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Viral Shedding 1 Year Following First-Episode Genital HSV-1 Infection

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IMPORTANCE Herpes simplex virus type 1 (HSV-1) is the leading cause of first-episode genital herpes in many countries.

OBJECTIVE To inform counseling messages regarding genital HSV-1 transmission, oral and genital viral shedding patterns among persons with first-episode genital HSV-1 infection were assessed. The trajectory of the development of HSV-specific antibody and T-cell responses was also characterized.

DESIGN, SETTING, AND PARTICIPANTS Prospective cohort followed up for up to 2 years, with 82 participants followed up between 2013 and 2018. Participants were recruited from sexual health and primary care clinics in Seattle, Washington. Persons with laboratory-documented first-episode genital HSV-1 infection, without HIV infection or current pregnancy, were referred for enrollment.

EXPOSURES First-episode genital HSV-1 infection.

MAIN OUTCOMES AND MEASURES Genital and oral HSV-1 shedding and lesion rates at 2 months, 11 months, and up to 2 years after initial genital HSV-1 infection. Participants self-collected oral and genital swabs for HSV polymerase chain reaction testing for 30 days at 2 and 11 months and up to 2 years after diagnosis of genital HSV-1. Blood samples were collected at serial time points to assess immune responses to HSV-1. Primary HSV-1 infection was defined as absent HSV antibody at baseline or evolving antibody profile using the University of Washington HSV Western Blot. HSV-specific T-cell responses were detected using interferon γ enzyme-linked immunospot.

RESULTS Among the 82 participants, the median (range) age was 26 (16-64) years, 54 (65.9%) were women, and 42 (51.2%) had primary HSV-1 infection. At 2 months, HSV-1 was detected from the genital tract in 53 participants (64.6%) and in the mouth in 24 participants (29.3%). Genital HSV-1 shedding was detected on 275 of 2264 days (12.1%) at 2 months and declined significantly to 122 of 1719 days (7.1%) at 11 months (model-predicted rate, 6.2% [95% CI, 4.3%-8.9%] at 2 months vs 3.2% [95% CI, 1.8%-5.7%] at 11 months; relative risk, 0.52 [95% CI, 0.29-0.93]). Genital lesions were rare, reported on 65 of 2497 days (2.6%) at 2 months and 72 of 1872 days (3.8%) at 11 months. Oral HSV-1 shedding was detected on 88 of 2247 days (3.9%) at 2 months. Persons with primary HSV-1 infection had a higher risk of genital shedding compared with those with nonprimary infection (model-predicted rate, 7.9% [95% CI, 5.4%-11.7%] vs 2.9% [95% CI, 1.7%-5.0%]; relative risk, 2.75 [95% CI, 1.40-5.44]). Polyfunctional HSV-specific CD4+ and CD8+ T-cell responses were maintained during the follow-up period.

CONCLUSIONS AND RELEVANCE Genital HSV-1 shedding was frequent after first-episode genital HSV-1, particularly among those with primary infection, and declined rapidly during the first year after infection.

Editorial

Supplemental content

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Both herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) cause genital herpes, a chronic infection characterized by recurrent, self-limited genital ulcers. Genital herpes is associated with large medical, social, and financial burdens. In 2018, there were an estimated 18.6 million prevalent and 572 000 incident HSV-2 infections among those aged 18 to 49 years in the US,[1] accounting for 27% of all prevalent sexually transmitted infections (STIs)[2]; millions of infections would be added if genital HSV-1 was included.[1] The lifetime medical cost of genital herpes due to HSV-2 acquired in 2018 alone in the US was estimated to be $90.7 million dollars.[3] These costs do not account for effects on sexual health, including stress associated with potential for transmission to sexual partners or neonates and social stigma.

HSV-1 is an increasing cause of genital herpes, especially in high-income countries that have a declining rate of oral HSV-1 acquisition during childhood,[4,5] leaving individuals susceptible at initiation of sexual activity. An "epidemiologic transition" of HSV-1 from oral to genital infection is predicted, with up to 25% of all HSV-1 acquisitions in the genital tract by 2050 in the US.[6] Given the changing epidemiology of HSV-1 infection, it is important for clinicians to understand outcomes of genital HSV-1 viral shedding and risk of recurrence over time.

Although HSV-1 is adapted to the trigeminal, superior cervical, and ciliary ganglia, where it establishes latency and reactivates causing orolabial ulcerations and asymptomatic shedding, the frequency of HSV-1 reactivation in the genital tract, the prevalence of concurrent oral and genital HSV-1 infection, and development of cellular and humoral responses to HSV-1 have not been determined. To answer these questions, a cohort of persons with first-episode genital HSV-1 infections was prospectively followed up.

Key Points

Question What are the rates of viral shedding after first-episode genital herpes simplex virus type 1 (HSV-1) infection?

Findings In this prospective cohort study, 82 participants with first-episode genital HSV-1 infection, of whom 42 had primary HSV-1 infection, self-collected oral and genital swabs daily for HSV polymerase chain reaction testing for two 30-day periods (2 months and 11 months after initial symptoms). Genital HSV-1 shedding was detected on 12.1% of days at 2 months and declined significantly to 7.1% of days at 11 months. Most genital shedding was asymptomatic; genital and oral lesions and oral shedding were rare.

Meaning Genital HSV-1 shedding was frequent after first-episode genital HSV-1 and declined rapidly during the first year of infection.

Methods

Participants and Study Design

All procedures were approved by the University of Washington Human Subjects Division. Participants provided written informed consent in English. Eligible participants had first-episode genital HSV-1 within the past 8 weeks, were seronegative for HSV-2 and HIV, and were not pregnant. First-episode genital HSV-1 was defined as detection of HSV-1 from genital ulcers among persons with no prior history of genital herpes. Participants were followed up between 2013 and 2018 and recruited from the Public Health-Seattle and King County Sexual Health Clinic, the University of Washington Healthcare System, and the surrounding community.

Antiviral therapy active against HSV was used for the initial episode and participants consented to avoid suppressive antiviral therapy during swabbing sessions. Participants collected daily oral and anogenital swabs and completed a diary of genital symptoms between 8 and 12 weeks (session 1) and between 48 and 52 weeks (session 2) after first-episode genital HSV-1.[7] Participants with detectable genital HSV-1 shedding during session 2 were invited to complete a third session 24 months after infection. Oral swabs were collected by rubbing a Dacron swab on the tonsils, tongue, hard palate, and gums. Anogenital swabs were collected by rotating a Dacron swab into the vagina, followed by swabbing the labia majora/minora, perianal area, and rectal area or swabbing the entire penile shaft, including underneath the foreskin if present, perianal area, and rectal area. Swabs were placed into 1-mL 1X polymerase chain reaction (PCR) buffer and stored at room temperature or 4 °C. Blood was drawn every 2 weeks during shedding sessions and at week 24, with additional blood draws at 2, 4, and 6 weeks among participants with primary genital HSV-1.

Participants were contacted monthly to assess for genital herpes recurrences and encouraged to come to the clinic during recurrences for laboratory confirmation.

Laboratory Assays

Immunology

Serology and Definition of Acquisition Type | Serum was tested for HSV-specific IgG antibodies at enrollment and at 12, 24, and 52 weeks using the University of Washington Western Blot, which differentiates between HSV-1 and HSV-2 infection.[8] Samples were considered HSV-1 or HSV-2 positive (4 bands that comigrated with HSV-1 or HSV-2 antigens), HSV-1 negative (no bands), or HSV-1 indeterminate (1-3 bands).[8] Participants were classified as having primary infection if they were HSV-1 seronegative or HSV-1 indeterminate at baseline and seroconverted or had maturation of HSV-1 antibody response over time. Persons who were HSV-1 seropositive at baseline and had blood drawn less than 42 days after the first episode had nonprimary infection and participants were classified as unknown if they did not demonstrate seroconversion or the first blood draw was 42 days or more after the first episode. A 3-person panel blinded to HSV-1 shedding rates adjudicated the HSV seroconversion of each participant.

T-Cell Responses | Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation and cryopreserved.[9] CD4+ T-cell responses were measured by

interferon γ enzyme-linked immunospot and stimulation with ultraviolet-irradiated HSV-1 strain E115 grown in Vero cells (ATCC) or ultraviolet-treated Vero cells as a negative control.[10] Intracellular cytokine staining (10^6 PBMC/well) was used to characterize the expression of interferon γ, interleukin 2, tumor necrosis factor α, and CD40L in single live CD3+CD4+CD8 T cells.[11] Samples that did not meet PBMC functional viability standards were censored.[11] CD8+ T-cell responses were measured in duplicate by interferon γ enzyme-linked immunospot by stimulating PBMCs (2.5×10^5 cells/well) from each time point with 117 HSV-1 peptide epitopes known to be recognized by CD8+ T cells in 3 pools.[10] For select samples responding to peptide pools, responses were deconvoluted to the single peptide level using methods analogous to those described for SARS-CoV-2.[12] Single peptides recognized by CD8+ T-cells and reactive in this study have been previously reported[13-16] with details documented in Immune Epitope Database and Analysis Resource.[17]

HSV-1 Detection

DNA was extracted and PCR testing was performed using type-common primers to the HSV glycoprotein B gene, with the first positive swab from each participant confirmed to be HSV-1 using a type-specific probe.[18] Swabs with at least 2.3 $\log_{10}$ HSV-1 copies/mL (3 copies/reaction) were considered positive. Swabs from genital and oral lesions were placed into a virus-transport medium and grown in cell culture as previously described.[19]

Full-genome HSV-1 DNA was sequenced from cultures on the Illumina platform[20] with assembly.[21] GenBank accession numbers are provided for newly and previously sequenced HSV-1 genomes (eTables 1-2 in the Supplement).

Statistical Analysis

Genital and oral shedding rates were compared in session 2 and session 1 and among participants with primary compared with nonprimary HSV-1 infection using a Poisson mixed model regression to calculate the relative risk (RR) of viral shedding. Mixed models accounted for association in outcomes between repeated sessions on the same individuals. The model included the number of days positive for HSV-1 shedding as the outcome, with log number of days with swabs as the offset. No assumption of linearity was included because all covariates were binary. The Poisson distribution was not assumed to determine the variance structure because the error variance was estimated empirically. Initial multivariable analyses included 5 terms: sex, age (<26 y vs ≥26 y), session number (1 vs 2), infection type (primary vs nonprimary), and the interaction between session and infection type. Backward elimination was used to select a final model. Similar analyses were performed for lesion frequency, using days with reported lesions as the outcome and log number of diary days recorded as the offset. The association between HSV-1 shedding rates and polyfunctional T-cell function was assessed using Spearmen correlation. Two-sided *P* values <.05 were considered statistically significant. Analyses were conducted using SAS, version 9.4, for Windows.

