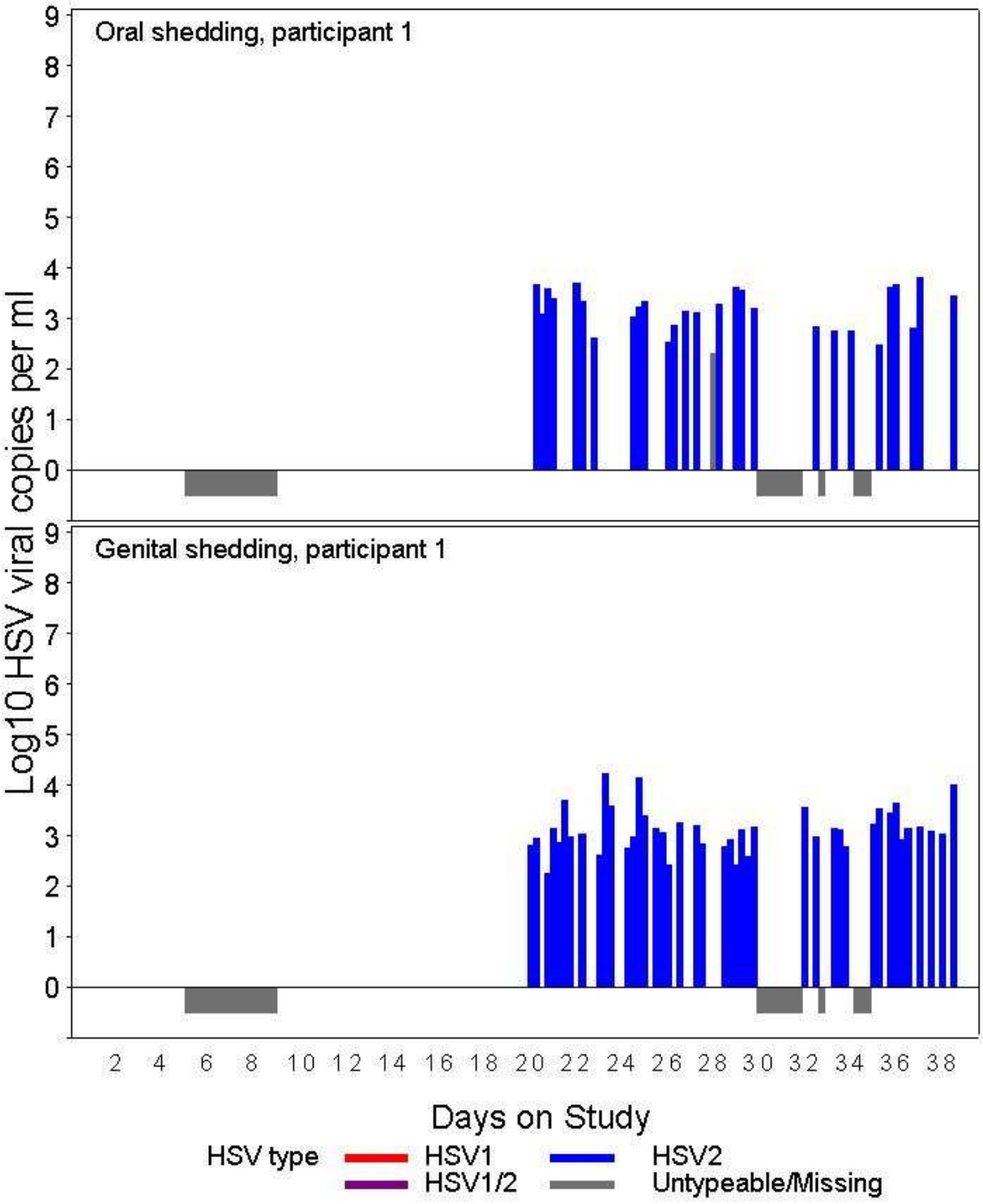
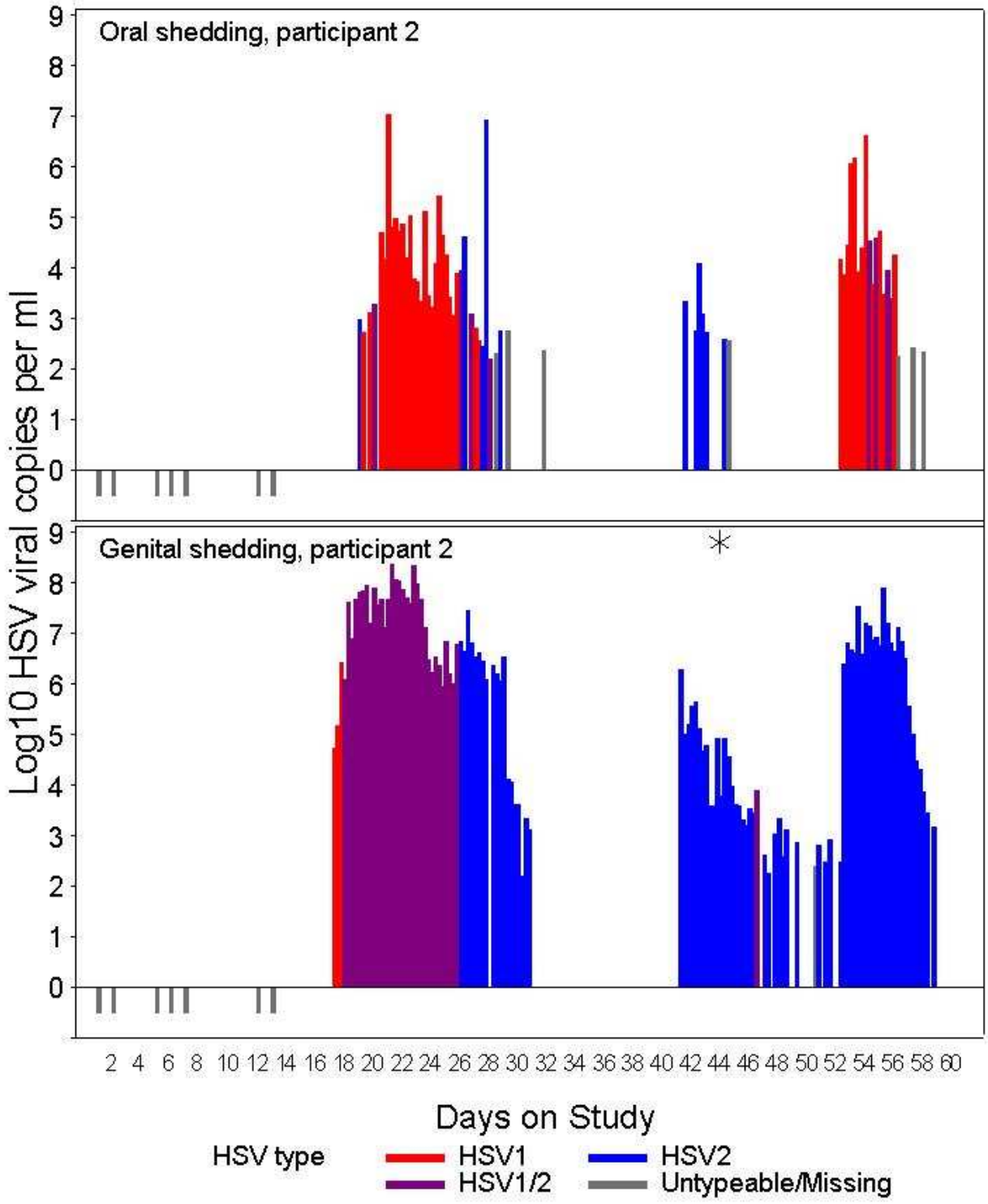

Oral shedding, participant 1
Genital shedding, participant 1
Log10 HSV viral copies per ml
0
1
2
3
4
5
6
7
8
9
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Days on Study
HSV type
HSV1
HSV1/2
HSV2
Untypeable/Missing

Figure 2B.


Oral shedding, participant 2
Genital shedding, participant 2
Log10 HSV viral copies per ml
0 1 2 3 4 5 6 7 8 9
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
Days on Study
HSV type
HSV1
HSV2
HSV1/2
Untypeable/Missing

REFERENCES

1. Siegal FP, Lopez C, Hammer GS, et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med.* Dec 10 1981;305(24):1439-1444.

2. Schacker T, Zeh J, Hu HL, Hill E, Corey L. Frequency of symptomatic and asymptomatic herpes simplex virus type 2 reactivations among human immunodeficiency virus-infected men. *J Infect Dis.* Dec 1998;178(6):1616-1622.

3. Kim HN, Meier A, Huang ML, et al. Oral herpes simplex virus type 2 reactivation in HIV-positive and -negative men. *J Infect Dis.* 2006;194(4):420-427. Epub 2006 Jul 2012.

4. Bagdades EK, Pillay D, Squire SB, O'Neil C, Johnson MA, Griffiths PD. Relationship between herpes simplex virus ulceration and CD4+ cell counts in patients with HIV infection. *AIDS.* Nov 1992;6(11):1317-1320.

5. Mark KE, Wald A, Magaret AS, et al. Rapidly cleared episodes of herpes simplex virus reactivation in immunocompetent adults. *J Infect Dis.* Oct 15 2008;198(8):1141-1149.

6. Lingappa JR, Kahle E, Mugo N, et al. Characteristics of HIV-1 discordant couples enrolled in a trial of HSV-2 suppression to reduce HIV-1 transmission: the partners study. *PLoS One.* 2009;4(4):e5272.

7. Wald A, Corey L, Cone R, Hobson A, Davis G, Zeh J. Frequent genital herpes simplex virus 2 shedding in immunocompetent women. Effect of acyclovir treatment. *J. Clin. Invest.* March 1, 1997 1997;99(5):1092-1097.

8. Wald A, Huang ML, Carrell D, Selke S, Corey L. Polymerase chain reaction for detection of herpes simplex virus (HSV) DNA on mucosal surfaces: comparison with HSV isolation in cell culture. *J Infect Dis.* Nov 1 2003;188(9):1345-1351.

9. Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. *J Clin Microbiol.* Apr 1988;26(4):662-667.

