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Frequent Recovery of HIV-1 From Genital Herpes Simplex Virus Lesions in HIV-1–Infected Men

Timothy Schacker, MD; Alexander J. Ryncarz, PhD; James Goddard; Kurt Diem; Mary Shaughnessy, RN; Lawrence Corey, MD

Context.—Genital ulcer disease has been epidemiologically linked as a risk factor in the transmission of the human immunodeficiency virus 1 (HIV-1). While herpes simplex virus 2 (HSV-2) is the most common cause of genital ulcers, no study has systematically evaluated the frequency or titer of HIV-1 virus in HSV-2 lesions.

Objective.—To compare lesional HIV-1 RNA levels during and after genital HSV-2 reactivation and to evaluate the frequency, titer, and duration of HIV-1 RNA shedding in lesions due to HSV-2.

Design.—Convenience sample.

Setting.—Sexually transmitted disease research clinic at the University of Washington, Seattle.

Patients.—Twelve HIV-infected men with a history of symptomatic HSV-2 infection who underwent daily sampling of genital lesions for HIV-1 RNA by polymerase chain reaction assay and HSV-2 by culture.

Main Outcome Measure.—Detection of lesional HIV RNA and HSV-2.

Results.—HIV-1 RNA was detected from lesional swabs in 25 of 26 consecutively studied HSV-2 episodes and on 67% of days in which genital lesions were noted. The HIV-1 RNA titers in lesional swabs exceeded 10 000 copies/mL of swab sample in 75% of samples (range, $2.2\text{--}3.2 \times 10^5$ copies/mL of swab sample). HIV-1 RNA in genital lesion swabs was seen in persons with high and low titers of plasma HIV-1 RNA and was not associated with plasma HIV-1 RNA levels.

Conclusions.—HIV-1 virions can consistently be detected in genital ulcers caused by HSV-2, which suggests that genital herpes infection likely increases the efficiency of the sexual transmission of HIV-1.

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SEVERAL epidemiologic studies have shown an association between the acquisition of the human immunodeficiency

virus 1 (HIV-1) and the reported presence of genital lesions in the presumed source contact.¹⁻⁶ In 1 study of discordant couples with HIV-1 infection, presence of a genital ulcer in the source partner was associated with a 5-fold greater risk in the transmission of HIV-1.⁷ Worldwide, the most common infectious etiologies of genital ulcers are herpes simplex virus (HSV), syphilis, and chancroid,^{8,9} with HSV the most common in North America and Europe, accounting for 75% to 80% of infectious genital ulcers.¹⁰ One of the first reports of a common source cluster of sexually transmitted HIV-1 infection in Europe emanated

from an HIV-1–infected individual who on retrospective questioning was noted to have genital HSV-2 infection.⁴ Recent studies have shown that genital herpes is the most frequent sexually transmitted disease among HIV-1–seropositive persons,¹¹ and most HSV-2–seropositive persons (whether HIV-1 infected or immunocompetent) intermittently reactivate the virus on mucosal surfaces.¹²⁻¹⁴ Clinically, HSV-2 causes lesions that are smaller and of shorter duration than those caused by chancroid or syphilis, and HSV-2 ulcerations often occur in locations difficult to visualize, such as the cervix or perineal region.^{13,15} As such, the true prevalence of clinically described genital lesions due to HSV among persons suspected of transmitting HIV-1 is likely to be underestimated. We undertook a study to evaluate the frequency, titer, and duration of HIV-1 in genital HSV-2 lesions; to relate lesional titers of HIV-1 RNA to plasma titers of HIV-1 RNA; to examine the association between lesional HIV-1 RNA with HSV-2 in culture; and to compare HIV-1 quasi species in lesions and plasma. As the HIV-1 epidemic in Seattle, Wash, is largely present in the homosexual/bisexual male population, our study enrolled gay men.

