HSV

Longitudinal study of herpes simplex virus type 2 infection using viral dynamic modelling

Catherine M Crespi, William G Cumberland, Anna Wald, Lawrence Corey, Sally Blower

Sex Transm Infect 2007;**83**:359–364. doi: 10.1136/sti.2006.022020

See end of article for authors' affiliations

Correspondence to: Dr Catherine M Crespi, Department of Biostatistics, UCLA School of Public Health, Los Angeles, CA 90095-1772; ccrespi@ucla.edu

Accepted 30 March 2007

Objectives: Rates of reactivation of herpes simplex virus type 2 (HSV-2) change over time and these changes affect transmission and clinical management strategies. We conducted a longitudinal study of HSV-2 infection to quantify rates of change in HSV-2 reactivation, mucosal shedding and recurrences of genital lesions, using a newly available model of HSV within-host dynamics.

Methods: A cohort of 18 women was studied at two time periods spaced 2 years apart. The cohort provided daily mucosal swabs for HSV PCR analysis for 10 weeks during each time period and recorded recurrences in diaries. We fit the model of HSV dynamics to the mucosal shedding data using Bayesian methods to produce estimates of HSV reactivation, shedding and longitudinal rates of change. The model was validated using a separate group of 67 individuals.

Results: According to the viral dynamic modelling results, rates of HSV-2 reactivation from latency in the ganglia varied >10-fold among the women, and were estimated to be ⩾10% higher than rates of mucosal shedding episodes for many individuals. The mucosal shedding associated with each reactivation typically lasted 1–3 days. Reactivation frequency was estimated to be declining by three reactivations a year on average. The median number of recurrences, based on patient diaries, declined from 6.8 per year to 2.1 per year over the 2-year period.

Conclusions: Rates of HSV-2 reactivation, shedding and recurrence generally decline over time but remain high in some individuals 4–5 years after primary infection. Viral dynamic modelling provides quantification of HSV infection that cannot be obtained by other methods.

Herpes simplex virus type 2 (HSV-2), the primary cause of genital herpes, has high prevalence in many countries.[1] The pathogenesis of HSV-2 involves acute infection, the establishment of latency in ganglia, and subsequent reactivation of virus with reinfection of epithelial cells and viral shedding on mucosal surfaces. HSV-2 reactivation is sporadic and frequently asymptomatic,[2] making it difficult to quantify. Long-term studies are particularly difficult to conduct and thus changes in HSV reactivation over time are incompletely characterised.

We conducted a longitudinal study of genital HSV-2 infection in order to characterise rates of changes in HSV-2 reactivation. For the study, we collected symptomatic recurrence data and daily swabs from the genital mucosa of 18 women during a 10-week baseline period close to primary infection, and a 10-week follow-up period 2 years later, and compared rates of HSV-2 mucosal shedding and recurrence across the two time periods.

To obtain additional insights on HSV-2 pathogenesis, we fit a newly available model of HSV within-host dynamics[3] to the mucosal shedding data. The model links HSV shedding from the mucosa with reactivation from latency in ganglia, providing estimates of the frequency of HSV reactivation from latency. The model also provides estimates of the frequency and duration of mucosal shedding episodes, which are uncertain when mucosal samples are taken at spaced time points. The model was fitted using Bayesian analysis with Markov chain Monte Carlo methods.[4 5] To validate the model, we compared shedding data collected from a separate group of 67 HSV-2-infected persons to computer simulations of HSV reactivation as postulated by the model.

METHODS

All subjects gave written informed consent. The protocols to collect the shedding data were approved by the University of Washington Human Subjects Review Committee. The modelling and statistical analyses were certified exempt by the UCLA Office for Protection of Research Subjects.