Table 1. Demographic and Clinical Characteristics of Participants in a Study of Viral Shedding 1 Year Following First-Episode Genital Herpes Simplex Virus Type 1 (HSV-1) Infection

Baseline characteristic	No. (%)	
	All enrolled (n = 82)	Completed 12-mo follow-up (n = 64)
Age, median (range), y	26 (16-64)	26 (16-64)
Age ≥26 y	42 (51.2)	33 (51.6)
Sex		
Women	54 (65.9)	43 (67.2)
Men	28 (34.2)	21 (32.8)
Race[a]		
American Indian/Alaska Native	0	0
Asian	2 (2.4)	1 (1.6)
Black	1 (1.2)	0
Native Hawaiian/Pacific Islander	0	0
White	66 (80.5)	53 (82.8)
More than 1 option	10 (12.2)	8 (12.5)
Other	3 (3.7)	2 (3.1)
Acquisition type[b]		
Primary[c]	42 (51.2)	35 (54.7)
Nonprimary[d]	23 (28.1)	17 (26.7)
Unknown[e]	17 (20.7)	12 (18.8)
Time since genital HSV acquisition if known, median (IQR), d	25 (16-42) [n = 82]	24 (16-59) [n = 64]
History of oral HSV[f]	10 (12.2)[g]	5 (8.3)
Days since oral HSV acquisition if known, median (range)	765 (54-7538) [n = 11]	65 (54-6059) [n = 6]
Method for genital HSV-1 diagnosis		
Only polymerase chain reaction	28 (34.2)	
Only culture	40 (48.8)	
Polymerase chain reaction and culture	14 (17.1)	
No. of sexual partners in past 4 wk, median (range)	1 (0-6) [n = 75]	1 (0-6) [n = 61]

[a] Selected by the participant from a closed list of options that included "other."

[b] Based on HSV serostatus using the HSV Western Blot at screening visit.

[c] Primary defined as HSV-1 seronegative or HSV-1 indeterminate at first blood draw or increasing antibody over time.

[d] Nonprimary defined as HSV-1 seropositive with first blood sample drawn <42 days after symptom onset.

[e] Unknown defined as HSV-1 seropositive with first blood sample drawn ≥42 days after symptom onset or seroconversion not observed.

[f] Oral HSV was defined as self-reported history of symptoms consistent with oral HSV infection.

[g] Three participants with primary HSV-1 reported history of oral HSV. Two participants had oral HSV symptoms with first-episode genital HSV-1. One participant reported a distant prior history of oral HSV symptoms; this participant had clear evidence of HSV-1 acquisition at the time of first-episode genital HSV-1 based on HSV serologic response, and thus was classified as having primary HSV-1.

Results

Participants

Ninety-two individuals were screened and 82 with first-episode genital HSV-1 were enrolled (**Table 1**; eFigure 1 in the Supplement). The median (range) age was 26 (16-64) years,

Table 2. Shedding Rates, Lesion Rates, and Quantity of Herpes Simplex Virus Type 1 (HSV-1) Detected

Outcome	No. (%)					
	8-12 wk after onset (session 1)			48-52 wk after onset (session 2)		
	All (n = 82)	Primary HSV-1 infection (n = 42)[a]	Prior or unknown HSV-1 infection (n = 40)[b,c]	All (n = 64)	Primary HSV-1 infection (n = 35)[a]	Prior or unknown HSV-1 infection (n = 29)[b,c]
HSV-1 DNA detected by polymerase chain reaction testing						
Persons with any genital shedding	53 (64.6)	32 (76.2)	21 (52.5)	21 (32.8)	14 (40.0)	7 (24.1)
Overall genital shedding rate[d]	275/2264 (12.1)	199/1156 (17.2)	76/1108 (6.9)	122/1719 (7.1)	79/933 (8.5)	43/786 (5.5)
Asymptomatic genital shedding[e]	239/2189 (10.9)	167/1099 (15.2)	72/1090 (6.6)	89/1637 (5.4)	48/871 (5.5)	41/766 (5.4)
Persons with any genital lesions	12 (14.6)	8 (19.1)	4 (10.0)	15 (23.4)	12 (34.3)	3 (10.4)
Days with genital lesions[f]	65/2497 (2.6)	60/1271 (4.7)	5/1226 (0.4)	72/1872 (3.8)	66/1042 (6.3)	6/830 (0.7)
Genital lesion shedding[g]	34/62 (54.8)	32/57 (56.1)	2/5 (40.0)	33/68 (48.5)	31/62 (50.0)	2/6 (33.3)
Persons with any oral shedding	24 (29.3)	16 (38.1)	8 (20.0)	17 (26.6)	11 (31.4)	6 (20.7)
Overall oral shedding rate[d]	88/2247 (3.9)	63/1140 (5.5)	25/1107 (2.3)	87/1714 (5.1)	64/925 (6.9)	23/789 (2.9)
Asymptomatic oral shedding[e]	87/2223 (3.9)	63/1140 (5.5)	24/1083 (2.2)	86/1690 (5.1)	64/919 (7.0)	22/771 (2.9)
Persons with any oral lesions	2 (2.4)	0	2 (5.0)	3 (4.7)	1 (2.9)	2 (6.9)
Days with oral lesions[f]	11/2497 (0.4)	0/1271	11/1226 (0.9)	10/1872 (0.5)	6/1042 (0.6)	4/830 (0.5)
Oral lesion shedding[g]	1/11 (9.1)		1/11 (9.1)	1/10 (10.0)	0/6	1/4 (25.0)
Log_{10} copies/mL HSV-1, median (IQR)						
Genital	2.9 (2.5-3.6) [n = 275]	2.9 (2.5-3.7) [n = 199]	2.8 (2.5-3.4) [n = 76]	4.4 (3.2-5.8) [n = 122]	4.5 (3.1-6.5) [n = 79]	4.4 (3.2-4.7) [n = 43]
When lesions present	5.1 (2.5-7.1) [n = 34]	5.2 (2.6-7.3) [n = 32]	2.6 (2.3-2.8) [n = 2]	6.1 (4.3-6.9) [n = 33]	6.1 (4.3-7.4) [n = 31]	5.0 (3.2-6.9) [n = 2]
When asymptomatic	2.8 (2.5-3.4) [n = 239]	2.8 (2.5-3.4) [n = 167]	2.8 (2.5-3.4) [n = 72]	4.2 (3.0-4.8) [n = 89]	3.9 (2.9-5.4) [n = 48]	4.4 (3.2-4.7) [n = 41]
Oral	3.5 (2.5-4.3) [n = 88]	3.4 (2.5-4.4) [n = 63]	3.5 (2.6-4.2) [n = 25]	3.6 (2.7-4.7) [n = 87]	3.5 (2.7-4.8) [n = 64]	3.8 (2.7-4.7) [n = 23]
When lesions present	3.0[h]		3.0[h]	2.2[h]		2.2[h]
When asymptomatic	3.5 (2.5-4.3) [n = 87]	3.4 (2.5-4.4) [n = 63]	3.6 (2.5-4.2) [n = 24]	3.6 (2.8-4.7) [n = 86]	3.5 (2.7-4.8) [n = 64]	3.9 (2.9-4.7) [n = 22]

[a] Primary defined as HSV-1 seronegative or HSV-1 indeterminate at first blood draw or increasing antibody over time.

[b] Nonprimary defined as HSV-1 seropositive with first blood sample drawn <42 days after symptom onset.

[c] Unknown defined as HSV-1 seropositive with first blood sample drawn ≥42 days after symptom onset or seroconversion not observed.

[d] Overall shedding includes all positive swabs over all swabs taken and includes all persons (days with shedding/total daily swabs).

[e] Asymptomatic shedding is defined as the proportion of days with shedding in the absence of genital lesions/total daily swabs collected in absence of lesions.

[f] The denominator for "days with [genital or oral] lesions" is obtained from diary data. There are slight discrepancies between days in which the diary was completed and days with swabs obtained.

[g] Lesion shedding is defined as the proportion of days that with shedding in the presence of genital lesions/total days with genital lesions.

[h] The CI was omitted because there was only 1 swab collected.

54 participants (65.9%) were women, and 66 (80.5%) self-identified as White. Ten participants (12.2%) reported a prior history of oral HSV infection. Eighteen participants (22.0%) were lost to follow-up or withdrew (eFigure 1 in Supplement); their baseline characteristics were similar to those who completed the study (Table 1; eTable 3 in the Supplement). Two participants had concurrent syphilis and 1 had chlamydia.

HSV Serostatus and Seroconversion

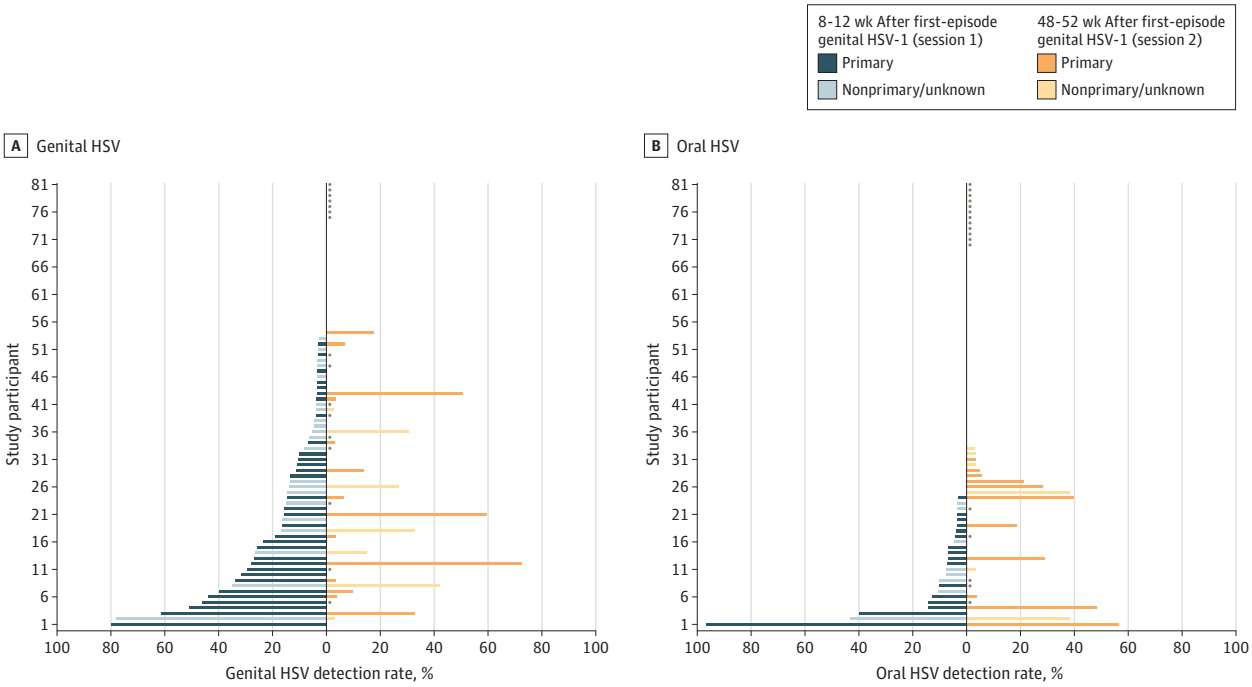
Overall, 42 participants (51.2%) had primary HSV-1 infection, 23 (28.1%) had nonprimary infection, and 17 (20.7%) were classified as unknown. Of 80 participants with sera available 12 weeks after the first symptoms of genital infection, 69 (86.3%) were HSV-1 seropositive, 2 (2.5%) were HSV-1 seronegative, and 9 (11.3%) were indeterminate. By 52 weeks, 60 of 63 participants (95.2%) were HSV-1 seropositive, 1 (1.6%) remained HSV-1 negative, and 2 (3.2%) remained indeterminate. All participants remained HSV-2 seronegative throughout the study.