METHODS

Patient Recruitment and Procedures

We recruited patients who were HIV-1 positive and had HSV-2 antibodies by Western blot assay through newspaper advertisements and referrals from local physicians into a protocol approved by the University of Washington Institutional Review Board. Patients were excluded at the screening visit if

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they had been receiving daily anti-HSV therapy in the previous 30 days. Of 33 persons referred to our clinic for the study, we enrolled 12. The reasons for lack of participation included desire to use antiviral therapy for herpes recurrences (n = 8), clinically unstable HIV-1 disease that required immediate use of other medications or new antiretrovirals (n = 7), or the patient felt the frequent visits to the clinic during HSV recurrences were too time-consuming (n = 6). We enrolled all patients who fit our serologic and clinical criteria. The study was conducted in 1995, when many HIV-1-positive patients were untreated or receiving monotherapy.

Upon entry, informed consent was obtained, and a standardized questionnaire describing the subject's history of HSV and HIV-1 infection was administered. Blood was obtained at entry for HSV and HIV-1 antibody assays, plasma HIV-1 RNA, and CD4 cell counts. Patients returned to the clinic within 24 hours of lesion onset and at every-other-day intervals until healed. At each visit a genital examination was done, including measurement of the lesion.¹⁶ Swabs of the lesion for HSV culture and HIV-1 polymerase chain reaction (PCR) assay were obtained at each clinic visit. Patients were also allowed to obtain cultures for HSV-2 at home on the days between visits. Home cultures for HIV-1 were not obtained, as the medium for HIV-1 RNA isolation contained guanidinium thiocyanate. The last 6 patients also collected swab samples from the genital region after the lesions healed.¹⁷

Sample Collection

Lesional swabs for HIV-1 RNA were obtained by rubbing a Dacron swab over the base of the lesion and immediately placing it in 500 mL of guanidinium thiocyanate denaturing buffer for transportation and storage. Samples were stored at -70°C within 3 hours of collection. A separate swab for HSV culture was obtained and placed into 1 mL of standard viral transport medium.¹⁸ In 2 patients, a separate lesional swab from the initial and subsequent 3 clinic visits during an HSV-2 reactivation was also collected for HIV-1 isolation.¹⁹

Laboratory Methods

HSV Serology, Isolation, and PCR.—The HSV antibody determinations were performed by Western blot analysis.¹⁵ Isolation of HSV from lesional swabs and detection of HSV DNA in lesional swabs by PCR were performed as previously described.²⁰

HIV-1 RNA Polymerase Chain Reaction and Culture.—Plasma HIV-1 RNA was assayed using the branched chain

DNA method (Chiron Diagnostics, Emeryville, Calif). This assay was performed according to the manufacturer's specifications and at the time of this study had a lower limit of detection of 10 000 copies/mL.^{21,22}

The HIV-1 RNA levels in HSV lesions were determined by reverse transcriptase-polymerase chain reaction (RT-PCR) with *gag*-specific primers and oligonucleotide probes using a modification of the assay described by Piatak et al.²³ Briefly, total RNA was extracted and complementary DNA (cDNA) was synthesized from the purified lesion RNA using random hexamers, prime inhibitor (5 Prime to 3 Prime), and Superscript II (SSII) (Gibco-BRL) reverse transcriptase in a SSII 1 × reaction buffer. The HIV-1 *gag*-specific primers (*gag* 4 and *gag* 6) were used to amplify (45 cycles) the HIV-1 cDNA.