The women in the longitudinal study ranged from 22 to 51 years of age (median age 27) at baseline, were seronegative for HSV-1 and were not taking antiviral therapy at the time of data collection for this analysis. They had enrolled in two clinical trials.[6 7] At baseline, the median time since HSV-2 acquisition was 8 months (range 2 months to 2 years), and at follow-up, the median time was 3.2 years (range 2–4.6 years). Acquisition of genital HSV-2 was defined clinically and all infections were confirmed virologically and serologically. The women collected separate cervicovaginal and vulvar swabs once daily by passing over these mucosal surfaces with a Dacron swab. The women collected mucosal swabs for 57–85 days (median 67.5 days) during the baseline period and 45–82 days (median 66 days) during the follow-up period. The swabs were analysed by a real-time quantitative HSV DNA PCR assay.[8] We defined a sample collection time point to be positive for shedding if ⩾500 HSV DNA copies/ml of specimen were detected in either specimen collected that day, and negative otherwise. The women also recorded recurrences of genital lesions in diaries. A recurrence was defined as an episode of genital lesions from the onset of the first lesion until all lesions were completely healed. We annualised recurrence rates as (number of recurrences)/(length of observation period). Symptomatic shedding was defined as an HSV positive result with lesions present; asymptomatic shedding was defined as an HSV positive result with no lesions present.

The numbers of women with HSV-2 shedding and recurrences at baseline and follow-up were compared using Fisher's exact test. Differences between baseline and follow-up in percentage of sample collection time points that were HSV positive, percentage of such time points that were asymptomatic,

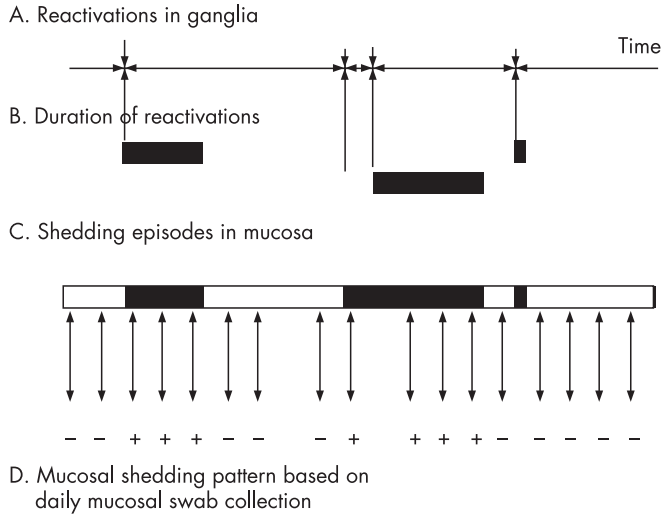

Figure 1 Model of HSV within-host dynamics. (A) Reactivations of HSV in the ganglia occur intermittently over time. (B) Each reactivation is associated with a short-term period of HSV shedding from the mucosa, the duration of which varies. (C) The reactivations create episodes of HSV shedding. On some occasions, additional reactivations can occur during a shedding episode. Thus, a shedding episode can involve multiple reactivations affecting different regions of the mucosa, as illustrated by the second shedding episode, which involves two reactivations overlapping in time. (D) Mucosal swabs are collected and assayed by HSV DNA PCR at a series of daily time points, yielding a pattern of positive and negative shedding results. In clinical studies of daily sampling of mucosal secretions, (D) is observed; (A), (B) and (C) are not. Model fitting procedures use the pattern of positive and negative shedding to estimate the individual's HSV-2 reactivation frequency and other quantities.

percentage of days with recurrences, and number of recurrences per year were assessed using the sign test for paired samples. The median duration of recurrences in each time period was estimated by aggregating all recurrences within the relevant time period.

Viral dynamic model

The model of HSV within-host dynamics, depicted in fig 1, was developed to describe HSV pathology[2 9] and links HSV reactivation from latency in the ganglia with shedding from the mucosa. The model postulates that each individual experiences intermittent reactivation of HSV-2 with a characteristic frequency. As reactivation is believed to be triggered by a variety of stimuli that tend to occur sporadically or randomly over time, we model it as a Poisson process.[10] Thus, the model postulates that an individual i has HSV-2 reactivations over time according to a Poisson process with rate λ_i, defined as the individual's mean number of reactivations per year. Figure 1A provides an example of the timing of reactivations.