Genital Shedding and Lesions

All participants completed session 1 and 64 (78.1%) completed session 2 (**Table 2** and **Figure 1**). Overall, 2170 of 2460 (88.2%) expected swabs were collected and 2350 of 2460 (95.5%) diary days were recorded. No participants had fewer than 13 swabs or 17 diary days and there was no antiviral use during either session.

During session 1, a total of 53 participants (64.6%) had genital HSV-1 detected on 275 of 2264 days (12.1%) (Table 2). Asymptomatic genital HSV-1 shedding occurred on 239 of 2189 days (10.9%). Twelve participants (14.6%) had genital lesions reported on 65 of 2497 days (2.6%) during session 1. Among 42 persons with primary HSV-1 infection, 32 (76.2%) had genital shedding, with an overall shedding rate of 17.2%, mostly due to asymptomatic shedding (15.2%) (Table 2). Genital lesions were present on 60 of 1271 days (4.7%). Of 40 participants with nonprimary infection, 21 (52.5%) had HSV-1 detected. The overall genital shedding rate was 6.9%, with an

Figure 1. Genital and Oral Herpes Simplex Virus Type 1 (HSV-1) Shedding Rates



All participants are included; those who completed only the first session are indicated by a dot for the second session. Twenty-eight participants did not have any genital shedding detected and 49 participants did not have any oral shedding detected. Shedding rates are calculated by dividing the number of days with HSV detected by the total number of days with swabs collected.

Study participants are plotted in reverse rank order from least to greatest shedding rate. The order of study participants is not equivalent in panels A and B, and the participant numbers do not relate directly to viral genome isolate numbers in eFigure 3 in the Supplement.

asymptomatic genital shedding rate of 6.6%. Lesions were reported on 5 of 1266 days (0.4%).

During session 2, HSV-1 was detected in 21 of 64 people (32.8%) on 122 of 1719 days (7.1%), with asymptomatic shedding detected on 89 of 1637 days (5.4%) (Table 2). Genital lesions were reported on 72 of 1872 days (3.8%). Among 35 persons with primary genital infection, 14 (40.0%) had genital shedding during session 2, with an overall genital shedding rate of 8.5%. Genital lesions were reported on 66 of 1042 days (6.3%). Among 29 persons with nonprimary infection, 7 participants (24.1%) had genital shedding and the overall shedding rate was 5.5%, with genital lesions reported on 6 of 830 days (0.7%). The median (IQR) quantity of genital HSV-1 detected was 2.9 (2.5-3.6) $\log_{10}$ copies/mL during session 1 and 4.4 (3.2-5.8) $\log_{10}$ copies/mL during session 2, a difference of 0.95 $\log_{10}$ copies/mL more during session 2 (95% CI, 0.60-1.31; $P < .0001$).

Oral Shedding and Lesions

Oral shedding and lesions were detected infrequently throughout the study. During session 1, a total of 24 participants (29.3%) had oral HSV-1 shedding. The oral shedding rate was 3.9% (88 of 2247 days). Oral lesions were reported on 11 of 2497 days (0.4%). Shedding rates were similar between the 2 sessions and in participants with primary and nonprimary infection. The median quantity of HSV-1 detected from the oral cavity was similar during both sessions (Table 2).

Genital and Oral Recurrences

Among 42 people with primary infection, 30 (71.4%) had recurrences over the first year, with a median (range) of 1 (0-7) genital recurrence in the first year. Of those with nonprimary infection, 13 (32.5%) had a recurrence, with a median (range) of 0 (range, 0-7) recurrences. Overall, 98 recurrences were reported among 43 study participants, with 82 recurrences (83.7%) in the genital area. Four people (9.5%) with primary infection and 6 (15.0%) with nonprimary infection reported oral recurrences. Twelve participants (14.6%) used suppressive antiviral therapy between session 1 and session 2.

Long-term HSV-1 Shedding

Eleven participants had shedding on at least 10.0% of days during session 2. Of these participants, 6 (54.5%) completed an additional 30 days of daily oral and genital swabbing at least 2 years after infection. Participants had genital shedding on 1.3% of days, while oral shedding rates were similar to prior sessions (3.2%). One participant (1.6%) had genital lesions; no oral lesions were reported.

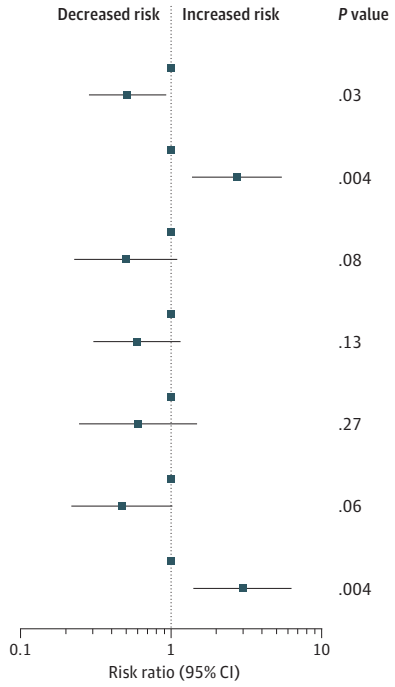
Predictors of Genital and Oral HSV-1 Shedding

In a bivariable model, there was a significant decrease in genital shedding between session 1 (predicted shedding rate, 6.2% [95% CI, 4.3%-8.9%]) and session 2 (predicted shedding rate, 3.2% [95% CI, 1.8%-5.7%]) (RR, 0.52 [95% CI, 0.29-0.93]; $P = .03$), with a negative but nonsignificant

Figure 2. Bivariable and Multivariable Risk Factors Associated With Genital and Oral Herpes Simplex Virus Type 1 (HSV-1) Shedding

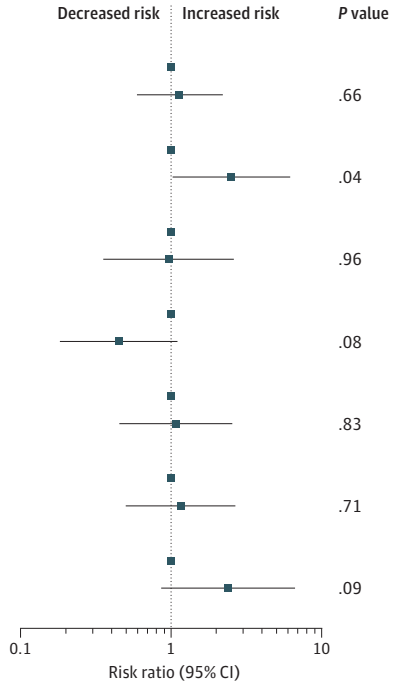
A Genital HSV-1 shedding

Effect	Model-predicted shedding rate (95% CI)	Regressions model	Risk ratio (95% CI)	P value
Session				
1	0.062 (0.043-0.089)	Bivariable	1 [Reference]	
2	0.032 (0.018-0.057)	Bivariable	0.52 (0.29-0.93)	.03
Infection type				
Nonprimary/unknown	0.029 (0.017-0.050)	Bivariable	1 [Reference]	
Primary	0.079 (0.054-0.117)	Bivariable	2.75 (1.40-5.44)	.004
Sex				
Women	0.062 (0.043-0.905)	Bivariable	1 [Reference]	
Men	0.031 (0.016-0.062)	Bivariable	0.50 (0.23-1.10)	.08
Age, y				
<26	0.064 (0.041-0.100)	Bivariable	1 [Reference]	
≥26	0.039 (0.024-0.064)	Bivariable	0.60 (0.31-1.16)	.13
Session among nonprimary infections				
1	0.034 (0.019-0.061)	Multivariable	1 [Reference]	
2	0.021 (0.009-0.050)	Multivariable	0.61 (0.25-1.48)	.27
Session among primary infections				
1	0.102 (0.067-0.156)	Multivariable	1 [Reference]	
2	0.049 (0.024-0.100)	Multivariable	0.48 (0.22-1.02)	.06
Infection type during session 1				
Nonprimary	0.034 (0.019-0.061)	Multivariable	1 [Reference]	
Primary	0.102 (0.067-0.156)	Multivariable	3.02 (1.45-6.29)	.004



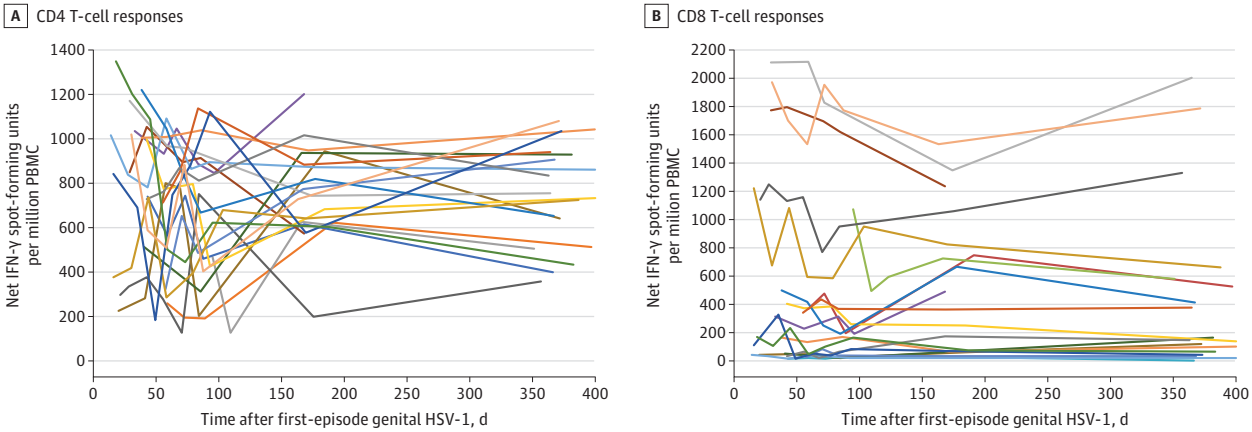
B Oral HSV-1 shedding

Effect	Model-predicted shedding rate (95% CI)	Regressions model	Risk ratio (95% CI)	P value
Session				
1	0.015 (0.009-0.025)	Bivariable	1 [Reference]	
2	0.017 (0.010-0.031)	Bivariable	1.15 (0.61-2.18)	.66
Infection type				
Nonprimary/unknown	0.010 (0.005-0.019)	Bivariable	1 [Reference]	
Primary	0.024 (0.014-0.042)	Bivariable	2.51 (1.03-6.10)	.04
Sex				
Women	0.016 (0.010-0.026)	Bivariable	1 [Reference]	
Men	0.016 (0.007-0.037)	Bivariable	0.97 (0.36-2.63)	.96
Age, y				
<26	0.023 (0.013-0.040)	Bivariable	1 [Reference]	
≥26	0.010 (0.005-0.021)	Bivariable	0.45 (0.19-1.09)	.08
Session among nonprimary infections				
1	0.009 (0.004-0.019)	Multivariable	1 [Reference]	
2	0.010 (0.004-0.024)	Multivariable	1.09 (0.47-2.56)	.83
Session among primary infections				
1	0.022 (0.011-0.045)	Multivariable	1 [Reference]	
2	0.026 (0.013-0.053)	Multivariable	1.17 (0.51-2.65)	.71
Infection type during session 1				
Nonprimary	0.009 (0.004-0.019)	Multivariable	1 [Reference]	
Primary	0.022 (0.011-0.045)	Multivariable	2.43 (0.88-6.69)	.09



