The Kinetics of Mucosal Herpes Simplex Virus–2 Infection in Humans: Evidence for Rapid Viral-Host Interactions

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(See the editorial commentary by Quinn, on pages 495–8.)

Background. Herpes simplex virus type 2 (HSV-2) reactivations in the genital tract are responsible for mucocutaneous lesions and transmission and manifest as discrete shedding episodes.

Methods. We analyzed duration, peak copy number, and expansion and decay rates of 1020 shedding episodes in 531 immunocompetent HSV-2–seropositive persons from whom daily swabs of genital secretions were collected.

Results. Viral quantity varied by as much as a multiple of 10 million in a single person over time. Peak episode copy number was distributed approximately evenly from $10^3$ through $10^8$ HSV DNA copies/mL. Median rate of increase was $10^{7.6}$ HSV DNA copies/day during the first 12 hours of an episode and $10^5$ copies/d from episode initiation to peak. These values depended only moderately on episode duration. Median decay rate was $-10^{6.2}$ HSV DNA copies/d during the final 12 hours of an episode and $-10^{3.6}$ copies/d from peak to termination. Episodes lasted a median of 3 days (interquartile range, 1–8 days). Prolonged (>5 days) episodes were associated with nonmonotonic decay.

Conclusions. HSV-2 shedding episodes are notable for rapid expansion and decay and extreme heterogeneity of duration and viral production. The net effect of these dynamic episodes is frequent shedding at high copy numbers.

Herpes simplex virus type 2 (HSV-2) infection is characterized by frequent reactivations [1–5] and resulting viral shedding that allows for transmission during coitus [6–8]. Although most shedding is asymptomatic [1, 4, 5], presence of HSV-2 is also associated with development of genital ulcers [9]. HSV-2 shedding is episodic, and episode duration and viral production vary greatly in an individual [1, 2, 5, 10, 11]. Shedding frequency is defined as percentage of genital swabs that contain HSV DNA above a threshold value, which we define as 150 HSV DNA copies/mL media [12]. Because the polymerase chain reaction (PCR) assay that we use has a linear threshold of 102–109 HSV DNA copies/mL media [12]. Because the polymerase chain reaction (PCR) assay that we use has a linear threshold of 102–109 HSV DNA copies/mL media, shedding can also be evaluated quantitatively. Studies using daily or more frequent sampling revealed a correlation between episode peak copy number and duration; episodes with higher peak copy also coincided with genital lesion formation [1], and high-copy shedding may enhance likelihood of sexual transmission.

We analyzed frequency histograms to describe HSV-2 shedding episode duration, peak HSV DNA copy number, expansion rate, and decay rate. Our findings show that episodes consist of extremely rapid expansion and decay phases in the immunocompetent host. The net effect of highly dynamic episodes is frequent high-level shedding, even though all episodes are effectively terminated.
METHODS

Study Participants
We explored data gathered from participants from whom swab samples of genital skin and mucosa were obtained daily for quantitative detection of HSV by PCR at the University of Washington Virology Research Clinic (Seattle) during 1992–2008. The University of Washington Institutional Review Board approved the studies. All participants signed informed consent. All participants were ≥18 years of age, in good general health, HIV seronegative, and HSV-2 seropositive. Recurrence history was not used as an inclusion or exclusion criterion for the cohort. No participant was using HSV antiviral therapies during swab sample collection. Participants swabbed the entire anogenital area regardless of the presence of visible lesions.

Laboratory Methods
HSV serologic testing was performed using Western blot [13]. Detection of HSV DNA by PCR was performed using a validated collection and detection method [1]. Swab samples of genital secretions were placed into vials containing 1 mL of PCR transport medium and were refrigerated until laboratory processing [14]. The PCR assay used type-common primers to the HSV gene encoding glycoprotein B [15]: Type-specific PCR assay was not performed, because in prior studies, <10% of swab samples were likely to test HSV-1 positive [1]. An internal control was included to ensure that negative swab samples were not caused by inhibition. Laboratory personnel were blinded to clinical data.