Each reactivation is associated with a short-term infection of the epithelium, during which HSV is shed. We define the duration of a reactivation as the duration of HSV shedding detectable through swabbing and PCR associated with the reactivation-inducing stimulus. The durations vary within individuals, and we model them as independent and identically distributed (iid) exponential random variables, with parameter θ_i corresponding to individual i's mean duration of a reactivation, measured in days. Figure 1B provides an illustration.

It has been observed that while shedding is occurring in one region of the mucosa, HSV can become newly detectable in another region.[7 11] This could be due to continued transport of HSV from the ganglia in response to the original stimulus, or might reflect the arrival of a new stimulus. The model encompasses both scenarios. In the first scenario, the shedding is subsumed into the duration of the original reactivation. To address the second scenario, the model postulates that stimuli continue to occur at the same rate, λ_i, during a shedding episode. Thus, another reactivation can occur before the shedding associated with previous reactivations has cleared, resulting in a shedding episode involving >1 reactivation. A shedding episode with overlapping reactivations is illustrated in fig 1C.

Figure 1D illustrates the collection of mucosal swabs at daily time points. Each swab pools secretions from the entire swabbed surface, abrogating the ability to identify the distinct areas where shedding is occurring. Thus the number of potentially overlapping reactivations cannot be determined directly from the mucosal swab data. However, it can be estimated using the model, as described below.

We fit the model to mucosal shedding data to estimate each individual's reactivation frequency λ_i, mean duration θ_i and other quantities using Bayesian analysis with Gibbs sampling as described elsewhere.[3] We allowed individuals to have different parameters during baseline and follow-up and regressed these parameters on time since HSV-2 acquisition using $\psi_i = (\log \lambda_{1i}, \log \theta_{1i}, \log \lambda_{2i}, \log \theta_{2i}) \sim$ iid $N(X_i\beta, \Sigma)$, where $(\lambda_{1i}, \theta_{1i})$ and $(\lambda_{2i}, \theta_{2i})$ are individual i's reactivation parameters during baseline and follow-up, X_i is a covariate matrix indicating individual i's time since HSV-2 acquisition, β is a regression coefficient vector, and Σ is a covariance matrix. Estimates of β, Σ, and ψ_i, i = 1,...,n, were obtained as posterior distributions, which we summarised using the median and interquartile range (IQR). Results are based on 15 000 posterior simulations following a burn-in of 5 000.

Transformations of ψ_i yielded estimates of the following quantities: frequency and mean duration of shedding episodes, $\lambda e^{-\lambda\theta/365}$ and $365(e^{\lambda\theta/365}-1)/\lambda$. These quantities are uncertain when swabs are collected at spaced time points, as the onset and resolution times of shedding episodes are not observed and episodes occurring between two sample points could be missed (fig 1D). Frequency and mean duration of reactivation from latency, λ and θ. This estimate is made under the assumption that reactivations continue to occur at the individual's characteristic rate regardless of whether the individual is currently shedding HSV as described above; thus, some reactivations can occur during shedding episodes. The percentage of reactivations that occurred during shedding episodes was modelled by $1-e^{-\lambda\theta/365}$. This is equivalent to the probability that a reactivation occurs while the individual is shedding and also equivalent to the percentage of time with mucosal shedding.