For this analysis, those with unknown acquisition type are grouped with those with nonprimary infection. For the comparison between first and second session, the model does not distinguish primary from nonprimary or unknown acquisition type. For the comparison between nonprimary unknown and primary the model does not distinguish the first from the second session. The multivariable model included an interaction term between session and acquisition type. In multivariable models including both age and sex, neither age nor sex contributed to the model in estimating shedding frequencies, so those measures were removed in backward elimination. Forest plots indicate the point estimate and 95% CI for each comparison. The reference is indicated by a box over the midline without a CI.

Figure 3. Cellular Immune Responses Measured Over Time for the First 20 Participants With Primary Genital Herpes Simplex Virus Type 1 (HSV-1)



Each line represents the time course for a single participant, and participants are represented by the same line color for both graphs. IFN indicates interferon; PBMC, peripheral blood mononuclear cells.

association for persons with primary and nonprimary infection (**Figure 2**). Participants with primary genital HSV-1 had a higher risk of genital HSV-1 shedding (model-predicted shedding rate, 7.9% [95%, 5.4%-11.7%]) compared with persons with nonprimary/unknown infections (model-predicted shedding rate, 2.9% [95% CI, 1.7%-5.0%]) (RR, 2.75 [95% CI, 1.40-5.44]; *P* = .004) (Figure 2) and an increased risk of genital lesions (model-predicted lesion rate of 2.1% [95% CI, 1.1%-3.8%] vs 0.3% [95% CI, 0.1%-0.7%]; RR, 6.50 [95% CI, 2.37-17.8]; *P* < .001) (eTable 4 in the Supplement). Sex and age were not significantly associated with genital shedding rates. In a multivariable model containing an interaction term between session and acquisition type, persons with primary infection had a higher risk of shedding during the first session compared with those with nonprimary infection (model-predicted shedding rate of 10.2% [95% CI, 6.7%-15.6%]vs 3.4% [95% CI, 1.9%-6.1%]; RR, 3.02 [95% CI, −1.45 to 6.29]; *P* = .004) (Figure 2).

In a multivariable model, oral shedding was not significantly different among persons with primary compared with nonprimary infection or among those with primary infection over time (Figure 2). A sensitivity analysis excluding 12 people who received suppressive antiviral therapy between session 1 and session 2 yielded similar results for all models (eTable 5 in the Supplement).

HSV-1 Sequencing

To determine whether HSV-1 strains found in the genital tract had unique genomic properties compared with HSV-1 sequences available in GenBank, all isolates from unique participants grown in culture were sequenced (n = 27) (eFigure 2 and eTable 1 in the Supplement). The genital HSV-1 genomes were dispersed throughout a network graph of a globally distributed collection of previously sequenced HSV-1 isolates.

T-cell Responses to HSV-1

Among a subset of participants with primary genital HSV-1, PBMC CD4+ and CD8+ T-cell responses to HSV-1 were performed at specified points during follow-up to determine the kinetics of the development of the cellular immune response (**Figure 3**). CD4+ T-cell responses to ultraviolet-irradiated HSV-1 were detected at the earliest time point available (2 weeks) after HSV-1 acquisition. All participants had detectable CD4+ T-cell responses, and the responses remained stable over the first year of infection for most participants. Regardless of quantity of HSV-1–specific CD4+ T cells, a substantial proportion were polyfunctional, as measured by expression of 3 or 4 of the functional markers interferon γ, interleukin 2, tumor necrosis factor-α, and CD40L. The proportion of cells expressing polyfunctionality did not change qualitatively over time (eFigure 3 in the Supplement). There was no association between genital or oral shedding rates and the proportion of cells that expressed 2, 3, or 4 cytokines in either session (eTable 6 in the Supplement). HSV-1–specific CD8+ T-cell responses were detected using peptide pools and were sustained, validated in select cases to single HSV-1 CD8+ T-cell peptide epitopes (eFigure 4 and eTable 7 in the Supplement).

Discussion

In this study, genital HSV-1 shedding was frequent after first-episode genital HSV-1, particularly among individuals with primary infection, and declined rapidly in the first year after infection. In contrast, symptomatic genital HSV-1 lesions and oral HSV-1 shedding and lesions were uncommon.

The prevalence of genital HSV-1 infection is difficult to determine because diagnosis often relies on HSV-1 antibody tests, which cannot differentiate between oral and genital infection, and there is no surveillance or reporting for genital herpes infections as is the case for other STIs. There were an estimated 192 million prevalent cases of genital HSV-1 infection in 2016 among people aged 15 to 49 years, mostly in the World Health Organization regions of the Americas and Europe.[22] HSV-1 has surpassed HSV-2 to become the leading

cause of first-episode genital herpes in some populations, and this is expected to increase over time. Although it is known that HSV-1 causes less-frequent genital recurrences than HSV-2,[23] this is the first study to our knowledge to comprehensively assess genital and oral HSV-1 viral shedding using PCR. Characterizing shedding rates is clinically important because patients with genital herpes are often concerned about transmission to sexual partners, which usually occurs in the absence of lesions.[24] A previous study using viral culture to assess genital HSV-1 shedding was small, involving only 14 women, and detected a shedding rate of 1.8%.[25] The current study showed that shedding rates detected by PCR, the current standard for viral diagnosis, were higher, particularly in the first months of infection, but declined substantially over the first year. However, these rates are lower than genital HSV-2 shedding rates, which is found on 33.6% of days in persons in the first year after the first clinical episode and persists at 16.7% of days even 10 years after genital HSV-2 infection.[26] Although the threshold quantity of virus for HSV-1 transmission is not known, quantities of HSV-2 greater than 4 $\log_{10}$ copies/mL have been modeled as leading to genital HSV-2 transmission.[27] The higher quantity of HSV-1 DNA detected from swabs in session 2 compared with session 1 likely reflects a larger proportion of swabs collected from lesions in session 2. Persons who lack HSV antibodies at presentation may be counseled to expect more frequent shedding and recurrences and may be candidates for suppressive antiviral therapy for the initial year of infection.

Whether the decline in genital HSV-1 shedding over time is due to virologic or immunologic factors is unclear. HSV reactivation patterns depend on anatomic site and viral type, with HSV-1 recurring more frequently in the oral compared with the genital niche.[28] Antibody responses to HSV-1 developed quickly over time, with most people considered HSV-1 seropositive or indeterminate by 12 weeks after first-episode genital HSV-1. A small rate of nonseroconversion at 1 year was found. This is in contrast with HSV-2, in which 100% seroconversion by 6 months has been previously reported.[29]

Cellular immunity is essential to contain HSV recurrences,[30] and this study demonstrated that polyfunctional HSV-1–specific CD4+ and CD8+ T cells developed early and were sustained after primary HSV-1. Tissue resident memory T cells may also be critical for containment of HSV-1, as has been shown for HSV-2 infection.[31] There was considerable heterogeneity between participants for levels of HSV-1–specific CD8+ T cells. The basis for this observation is unknown, but may be linked to the very large genome size of HSV and the set of 117 proven CD8+ T cell epitopes chosen. Understanding effective immune responses to contain genital HSV-1 infection may inform future development of HSV vaccines.

Although neonatal herpes is rare, with an estimated 10 cases per 100 000 livebirths globally, mortality, morbidity, and cost remain high.[32,33] Genital HSV-1 is estimated to contribute more cases to neonatal herpes than HSV-2 in the World Health Organization regions of the Americas, Europe, and Western Pacific.[32] The finding that genital HSV-1 shedding occurred at a high rate 2 to 3 months after first-episode genital herpes, particularly among those with primary infection, is consistent with the increased risk of neonatal herpes observed after first-episode genital HSV in pregnancy.[34] Identifying pregnant people at risk of genital HSV-1 acquisition or who acquire HSV-1 during pregnancy is a high priority for prevention of neonatal herpes.

This study provides a large contribution of genital HSV-1 specimens to the HSV-1 genomic sequence database. There was no clustering of sequences on the whole genome level to suggest that HSV-1 strains inhabiting the genital niche have unique genetic features. In addition, there was no evidence of transmission clusters in the Seattle area over a 4-year period, suggesting that transmitted HSV-1 infections are from latently infected hosts. Whether people with both oral and genital HSV-1 infection are infected with the same strain simultaneously or can be infected with 2 different strains at the different sites requires additional analysis of the genomic data. Given that some people presented with newly symptomatic genital HSV-1 infection in the setting of fully developed HSV-1 antibody, new genital HSV-1 infection appears possible in persons with prior oral HSV-1.

Limitations

This study has several limitations. First, there was a 22% loss to follow-up at the end of year 1. Second, the study was conducted in a single geographic location in the US and the population comprised predominantly White individuals. This may reflect higher HSV-1 prevalence in childhood among racial and ethnic minority populations, likely due to social determinants of health.[35,36] Third, although no participants used antiviral medication during the swabbing periods, the use of antiviral medications was incompletely captured between the swabbing periods, and recurrences were captured by monthly self-report. Therefore, the number of oral and genital recurrences may be underestimated. Fourth, there were a limited number of participants who were followed up beyond 1 year.

Conclusions

Genital shedding was frequent after first-episode genital HSV-1, particularly among those with primary infection, and declined rapidly in the first year of infection.