10. Jerome KR, Huang ML, Wald A, Selke S, Corey L. Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. *J Clin Microbiol.* 2002;40(7):2609-2611.

11. Magaret A, Wald A, Huang M, Selke S, Corey L. Optimizing PCR positivity criterion for detection of HSV DNA on skin and mucosa. *J Clin Microbiol.* 2007;45(5):1618-1620.

12. Corey L, Huang ML, Selke S, Wald A. Differentiation of herpes simplex virus types 1 and 2 in clinical samples by a real-time taqman PCR assay. *J Med Virol.* Jul 2005;76(3):350-355.

13. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep.* Dec 18 1992;41(RR-17):1-19.

14. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *N Engl J Med.* Mar 23 2000;342(12):844-850.

15. Gray RH, Wawer MJ, Brookmeyer R, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet.* Apr 14 2001;357(9263):1149-1153.

16. Mosca JD, Bednarik DP, Raj NB, et al. Herpes simplex virus type-1 can reactivate transcription of latent human immunodeficiency virus. *Nature.* Jan 1-7 1987;325(6099):67-70.

17. Zhu J, Koelle DM, Cao J, et al. Virus-specific CD8+ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. *J. Exp. Med.* March 19, 2007 2007;204(3):595-603.

18. Zhu J, Hladik F, Woodward A, et al. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med.* Aug 2009;15(8):886-892.

19. Celum C, Wald A, Lingappa J, et al. Twice-daily acyclovir to reduce HIV-1 transmission from HIV-1/HSV-2 co-infected persons within HIV-1 serodiscordant couples: a randomized, double-blind, placebo-controlled trial. *5th IAS Conference on HIV Pathogenesis, Treatment, and Prevention*. Cape Town, South Africa; 2009.

20. Wald A, Zeh J, Barnum G, Davis LG, Corey L. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. *Ann Intern Med.* Jan 1 1996;124(1 Pt 1):8-15.

Chapter 3: Three Phase III Randomized Controlled Trials of Topical Resiquimod 0.01% Gel to Reduce Anogenital Herpes Recurrences

ABSTRACT

Background: Resiquimod, a toll-like receptor 7 and 8 agonist, stimulates production of cytokines that promote an antigen-specific T helper type 1 acquired immune response. Animal and phase II human trials showed post-treatment efficacy in reducing recurrent herpes lesion days and/or time to first recurrence.

Methods: Three phase III randomized, double-blind, vehicle-controlled trials of topical resiquimod to reduce anogenital herpes recurrences were conducted in healthy adults with ≥4 recurrences within the prior year. Participants applied resiquimod 0.01% or vehicle gel 2 times per week for 3 weeks to each recurrence for 12 months. Trials 1 and 2 had 2:1 resiquimod:vehicle randomization. Trial 3 had 1:1:1 randomization for resiquimod plus valacyclovir 500 mg orally twice daily for 5 days (RESI/VAL), resiquimod plus oral placebo (RESI/PLA), and vehicle plus oral placebo (VEH/PLA).

Results: Median time to first recurrence was similar for resiquimod and vehicle [Trial 1: 60 and 56 days, p=0.7; Trial 2: 54 and 48 days, p=0.47; Trial 3: 51 (RESI/VAL), 55 (RESI/PLA), and 44 (VEH/PLA) days, p=NS]. Median time to healing of initial treated recurrence was longer for resiquimod [Trial 1: 18 versus 10 days, p<0.001; Trial 2: 19 versus 13 days, p=0.16; Trial 3: 14 (RESI/VAL), 16 (RESI/PLA), and 8 (VEH/PLA) days, p<0.001]. In Trials 1 and 2, moderate to severe erythema and erosion/ulceration at the application site were more common in resiquimod recipients.

Conclusions: No post-treatment efficacy of resiquimod 0.01% gel was observed. Increased application site reactions and initial recurrence healing time are consistent with resiquimod-induced cytokine effects.

INTRODUCTION

Approximately 3 percent of the United States (US) adult population has been diagnosed with anogenital herpes [1]. Although anogenital herpes recurrences can be treated with episodic antiviral use, and reduced with daily suppressive antiviral use, no currently available treatment impacts the natural history of the disease by reducing subsequent recurrences once nucleoside analogue therapy is stopped.

Resiquimod (R-848), an imidazoquinolinamine, is an investigational toll-like receptor 7 and 8 agonist which stimulates an innate immune response with production of cytokines that lead to a subsequent antigen-specific T helper type 1 acquired immune response [2-5]. Application of topical resiquimod to anogenital herpes lesions is hypothesized to act as an endogenous therapeutic HSV vaccine, with resiquimod acting like an adjuvant in the presence of endogenous antigen (HSV). The induced immunity could potentially delay or reduce future HSV recurrences. Resiquimod has been shown in an animal model [6] and phase 2 human trials [7, 8] to have some efficacy in reducing anogenital herpes recurrences. We report the results of three Phase III randomized controlled trials of topical resiquimod to reduce anogenital herpes recurrences.

METHODS

Participants applied resiquimod 0.01% or vehicle gel 2 times per week for 3 weeks to each recurrence of anogenital herpes for 12 months. In Trial 1, conducted in 38 European centers, and Trial 2, conducted in 19 US centers, both from 2000-2002, participants were randomized 2:1 to resiquimod versus vehicle. In Trial 3, conducted in 18 US and 7 Canadian centers from 2001-2002, participants were randomized 1:1:1 to 1) resiquimod 2 times per week for 3 weeks and valacyclovir 500 mg caplet orally BID for 5 days, 2) resiquimod 2 times per week for 3 weeks plus oral placebo BID for 5 days, or 3) vehicle 2 times per week for 3 weeks and oral placebo BID for 5 days. Each trial was approved by each trial center's Independent Ethics Committee, and all participants gave written informed consent.

Participants in all 3 trials were healthy, 18-65 years of age, HIV-negative, and had a history of frequently recurrent anogenital herpes. For inclusion in any of the 3 trials, participants had to have had ≥4 genital herpes recurrences per year in the past year or, if on suppressive oral nucleoside therapy, prior to starting suppressive therapy, and at least one recurrence in the 3 months prior to screening, and were willing to refrain from using topical or oral antivirals during the eligibility period and to use them only as directed by the investigator during the treatment period. Exclusion criteria included ever having used resiquimod (R-848) or imiquimod, pregnancy or breastfeeding, receipt of HSV vaccine within the past 2 years, treatment of anogenital warts within 4 weeks prior to enrollment or expected during the trial period, allergy to study gel excipient, hemoglobin ≤ 9.4 g/dL, granulocyte count ≤ 1.5×10^9 cells/L, platelet count ≤ 100,000 platelets per microliter, any serum chemistry value of Grade 2 (moderate) or higher as defined in the protocol, or, in the 4 weeks prior to the screening visit, any use of an investigational or cytotoxic drug; interferon therapy or inducer; immunomodulator; systemic

antiviral drug other than acyclovir, valacyclovir, or famciclovir; or systemic or high-dose inhaled corticosteroid.

After the screening visit, participants entered a 12-week eligibility period during which they had to have a qualifying recurrence to continue. Within 36 hours after development of the qualifying recurrence, participants presented to the trial center, were randomized, and received blinded study drug. Participants treated each recurrence with the same regimen during the 12 month trial.

Randomization was in blocks of 6 and stratified by sex and trial center. To ensure proper randomization by trial center, subjects presenting with a qualifying recurrence were given a unique subject identification number in consecutive numerical sequence within that trial center, and study drug kits were issued in sequence. Study drug (resiquimod or vehicle gel), supplied in single-use sachets containing 225 mg of gel, was applied topically to lesions just before bedtime and washed off after 8 to 10 hours. During the initial treatment cycle, participants returned to the trial center on days 3, 8, 15, and 22. On day 22, they received study drug for use during the next recurrence. For subsequent recurrences, participants self-initiated treatment within 24 hours of lesion recurrence (prodrome-only and erythema-only events were not treated) and returned to the trial center within 72 hours after onset for clinical confirmation. For the first recurrence after the initial treated recurrence, participants were seen on days 1, 8, 15, and 22. For all other subsequent recurrences participants were seen on day 1 and 1-3 days after their last application of study drug. Additional scheduled visits were at weeks 12, 24, 36, and 52, unless these visits overlapped with a treatment cycle. Recurrences were assessed independently by participant diary and by the investigator during each clinic visit. Compliance with study drug was measured by participant diary and return of used and unused study drug sachets. Safety was measured by

evaluation of adverse events, anogenital site assessments (local skin signs and local symptoms), and clinical laboratory tests. Anogenital site assessments occurred at each clinic visit during the initial and first subsequent treatment cycles, at the day 1 and 1-3 days post last dose visits for other subsequent recurrences, and at the last trial visit. During anogenital site assessments, investigators recorded the most severe signs for each category: erythema, edema, vesicles, erosion/ulceration, and scabbing (investigator-assessed signs). Participants were asked, without prompting for specific terms, if they had anogenital site signs since their last clinic visit (subject-reported signs). Size and number of lesions were collected as part of lesion assessments.

Laboratory methods

HSV serology was done at the University of Washington by Western blot [9]. HSV PCR was done at Pasteur, Cerba, France (Trial 1), at Children's Hospital Medical Center, Cincinnati, Ohio, USA (Trial 2), and at Viridae Clinical Sciences, Vancouver, British Columbia, Canada (Trial 3). HSV PCR swabs were collected at the day 1 visit for all recurrences.

Statistical analysis

A clinical recurrence was defined as a period of days with lesions preceded and followed by at least 1 day without lesions. For Trials 1 and 2, the primary endpoint was time to first subject-reported recurrence; for Trial 3, it was annual recurrence rate. Secondary endpoints, depending on trial, included time to first recurrence, annual recurrence rate, percent of subjects who had no subsequent recurrences after the qualifying recurrence, total number of days the subject had a lesion present (lesion days), and time interval between the first and second recurrence. Recurrences were measured 3 ways: 1) subject-reported recurrences based on dates

of lesion onset and resolution, 2) investigator-confirmed recurrences, and 3) PCR-confirmed recurrences. Kaplan-Meier analysis was used to calculate median time to first recurrence, defined as time from qualifying recurrence healing date to start date of the next recurrence, with the log-rank test used to assess difference between groups. If randomization recurrence healing date was missing, we used randomization recurrence start date. Persons were censored on their last visit date. A Cox proportional hazards model stratified by site was used to test the treatment effect. Covariates considered included sex, age (≤40 versus >40 years), race (White vs non-White), baseline HSV-1 serostatus, and number of recurrences in the past year (≤ 8 versus >8).