Amplified HIV-1 *gag* sequences were detected by liquid hybridization using a phosphorus 32-labeled oligonucleotide probe (5'-CCAGGCCAGATGAGAGAACCAAGGG-3') specific for a conserved internal region of the amplified HIV-1 *gag* PCR product. Electrophoresis was performed with 20 µL of the hybridization products in a 6% polyacrylamide gel. Visual inspection of the autoradiographs, along with comparisons to known amounts of amplified HIV-1 cDNA, were used to determine the approximate original number of HIV-1 RNA copies in the lesion swab. Each autoradiograph band signifying a positive PCR assay was assigned a score of 1 to 4+ approximating the concentrations of a dilution curve containing from 5 to 5000 copies of HIV-1 RNA, respectively. Each cDNA and PCR reaction contained both positive and negative controls. Known amounts of HIV-1 RNA and cDNA were used for positive controls and for quantification. All samples that were PCR negative for HIV-1 were confirmed to be true negatives, not attributable to nonspecific inhibition of the reaction by performing an additional PCR with 10³ copies of HIV-1 cDNA. Samples that were unable to support amplification of the input substrate were denoted as inhibitory. All others were reported as samples void of HIV-1 RNA. For calculations estimating HIV-1 copies per 200 µL of specimen by PCR, samples with titers between 500 and 5000 were given a titer of 2500, those between 50 and 500, a titer of 250, and those below 50, a titer of 25.

Heteroduplex Tracking Assays.—Heteroduplex tracking assays (HTAs) to HIV-1 envelope were performed to compare viral quasi species in genital lesions and plasma.²⁴⁻²⁷ Nested PCR primers and amplification conditions were

performed as previously described.^{24,25} Primer ED-12 was used for reverse transcription. Primers ED-5 and ED-12 were used for the first round and primers ES-7 and ES-8 for the second round of PCR amplification.^{26,27}

Probes were generated and then reannealed with the PCR products. The resulting reactions were run on 6% non-denaturing polyacrylamide gels, autoradiographed, scanned using an Agfa Arcus II scanner, and analyzed with National Institutes of Health image software.

Plasma and Lesion Cloning.—The ES-7- and ES-8-derived PCR products from lesion samples or plasma samples in which HSV-1 cDNA was detected were ligated into the PCR 2.1 vector (Invitrogen, Carlsbad, Calif) and transformed into *Escherichia coli* with blue/white screening according to the manufacturer's directions. Plasmid DNA was isolated by standard technique. Clones were confirmed by PCR with ES-7 and ES-8 primers.

The PCR products were cloned into a PCR 2.1 vector and then reassayed in the HTA to ensure their similar mobility to the parent clone.

Statistical Methods

Statistical analyses were performed using χ^2 and nonparametric methods as described.

RESULTS

Study Population

We studied 12 male subjects; their mean age was 39 years (range, 30-56 years), and all were men who had sex with men. The CD4 cell counts at entry ranged from 0.018 to 0.541 × 10³/L (18-541/µL); 3 of the 12 were receiving antiretroviral therapy at entry (Table 1). All 12 patients were HSV-2 seropositive, 10 (83%) of 12 also had HSV-1 antibodies. While 9 reported a history of clinically diagnosed genital or anal HSV at the enrollment visit, the other 3 subjects who denied any history of clinically diagnosed genital herpes reported intermittent episodes of genital ulcerations that would spontaneously heal within 5 to 14 days. No patient was receiving suppressive therapy for HSV infection, and none routinely used antiviral therapy for their herpetic recurrences. The median plasma HIV-1 RNA level at entry was 35 000 copies/mL (range, 13 000-1 600 000 copies/mL) (Table 2).

Frequency and Location of HSV-2 Reactivation

The 12 patients were followed up through 26 episodes of HSV-2 reactivation; 8 through a single HSV-2 episode, 1 during 2 episodes, 1 during 4 episodes, and 2 during 6 consecutive episodes. The anatomic site of reactivation of the 26

episodes was penile shaft in 4 episodes (15%), perirectal or inner gluteal fold region in 19 episodes (73%), facial region in 1 episode (4%), and the outer buttock or lumbosacral region in 2 episodes (8%). The size of lesions (median, 47.5 mm²) and the duration of the episode (median, 15 days) were typical for genital lesions for mildly immunocompromised persons.¹³ HSV-2 was isolated from the lesions in 24 (92%) of the 26 episodes; the 2 HSV culture-negative episodes had HSV-2 DNA detected by PCR in the lesional swab at the initial visit for that episode. Thus, all 26 episodes sampled were due to HSV-2; of the 26 episodes, 23 healed without antiviral therapy.