ARTICLE INFORMATION

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REFERENCES

1. Spicknall IH, Flagg EW, Torrone EA. Estimates of the prevalence and incidence of genital herpes, United States, 2018. *Sex Transm Dis*. 2021;48(4):260-265. doi:10.1097/OLQ.0000000000001375

2. Kreisel KM, Spicknall IH, Gargano JW, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2018. *Sex Transm Dis*. 2021;48(4):208-214. doi:10.1097/OLQ.0000000000001355

3. Chesson HW, Spicknall IH, Bingham A, et al. The estimated direct lifetime medical costs of sexually transmitted infections acquired in the United States in 2018. *Sex Transm Dis*. 2021;48(4):215-221. doi:10.1097/OLQ.0000000000001380

4. Ryder N, Jin F, McNulty AM, Grulich AE, Donovan B. Increasing role of herpes simplex virus type 1 in first-episode anogenital herpes in heterosexual women and younger men who have sex with men, 1992-2006. *Sex Transm Infect*. 2009;85(6):416-419. doi:10.1136/sti.2008.033902

5. Bradley H, Markowitz LE, Gibson T, McQuillan GM. Seroprevalence of herpes simplex virus types 1 and 2–United States, 1999-2010. *J Infect Dis*. 2014;209(3):325-333. doi:10.1093/infdis/jit458

6. Ayoub HH, Chemaitelly H, Abu-Raddad LJ. Characterizing the transitioning epidemiology of herpes simplex virus type 1 in the USA: model-based predictions. *BMC Med*. 2019;17(1):57. doi:10.1186/s12916-019-1285-x

7. 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;342(12):844-850. doi:10.1056/NEJM200003233421203

8. 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;26(4):662-667. doi:10.1128/jcm.26.4.662-667.1988

9. Koelle DM, Corey L, Burke RL, et al. Antigenic specificities of human CD4+ T-cell clones recovered from recurrent genital herpes simplex virus type 2 lesions. *J Virol*. 1994;68(5):2803-2810. doi:10.1128/jvi.68.5.2803-2810.1994

10. Ott M, Jing L, Lorenzo L, Casanova J-L, Zhang S-Y, Koelle DM. T-cell responses to HSV-1 in persons who have survived childhood herpes simplex encephalitis. *Pediatr Infect Dis J*. 2017;36(8):741-744. doi:10.1097/INF.0000000000001631

11. Dropulic LK, Oestreich MC, Pietz HL, et al. A randomized, double-blinded, placebo-controlled, phase 1 study of a replication-defective herpes simplex virus (HSV) type 2 vaccine, hsv529, in adults with or without HSV infection. *J Infect Dis*. 2019;220(6):990-1000. doi:10.1093/infdis/jiz225

12. Jing L, Wu X, Krist MP, et al. T cell response to intact SARS-CoV-2 includes coronavirus cross-reactive and variant-specific components. *JCI Insight*. 2022;7(6):e158126. doi:10.1172/jci.insight.158126

13. Jing L, Haas J, Chong TM, et al. Cross-presentation and genome-wide screening reveal candidate T cells antigens for a herpes simplex virus type 1 vaccine. *J Clin Invest*. 2012;122(2):654-673. doi:10.1172/JCI60556

14. Koelle DM, Liu Z, McClurkan CL, et al. Immunodominance among herpes simplex virus-specific CD8 T cells expressing a tissue-specific homing receptor. *Proc Natl Acad Sci U S A*. 2003;100(22):12899-12904. doi:10.1073/pnas.2131705100

15. Srivastava R, Khan AA, Spencer D, et al. HLA-A02:01-restricted epitopes identified from the herpes simplex virus tegument protein VP11/12 preferentially recall polyfunctional effector memory CD8+ T cells from seropositive asymptomatic individuals and protect humanized HLA-A*02:01 transgenic mice against ocular herpes. *J Immunol*. 2015;194(5):2232-2248. doi:10.4049/jimmunol.1402606

16. Dervillez X, Qureshi H, Chentoufi AA, et al. Asymptomatic HLA-A*02:01-restricted epitopes from herpes simplex virus glycoprotein B preferentially recall polyfunctional CD8+ T cells from seropositive asymptomatic individuals and protect HLA transgenic mice against ocular herpes. *J Immunol*. 2013;191(10):5124-5138. doi:10.4049/jimmunol.1301415

17. Martini S, Nielsen M, Peters B, Sette A. The Immune Epitope Database and Analysis Resource Program 2003-2018: reflections and outlook. *Immunogenetics*. 2020;72(1-2):57-76. doi:10.1007/s00251-019-01137-6

18. Ryncarz AJ, Goddard J, Wald A, Huang ML, Roizman B, Corey L. Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. *J Clin Microbiol*. 1999;37(6):1941-1947. doi:10.1128/JCM.37.6.1941-1947.1999

19. 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*. 2003;188(9):1345-1351. doi:10.1086/379043

20. Shipley MM, Renner DW, Ott M, et al. Genome-wide surveillance of genital herpes simplex virus type 1 from multiple anatomic sites over time. *J Infect Dis*. 2018;218(4):595-605. doi:10.1093/infdis/jiy216

21. Rathbun MM, Shipley MM, Bowen CD, et al. Comparison of herpes simplex virus 1 genomic diversity between adult sexual transmission partners with genital infection. *PLoS Pathog*. 2022;18(5):e1010437. doi:10.1371/journal.ppat.1010437

22. James C, Harfouche M, Welton NJ, et al. Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bull World Health Organ*. 2020;98(5):315-329. doi:10.2471/BLT.19.237149

23. Benedetti JK, Zeh J, Corey L. Clinical reactivation of genital herpes simplex virus infection decreases in frequency over time. *Ann Intern Med*. 1999;131(1):14-20. doi:10.7326/0003-4819-131-1-199907060-00004

24. Mertz GJ, Schmidt O, Jourden JL, et al. Frequency of acquisition of first-episode genital infection with herpes simplex virus from symptomatic and asymptomatic source contacts. *Sex Transm Dis*. 1985;12(1):33-39. doi:10.1097/00007435-198501000-00007

25. Wald A, Zeh J, Selke S, Ashley RL, Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. *N Engl J Med*. 1995;333(12):770-775. doi:10.1056/NEJM199509213331205

26. Phipps W, Saracino M, Magaret A, et al. Persistent genital herpes simplex virus-2 shedding years following the first clinical episode. *J Infect Dis*. 2011;203(2):180-187. doi:10.1093/infdis/jiq035

27. Schiffer JT, Mayer BT, Fong Y, Swan DA, Wald A. Herpes simplex virus-2 transmission probability estimates based on quantity of viral shedding. *J R Soc Interface*. 2014;11(95):20140160. doi:10.1098/rsif.2014.0160

28. 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;316(23):1444-1449. doi:10.1056/NEJM198706043162304

29. Ashley-Morrow R, Krantz E, Wald A. Time course of seroconversion by HerpeSelect ELISA after acquisition of genital herpes simplex virus type 1 (HSV-1) or HSV-2. *Sex Transm Dis*. 2003;30(4):310-314. doi:10.1097/00007435-200304000-00007

30. Koelle DM, Posavad CM, Barnum GR, Johnson ML, Frank JM, Corey L. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. *J Clin Invest*. 1998;101(7):1500-1508. doi:10.1172/JCI1758

31. Zhu J, Peng T, Johnston C, et al. Immune surveillance by CD8αα+ skin-resident T cells in human herpes virus infection. *Nature*. 2013;497(7450):494-497. doi:10.1038/nature12110

32. Looker KJ, Magaret AS, May MT, et al. First estimates of the global and regional incidence of neonatal herpes infection. *Lancet Glob Health*. 2017;5(3):e300-e309. doi:10.1016/S2214-109X(16)30362-X

33. Lee SWH, Gottlieb SL, Chaiyakunapruk N. Healthcare resource utilisation pattern and costs associated with herpes simplex virus diagnosis and management: a systematic review. *BMJ Open*. 2022;12(1):e049618. doi:10.1136/bmjopen-2021-049618

34. Brown ZA, Wald A, Morrow RA, Selke S, Zeh J, Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *JAMA*. 2003;289(2):203-209. doi:10.1001/jama.289.2.203

35. Xu F, Lee FK, Morrow RA, et al. Seroprevalence of herpes simplex virus type 1 in children in the United States. *J Pediatr*. 2007;151(4):374-377. doi:10.1016/j.jpeds.2007.04.065

36. Delaney AS, Thomas W, Balfour HH Jr. Coprevalence of Epstein-Barr virus, cytomegalovirus, and herpes simplex virus type-1 antibodies among United States children and factors associated with their acquisition. *J Pediatric Infect Dis Soc*. 2015;4(4):323-329. doi:10.1093/jpids/piu076