Model validation

Our model validation methods followed recommendations for posterior predictive model checking for Bayesian data analysis.[5 12] Our model validation approach differs from procedures that fit the model to one set of individuals and use the estimates to predict outcomes for another set of individuals. This type of prediction is not possible with our model because our model postulates that each individual has his/her own characteristic HSV-2 reactivation frequency and duration, and it is not possible under the current state of knowledge to predict a particular individual's frequency and duration from an analysis of an independent group of subjects. Rather, our objective was to examine whether the model assumptions, that reactivations occur according to a Poisson process and have durations that are iid exponential, provide a reasonable explanation of observed patterns of shedding. Thus, our approach was to simulate HSV reactivation as postulated by the model for a diverse group of individuals, producing simulated mucosal shedding data that show what the data

Table 1 Characteristics of HSV-2 mucosal shedding and recurrences of genital lesions among a cohort of 18 women

Characteristic	Baseline	Follow-up
Women with HSV-2 mucosal shedding during the sampling period (number, percentage)	16 (89%)	15 (83%)
Percentage of sample collection time points that were HSV positive (median, range)	9.9% (0–100%)	6.3% (0–70%)
Geometric mean HSV DNA copies/ml of specimen, vulva (median, range)	$10^{4.2}$ ($10^{2.9}$ to $10^{6.6}$)	$10^{4.8}$ ($10^{2.9}$ to $10^{5.7}$)
Geometric mean HSV DNA copies/ml of specimen, cervix (median, range)	$10^{3.8}$ ($10^{2.9}$ to $10^{6.0}$)	$10^{4.2}$ ($10^{3.6}$ to $10^{5.2}$)
Percentage of HSV positive sample collection time points that were asymptomatic (median, range)*	57% (0–100%)	81% (30–100%)
Women with recurrences during the sampling period (number, percentage)*	14 (78%)	9 (50%)
Percentage of days with recurrences (median, range)	21% (0–87%)	7.5% (0–53%)
Number of recurrences/year (median, range)	6.8 (0–25)	2.1 (0–25)
Duration of recurrences, days (median, range)	7 (2–32)	7 (3–19)

*$p<0.05$.

would look like if the model were valid. We then compared the simulated data to actual mucosal shedding data collected from the individuals.

The individuals used for model validation were different from the individuals in the longitudinal study and included 27 men and 40 women with genital HSV-2 infection who had either recent diagnosis of genital herpes (n = 25, median time since acquisition 5.8 months, range 2–8.5 months) or established HSV with frequent recurrences (n = 42, median time since acquisition 5.5 years, range 9 months to 20 years). The subjects had enrolled in a clinical trial,[13] and included 22 women and 16 men seropositive for HSV-2 only, and 18 women and 11 men seropositive for HSV-1 and 2. Patients were not taking antiviral therapy at the time of data collection for this analysis, and provided mucosal swabs for a median of 55 days (range 39–63 days). The individuals collected one daily specimen by swabbing the entire cervicovaginal, vulvar and perianal areas (women), or penile skin and perianal areas (men).

To produce simulated mucosal shedding data, we first obtained estimates of each individual's reactivation frequency and duration by fitting the model as described above, using $\psi_i = (\log \lambda_i, \log \theta_i)$. For each individual i, we drew a reactivation frequency and mean duration $(\lambda_i^{(m)}, \theta_i^{(m)})$ from his/her posterior distribution and simulated reactivation times $r_1, r_2, \ldots$, with $r_1 \sim \text{Exp}(\lambda_i^{(m)})$ and $r_{j+1} = r_j + \text{Exp}(\lambda_i^{(m)})$ for j = 2, 3, ..., and associated durations $d_1, d_2, \ldots \sim \text{Exp}(1/\theta_i^{(m)})$. We superimposed the reactivations to obtain a continuous-time mucosal shedding pattern as in fig 1C, then drew a random start time and ascertained the positive/negative shedding state at time points equal in number and spacing to the individual's actual swab collection time points, producing simulated data as in fig 1D. We produced 300 sets of simulated data for each individual, using a different draw of $(\lambda_i^{(m)}, \theta_i^{(m)})$ from the posterior distribution for each replication, in order to account for random variation in the reactivation process and uncertainty in parameter values.