Shedding Episode Characteristics
We defined each shedding episode according to peak copy number, expansion rate, and decay rate and used frequency histograms to describe ranges of values for all episodes [16]. For each measure, we only included episodes of known duration (preceded and followed by at least 2 negative swab results). We estimated duration by the number of consecutive swab samples containing at least 150 copies/mL of HSV (preceded and followed by 2 negative swab results). Because swab samples were obtained every 24 hours, episodes could have initiated within 0–24 hours before the first positive swab result of the episode and terminated within 0–24 hours after the last positive swab result. We assumed that swab timing was independent from timing of the episode and, therefore, that the midpoint of this interval would provide an unbiased estimate of duration [17]. Therefore, for duration, we assumed that episodes began 12 hours before the first positive swab result and ended 12 hours after the last positive swab result. Occasionally, only missed swab samples separated successive episodes; using survival analysis with interval censoring, we combined such episodes to form a single episode with longer maximum duration. Survival analysis was used because the longest episodes were most likely to have missing swab samples.

Shedding Episode Peak, Expansion, and Decay
We defined peak episode copy number as the greatest quantity of HSV DNA copies/mL during an episode. Because of rapid kinetics of viral expansion, values for the first positive swab result were, in part, a reflection of duration of the episode. Therefore, we assumed that median episode duration was 12 hours at the first positive swab result. We calculated median exponential expansion slope during the first 12 hours of an episode by dividing the median value for the first positive swab result by 0.5 days. Because of rapid kinetics of viral decay, values for the last positive swab result reflected remaining time in the episode at the time of the swab. Exponential slope of decay during the final 12 hours of an episode was calculated by dividing the median last positive swab result value by 0.5 days.

We calculated rate of increase from initiation to peak of each episode by computing linear regression line slope over copy numbers up to and including maximum copy and setting time of the most proximal negative swab result at 0.5 days before the first positive. We calculated rate of decrease from peak to termination by setting time of termination to 0.5 days after the last positive result. We calculated a median for each slope on the basis of results from all episodes.

We performed separate analyses of episode characteristics for episodes with and without nonmonotonic decay (defined as an increase in HSV load by 0.5 logs after a prior decrease in the episode of at least 0.5 logs) and episodes with and without associated genital lesions.

We used generalized estimating equations to assess associations between episode duration, maximum copy number, and rate of increase and decrease in the same individual [18]. We measured individual correlation for shedding characteristics with use of variance components analysis and examined episode characteristics with use of covariates, including sex, time since acquisition, and HSV-1 coinfection. Noncentrally distributed measures were log transformed before analysis.

RESULTS

Study Participants
We included 531 HSV-2–seropositive persons who contributed at least 30 days of genital swabbing and diaries. Two hundred twenty-one participants (42%) were men. The median age was 39 years (interquartile range [IQR], 31–48 years; range, 20–76 years). Two hundred thirty-three participants (44%) were also infected with HSV-1, whereas 437 (82%) reported a history of recognized genital lesions. The median time since acquisition for persons with recollection of their first episode of genital herpes was 8 years (IQR, 2–16 years; range, 0–38 years). The
cohort was sampled for 106 person-years (per-person median, 62 days; IQR, 56–99 days), and genital swab samples were available from 14,685 days (92%). Sensitivity analyses were performed, limiting included data to a maximum of 30 days per participant (15,930 study days).

**Summary of Shedding Episodes**

Of 531 participants, 381 (72%) had at least 1 episode. We identified 1,809 separate episodes, of which 1,020 were of certain duration, and 1,695 were included after interval censoring. We separated episodes into subsets according to duration in days, and calculated median HSV DNA load for each day of the episode, to generate generic episode curves for episodes. Shedding episodes had a characteristic morphology with steep upward slope, sharp peak, and more gradual decrease (Figure 1) irrespective of maximal genomic copy or duration.

We demonstrated the cumulative effect of all episodes in a histogram showing shedding frequency in quantitative strata. Of 14,685 total swab samples, 2,658 (18%) contained HSV DNA. A similar proportion of swab samples (~3%) contained $10^2$–$10^3$, $10^3$–$10^4$, $10^4$–$10^5$, or $10^5$–$10^6$ HSV DNA copies/mL; 3.48% of swab samples contained $10^5$–$10^6$ HSV DNA copies/mL, and only 0.5% of swab samples contained $>10^8$ HSV DNA copies/mL (Figure 2).