Annualized recurrence rate was calculated by counting the total number of clinical recurrences occurring after the randomization recurrence and dividing by observation time, defined as time in years from randomization recurrence clear date (or randomization recurrence start date if clear date was unavailable) to last visit date. Recurrence length was defined as the number of days during which lesions were present during a clinical recurrence. Recurrences of uncertain duration were those which did not have a clear date. To calculate a minimum recurrence length for these recurrences, we substituted the last date during that recurrence when the participant was seen for the clear date. Per person lesion rates were calculated as the number of days with lesions divided by the number of days of observation, defined as the number of days from the start of the initial randomization recurrence to the last day of observation. The Wilcoxon rank sum test (Trials 1 and 2) and the Kruskal-Wallis test (Trial 3) were used to compare, by treatment arm, the median annualized subject-reported recurrence rate, median recurrence length, and median lesion rate. Fisher's Exact test was used to compare, by treatment arm, the percent of participants without subsequent recurrences. The Prentice, Williams, and Peterson (PWP) Gap Time model[14] adjusted for treatment, sex, age, race, number of

recurrences in the past year (≤8 versus >8 recurrences/year), and baseline HSV-1 serostatus was used to model the time interval between the first and second subsequent subject-reported recurrences. In this model, the analysis of the second subsequent recurrence was necessarily restricted to subjects who had a first subsequent recurrence.

Power calculations were obtained using a simulation of 5000 trials. For each subject, the gamma distribution was chosen to represent the number of days to each subsequent recurrence. The vehicle group was assumed to have a gamma distribution with a shape parameter of 2 and a scale parameter of 42, corresponding to a median time to first recurrence of 70 days and approximately 4 recurrences after the initial treated recurrence during the 1 year trial period. The resiquimod treatment group was assumed to have a shape parameter of 2 and a scale parameter of 63, corresponding to a median time to first recurrence of 105 days and approximately 2.6 recurrences after the initial treated recurrence. It was assumed that 40% of subjects would discontinue prior to completing the treatment period, and these subjects were assigned a value of 18 recurrences. For trials 1 and 2, 20% of subjects were assumed to have dropped out prior to experiencing a recurrence. For trials 1 and 2, 160 resiquimod and 80 vehicle subjects provided 96% power to detect a 50% difference in the median number of days to first subsequent recurrence and 92% power to detect a 50% difference in total number of recurrences. For trial 3, 105 subjects per treatment arm provided 81% power to detect a 50% difference in total number of recurrences. A two-sided alpha of 0.05 was used for each power calculation. Unless otherwise stated, all analyses were intention-to-treat, including all randomized subjects who were dispensed study drug.

Safety was measured by evaluations of adverse events, local skin signs and symptoms, time to recurrence healing, and size of lesions. Local skin signs (erythema, edema, vesicles,

scabbing, and erosion/ulceration, as assessed by the investigator and participant) and local symptoms (pain, numbness/tingling, burning, and pruritis/itching, as assessed only by the participant) were summarized by maximum severity during the initial treatment cycle. The Kruskal-Wallis test was used to compare maximum severity scores across treatment groups. The Wilcoxon rank sum test was used to compare the median total investigator-confirmed lesion size during the initial treatment cycle between treatment groups. Time to healing of the qualifying recurrence was summarized using Kaplan-Meier survival methods. The Kruskal-Wallis test was also used to compare across all 3 trials by treatment group both trial and treatment completion rates and the proportion of participants who discontinued treatment due to adverse events or local skin symptoms or signs.

RESULTS

The numbers of people who were assessed for eligibility, randomized, completed treatment, and completed trial are shown by trial and randomization arm in Figure 1. Among those randomized, trial completion rates were 70%, 72%, and 70% in Trials 1, 2, and 3 respectively, including 67%, 68%, and 69% who completed both trial and treatment and 3%, 3%, and 2% who completed trial but discontinued treatment during the trial, respectively (Fig. 1). Across trials, 307/451 (68%) resiquimod, 198/264 (75%) vehicle, and 73/103 (71%) RESI/VAL recipients completed the trial (p=0.14). In all 3 trials, the median percent of expected doses applied was 100% in all treatment groups. Across trials, the percentage of persons that applied all 6 doses during the initial treatment cycle was 89-94% in the resiquimod, 89-93% in the vehicle, and 89% in the RESI/VAL arm.

Within each of the 3 trials, resiquimod, vehicle, and RESI/VAL participants were similar in baseline characteristics (Table 1). Consistent with previously published data [10, 11], male circumcision rates in the European trial (Trial 1) were lower than in the North American trials (Trials 2 and 3). Observation time (from start date of randomization recurrence to the later of healing date of first recurrence or last visit) was similar in all arms of all trials, with an overall median of 367 days (range 1-473 days, IQR 262-378 days).

Across all 3 trials, 818 participants had 3,779 clinical recurrences of anogenital herpes. Participants had a median of 3 recurrences (range 1-22) per person. Recurrence duration was known for all but 174 (4.6%) recurrences, and was a median of 11 (range 1-75) days. The median minimum duration for the 174 recurrences of uncertain duration was 17 days (range 1-31 days), and the median duration for all 3,779 recurrences (using the minimum duration if the total duration was unknown) was 11 (range 1-75) days. Of all 3,779 recurrences, 1,952 (52%) were

HSV positive, 867 (23%) were HSV negative, and 960 (25%) did not have a swab collected for virologic evaluation. PCR positive recurrences had a longer median duration than both PCR negative recurrences and recurrences from which no PCR swab was collected (13, 11, and 8 days respectively, $p<0.001$).

Time to first recurrence and between the first and second subsequent recurrences

Time to first subject-reported recurrence after the initial randomization recurrence was similar in all trial arms in all 3 trials (Table 2 and Figure 2a, 2b, and 2c). The Cox proportional hazards model for time to first subject-reported recurrence, both the crude unadjusted model and after adjusting for sex, age, race, number of recurrences in the past year, and baseline HSV-1 serostatus, also showed no statistically significant treatment difference with respect to time to first subject-reported recurrence in any of the 3 trials (data not shown). Results were similar when investigator-confirmed or virologically-confirmed recurrences were used as the endpoint. No treatment differences were detected for the time interval between the first and second subsequent recurrences in any of the three trials.

Annualized recurrence rate, lesion rate, and recurrence duration

The median annualized recurrence rate was also similar in all trial arms in all 3 trials (Table 2). The percent of trial subjects with no recurrence after the initial randomization recurrence was similar between the randomized groups in all 3 trials (Trial 1: resiquimod 16% versus vehicle 14%, $p=0.40$; Trial 2: resiquimod 16% versus vehicle 11%, $p=0.33$; Trial 3: 15% RESI/PLA, 16% VEH/PLA, 17% RESI/VAL, $p=0.98$). The median lesion rate (percent of days with lesions) was higher in the resiquimod than the vehicle group in Trial 1 (20% versus 14%,

p<0.001), higher in the RESI/VAL and RESI/PLA groups than in the VEH/PLA group in Trial 3 (22%, 21%, and 16% respectively, p=0.007), and similar in the resiquimod and vehicle groups in Trial 2 (20% versus 19%, p=0.24). The median recurrence duration was longer in the resiquimod than vehicle groups in both Trials 1 and 2 (Table 2), and longest in the RESI/PLA group, second longest in the RESI/VAL group, and shortest in the VEH/PLA group in Trial 3 (13, 10, and 9 days respectively, p<0.001).

Pooled, subgroup, and sensitivity analyses

Analysis of pooled data from the resiquimod and vehicle arms of Trials 1, 2, and 3 also showed no difference in median days to first recurrence between the resiquimod and vehicle arms (44 versus 34 days respectively, p = 0.35). Using the pooled data, we conducted subgroup analyses to examine median days to first recurrence by treatment arm among all men, circumcised men, uncircumcised men, and all women. There was no statistically significant difference in time to first recurrence by treatment arm among any of these subpopulations, although there was a trend towards longer time to first recurrence among men receiving resiquimod versus vehicle (55 versus 48 days respectively, p = 0.08), which was limited to circumcised men (64 versus 44 days respectively, p = 0.10) with no difference seen among uncircumcised men (48 versus 52 days respectively, p = 0.45).

Across all 3 trials, 78 persons (9.5%) were observed for <60 days. Because theoretically these participants randomized to resiquimod may have had time to experience the cytokine-mediated inflammation and delayed healing of the randomization recurrence without having had enough time under observation to experience the potential benefits of resiquimod in delaying subsequent recurrences, we conducted a post-hoc sensitivity analysis excluding these 78 persons

and then re-examined lesion rate and median recurrence duration by trial and randomization group, and number and percent of trial participants with no subsequent recurrences after the initial randomization recurrence. The results for lesion rate for Trials 1 and 3 were similar to the intent to treat analysis but in Trial 2 the difference in lesion rate between arms was no longer statistically significant (median lesion rate 19% in resiquimod group and 17% in vehicle group, p = 0.17). The results for recurrence duration were identical to the intent to treat analysis. In all three trials, a similar percentage of trial participants had no subsequent recurrences after the initial randomization recurrence (Trial 1: 8.6% resiquimod versus 8.9% placebo, p = 1.0; Trial 2: 8.3% resiquimod versus 2.9% placebo, p = 0.24; Trial 3: 8.7% RESI/VAL, 6.1% RESI/PLA, 9.6% VEH/PLA, p = 0.68).

Duration of initial treated recurrence

Median days to healing of randomization recurrence was 6-8 days longer in the resiquimod than vehicle-treated groups in all 3 trials, although this reached statistical significance only in Trials 1 and 3 (Table 2 and Figure 2d, 2e, and 2f). There was no statistically significant difference in days to healing of the initial recurrence between the RESI/VAL and RESI/PLA groups in Trial 3 (14 versus 16 days, p=0.35), although both were longer than in the VEH/PLA group (8 days, $p<0.001$ for comparison with both RESI/VAL and RESI/PLA).