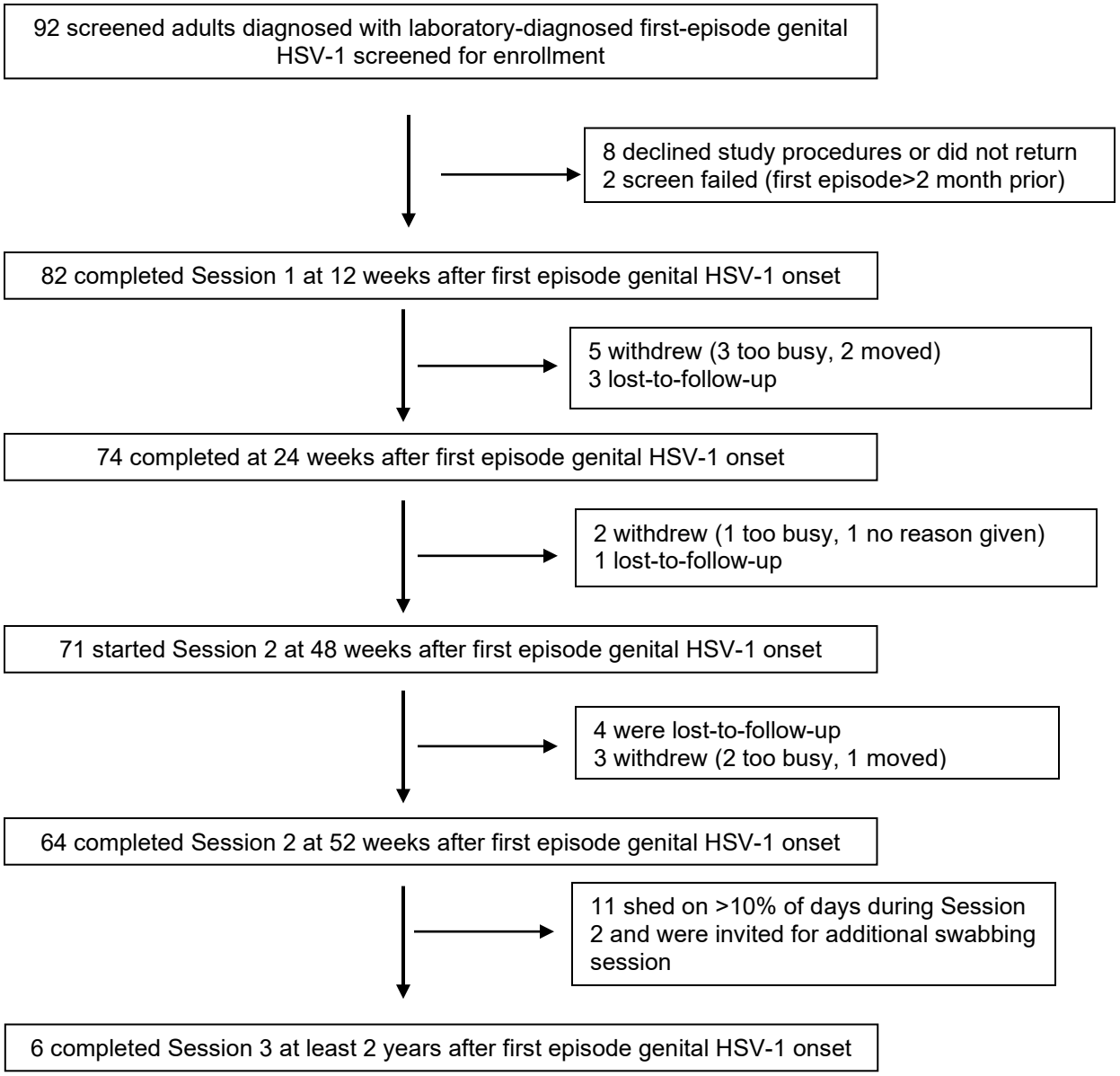
Supplemental Online Content

Johnston C, Magaret A, Son H, et al. Viral Shedding 1 Year Following First-Episode Genital HSV-1 Infection. *JAMA.* Published online October 22, 2022. doi:10.1001/jama.2022.19061

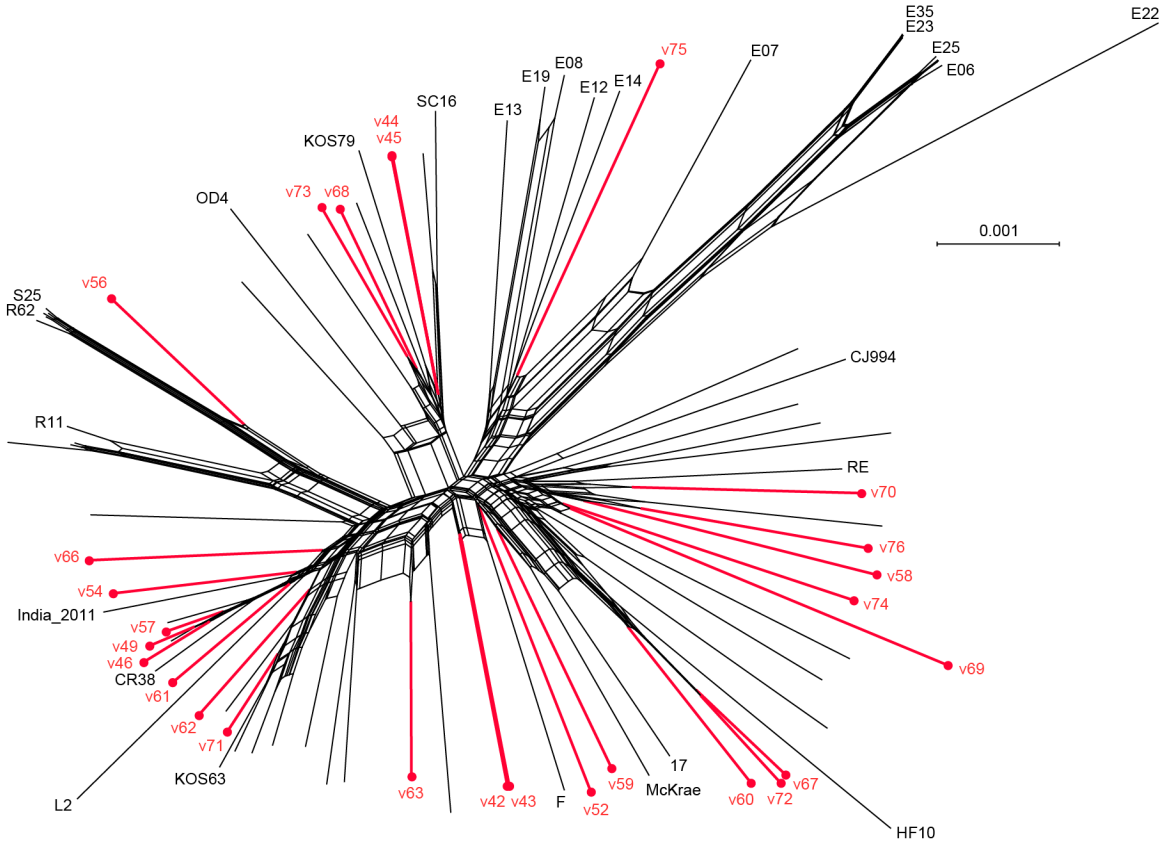
eFigures
eTables
eMethods

This supplemental material has been provided by the authors to give readers additional information about their work.

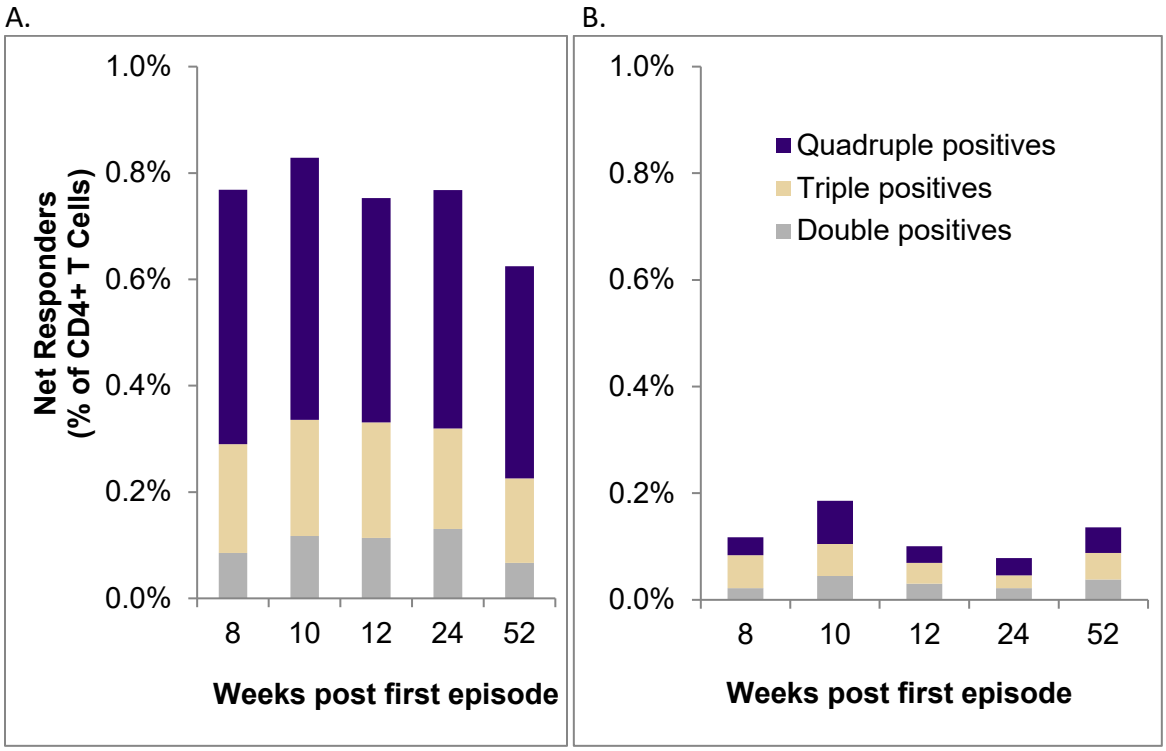
eFigure 1: Participant Flow.



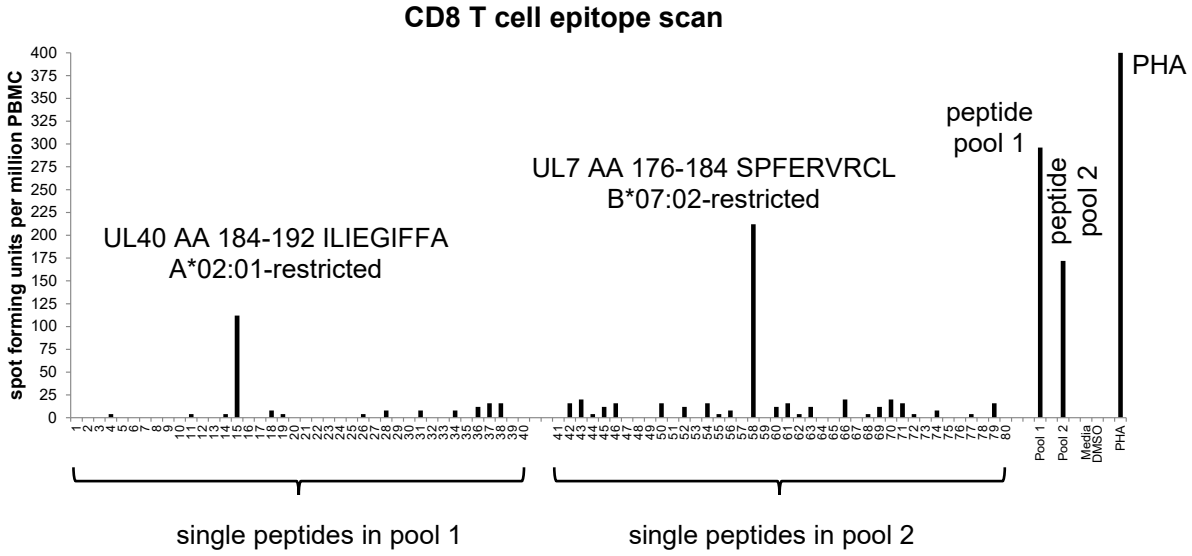
eFigure 2. Genital HSV-1 genomes are distributed among the previously known genetic diversity of HSV-1 genomes. Most previously sequenced HSV-1 genomes were derived from non-genital sources, including herpes labialis (oral), eczema herpeticum (skin), and herpes keratitis (ocular). The network graph of genital HSV-1 genomes from the present study (n=27) are shown in red, with the remaining strains from GenBank in black. Closely paired genomes (v44-v45, v42-v43) were collected from transmission pairs, as previously described[1] SplitsTree (version 4.14.5) was used to create the network graph. See Supplemental Table 1 & 2 for a list of GenBank Accessions for the new and prior genomes.



eFigure 3. Representative time course in two participants with primary genital HSV-1 with high (A) and low (B) proportion of HSV-1 specific CD4 T cells in PBMC. Expression of IFN-γ, IL-2, TNF-α and CD40L was tested. Bar colors reflect the sum of possible combinations of net rates for two or three effector molecules of the abundance of CD4 T cells with all 4 responses.