**Duration and Genomic Copy Number of Shedding Episodes**

We observed significant heterogeneity in episode duration (Figure 3). Of 1,695 episodes analyzed, the median duration was 3 days (IQR, 1–8 days), with 28.8% lasting 1 day and 19.5% lasting >9 days. There was a wide range of peak viral production with median peak copy number of $10^{6.8}$ HSV DNA copies/mL (geometric mean, $10^{6.9}$ HSV DNA copies/mL). Of 1,020 episodes of known duration, 27% peaked at $<10^3$ HSV DNA copies/mL, more first positive swab samples contained $10^2$–$10^3$ HSV DNA copies/mL (34%) than $10^3$–$10^6$ HSV DNA copies/mL (17%). This may also suggest that viral expansion is slower during the initial hours of an episode and increases until HSV DNA copy number surpasses $10^3$ copies/mL (Figure 4, A and B). A lower frequency of first positive swab samples was in the strata with $>10^6$ HSV DNA copies/mL. If viral expansion hypothetically remained constant throughout the first 24 hours at a mean rate of 7.6 logs per day, there would have been an equivalent proportion of swab samples during the early stratum ($10^3$–$10^4$ HSV DNA copies/mL) and the later stratum ($10^5$–$10^9$ HSV DNA copies/mL). The decrease in frequency from 17% to 2% between these 2 strata suggests that exponential viral expansion rate rapidly decreases during the first 24 hours of an episode. For the 1,020 episodes, median regression slope from 12 hours before episode initiation to episode peak was 5.0 logs per day (7.6 logs per day during the first 12 hours of an episode), indicating a decreasing viral expansion rate after the first 12 hours of an episode. Decelerating exponential expansion is also evident in Figure 1.

Figure 1. Rapid expansion, slightly slower decay, and a sharp peak define typical shedding episode morphology. A, We separated 1,020 episodes by duration and took median HSV DNA quantity obtained at each time point during episodes to generate stereotypical curves for each of the durations. Episodes are assumed to start at 12 hours before first positive swab result and to end 12 hours after the last positive swab result. B, Connected percentiles for episodes of 4 days duration, including median values (solid line), and 5th and 95th percentiles (dotted lines).
For the 1020 episodes, the median last positive copy number was $10^{3.1}$ HSV DNA copies/mL. The calculated mean rate of decay was $-6.2$ logs per day during the final 12 hours of the episode. A greater proportion of last positive swab samples contained $10^2–10^3$ HSV DNA copies/mL (48%) than $10^3–10^4$ HSV DNA copies/mL (23%), which may suggest that viral decay rate is slower during the final few hours than during the final 12 hours of an episode. This may also reflect that many episodes never exceed $10^3$ HSV DNA copies/mL. The frequency of last positive swab samples decreased at each successive strata $10^4$ HSV DNA copies/mL, indicating that exponential viral decay rate continually decreased during the last 24 hours. Nevertheless, decay rate remained sufficiently high to terminate each episode (Figure 5, A and B). The median regression slope from peak to 12 hours after the last positive swab result was $-3.6$ logs per day (smaller absolute value than $-6.2$ logs during the last 12 hours of an episode). Therefore, decay rate increased substantially from peak to termination (Figure 1), despite an apparent slight decrease during the final 24 hours.

Episode decay occurred more slowly than expansion (Figure 1). Juxtaposed histograms of first and last positive swab copy number revealed that a larger proportion of first positive swab results occurred at higher copy numbers (Figure 5, A and B). Moreover, the absolute value of median slope from initiation to peak was greater than from peak to termination ($5.0$ vs $-3.6$ logs/d).

Nonmonotonic Episode Decay
Long episodes often had multiple peaks as an important feature (Figure 6A). Of 1020 episodes, 198 (19%) had nonmonotonic decay (decrease of at least 0.5 log, followed by increase of at least 0.5 log). Episode duration correlated with likelihood of nonmonotonic decay (Figure 6B). Episodes with nonmonotonic decay were longer (mean, 10.7 vs 2.5 days), had higher peaks (mean, 7.0 vs 4.4 log 10 HSV DNA copies/mL), higher first swab copy number (mean, 5.5 vs 3.2 log 10 HSV DNA copies/mL), and lower peak to termination slope (mean, $-1.0$ vs $-4.6$ logs per day), despite no difference in last swab copy number (mean, 3.4 vs 3.4 log 10 HSV DNA copies/mL).