Adverse effects

Overall, topical application of resiquimod to anogenital herpes lesions was generally well tolerated. There were no deaths, and all serious adverse events (6 in Trial 1, 9 in Trial 2, and 8 in Trial 3) were considered by the investigator to be probably not related to resiquimod. No

clinically meaningful differences between treatment groups in the number of subjects reporting systemic adverse events, and no increases in adverse events compared with the vehicle group, were observed that might be associated with systemic exposure to resiquimod.

The percent of participants with moderate or severe investigator-assessed local signs during the initial treatment cycle was higher in the resiquimod than vehicle group for erythema and erosion/ulceration in Trials 1 and 2 but not Trial 3 (Table 3), and for scabbing in Trial 2 only. No differences were seen for edema or vesicles, nor were differences seen in subject assessments of local skin sign severity or the percent of subjects who graded local symptoms as moderate or severe during the initial treatment cycle. The median investigator-confirmed total lesion size during the initial treatment cycle was greater at days 3, 8, and 15 for resiquimod as compared with vehicle (Trials 1 and 2 only). Combining results from all 3 trials, resiquimod recipients were most likely to discontinue drug, followed by RESI/VAL recipients and then vehicle recipients [161/451 (36%), 30/103 (29%), and 70/264 (27%), respectively; p=0.03]. Overall, 5 resiquimod, 1 vehicle, and 2 RESI/VAL participants discontinued drug due to adverse events (p=0.35), and 11 resiquimod, 2 vehicle, and 1 RESI/VAL participant discontinued drug due to local skin reaction (p=0.55).

DISCUSSION

These three randomized controlled trials of resiquimod 0.01% gel applied topically to lesions of recurrent anogenital herpes two times per week for 3 weeks for each recurrence over 12 months did not show any post-treatment efficacy of resiquimod as measured by time to first recurrence, annualized recurrence rate, or lesion rate. Although systemically well tolerated, use of topical resiquimod resulted in increased application site reactions which were only partially ameliorated by valacyclovir, and also increased time to healing. These findings are consistent with pro-inflammatory effects of resiquimod-induced cytokines.

In a guinea pig model of anogenital herpes, subcutaneous resiquimod reduced recurrent lesion days both during treatment and after treatment discontinuation [6]. Three human, phase 2, randomized, double-blind, vehicle-controlled trials of resiquimod have been published [7, 8, 12]. In the first, resiquimod recipients applied resiquimod gel 0.05% once or twice weekly or 0.01% twice or thrice weekly topically to anogenital herpes lesions. Those assigned to resiquimod had a longer median time to first recurrence than those assigned to vehicle (169 versus 57 days; p=0.006), with the group receiving 0.01% thrice weekly having the longest median time to first recurrence (>195 days) [7]. In the second trial, participants with anogenital herpes applied resiquimod 0.01% or vehicle gel topically to herpes lesions 2 times weekly for 3 weeks and then collected daily anogenital swabs for 60 days for HSV detection. Recurrences during the subsequent 7 months were treated with study gel. During the final treatment-free 60 days, participants again collected daily swabs to assess shedding. The median lesion and shedding rates were statistically significantly lower for resiquimod compared with vehicle recipients during the initial and final sampling periods, and resiquimod recipients tended to have a longer time to first recurrence than vehicle recipients (median 41 versus 28 days, p=0.25) [8]. These

data from smaller trials suggested that resiquimod modifies the natural history of established HSV infection. In the third trial, participants with anogenital herpes applied resiquimod 0.01% or vehicle gel topically to herpes lesions 2 times weekly for 3 weeks within 24 hours of recurrence onset and had daily lesion assessments and sampling for HSV DNA PCR for 21 days or until investigator-determined healing. No difference was observed between arms in median time to healing, maximum severity scores for investigator- or participant-assessed local skin signs/symptoms, or time to cessation of viral shedding [12].

Why were the Phase III results different from the published results of 3 phase II trials of topical resiquimod [7, 8, 12]? Differences between the trials in dosing methodology, timing, proportion of recurrences which were HSV positive by PCR, or severity of disease in participants could be partially or completely responsible for the different findings. In the phase II efficacy trials, participants reported to the trial center within 24 hours of the qualifying recurrence and received investigator-measured amounts of study drug dispensed from a tube in the center during the day, whereas in the Phase III trials participants reported to the trial center within 36 hours of the qualifying recurrence and applied study drug later that evening at home from a single-use sachet. The increased delay between epithelial viral replication and drug application could have adversely affected drug efficacy in the Phase III trials, especially if it resulted in less time with both resiquimod and HSV present together on the anogenital mucosa. The Phase III trials also had a lower day 1 virological yield than the Phase II trials (74% in Trial 1, 48% in Trial 2, and 69% in the RESI/VAL and 77% in the RESI/PLA groups in Trial 3, compared with 85% by culture in the Spruance Phase II trial) despite a more sensitive method of detecting HSV, although the Phase III trial PCR tests were performed in different labs for each trial hampering direct comparison. However, the lack of efficacy seen in the Phase III trials

even when restricted to virologically-confirmed recurrences suggests that lower virologic yield in the Phase III compared to Phase II trials is not responsible for the different outcomes. Reported adherence, measured by participant diary and return of used and unused study drug sachets, was quite good in the Phase III trials, with ≥ 89% of all trial participants in all 3 trials in all randomized groups applying all 6 topical doses during the initial treatment cycle. Thus inadequate adherence is unlikely to account for lack of efficacy, although it is possible that self-application of the study drug in the Phase III trials had an adverse effect on efficacy if participants did not effectively apply an adequate amount of drug directly to the herpes lesions. It is also possible that the relatively high dropout rate in the Phase III trials masked a small effect, or that the favorable results in the 2 Phase II trials with efficacy assessments were due to chance alone.

The results are also consistent with resiquimod having a small effect on HSV-2 reactivation after being applied directly to anogenital mucosa with HSV-2 present. Because HSV shedding occurs more frequently than clinical reactivation of anogenital herpes [13], shedding may be a more sensitive (and objective) measure of the pharmacologic efficacy of resiquimod at reducing future recurrences. Additional studies would need to be conducted to optimize this antiviral effect and translate it into clinical benefit. Thus further investigation of immunomodulation, perhaps with better TLR 7 and 8 agonists or other agents, as a treatment strategy for anogenital herpes is warranted.

REFERENCES

1. Seroprevalence of herpes simplex virus type 2 among persons aged 14-49 years--United States, 2005-2008. MMWR Morb Mortal Wkly Rep Apr 23;59(15):456-9.
2. Ahonen CL, Gibson SJ, Smith RM, et al. Dendritic cell maturation and subsequent enhanced T-cell stimulation induced with the novel synthetic immune response modifier R-848. Cellular Immunology **1999** 1999/10/10;197(1):62-72.
3. Wagner TL, Ahonen CL, Couture AM, et al. Modulation of TH1 and TH2 cytokine production with the immune response modifiers, R-848 and imiquimod. Cellular Immunology **1999** 1999/1/10;191(1):10-9.
4. Hemmi H, Kaisho T, Takeuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol **2002** Feb;3(2):196-200.
5. Jurk M, Heil F, Vollmer J, et al. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. Nat Immunol **2002** 2002/06//print;3(6):499.
6. Bernstein DI, Harrison CJ, Tomai MA, Miller RL. Daily or weekly therapy with resiquimod (R-848) reduces genital recurrences in herpes simplex virus-infected guinea pigs during and after treatment. J Infect Dis **2001** Mar 15;183(6):844-9.
7. Spruance SL, Tyring SK, Smith MH, Meng TC. Application of a topical immune response modifier, resiquimod gel, to modify the recurrence rate of recurrent genital herpes: a pilot study. J Infect Dis **2001** Jul 15;184(2):196-200.

8. Mark K, 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):1324-31.

9. Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol **1988** Apr;26(4):662-7.

10. Xu F, Markowitz LE, Sternberg MR, Aral SO. Prevalence of circumcision and herpes simplex virus type 2 infection in men in the United States: the National Health and Nutrition Examination Survey (NHANES), 1999-2004. Sex Transm Dis **2007** Jul;34(7):479-84.

11. Alanis MC, Lucidi RS. Neonatal circumcision: a review of the world's oldest and most controversial operation. Obstet Gynecol Surv **2004** May;59(5):379-95.

12. Fife KH, Meng TC, Ferris DG, Liu P. Effect of resiquimod 0.01% gel on lesion healing and viral shedding when applied to genital herpes lesions. Antimicrob Agents Chemother **2008** Feb;52(2):477-82.

13. Wald A, Zeh J, Selke S, Ashley RL, Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. N Engl J Med **1995** Sep 21;333(12):770-5.

14. Prentice RL, Williams BJ, Peterson AV. On the regression analysis of multivariate failure time data. Vol. 68, **1981**:373-9.

Table 1. Summary of trials and demographic characteristics of participants.