eFigure 4. Representative ELISPOT data from an HLA-A*02:01/HLA-B*07:02 participant in the current study documenting reactivity with peptide pools and individual HSV-1 peptide epitopes. PBMC from representative participant with primary genital HSV-1 infection) were tested in IFN-γ ELISPOT with pooled or single known HSV-1 CD8 T cell peptides at 1 µg/ml concentration. Identity of reactive peptides is indicated. Reactivity to peptide pools 1 and 2, negative controls media and DMSO, and positive control PHA are shown at right.



eTable 1: 27 new genital HSV-1 genomes from viral cultures

Viral genome ID	Full viral genome name	GenBank Accession	Culture at # days post-primary infection	Infection status at screening
v42	v42_d338_cu_gen_les	OP297869	338	Unable to determine
v43	v43_d17_cu_gen_les	OP297878	17	Primary
v44	v44_d2_cu_gen_les	OP297865	2	Non-primary
v45	v45_d4_cu_gen_les	OP297881	4	Primary
v46	v46_d349_cu_gen_les	OP297868	349	Primary
v49	v49_d257_cu_gen_les	OP297875	257	Primary
v52	v52_d84_cu_gen_les	OP297867	84	Primary
v54	v54_d71_cu_gen_les	OP297873	71	Primary
v56	v56_d6_cu_gen_les	OP297864	6	Primary
v57	v57_d3_cu_gen_les	OP297884	3	Primary
v58	v58_d1_cu_gen_les	OP297870	1	Non-primary
v59	v59_d121_cu_gen_les	OP297876	121	Primary
v60	v60_d3_cu_gen_les	OP297860	3	Primary
v61	v61_d2_cu_gen_les	OP297863	2	Primary
v62	v62_d3_cu_gen_les	OP297872	3	Primary
v63	v63_d284_cu_oral	OP297879	284	Primary
v66	v66_d6_cu_gen	OP297877	6	Primary
v67	v67_d346_cu_gen_les	OP297885	346	Primary
v68	v68_d8_cu_gen_les	OP297880	8	Primary
v69	V69_d2_cu_gen_les	OP297871	2	Unable to determine
v70	v70_d1_cu_gen_les	OP297861	1	Primary
v71	v71_d395_cu_gen_les	OP297866	395	Primary
v72	v72_d53_cu_gen_les	OP297886	53	Non-primary
v73	v73_d4_cu_gen_les	OP297862	4	Primary
v74	v74_d193_cu_gen	OP297874	193	Primary
v75	v75_d23_cu_gen_les	OP297883	23	Non-primary
v76	v76_d266_cu_gen_les	OP297882	266	Primary

eTable 2: HSV-1 genomes used for network graph analysis

Virus Isolate	Country (with location detail, if available)	GenBank Accession #	References
H1211_F-11	Finland	MH999843	[2,3]
H1215_M-15	Finland	MH999846	[2,3]
H12113_ F-13	Finland	MH999842	[3]
H12114_F-14g	Finland	MH999844	[2,3]
H12117_F-17	Finland	MH999845	[2,3]
H12118_F-18g	Finland	MH999847	[2,3]
H1311_F11/	Finland	MH999848	[3]
H1312_M-12	Finland	MH999849	[3]
H1412_F-12g	Finland	MH999851	[3]
H15119_M-19	Finland	MH999850	[3]
SC16	Spain (Madrid)	KX946970	[4]
172_2010	Jena, Germany	LT594105	[5]
2158_2007	Jena, Germany	LT594106	[5]

3083_2008	Jena, Germany	LT594107	[5]
1319_2005	Germany	LT594108	[5]
270_2007	Manebach, Germany	LT594109	[5]
66_2007	Jena, Germany	LT594110	[5]
1394_2005	Germany	LT594111	[5]
369_2007	Jena, Germany	LT594112	[5]
160_1982	Erfurt, Germany	LT594192	[5]
132_1998	Gelsenkirchen, Germany	LT594457	[5]
L2	Russia (Moscow)	KT780616	[6]
H193	U.S.A.	KT425108	[7]
KOS63	U.S.A. (Houston, TX)	KT425110	[8]
KOS79	U.S.A. (Madison, WI)	KT425109	[8]
CJ994	U.S.A. (Madison, WI)	KR011283	[9]
HSV-1/0116209/India/2011	India	KJ847330	[10]
H166	U.S.A.	KM222726	[11]
H166syn	U.S.A.	KM222727	[11]
RE	New Orleans, U.S.A.	KF498959	n/a
OD4	U.S.A. (Madison, WI)	JN420342	[12]
17	U.K. (Glasgow)	JN555585	[13]
CR38	China (Shenyang)	HM585508	[13]
E06	Kenya (Nairobi)	HM585496	[13]
E07	Kenya (Nairobi)	HM585497	[13]
E08	Kenya (Nairobi)	HM585498	[13]
E10	Kenya (Nairobi)	HM585499	[13]
E11	Kenya (Nairobi)	HM585500	[13]
E12	Kenya (Nairobi)	HM585501	[13]
E13	Kenya (Nairobi)	HM585502	[13]
E14	Kenya (Nairobi)	HM585510	[13]
E15	Kenya (Nairobi)	HM585503	[13]
E19	Kenya (Nairobi)	HM585511	[13]
E22	Kenya (Nairobi)	HM585504	[13]
E23	Kenya (Nairobi)	HM585505	[13]
E25	Kenya (Nairobi)	HM585506	[13]
E35	Kenya (Nairobi)	HM585507	[13]
R11	South Korea (Seoul)	HM585514	[13]
R62	South Korea (Seoul)	HM585515	[13]
S23	Japan (Sapporo)	HM585512	[13]
S25	Japan (Sapporo)	HM585513	[13]
F	U.S.A. (Chicago, IL)	GU734771	[14]
H129	U.S.A. (San Francisco, CA)	GU734772	[14]
McKrae	U.S.A. (Gainesville, FL)	JX142173	[13]
HF10	U.S.A. (New York, NY)	DQ889502	[15]
Ty_25	Japan	MH999840	n/a
Ty_148	Japan	MH999841	n/a
K_86	Japan	MH999839	n/a
K_47	Japan	MH999838	n/a

eTable 3. Demographic and clinical characteristics of people enrolled by acquisition type.

Baseline Characteristic	Non-primary (n=23)	Primary acquisition (N = 42)	Unknown acquisition (n=17)
Median age, years (range)	25 (19, 57)	26 (16, 64)	29 (19, 47)
Sex, N (%)			
Female	13 (57%)	31 (74%)	10 (59)
Male	10 (43%)	11 (26%)	7 (41%)
Race, N (%)			
White	19 (83%)	36 (86%)	11 (65%)
Black	0 (0%)	0 (0%)	1 (6%)
Asian	0 (0%)	0 (0%)	2 (12%)
Other	1 (4%)	2 (5%)	0 (0%)
Mix/multiple	3 (13%)	4 (10%)	3 (18%)
Median days since genital HSV acquisition at enrollment, if known (range)	58 (46, 70) [n=23]	58 (54, 95) [n=42]	63 (57, 119) [n=17]
Hx of oral HSV	5 (22%)	3 (7%)	2 (12%)
Median days since oral HSV acquisition, if known (range)	765 (58, 5127) [n=5]	3058 (54, 7538) [n=4]	2302 (71, 4532) [n=2]

eTable 4. Bivariable (B) and multivariable (M)[a] risk factors associated with genital lesions

Effect	Model-predicted Shedding rate (95% CI)	Regressions		
		Model	Risk ratio (95% CI)	p-value
1st session	0.007 (0.003, 0.015)	B	Ref	--
2nd session	0.014 (0.007, 0.026)	B	1.96 (0.77 to 4.98)	0.15
Non-primary/unknown acquisitions	0.003 (0.001, 0.007)	B	Ref	--
Primary acquisitions	0.021 (0.011, 0.038)	B	6.50 (2.37, 17.8)	<0.001
Female	0.012 (0.007, 0.022)	B	Ref	--
Male	0.006 (0.002, 0.016)	B	0.52 (0.17 to 1.61)	0.25
Age < 26	0.014 (0.007, 0.027)	B	Ref	--
Age ≥ 26	0.007 (0.003, 0.015)	B	0.50 (0.18, 1.39)	0.18
1st session among non-primary	0.002 (0.001, 0.006)	M	Ref	--
2nd session among non-primary	0.004 (0.015, 0.012)	M	1.83 (0.57 to 5.86)	0.30
1st session among primary	0.015 (0.006, 0.035)	M	Ref	--
2nd session among primary	0.028 (0.014, 0.060)	M	1.95 (0.71 to 5.37)	0.19
Non-primary during 1st session	0.002 (0.001, 0.006)	M	Ref	--
Primary during 1st session	0.015 (0.006, 0.035)	M	6.21 (1.72 to 22.4)	0.006

[a] For this analysis, those with unknown acquisition type are grouped with non-primaries. For the comparison between first and second session, the model does not distinguish primary from non-primary or unknown acquisition type. For the comparison between non-primary unknown and primary the model does not distinguish 1st session from 2nd session. The **multivariable model** included an interaction term between session and acquisition type. In **multivariable models** including both age and gender, neither age nor gender contributed to the model in estimating shedding frequencies, so those measures were removed in backward elimination

eTable 5. Bivariable (B) and multivariable (M) risk factors associated with genital and oral HSV-1 shedding among 70 people who did not receive suppressive antiviral therapy between Session 1 and Session 2. For this analysis, those with unknown acquisition type are grouped with non-primaries. For the comparison between first and second session, the model does not distinguish primary from non-primary or unknown acquisition type. For the comparison between non-primary unknown and primary the model does not distinguish 1st session from 2nd session. The multivariable model included an interaction term between session and acquisition type. In multivariable models including both age and gender, neither age nor gender contributed to the model in estimating shedding frequencies, so those measures were removed in backward elimination.