We compared the simulated and observed data in terms of percent of time with mucosal shedding and the dispersal of shedding over time. To characterise dispersal, we tabulated the number and average length of HSV positive "runs", where an HSV positive "run" is defined as a sequence of HSV positive results preceded and succeeded by HSV negative results. The concept of a run is widely used in statistics to characterise patterns in sequences of data.[14]

RESULTS

Mucosal shedding and recurrence data

Table 1 compares mucosal shedding and recurrences between the two time periods, obtained directly from the data without modelling. Most women shed HSV during baseline and follow-up. The median percentage of sample time points that were HSV positive declined by a median of 36% from baseline to follow-up, but the range in the study population remained broad, from 0% to 70%. The percentage of these time points that were asymptomatic increased (p = 0.039). HSV DNA copy number per ml was not significantly different between baseline and follow-up.

The number of women with recurrences and percent of days with recurrences both declined; however, half of the women experienced one or more recurrences during the follow-up period. The frequency of recurrences also declined (p = 0.057); however, the range of recurrence rates remained unchanged at 0–25/year. The median duration of recurrences was also unchanged at 7.0 days.

Model validation

Figure 2 displays the concordance between the simulated and observed data for the 67 individuals in the model validation. The correlation between the observed percent of time with mucosal shedding and the median percent in the simulated data was 0.99 (fig 2A). The simulated and observed data also agreed well in terms of the number and average length of HSV positive runs (fig 2B and 2C), with correlations of 0.96 and 0.93, respectively. There was a slight tendency for the model to underpredict the number of HSV positive runs for individuals with very frequent runs.

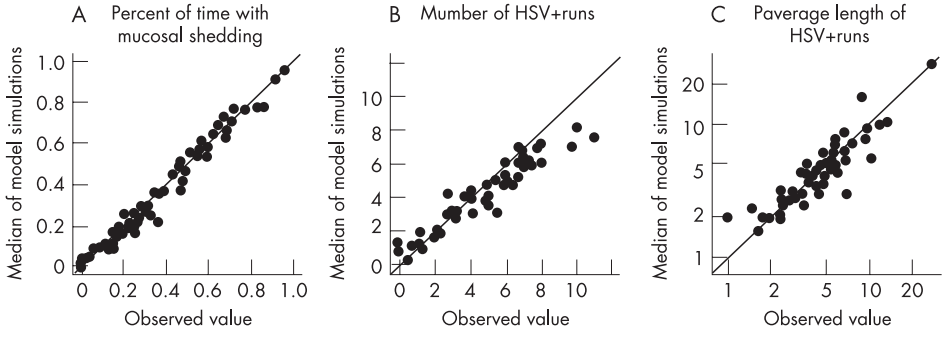

Figure 2 HSV mucosal shedding data for 67 individuals compared to data simulated under model assumptions. Solid circles plot the observed value from mucosal swabs against the median of the 300 model simulations for each person. Diagonal lines represent perfect agreement.