	Trial 1		Trial 2		Trial 3		
Location	Europe		United States		United States and Canada		
Randomization	2:1 RESI:VEH		2:1 RESI:VEH		1:1:1 RESI/VAL: RESI/PLA: VEH/PLA		
Total number of participants	255		246		317		
Randomization group	RESI (n=170)	VEH (n=85)	RESI (n=171)	VEH (n=65)	RESI/VAL (n=110)	RESI/PLA (n=104)	VEH/PLA (n=103)
Age in years, median (range)	40 (19-65)	41 (21-64)	38 (18-64)	37 (19-64)	37 (19-67)	36 (19-62)	38 (20-62)
Men, n (%)	82 (48)	38 (45)	66 (39)	28 (37)	36 (33)	36 (35)	34 (33)
Race, n (%)							
White	160 (94)	76 (89)	142 (83)	66 (88)	99 (90)	94 (90)	92 (89)
Black	5 (3)	7 (8)	27 (16)	9 (12)	10 (9)	9 (9)	9 (9)
Native American	1 (1)	1 (1)	0	0	0	0	1 (1)
Asian/Pacific Islander	4 (2)	1 (1)	2 (1)	0	1 (1)	1 (1)	1 (1)
Circumcised, n (% of men)	13 (16)	7 (18)	54 (82)	26 (93)	28 (78)	29 (81)	24 (71)
Annual anogenital herpes recurrences, median (range)	7 (3-24)	8 (4-30)	6 (3-36)	7 (4-36)	8 (4-40)	6.5 (4-28)	8 (4-24)
HSV-1 seropositive, n (%)	104 (61)	49 (58)	78 (46)	49 (65)	52 (50)	72 (65)	53 (51)
HSV-2 seropositive, n (%)	156 (92)	78 (92)	157 (92)	72 (96)	98 (95)	105 (95)	101 (97)
Positive HSV PCR swab, qualifying recurrence, n (%)	126 (74)	62 (73)	78 (46)	41 (55)	71 (69)	85 (77)	75 (72)
Positive HSV PCR swab, any time during trial, n (%)	150 (88)	76 (89)	127 (74)	58 (77)	94 (90)	98 (89)	88 (85)

Abbreviations: RESI: resiquimod
VEH: vehicle
VAL: valacyclovir

PLA: oral placebo
HSV: herpes simplex virus
PCR: polymerase chain reaction

Table 2. Efficacy of resiquimod compared to vehicle in 3 randomized clinical trials (intent-to-treat analysis).

	Trial 1			Trial 2			Trial 3			
Outcome	RESI	VEH	p value	RESI	VEH	p value	RESI/ VAL	RESI/ PLA	VEH/ PLA	p value
Median days to first recurrence	47	41	0.13	44	33	0.44	35	39	34	0.87
Median annualized recurrence rate	3.1	3.2	0.77	3.7	4.1	0.11	4.2	4.2	4.3	0.90
Median lesion rate (%)	20	14	<**0.001**	20	19	0.24	22	21	16	**0.007**
Median recurrence duration (days)	15	9	<**0.001**	13	9	<**0.001**	10	13	9	<**0.001**
Median days to healing of initial recurrence	18	10	<**0.001**	19	13	0.16	14	16	8	<**0.001**

Abbreviations: RESI: resiquimod
VEH: vehicle
VAL: valacyclovir
PLA: oral placebo

Table 3. Adverse events: Investigator-assessed local skin signs at site of drug application during initial treatment cycle.

	Trial 1			Trial 2			Trial 3			
Moderate to severe local skin sign	RESI	VEH	p value	RESI	VEH	p value	RESI/ VAL	RESI/ PLA	VEH/ PLA	p value
Erythema	57%	41%	**0.017**	47%	32%	**0.006**	38%	42%	30%	NS
Scabbing	14%	8%	**0.044**	16%	21%	NS	15%	23%	16%	NS
Erosion/ulceration	47%	34%	**0.007**	28%	20%	**0.003**	31%	32%	25%	NS

Abbreviations: RESI: resiquimod
VEH: vehicle
VAL: valacyclovir
PLA: oral placebo

Figure 1. Participant flow diagram for 3 randomized controlled trials.

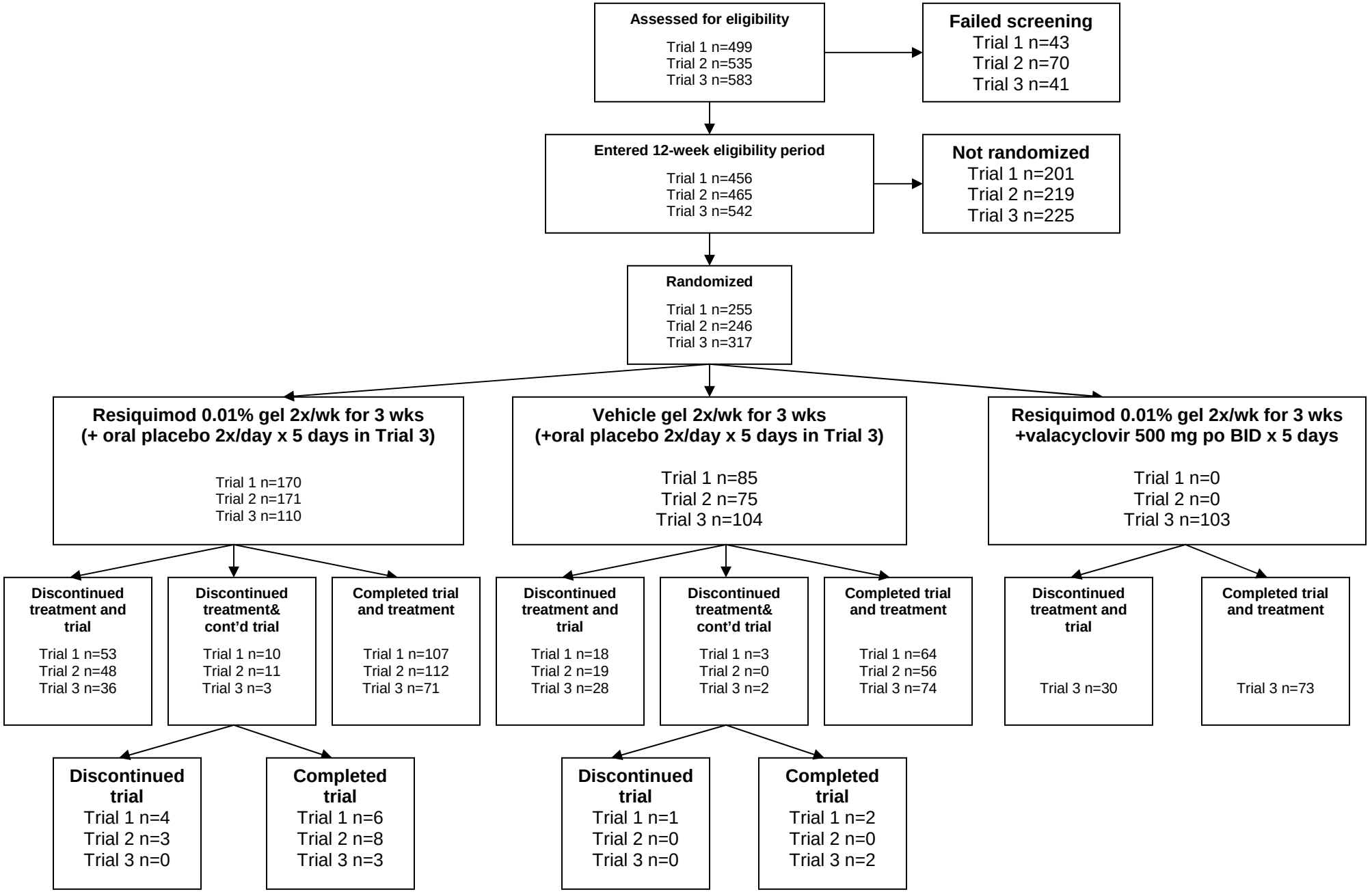
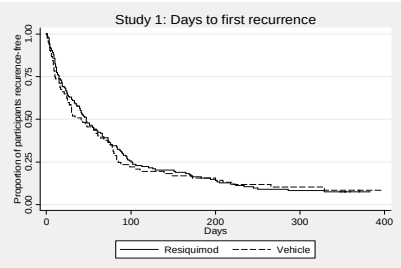
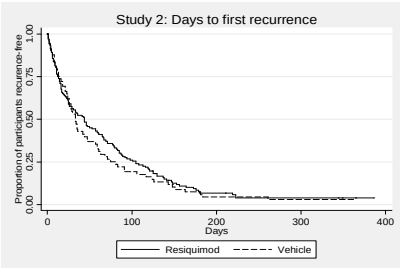


Figure 2. Days to first herpes recurrence in Trial 1 (a), Trial 2 (b), and Trial 3 (c), and days to resolution of randomization recurrence in Trial 1 (d), Trial 2 (e), and Trial 3 (f).

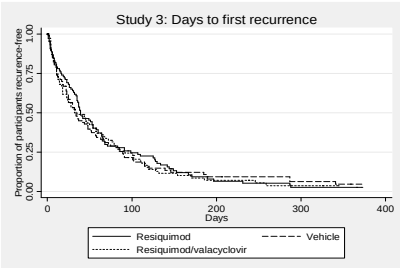
a.



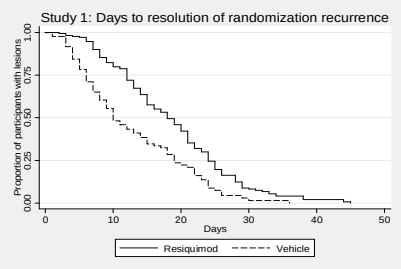
b.



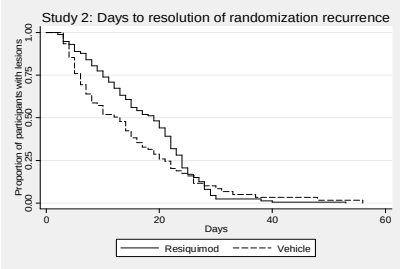
c.



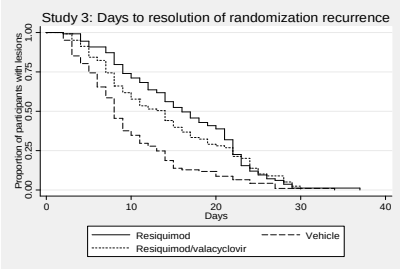
d.



e.



f.



Chapter 4: Incidence and Clinical Management of Newly Diagnosed Symptomatic Genital Herpes in Western Washington, 2008-2009

ABSTRACT

Background: Incidence and clinical management of newly diagnosed symptomatic genital herpes is ill-defined.

Methods: We used surveillance data collected in King and Pierce Counties, Washington, to identify persons ≥ 18 years of age with newly diagnosed symptomatic genital herpes, and interviewed patients and their clinicians regarding treatment. Patients were eligible if they had newly diagnosed symptomatic genital herpes, spoke English, and their provider agreed.