Genital Shedding				
Effect	**Model-predicted Shedding rate (95% CI)**	**Regressions**		
		Model	**Risk ratio (95% CI)**	**p-value**
1st session	0.054 (0.036, 0.083)	B	Ref	--
2nd session	0.028 (0.014, 0.054)	B	0.52 (0.26, 1.01)	0.05
Non-1° acquisitions	0.025 (0.014, 0.045)	B	Ref	--
1° acquisitions	0.079 (0.049, 0.126)	B	3.14 (1.47, 6.73)	0.004
Female	0.053 (0.034, 0.084)	B	Ref	--
Male	0.030 (0.014, 0.063)	B	0.57 (0.24, 1.35)	0.19
Age < 26	0.060 (0.035, 0.100)	B	Ref	--
Age ≥ 26	0.032 (0.018, 0.057)	B	0.54 (0.25, 1.17)	0.12
1st session among non-1°	0.031 (0.016, 0.058)	M	Ref	--
2nd session among non-1°	0.016 (0.006, 0.043)	M	0.53 (0.20, 1.41)	0.20
1st session among 1°	0.010 (0.060, 0.166)	M	Ref	--
2nd session among 1°	0.051 (0.022, 0.118)	M	0.51 (0.21, 1.23)	0.13
non-1° during 1st session	0.031 (0.016, 0.058)	M	Ref	--
1° during 1st session	0.100 (0.060, 0.166)	M	3.25 (1.44, 7.35)	0.005
Oral Shedding				
1st session	0.015 (0.008, 0.027)	B	Ref	--
2nd session	0.016 (0.008, 0.031)	B	1.08 (0.50, 2.37)	0.83
Non-1° acquisitions	0.008 (0.004, 0.016)	B	Ref	--
1° acquisitions	0.029 (0.015, 0.055)	B	3.53 (1.41, 8.81)	0.008
Female	0.015 (0.009, 0.026)	B	Ref	--
Male	0.016 (0.006, 0.039)	B	1.06 (0.37, 3.04)	0.91
Age < 26	0.021 (0.012, 0.037)	B	Ref	--
Age ≥ 26	0.011 (0.005, 0.024)	B	0.51 (0.19, 1.37)	0.18
1st session among non-1°	0.007 (0.003, 0.017)	M	Ref	--
2nd session among non-1°	0.009 (0.003, 0.031)	M	1.29 (0.26, 6.40)	0.75

1st session among 1°	0.028 (0.013, 0.060)	M	Ref	--
2nd session among 1°	0.029 (0.013, 0.065)	M	1.04 (0.44, 2.47)	0.93
non-1° during 1st session	0.007 (0.003, 0.017)	M	Ref	--
1° during 1st session	0.028 (0.013, 0.060)	M	3.87 (1.22, 12.25)	0.022

eTable 6. Association between shedding rates and polyfunctional cytokine expression

Spearman correlations (two-sided p-value)			% Cytokine expressed		
			At least 2	At least 3	4
Shedding rates	Genital	1st session	-0.09 (0.68)	-0.12 (0.61)	-0.11 (0.61)
		2nd session	0.46 (0.06)	0.29 (0.25)	0.25 (0.31)
	Oral	1st session	0.07 (0.76)	0.06 (0.77)	0.12 (0.58)
		2nd session	0.32 (0.20)	0.34 (0.17)	0.36 (0.15)

eTable 7. HSV-1 peptides found to be immunogenic in IFN-γ ELISPOT in one or more subjects in this study.		
peptide (HSV-1 open reading frame_amino acids)	**sequence**	**HLA restricting allele**
UL40_184-192	ILIEGIFFA	A*02:01
UL48_479-488	FTDALGIDEY	A*01:01
UL49_281-290	RPTERPRAPA	B*07:02
UL27_17-25	ALLGLTLGV	A*02:01
UL27_561-569	RMLGDVMAV	A*02:01
UL46_702-710	ALSALLTKL	A*02:01
UL46_226-234	AYVSVLYRW	A*24:02
RL2_698-706	VPGWSRRTL	B*07:02
UL7_176-184	SPFERVRCL	B*07:02
UL47_286–294	FLADAVVRL	A*02:01
UL47_544–552	RLLGFADTV	A*02:01
UL48_90-99	SALPTNADLY	A*01:01

eMethods

Viral culture expansion, nucleocapsid DNA isolation, and deep sequencing

A viral master stock was created from each culture-positive swab (27 total; see Supplemental Table 1 below), by expansion on Vero (African green monkey kidney) cells (ATCC, CCL-81). The titer of each stock was determined by limiting dilution on Vero cell monolayers under methylcellulose. To collect viral nucleocapsid DNA, each master stock was used to infect Vero cells at an MOI of 5. From this infection, DNA was isolated according to previously described methods. using Freon-based separation, proteinase K digestion, phenol-chloroform DNA extraction, and ethanol precipitation[16]. Viral nucleocapsid DNA was sheared on a Covaris M220 (parameters: 60-s duration, peak power of 50, 10% duty cycle, 4°C) and used to create barcoded Illumina TruSeq DNA sequencing libraries according to manufacturer's protocols. Libraries were checked by Qubit (Invitrogen, CA), Bioanalyzer (Agilent), and quantitative PCR (KAPA Biosystems), before paired-end sequencing (2 × 300 bp; v3 chemistry) on our in-house Illumina MiSeq.

De novo assembly and network graph analysis of viral genomes

First, HSV-specific reads were selected from by BLAST-based comparison of all Illumina sequence data (FASTQ files) against a database of all HSV genes and genomes in GenBank. The resulting sequence reads were quality-controlled using our published Viral Genome Assembly (VirGA) pipeline[17], which includes adaptor trimming via Trimmomatic[18], and removal of low quality bases (minimum Phred score 30, over a 15 bp window size), short read fragments (minimum size 30 bp), and unpaired reads. The resulting paired-end reads were used for viral genome *de novo* assembly via MetaSpades v.3.14.0 (parameters: *spades.py -k 21, 33, 55, 77 --meta -1 $R1 -2 $R2*)[19]. The resulting MetaSpades contigs were compiled into full-length consensus genomes using VirGA, and annotated by comparison to the HSV1 reference genome (strain 17, GenBank JN555585)[17,20]. These 27 viral genomes were compared to a globally-representative set of 60 previously sequenced viral genomes[3] (see Supplemental Table 2 for strain names, source locations, GenBank accessions, and references). Trimmed viral genomes (excluding the terminal copies of the repeat regions) were aligned using

MAFFT v7.394 with default parameters[21]. Network graphs were constructed using SplitsTree v4 (version 4.14.5; uncorrected P-distance, gaps excluded)[22].

References

1. Rathbun MM, Shipley MM, Bowen CD, et al. Comparison of herpes simplex virus 1 genetic diversity between adult sexual transmission partners with genital infection. *PLOS Pathog*. Published online May 19, 2022. doi:10.1371/journal.ppat.1010437

2. Paavilainen H, Lehtinen J, Romanovskaya A, et al. Inhibition of clinical pathogenic herpes simplex virus 1 strains with enzymatically created siRNA pools. *J Med Virol*. 2016;88(12):2196-2205. doi:10.1002/jmv.24578

3. Bowen CD, Paavilainen H, Renner DW, et al. Comparison of herpes simplex virus 1 strains circulating in Finland demonstrates the uncoupling of whole-genome relatedness and phenotypic outcomes of viral infection. Longnecker RM, ed. *J Virol*. 2019;93(8):e01824-18. doi:10.1128/JVI.01824-18

4. Rastrojo A, López-Muñoz AD, Alcamí A. Genome Sequence of Herpes Simplex Virus 1 Strain SC16. *Genome Announc*. 2017;5(4):e01392-16. doi:10.1128/genomeA.01392-16

5. Pfaff F, Groth M, Sauerbrei A, Zell R. Genotyping of herpes simplex virus type 1 (HSV-1) by whole genome sequencing. *J Gen Virol*. Published online August 24, 2016. doi:10.1099/jgv.0.000589

6. Skoblov MYu, Lavrov AV, Bragin AG, et al. The genome nucleotide sequence of herpes simplex virus 1 strain L2. *Russ J Bioorganic Chem*. 2017;43(2):140-142. doi:10.1134/S1068162016060133

7. Dweikat SN, Renner DW, Bowen CD, Szpara ML. Multi-phenotype analysis for enhanced classification of 11 herpes simplex virus 1 strains. *J Gen Virol*. 2022;in press. doi:10.1099/jgv.0.001780

8. Bowen CD, Renner DW, Shreve JT, et al. Viral forensic genomics reveals the relatedness of classic herpes simplex virus strains KOS, KOS63, and KOS79. *Virology*. 2016;492:179-186. doi:10.1016/j.virol.2016.02.013

9. Lee K, Kolb AW, Sverchkov Y, Cuellar JA, Craven M, Brandt CR. Recombination Analysis of Herpes Simplex Virus 1 Reveals a Bias toward GC Content and the Inverted Repeat Regions. Longnecker RM, ed. *J Virol*. 2015;89(14):7214-7223. doi:10.1128/JVI.00880-15

10. Bondre VP, Sankararaman V, Andhare V, Tupekar M, Sapkal GN. Genetic characterization of human herpesvirus type 1: Full-length genome sequence of strain obtained from an encephalitis case from India. *Indian J Med Res*. 2016;144(5):750-760. doi:10.4103/ijmr.IJMR_747_14

11. Parsons LR, Tafuri YR, Shreve JT, et al. Rapid Genome Assembly and Comparison Decode Intrastrain Variation in Human Alphaherpesviruses. *mBio*. 2015;6(2):e02213-14. doi:10.1128/mBio.02213-14

12. Kolb AW, Adams M, Cabot EL, Craven M, Brandt CR. Multiplex sequencing of seven ocular herpes simplex virus type-1 genomes: phylogeny, sequence variability, and SNP distribution. *Invest Ophthalmol Vis Sci*. 2011;52(12):9061-9073. doi:10.1167/iovs.11-7812

13. Szpara ML, Gatherer D, Ochoa A, et al. Evolution and diversity in human herpes simplex virus genomes. *J Virol*. 2014;88(2):1209-1227. doi:10.1128/JVI.01987-13

14. Szpara ML, Parsons L, Enquist LW. Sequence variability in clinical and laboratory isolates of herpes simplex virus 1 reveals new mutations. *J Virol*. 2010;84(10):5303-5313. doi:10.1128/JVI.00312-10

15. Ushijima Y, Luo C, Goshima F, Yamauchi Y, Kimura H, Nishiyama Y. Determination and analysis of the DNA sequence of highly attenuated herpes simplex virus type 1 mutant HF10, a potential oncolytic virus. *Microbes Infect*. 2007;9(2):142-149. doi:10.1016/j.micinf.2006.10.019

16. Szpara ML, Tafuri YR, Enquist LW. Preparation of viral DNA from nucleocapsids. *J Vis Exp JoVE*. 2011;(54):2-7. doi:10.3791/3151

17. Parsons LR, Tafuri YR, Shreve JT, et al. Rapid genome assembly and comparison decode intrastrain variation in human alphaherpesviruses. *mBio*. 2015;6(2):e02213-14. doi:10.1128/mBio.02213-14

18. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114-2120. doi:10.1093/bioinformatics/btu170

19. Bankevich A, Nurk S, Antipov D, et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J Comput Biol*. 2012;19(5):455-477. doi:10.1089/cmb.2012.0021

20. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9(4):357-359. doi:10.1038/nmeth.1923

21. Katoh K, Misawa K, Kuma K ichi, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*. 2002;30(14):3059-3066.

22. Huson DH. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics*. 1998;14(1):68-73. doi:10.1093/bioinformatics/14.1.68