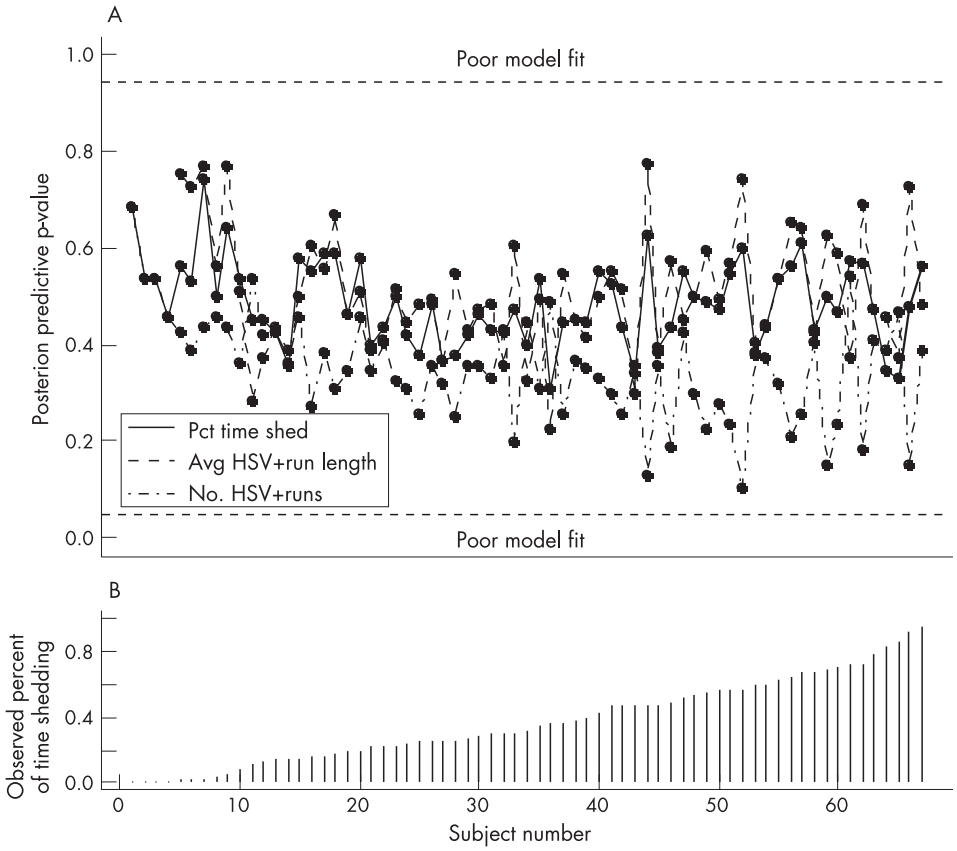

Figure 3 Person-by-person model fit for the 67 individuals in the model validation study. (A) Three indices of model fit, calculated as posterior predictive p values, are plotted. Values outside the range of 0.05–0.95 indicate poor model fit. (B) Observed percent of mucosal swabs that were HSV positive, showing the range of shedding rates considered in the model validation.

A person-by-person model fit is provided in fig 3. The figure plots posterior predictive p values, indices of model fit calculated as the proportion of simulated data sets that are more extreme than the observed data. The figure shows acceptable model fit for all 67 individuals. The plot again shows a slight tendency for the model to predict fewer HSV positive runs than were observed for individuals with high shedding percentages, but the model fit is still well within the acceptable range for these individuals.

Estimates from viral dynamic modelling

Table 2 provides estimates obtained by fitting the viral dynamic model to the cohort's mucosal shedding data. The median frequency of mucosal shedding episodes declined by a median of 4 per year from baseline to follow-up, while the duration of shedding episodes increased by a median of 0.4 days. Reactivations were more frequent than shedding episodes. The difference is explained by the estimated percentages of reactivations that occurred during shedding episodes and thus did not initiate a new shedding episode. This percentage was estimated to have exceeded 10% and 7% in half the women at baseline and follow-up, respectively.

By regressing reactivation frequency and duration on time since acquisition as described in Methods, we estimated that reactivation frequency was declining at the rate of 3.2 reactivations/year at 2 years post-acquisition (IQR 2.2 to 3.5) and reactivation duration was increasing at the rate of 0.18 days/year (IQR 0.04 to 0.43).

Figure 4 shows the heterogeneity in reactivation and shedding rates and explores the relationship between them. The figures combine estimates from baseline and follow-up. Figure 4A shows that reactivation frequency ranged over almost two orders of magnitude and increased as the percent of time with shedding increased. The duration of a reactivation (fig 4B) averaged 1–3 days and did not vary much among the cohort or with percent of time shedding.

For women with high percentages of time shedding, the estimates of reactivation and shedding diverged. The modelling results suggested that these women had more reactivations than shedding episodes, and that their shedding episodes were longer than their individual reactivations. The divergence can be explained by the fact that when shedding occurs at higher percentages, reactivation-inducing stimuli become increasingly likely to occur during an ongoing shedding episode (fig 4C).