Results: We interviewed clinicians regarding 496 (79%) of 627 reported eligible cases, and 240 (38%) case patients. Among all reported cases, the median age was 27 (range 18-93) years; 75% were female, 66% White, 15% Black, and 94% heterosexual. Incidence decreased with age and was almost 3 times higher in women than men and in Blacks than Whites. Among 472 cases with a lesion culture and/or PCR result reported, 60% revealed HSV-2 (of which 37% were seronegative for HSV-2), 35% HSV-1 (of which 55% were seronegative for HSV-1), and 1% both. Patients reported condom use was discussed in 75% of clinical encounters, suppressive therapy in 69%, and suppressive therapy to decrease transmission in 39%; 30% reported taking suppressive therapy. Only 26% of patients correctly responded that condoms are ~50% effective in preventing herpes transmission, and only 16% that suppressive therapy is ~50% effective in preventing transmission, with most (55%) unsure of the effect of suppressive therapy on transmission. Both discussion of suppressive therapy and discussion of suppressive therapy for

transmission prevention were associated with suppressive therapy use ($p<0.001$ for both), as was HSV type 2 as opposed to type 1 ($p=0.016$).

Conclusions: Although clinicians usually discuss condoms and suppressive therapy with patients diagnosed with genital herpes, only a minority discuss suppressive therapy to prevent transmission and only 30% of patients take suppressive therapy. Most patients could not estimate the effectiveness of condoms or suppressive therapy in preventing transmission.

INTRODUCTION

Genital herpes is the most prevalent STD in the United States [1] with at least 50 million Americans infected with HSV-2 and many others infected with HSV-1 in the genital area [2]. Although an increasing proportion of diagnosed symptomatic first episode genital herpes is caused by HSV-1 [3-8], most recurrent genital herpes is caused by HSV-2 as HSV-1 is less likely to recur symptomatically in the genital area [9, 10]. Unlike most states, in Washington State an initial episode of symptomatic genital herpes is a reportable disease. Only two other states (Arizona and Nebraska) require name-based reporting of adults with genital herpes. No population-based descriptions of persons newly diagnosed with genital herpes have been published, thus the current incidence and community standard-of-care in clinical management of newly diagnosed symptomatic genital herpes is ill-defined. Moreover, it is unknown to what extent community clinicians discuss preventive strategies with patients newly diagnosed with genital herpes. Understanding clinician practices related to genital herpes diagnosis, clinical care, and prevention is an important step in creating an effective public health program to control genital herpes. We sought to describe the demographic characteristics and sexual history of a population-based sample of persons newly diagnosed with genital herpes in western Washington, and to examine patient, partner, and clinician characteristics associated with suppressive antiviral therapy use.

METHODS

Using public health case reports, we prospectively identified persons newly diagnosed with symptomatic genital herpes ≥ 18 years of age who were able to understand and speak English and were reported to Public Health—Seattle & King County with a diagnosis date between September 1, 2008 and August 31, 2009 or to the Pierce County Health Department with a diagnosis date between December 1, 2008 and August 31, 2009, and who resided in each respective Washington State county. Persons who had mistakenly reported recurrent genital herpes, or those with asymptomatic HSV infection diagnosed by serologic assay, were excluded. The clinician reporting each case was contacted by telephone and asked to complete a short supplemental questionnaire, administered over the telephone or faxed, with additional details regarding diagnosis and treatment of the reported case. The clinician was also asked to give permission for public health officials to contact the patient for a telephone interview. Because some clinicians gave blanket permission for public health officials to contact any of their reported cases, a few patient interviews did not have an accompanying clinician questionnaire regarding the interviewed patient.

If permission was obtained, the patient was contacted by telephone and, using a standardized questionnaire, information was elicited about demographic characteristics, past history of genital herpes and any subsequent herpes outbreaks, and treatment, including treatment of the initial outbreak and any recurrences thus far. Participants were also asked what treatment options (episodic therapy, suppressive therapy, or no therapy) were discussed with them by the diagnosing clinician and any clinicians subsequently seen. In addition, participants were asked about the number and type of sex partners in the 1 month prior to the beginning of lesions and about condom use and subsequent disclosure of infection to these partners. Because

this was a public health surveillance project, institutional review board approval was not required nor obtained, but all patients gave verbal informed consent prior to the interview.

Statistical analysis

The annual incidence of newly diagnosed symptomatic genital herpes among King County residents ≥ 18 years of age was calculated by dividing the number of newly diagnosed and reported symptomatic cases ≥ 18 years of age over the 12 month period by the July 1, 2009 United States census population estimate for persons ≥ 18 years of age for King County, with a similar method used for Pierce County except that the 9 months of cases was annualized by multiplying the Pierce County case counts by 1.33 to obtain 12 month estimates for Pierce County case counts [11]. For incidence calculations, the two cases with missing county of residence were distributed between the two counties based on the relative frequency of King versus Pierce County cases. Similar methods were used to calculate incidence by age group, sex, and race/ethnicity, with missing race/ethnicity imputed based on the distribution of cases of known race/ethnicity by county. The Wilcoxon rank-sum test was used to test whether duration of symptoms prior to herpes diagnosis differed between those with documented new versus old HSV infection. Pearson chi-square tests were used to test the significance of patient, partner, and clinician characteristics associated with suppressive antiviral therapy use, with a p value < 0.05 considered significant. All statistical analyses were done using Intercooled Stata 9.1 (College Station, TX).

RESULTS

Seven hundred one cases ≥ 18 years of age were reported to Public Health—Seattle & King County with a diagnosis date between September 1, 2008 and August 31, 2009 or to the Pierce County Health Department with a diagnosis date between December 1, 2008 and August 31, 2009. Of these 701 cases, 34 had been previously diagnosed with symptomatic genital herpes and 40 were diagnosed by serology in the absence of symptoms. Of the remaining 627 symptomatic newly diagnosed and reported cases ≥ 18 years of age, 496 (79%) cases had completed clinician questionnaires and 240 (38%) cases had completed patient interviews, including 215 cases with both a completed clinician questionnaire and patient interview, 281 with a completed clinician questionnaire only, and 25 with a patient interview only. Of the 281 cases with a completed clinician questionnaire and no patient interview, reasons for the lack of patient interview included unable to contact the patient (n = 173), patient refused (n = 53), clinician refused (n = 40), patient did not speak English (n = 14), and unknown (n = 1). Demographic characteristics of cases with completed clinician questionnaires and those with completed patient interviews were similar to all reported cases (Table 1). The median age of all reported cases was 27 years (range 18-93 years). Of all reported cases, 75% were women and 94% were heterosexual.

Incidence of genital herpes

The annual incidence rate of newly diagnosed, symptomatic genital herpes among adults was 30.1 cases per 100,000 in King County and 39.0 cases per 100,000 in Pierce County (Table 1). In both counties, incidence decreased with age and was 7 times higher in 18-24 year olds and 4 times higher in 25-29 year olds than in persons 30 years of age or older. Incidence was almost

3 times higher in women than men, almost 3 times higher in Blacks than Whites, and in Asian/Pacific Islanders half that of Whites.

Diagnosis and treatment of initial episode of genital herpes

Among 496 cases with completed clinician questionnaires, 475 (96%) patients had herpes lesions present at the time of clinical evaluation, for a median of 4 days (Table 2). Of those with lesions, most (n = 414, 87%) had genital lesions, 17 (4%) had perirectal lesions, lesion location was unknown for 33 (7%), and the remainder had lesions in less common places (buttocks or sacrum, n = 7; suprapubic, n = 2; cervix only, n = 1; and posterior thigh, n = 1). Those without lesions had other symptoms consistent with herpes such as dysuria, hemorrhoidal symptoms, vaginal pruritis, or vulvar pain.

Thirty-six (7%) patients were diagnosed by physical exam only, 473 (95%) patients had a viral culture obtained, 17 (3%) had an HSV PCR obtained, and 160 (32%) had an HSV serology obtained. Among 472 cases with a viral culture and/or HSV PCR result reported, 166 (35%) cases had HSV-1 isolated, 282 (60%) cases HSV-2, 6 (1%) both HSV-1 and HSV-2, and 10 (2%) untyped HSV.

To determine what proportion of persons with newly diagnosed symptomatic genital herpes had a new HSV infection as opposed to a first symptomatic episode of reactivation after an asymptomatic initial infection, we examined the subset of cases with both serology and viral culture or PCR results available. Of 49 cases with a type-specific serologic result and a culture or PCR result positive for HSV-1 only, HSV serology was negative for 26 (53%) and positive for HSV-2 only for 2 (4%) indicating recent HSV-1 infection for 57%. The remaining 21 (43%) patients with a culture or PCR result positive for HSV-1 only also had serologic evidence of

HSV-1 infection. Of 77 cases with a type-specific serologic result and a culture or PCR positive for HSV-2, HSV serology was negative for 20 (26%) and positive for HSV-1 only for 9 (12%) indicating recent HSV-2 infection in 38%. The remaining 48 (62%) patients with a culture or PCR positive for HSV-2 also had serologic evidence of HSV-2 infection. Thus overall, 57 (45%) of 126 evaluable infections were new HSV infections, with the remainder being first symptomatic episodes of older infections. The median duration of symptoms prior to herpes diagnosis was similar in those with new and old infections (4 versus 5 days respectively, $p = 0.15$).

Of 485 case patients for whom information was available on acute antiviral treatment of the initial episode, clinicians reported treating 425 (88%) with the antivirals shown in Table 2. Written information on genital herpes was provided to 64% of patients. Only 29% of patients had a follow up appointment scheduled, with an additional 8% referred elsewhere for follow up.

Education on transmission prevention and suppressive therapy

Among 240 case patients who completed the interview, 180 (75%) reported that the clinician discussed condom use, 154 (64%) that they were counseled to notify recent and 126 (53%) to tell future sex partners about their diagnosis. One hundred fifty-three (64%) reported that the clinician discussed suppressive therapy with them, 93 (39%) specifically as a way to reduce transmission of genital herpes. Ninety-three (39%) case patients reported that the clinician offered to prescribe suppressive therapy and 72 (30%) reported taking suppressive therapy. Of 237 case patients who answered the question, “How effective do you think condoms are in transmission prevention?” only 61 (26%) correctly replied that condoms were about 50% effective in preventing transmission of genital herpes, while 102 (43%) incorrectly thought

condoms were close to 100% effective, 31 (13%) thought condoms were less than 50% effective, and 43 (18%) didn't know. The majority of case patients [130 (55%) of 235 who responded)] didn't know how effective suppressive therapy is for transmission prevention, while 54 (23%) thought incorrectly that it was close to 100% effective, 37 (16%) correctly identified that it is about 50% effective, and 14 (6%) incorrectly stated that it is less than 50% effective.