Lesional and Nonlesional Episodes
Three hundred thirty-six (33%) of 1020 episodes occurred while genital lesions were present; genital lesions were noted on 6158 (11%) of 58299 days and on 2835 (38%) of 7248 days when swab samples were positive for HSV DNA. Shedding episodes with lesions were longer (median, 5 vs 1 days; mean, 6.8 vs 2.9 days; $P < .001$) and had higher peak copy number (median, 6.7 vs 3.6 log$_{10}$ HSV DNA copies/mL; $P < .001$), first copy number (median, 5.5 vs 3.2 log$_{10}$ HSV DNA copies/mL; $P < .001$), and last copy number (median, 3.5 vs 2.9 log$_{10}$ HSV DNA copies/mL; $P < .001$), compared with nonlesional episodes.

Figure 2. Frequency histograms of shedding frequency. We analyzed 14 685 swab samples obtained daily. A total of 12 027 (81.82%) contained no HSV DNA or $<150$ DNA copies/mL. A, Frequencies of log$_{10}$ HSV DNA copy number for shedding. B, Cumulative frequencies of log$_{10}$ HSV DNA copy number for shedding. Bars represent 95% confidence intervals.

Figure 3. Frequency histogram of episode duration. We analyzed 1695 episodes with daily sampling.
Consistency of Expansion and Decay Rates
Using a generalized estimating equation, we explored whether expansion and decay rates can be described generally for all episodes or whether rates vary by episode duration and must be described separately for longer and shorter episodes. Among episodes without multiple peaks, expansion and decay rates were mildly associated with episode duration. Among 299 episodes lasting for >2 days with only monotonic decay, regression expansion rate decreased by 0.9 log per day for each 1-day increase in episode duration (from 6.6 at 3 days duration to 4.8 at 5 days duration; \( P < .001 \)). Decay rate also decreased by 0.5 logs per day for each 1-day increase in episode duration (from -3.3 at 3 days duration to -2.2 at 5 days duration; \( P < .001 \)).

Shedding Characteristics According to Demographic and Clinical Participant Features
Episode characteristics (first, last, and peak HSV DNA copy number; expansion and decay rate [logs/d]; duration; and monotonicity) did not differ between HSV-1–seropositive and –seronegative persons or between men and women, with the exception of last swab copy number, which was 0.22 logs lower in men than in women (95% CI, -0.38 to -0.06; \( P = .007 \)). All episode characteristics, including expansion and decay rates, were similar according to time since acquisition (\( \leq 1 \) year vs >1 year). Only a small percentage of each characteristic’s variability (first [1.9%], last [13.0%], and peak [3.7%] copy number; expansion [9.3%] and decay rate [8.3%]; duration [1.2%]; and probability of monotonicity [2.5%]) could be attributed to individual characteristics.

DISCUSSION
Our analysis of a large, diverse cohort of patients with HSV-2 infection provides, to our knowledge, the first detailed kinetic evaluation of mucosal HSV-2 infection in the healthy host.
HSV-2 infection reactivations vary substantially in and among individuals according to duration and peak HSV DNA copy number, 2 measures that strongly correlate. Episodes with higher viral production are more commonly associated with genital lesion formation and nonmonotonic viral decay [1]. Regardless of peak copy number, episodes expand extremely rapidly, with subsequent rapid deceleration, followed by sharp decay, leading to a stereotypical episode appearance with sharp peaks. Duration of the expansion phase varies among episodes, and some episodes are eliminated within hours [1]. However, even during prolonged lesional episodes, the exponential rate of expansion invariably decelerates during the first 24 hours. Moreover, exponential decay rate increases dramatically from episode peak to termination. During the final 24 hours of an episode, exponential decay rate may actually decrease slightly, although decay rate remains sufficiently high to ensure termination of viral replication.

Although these observations most obviously pertain to viral kinetics, they indirectly highlight the importance of the mucosal host immune response in containment of viral shedding. The rapid cessation of expansion phase and accelerated decay phase of episodes suggest that the peripheral immune response must be continually primed to rapidly eliminate HSV-infected cells. In a previous study [11], we used a mathematical model to indicate that the most likely mechanism to explain high frequency of annual genital episodes in HSV-2–infected persons is nearly constant release of low numbers of viral particles from sensory nerve endings at the dermal-epidermal junction in the genital tract. After a single epithelial cell is productively infected, viral production is extremely rapid (>10 000 HSV DNA copies/d/cell), and in the absence of an immediate immune response, spread to contiguous epidermal cells occurs within hours. Thus, in 1 day, thousands of cells may harbor replicating HSV.