Table 2 Summary of HSV-2 reactivation and shedding estimates for the cohort of 18 women obtained using the model of HSV-2 within-host dynamics

	Median (range) of 18 point estimates	
Quantity	**Baseline**	**Follow-up**
Mucosal shedding episodes/year	16 (8–48)	12 (6–43)
Duration of mucosal shedding episodes, days	1.7 (1.2–39)	2.1(1.4–11)
Reactivations/year	21 (8–234)	13 (7–94)
Duration of reactivations, days	1.6 (1.1–5.1)	2.0 (1.3–5.2)
Percentage of reactivations occurring during shedding episodes	10% (0–96%)	7% (0–73%)

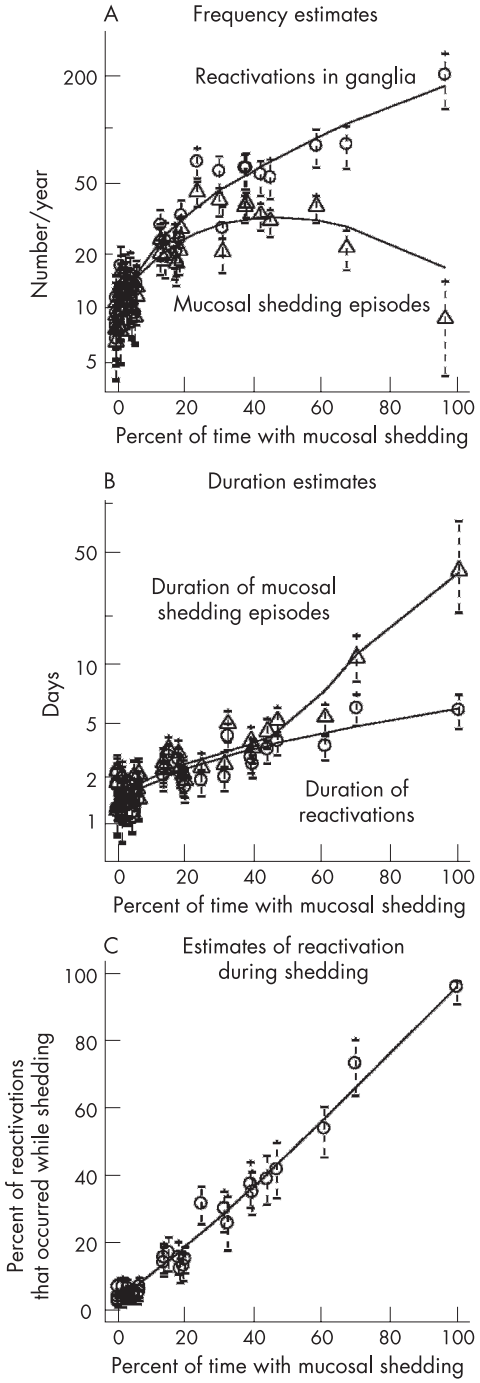

Figure 4 Estimates obtained by fitting the model of HSV dynamics to mucosal swab data, suggesting divergence between reactivation and mucosal shedding in individuals with high percentages of time shedding. Estimates of reactivation (circles) and shedding (triangles) for each of the 18 women in the longitudinal study are displayed with dashed vertical lines indicating the interquartile range of the estimates. Estimates for baseline and follow-up are included. Smooth lines are splines showing relationship with percent of time shedding.

Thus these individuals have the potential to experience episodes of mucosal shedding that involved multiple reactivations.

DISCUSSION

Our findings characterise patterns of HSV-2 reactivation and quantify rates of change that are important for understanding transmission and guiding clinical management. We found that among this group of women in the early years of infection, HSV-2 reactivation rates varied >tenfold. The duration of HSV-2 mucosal shedding associated with each distinct reactivation was typically 1–3 days, and this duration varied little among the individuals in the study. Thus, most of the variation in HSV-2 reactivation patterns was due to differences in frequency rather than duration of viral reactivation. Our results further suggested that high frequency of reactivation could lead to overlapping of reactivations, with extended mucosal shedding episodes composed of two or more reactivations. The frequency of reactivation and recurrences generally waned over time, but some individuals continued to experience high rates 4–5 years after primary infection.