Factors associated with taking suppressive therapy

Among 212 patients with both a completed clinician and case interview and a response to the suppressive therapy question, taking suppressive therapy was more common among those with a lesion culture or PCR positive for HSV-2 than HSV-1 (36% versus 20%, $p = 0.016$), among those diagnosed by someone other than their primary care provider as opposed to their primary care provider (35% versus 20%, $p = 0.022$), and among those whose clinician discussed suppressive therapy with them (40% versus 11%, $p < 0.001$) and whose clinician discussed suppressive therapy specifically as a way to prevent giving other people herpes (45% versus 18%, $p < 0.001$) (Table 3). Patient demographics and recent number of sexual partners were not associated with suppressive therapy use, nor were other clinician demographic or practice setting characteristics.

Sexual history and partner notification

Among 231 interviewed patients who responded to questions about recent vaginal, anal, and oral sex partners, 192 (83%) reported only one partner (the likely source partner) in the one month prior to first developing symptoms of genital herpes. Seventeen (7%) patients reported 2 partners over the same time period, and 6 (3%), 3 (1%) and 3 (1%) patients reported 3, 4, and 5

sex partners over the same time period, respectively. Ten (4%) patients reported no partners in the one month prior to developing symptoms; of these, 2 had serologically documented old infection and 8 had no serologic results. Nine had a positive lesion culture or PCR result showing HSV-2, and one HSV-1.

The 240 interviewed index patients reported a total of 271 partners in the one month prior to HSV diagnosis; detailed information was available for 254 partners. Index patients reported that 53 (21%) partners had a prior herpes diagnosis, of whom 50 were told by the index patient about the index patient's new herpes diagnosis. An additional 162 (64%) partners without a known prior herpes diagnosis were notified by the index patient of the index patient's diagnosis, 35 (14%) partners were not notified, and partner notification was unknown for 4 (2%).

DISCUSSION

We provide the first truly population-based description of adults newly diagnosed with symptomatic genital herpes, including incidence, virologic and serologic characteristics, and current community-based clinical practices around diagnosis, clinical management, and discussion of transmission prevention strategies. Our findings confirm and extend those of many prior studies and provide additional information helpful towards developing a public health approach to genital herpes prevention.

In the HERPEVAC phase 3 herpes vaccine study for women conducted at 50 sites in the United States and Canada [12], 18-30 year old healthy women seronegative for HSV-1 and HSV-2 who received the control hepatitis A vaccine (Havrix) had a rate of symptomatic genital herpes caused by HSV-1 of 0.5 per 100 person-years and by HSV-2 of 0.4 per 100 person-years, for a total rate of symptomatic genital herpes of 0.9 per 100 person-years [13]. Fifty-seven percent of symptomatic genital cases were attributed to HSV-1 and 43% to HSV-2 [13]. By comparison, among the general population of 18-29 year old women in the two western Washington counties in our study, we found an annual incidence rate of newly diagnosed symptomatic genital herpes of 140.4 cases per 100,000 population (approximately 0.14 per 100 person-years), with approximately one-third attributed to HSV-1 and two-thirds to HSV-2. Our incidence rate approximately 6 times lower than that found in the HERPEVAC trial and our higher proportion of genital HSV-2 cases might be due to a number of factors, including 1) the HERPEVAC trial enrolled only HSV seronegative women, whereas 57.1% of the general female United States population aged 20-29 years is seropositive for HSV-1 and 15.6% for HSV-2 [14] and therefore no longer at risk for acquisition of these viruses; 2) the HERPEVAC population may have been at higher risk than the general population of healthy young women because they

choose to enroll in a herpes vaccine study; 3) some newly diagnosed symptomatic genital herpes cases who presented for care may not have been reported to the Seattle & King County and Pierce County health departments; and 4) western Washington may have a lower incidence of newly diagnosed symptomatic genital herpes than the rest of the United States population, in part due to demographic differences (western Washington has a lower percentage of Blacks and a higher percentage of Asian/Pacific Islanders than the general United States population).

Similar to our study, the HERPEVAC study found a higher incidence of symptomatic genital HSV-2 infections among Blacks than Whites. United States population-based HSV-2 seroprevalence data also show an HSV-2 seroprevalence 3 times higher among non-Hispanic Blacks than among non-Hispanic Whites, similar HSV-2 seroprevalence among Whites and Mexican Americans, and 2 times higher among women than men [14]. Very little published data on HSV or genital herpes incidence or seroprevalence among United States Asian/Pacific Islanders exist, although a prenatal clinic-based HSV-2 seroprevalence study in London showed an HSV-2 seropositivity rate among Britons of Asian descent was half that of White Britons [15], and HSV-2 seropositivity rates have been described to be lower in Asian than African or Central or South American countries [16].

Among reported newly diagnosed symptomatic genital herpes cases in western Washington, we found that one-third were caused by HSV-1 and two-thirds by HSV-2. The proportion of newly diagnosed genital herpes cases attributable to HSV-1 varies from 14%-78% among studies [8, 17]. Also similar to other studies [13, 18-21], we found that many first symptomatic episodes of genital herpes represented reactivation disease rather than primary infection, with only 57% of persons in our study with a first symptomatic episode of genital

HSV-1 infection being HSV-1 seronegative and only 38% of persons with a first symptomatic episode of genital HSV-2 infection being HSV-2 seronegative.

Our study showed that diagnostic testing and treatment among reported cases appeared to be appropriate, and that most clinicians discussed condoms (75%), suppressive therapy (69%), and notification of recent (64%) and future (53%) sex partners with the patient. However, a sizeable minority did not. Only a minority of patients (37%) were offered routine follow up care and only 30% took suppressive therapy. Suppressive therapy was more often taken by patients with newly diagnosed genital HSV-2 than HSV-1 infection (36% versus 20%, $p = 0.016$), consistent with both the increased likelihood of symptomatic recurrence of genital HSV-2 compared with genital HSV-1 [10] and the documented efficacy of suppressive antiviral therapy at preventing genital HSV-2 transmission [22]. However, patient knowledge of transmission prevention modalities was incomplete, as only 26% of patients correctly responded that condoms are approximately 50% effective in preventing herpes transmission, and only 16% that suppressive therapy is approximately 50% effective in preventing transmission, with most (55%) unsure of the effect of suppressive therapy on transmission. Most patients (83%) reported notifying prior partners about their new genital herpes diagnosis. Several partially protective strategies exist of the prevention of HSV-2 transmission among sexually active persons, including antiviral therapy of the source partner [22], condom use [23-25], disclosure of HSV-2 serostatus to sex partners [26], and limiting the frequency of sexual encounters or the number of partners. However, these strategies can only be used effectively if patients are made aware of them.

Strengths of this study include both its population-based sample and that information was obtained from both the treating clinician and the newly diagnosed patient. Limitations include

cases from only one geographic region (western Washington) and thus findings may not be generalizable to the entire United States population or other regions, and the uncertainty regarding how representative reported cases are of all diagnosed cases. Although our response rate for the case interviews was sub-optimal, our analysis of baseline demographic characteristics suggests that interviewed cases were representative of all reported cases.

Our findings suggest that although many clinicians are providing excellent care for newly diagnosed genital herpes, significant gaps in care remain, including low utilization of suppressive therapy and limited discussions related to partner notification and disclosure of HSV status to future partners. Further research is needed to identify economical ways to improve the care and education of persons diagnosed with genital herpes; public health departments may be able to assist by promoting higher standards of genital herpes care in their communities.

REFERENCES

1. Patrick DM, Money D. Debate: the argument for. Should every STD clinic patient be considered for type-specific serological screening for HSV? Herpes **2002** Jul;9(2):32-4.
2. Corey L, Handsfield HH. Genital herpes and public health: addressing a global problem. JAMA **2000** February 9, 2000;283(6):791-4.
3. Lafferty WE, Downey L, Celum C, Wald A. Herpes simplex virus type 1 as a cause of genital herpes: impact on surveillance and prevention. J Infect Dis **2000** Apr;181(4):1454-7.
4. Lowhagen GB, Tunback P, Andersson K, Bergstrom T, Johannisson G. First episodes of genital herpes in a Swedish STD population: a study of epidemiology and transmission by the use of herpes simplex virus (HSV) typing and specific serology. Sex Transm Infect **2000** Jun;76(3):179-82.
5. Nilsen A, Myrmel H. Changing trends in genital herpes simplex virus infection in Bergen, Norway. Acta Obstet Gynecol Scand **2000** Aug;79(8):693-6.
6. Tran T, Druce JD, Catton MC, Kelly H, Birch CJ. Changing epidemiology of genital herpes simplex virus infection in Melbourne, Australia, between 1980 and 2003. Sex Transm Infect **2004** Aug;80(4):277-9.
7. Ribes JA, Steele AD, Seabolt JP, Baker DJ. Six-year study of the incidence of herpes in genital and nongenital cultures in a central Kentucky medical center patient population. J Clin Microbiol **2001** Sep;39(9):3321-5.
8. Roberts CM, Pfister JR, Spear SJ. Increasing proportion of herpes simplex virus type 1 as a cause of genital herpes infection in college students. Sex Transm Dis **2003** Oct;30(10):797-800.

9. Engelberg R, Carrell D, Krantz E, Corey L, Wald A. Natural history of genital herpes simplex virus type 1 infection. Sex Transm Dis **2003** Feb;30(2):174-7.

10. Lafferty WE, Coombs RW, Benedetti J, Critchlow C, Corey L. Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. N Engl J Med **1987** Jun 4;316(23):1444-9.

11. Intercensal Estimates of the Resident Population by Age, Sex, Race, and Hispanic Origin for Counties in Wasington State: April 1, 2000 to July 1, 2010. U.S. Census Bureau, Population Division, **Release Date: October 2012**.