However, all episodes are cleared in immunocompetent persons despite explosive viral expansion. Peak episode copy numbers almost never exceed $10^9$/mL, and despite an abundance of skin epithelial cells, most HSV-2 infection recurrences lead to vesicles and ulcers that are only several millimeters in diameter [9]. Therefore, in contrast to HIV-1 [19], hepatitis B and C [20, 21], and influenza [22] infections, target cell saturation is not hypothesized to play a role in determining peak viral load. The deceleration of expansion during the initial 24 hours suggests early immune influence on viral replication and spread, perhaps because of innate pressure from local interferon production [23]. Our modeling and experimental data also suggest that an intense, localized acquired mucosal immune response limits the extent of each episode and may promote early containment. HSV-2–specific CD4^+^ and CD8^+^ T cells form dense infiltrates at recurrence sites in genital skin and persist for months at the dermal-epidermal junction where HSV-2 is released from neurons [24–26]. Presumably, these lymphocytes participate in immunosurveillance, and their density in the genital mucosa at episode initiation is likely to play an important role in determining extent of viral production before clearance [26]. For these reasons, we speculate that viral decay kinetics may be extremely different in the immunocompromised host.

Our data suggest a decreased rate of decay toward the completion of an episode, an observation that initially seems counterintuitive. Mathematical models of HIV-1 decay during antiretroviral therapy document differential decay kinetics of virus in different cellular compartments [19, 27–29]. In the case of HSV, the cytotoxic immune response effectively clears infected epidermal cells. If decay of remaining free virus is slower than that of infected cells, viral decay rate may decrease after infected cells are no longer present.

Episode prolongation is an important strategy used by HSV-2, presumably to enhance transmission, because likelihood of transmission is probably a function of shedding frequency and quantity. Prolonged episodes are associated with higher peak copy number [1], and increased inoculum at time of coitus is likely to correlate with higher probability of transmission. It is therefore an important finding of our study that prolonged episodes often have nonmonotonic decay or re-expansion, of virus.

Mechanisms of viral re-expansion during an episode are not currently understood. One hypothesis for re-expansion...
proposed for acute influenza infection is that antiviral cytokines fail to clear all infected cells of a given plaque and have a short half-life in tissue, allowing for bimodal episodes [22]. Similarly, with HSV-2 infection, local innate immune mechanisms may be inadequate, because acquired immune cells must arrive from external sites to achieve episode containment. Alternatively, the common appearance of secondary crops of vesicles during prolonged HSV-2 infection recurrences [9] suggests that new plaque formation at spatially distinct sites (via cell-free particles or separate neuronal reactivations) may allow for evasion of the localized immune response. The recent findings that HSV-2 is commonly detected throughout the genital tract during focal genital lesions and that maximum genomic copy number is associated with wider spatial spread support the hypothesis that HSV-2 infects new sites to avoid an intense but highly localized immune response at the primary plaque of infection [30].

An important limitation of our study is that sampling occurred every 24 hours. In studies using swabs every 6 hours, 2 of 3 HSV-2 infection episodes lasted <24 hours; median duration was 13 hours rather than 3 days [1]. Brief episodes are largely asymptomatic and peak at low HSV DNA copy numbers. Therefore, our study overestimates proportion of episodes with high copy number, long duration, and associated lesions. Moreover, our data are from studies in which participants performed mixed swabs of the anogenital tract. We believe that episodes might be shorter and without re-expansion if we localized swabs to single vesicles in lesions. Finally, we measured viral quantity with use of genome equivalent copies, a more sensitive method than viral isolation; prior studies indicate that high DNA copy numbers correlate with presence of infectious viral particles [2].

Detailed examination of HSV-2 shedding episodes reveals a remarkable compromise between virus and host immunity. Viral replication and spread is explosive during the initial hours after episode initiation because of infection of a single epithelial cell. Nevertheless, in immunocompetent hosts, immune cells clear all episodes and the majority of episodes are terminated in <5 days. Most shedding is asymptomatic, and most recurrences, although a clinical nuisance, are not harmful to the infected host. Nevertheless, the cumulative effect of these episodes is that virus is present for enough of the time to facilitate transmission after relatively few coital acts [31], which in turn explains high worldwide seroprevalence [32]. Therefore, frequent, highly dynamic shedding episodes are an extremely effective strategy for HSV-2.

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**References**