Our use of the model of HSV within-host dynamics provided several benefits. First, the model provided estimates of the frequency and duration of episodes of mucosal shedding, which are uncertain when mucosal specimens are collected at spaced time points. Second, the model provided estimates of the rate of HSV reactivation in ganglia, which is not feasible to measure directly. In order to obtain these estimates, the model assumes that individuals continue to experience reactivations of HSV from latency at their characteristic rate regardless of whether they are currently shedding HSV from the mucosa. The validity of this assumption is difficult to confirm with laboratory or clinical studies. However, our model validation showed that mucosal shedding data computer-simulated using this assumption closely matched observed mucosal shedding patterns, supporting the plausibility of the model. Studies are planned to further evaluate the model.

The modelling results suggest that reactivations occur at a higher frequency but shorter duration than mucosal shedding episodes, with the implication that some episodes of mucosal shedding are a composite of two or more distinct reactivation events. This has several implications. It suggests that true rates of HSV reactivation from latency could be higher than previously surmised based on the frequency of mucosal shedding episodes. This in turn implies that the stimuli that trigger reactivation could be more numerous and diverse than currently appreciated. These stimuli are still incompletely characterised. Because we are able to quantify reactivation rates, our methods could be used to better characterise the determinants of reactivation frequency and the effect of antiviral therapy on HSV-2 shedding.

Identifying frequent reactivators and targeting them for antiviral therapy can be an effective public health strategy for reducing transmission of HSV-2.[15] Here we have quantified biological heterogeneity in reactivation frequency and the rate of waning over time. This information can be used to identify the virological core groups that are disproportionately responsible for transmission, leading to more effective control.

Key messages

- Viral dynamic modelling of clinical data suggests that single episodes of HSV-2 mucosal shedding could involve multiple reactivations in the ganglia. Consequently, rates of HSV-2 reactivation could be higher than suggested by mucosal shedding rates.
- The frequency of HSV-2 reactivation varies widely among individuals and decreases over time; in contrast, the duration of mucosal shedding associated with a reactivation varies little and does not appear to decrease over time.
- Modelling methods can provide insight into viral pathogenesis and suggest novel approaches for experimental biology.

Our study is the first to apply viral dynamic modelling to longitudinal data on HSV infection. Applying these techniques to our sample of 18 women, the largest repeated longitudinal data set currently available, is sufficient to demonstrate the feasibility and benefits of these new methods and provide insights into typical HSV reactivation rates and patterns. Future studies with larger and more diverse samples, including men, will allow inferences about a wider population of infected patients.

Our viral dynamic model decomposes HSV pathogenesis into two distinct processes, reactivation of HSV in the ganglia and subsequent shedding from the mucosa, and provides distinct estimates of these processes for each individual. Because of this approach, our methods can be used to evaluate the differential efficacy of antiviral therapies on the two processes. This approach could facilitate the development of treatments that are targeted to a specific process or compartment.

Authors' affiliations

Catherine M Crespi, William G Cumberland, Department of Biostatistics, University of California, Los Angeles, California, USA
Anna Wald, Departments of Medicine, Epidemiology and Laboratory Medicine, University of Washington, Seattle, Washington, USA
Lawrence Corey, Departments of Medicine and Laboratory Medicine, University of Washington, and Program in Infectious Diseases, Fred Hutchinson Cancer Center, Seattle, Washington, USA
Sally Blower, Semel Institute of Neuroscience and Human Behavior, David Geffen School of Medicine at University of California, Los Angeles, California, USA

Financial Support: National Institutes of Health (grants T32 AI007370 and AI-30731).

Competing interests: None declared.

CMC conducted the analyses and had primary responsibility for drafting the paper. WCG provided guidance on the analyses and presentation and interpretation of results. AW and LC conceived the study, supervised the data collection and contributed to the writing. SB contributed to the interpretation and discussion of results.

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