12. Belshe RB, Leone PA, Bernstein DI, et al. Efficacy results of a trial of a herpes simplex vaccine. N Engl J Med Jan 5;366(1):34-43.

13. Bernstein DI, Bellamy AR, Hook EW, 3rd, et al. Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. Clin Infect Dis Feb;56(3):344-51.

14. 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** August 23, 2006;296(8):964-73.

15. Ades AE, Peckham CS, Dale GE, Best JM, Jeansson S. Prevalence of antibodies to herpes simplex virus types 1 and 2 in pregnant women, and estimated rates of infection. J Epidemiol Community Health **1989** Mar;43(1):53-60.

16. Weiss H. Epidemiology of herpes simplex virus type 2 infection in the developing world. Herpes **2004** Apr;11 Suppl 1:24A-35A.

17. Bruisten SM, Cairo I, Fennema H, et al. Diagnosing genital ulcer disease in a clinic for sexually transmitted diseases in Amsterdam, The Netherlands. J Clin Microbiol **2001** Feb;39(2):601-5.

18. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. N Engl J Med **2000** Mar 23;342(12):844-50.

19. Langenberg AGM, Corey L, Ashley RL, Leong WP, Straus SE, The Chiron HSV Vaccine Study Group. A prospective study of new infections with herpes simplex virus type 1 and type 2. N Engl J Med **1999** November 4, 1999;341(19):1432-8.

20. Bernstein DI, Lovett MA, Bryson YJ. Serologic analysis of first-episode nonprimary genital herpes simplex virus infection. Presence of type 2 antibody in acute serum samples. Am J Med **1984** Dec;77(6):1055-60.

21. Diamond C, Selke S, Ashley R, Benedetti J, Corey L. Clinical course of patients with serologic evidence of recurrent genital herpes presenting with signs and symptoms of first episode disease. Sex Transm Dis **1999** Apr;26(4):221-5.

22. 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** January 1, 2004;350(1):11-20.

23. Wald A, Langenberg AG, Link K, et al. Effect of condoms on reducing the transmission of herpes simplex virus type 2 from men to women. JAMA **2001** Jun 27;285(24):3100-6.

24. Gottlieb SL, Douglas JM, Jr., Foster M, et al. Incidence of herpes simplex virus type 2 infection in 5 sexually transmitted disease (STD) clinics and the effect of HIV/STD risk-reduction counseling. J Infect Dis **2004** Sep 15;190(6):1059-67.

25. Wald A, Langenberg AG, Krantz E, et al. The relationship between condom use and herpes simplex virus acquisition. Ann Intern Med **2005** Nov 15;143(10):707-13.

26. Wald A, Krantz E, Selke S, Lairson E, Morrow RA, Zeh J. Knowledge of partners' genital herpes protects against herpes simplex virus type 2 acquisition. J Infect Dis **2006** July 1, 2006;194(1):42-52.

Table 1. Demographic characteristics of patients ≥ 18 years of age reported with newly diagnosed symptomatic genital herpes, King and Pierce Counties, western Washington, 9/1/08 – 8/31/09.

Demographic characteristic	All reported cases (n = 627) n	(%)	Annual incidence per 100,000 population	Incidence rate ratio	Cases with completed clinician interviews (n = 496) n	(%)	Cases with completed case interviews (n = 240) n	(%)
Age								
18-19 years	68	(11)	106.6	6.75	51	(10)	29	(12)
20-24 years	191	(30)	114.5	7.25	147	(30)	68	(28)
25-29 years	127	(20)	62.5	3.96	102	(21)	51	(21)
30+ years	241	(38)	15.8	ref	196	(40)	92	(38)
Sex								
Female	471	(75)	48.1	2.86	378	(76)	189	(79)
Male	156	(25)	16.8	ref	118	(24)	51	(21)
Race/ethnicity*								
White	362	(66)	30.5	ref	305	(68)	165	(69)
Black	82	(15)	85.4	2.80	61	(14)	32	(13)
Hispanic	39	(7)	32.9	1.08	33	(7)	17	(7)
Asian/Pacific Islander	36	(7)	16.7	0.55	29	(6)	13	(5)
Other/multiracial	28	(5)	45.7	1.50	23	(5)	11	(5)
Sexual orientation†								
Heterosexual	501	(94)	§	§	412	(93)	230	(97)
MSM/MSB	14	(3)	§	§	13	(3)	3	(1)
WSW/WSB	20	(4)	§	§	18	(4)	4	(2)
County of residence‡								
King	451	(72)	30.1	ref	388	(79)	184	(77)
Pierce	174	(28)	39.0	1.30	106	(21)	56	(23)

*Missing for 80 (13%) all reported cases, 45 (9%) cases with completed clinician interviews, and 2 (1%) cases with completed case interviews.

†Missing for 92 (15%) all reported cases, 53 (11%) cases with completed clinician interviews, and 3 (1%) cases with completed case interviews.

‡Missing for 2 all reported cases and 2 cases with completed clinician interviews.

§Unable to calculate due to lack of denominator data.

MSM/MSB: Men who have sex with men/men who have sex with both men and women.

WSW/WSB: Women who have sex with women/women who have sex with both women and men.

Table 2. Diagnosis and acute treatment of newly diagnosed symptomatic genital herpes, King and Pierce Counties, western Washington, 9/1/08 – 8/31/09.

Diagnosis and treatment	Cases with completed clinician interviews (n = 496) n	(%)
Herpes lesions present at time of clinical evaluation?	475	(96)
Diagnostic method		
Physical exam only	36	(7)
Lesion culture obtained	473	(95)
Lesion PCR obtained	17	(3)
Serology obtained	160	(32)
Lesion culture/PCR result[*]		
HSV-1	166	(35)
HSV-2	282	(60)
Both HSV-1 and HSV-2	6	(1)
Positive, not type-specific	10	(2)
Negative	8	(2)
Treatment of current episode[†]		
Acyclovir	297	(61)
Valacyclovir	117	(24)
Famciclovir	11	(2)
Other (antibacterial, antifungal, or analgesic)	9	(2)
None	51	(11)
Written information given to patient[‡]	263	(64)
Follow-up appointment scheduled[§]		
Yes	135	(29)
No	298	(63)
No, but referred elsewhere for follow-up	37	(8)

[*]Among 472 cases with a lesion culture and/or PCR result reported.
[†]Missing for 11 cases.
[‡]Missing/unknown for 82 cases.
[§]Missing/unknown for 26 cases.
PCR: polymerase chain reaction
HSV-1: herpes simplex virus type 1
HSV-2: herpes simplex virus type 2

Table 3. Univariate predictors of taking suppressive therapy among 212 participants with completed clinician and case interviews and response to suppressive therapy question, King and Pierce Counties, western Washington, 9/1/08 – 8/31/09.

	Total		Took suppressive therapy		
	n	(%)	n	(%)	p value
Patient characteristics					
Age					0.18
18-19 years	26	(12)	7	(27)	
20-24 years	60	(28)	21	(35)	
25-29 years	44	(21)	17	(39)	
≥30 years	82	(39)	18	(22)	
Sex					0.25
Male	44	(21)	10	(23)	
Female	168	(79)	53	(32)	
Race/ethnicity[*]					0.38
White	147	(70)	46	(31)	
Black	27	(13)	10	(37)	
Hispanic	14	(7)	2	(14)	
Asian	13	(6)	2	(15)	
Multiracial/other	10	(5)	2	(20)	
Highest level of education completed					0.99
Less than high school	13	(6)	4	(31)	
High school graduate	48	(23)	14	(29)	
Some college	90	(42)	26	(29)	
Four year college graduate or higher	61	(29)	19	(31)	
Marital status					0.67
Single	115	(54)	36	(31)	
Living with a partner	39	(18)	13	(33)	
Married	37	(17)	8	(22)	
Separated/divorced/widowed	21	(10)	6	(29)	
County of residence					0.97
King	172	(81)	51	(30)	
Pierce	40	(19)	12	(30)	
Number of sex partners in the one month prior to first lesions[†]					0.33
Zero or one partner	182	(88)	53	(29)	
More than one partner	26	(13)	10	(38)	
Clinician characteristics					
Clinician type[‡]					0.39
Nurse practitioner	98	(50)	33	(34)	
Physician	77	(39)	20	(26)	

Physician's assistant	20	(10)	4	(20)	
Other	2	(1)	0	(0)	
Clinician sex[§]					0.25
Male	47	(22)	11	(23)	
Female	162	(78)	52	(32)	
Years since completion of training[¶]					0.12
0-10 years	85	(48)	31	(36)	
11-20 years	43	(24)	15	(35)	
21+ years	50	(28)	10	(20)	
Practice setting in which herpes diagnosed[□]					0.33
Primary care clinic	70	(33)	15	(21)	
STD/family planning clinic	86	(41)	27	(31)	
Urgent care/emergency room	17	(8)	7	(41)	
Obstetrics and gynecology office/clinic	24	(11)	9	(38)	
Other	13	(6)	5	(38)	
Primary care provider diagnosed patient?					0.022
Yes	75	(35)	15	(20)	
No	137	(65)	48	(35)	
HSV type by lesion culture or PCR[**]					0.016
Type 1	76	(39)	15	(20)	
Type 2	120	(61)	43	(36)	
Patient report of suppressive therapy discussion					
Clinician discussed suppressive therapy when patient first diagnosed with herpes[††]					<0.001
Yes	139	(67)	55	(40)	
No	70	(33)	8	(11)	
Clinician discussed suppressive therapy to prevent giving other people herpes[‡‡]					<0.001
Yes	86	(44)	39	(45)	
No	111	(56)	20	(18)	

[*]Missing for 1 case.
[†]Missing for 4 cases.
[‡]Missing for 15 cases.
[§]Missing for 3 cases.
[¶]Missing or unknown for 34 cases.
[□]Missing for 2 cases.
[**]Missing for 16 cases.
[††]Missing or unknown for 4 cases.
[‡‡]Missing or unsure for 16 cases.
STD: sexually transmitted disease
HSV: herpes simplex virus
PCR: polymerase chain reaction