HIV-1 in Genital HSV Lesions

A total of 175 lesional swab samples were collected at the clinic visits (median, 5.5 samples per episode; range, 2-18 samples per episode). Five lesional swabs from 5 separate persons repeatedly inhibited the PCR reaction and were thus not evaluable for HIV-1 RNA detection. HIV-1 RNA was detected in lesional

swabs in 25 of the 26 episodes of genital herpes (Table 2), and HIV-1 RNA was detected in 108 (64%) of the 169 assayable lesional swab samples. The median percentage of days that HIV-1 RNA was detected from lesional swabs was 66.7%; HIV-1 RNA was detected at a level of 10 000 copies/mL or higher in 75% of the of HIV-1 RNA-positive lesional swab samples. To estimate the maximal titer of HIV-1 RNA in the lesion samples with more than 10 000 copies/mL in the initial screening assay, additional 10-fold dilution of the cDNA isolated from the swabs was performed on 8 samples from 6 patients who had a lesional swab titer of more than 5000 copies/200 µL of swab specimen. The titer of HIV-1 RNA per milliliter of swab sample in the 8 samples ranged from 20 000 to 320 000 copies/mL of swab specimen (median, 40 000 copies/mL) with 3 of the samples having end point titers between 2.2×10^5 and 3.2×10^5 copies of HIV-1 in the lesional swab samples. These titers of HIV-1 RNA in the lesional swab specimens exceeded the patient's plasma RNA taken

at the same time by at least 2-fold in 4 of the 6 patients.

A representative autoradiogram from the lesional swabs is shown in Figure 1. The PCR reaction was performed both with and without the addition of RT. Even among samples in which the level of HIV-1 RNA was greater than or equal to 5000 copies/200 µL of lesion swab material, a signal was detected only in the reaction in which RT was placed in the sample, indicating that all the PCR signal was attributable to the presence of virion RNA (Figure 1). Proviral DNA was not detected in any of the 14 HIV-1 RNA-positive samples run in parallel with and without RT.

HIV-1 RNA was detected at equal frequency in all anatomic areas sampled, and all 4 patients who were sampled through multiple recurrences of HSV-2 had HIV-1 RNA detected from lesional swab samples in all the recurrences. A representative autoradiogram of the lesion samples from sequential episodes of 1 of these 4 patients is shown in Figure 2.

We next evaluated whether HIV-1 RNA could be detected on intact genital skin from a recently healed HSV-2 episode. We tested 9 swabs obtained from 3 of the 6 patients who collected these samples after lesional healing. All samples were negative for HIV-1 by viral isolation, and 8 of the 9 swabs from intact skin were also negative for HIV-1 RNA by PCR. One swab had approximately 10 copies of HIV-1 RNA detected in the swab sample obtained from a site where a herpes lesion had healed within the previous 4 days.

Swabs were submitted for HIV-1 isolation in cell culture in 2 patients (4 swabs each). No HIV-1 was isolated from any of the lesional swabs. HIV-1 RNA was, however, detected in all 8 of these same samples (4 swabs were associated with

Table 1.—CD4 Cell Counts, Antiretroviral Therapy, and Genital HSV History in Patients Enrolled*

Patient No.	CD4 Cell Count at Entry, $\times 10^6/L$	Duration of HIV-1 Diagnosis, y	Antiretroviral Therapy on Entry	HSV Type Antibody Status	Duration of Symptomatic Anogenital HSV, y	No. of Reported Genital HSV Episodes in Past 12 mo
1	0.101	12	Zidovudine, saquinovir	1, 2	3	12
2	0.069	11	Zidovudine, didanosine	1, 2	>1	14
3	0.472	9	No	2	14	3
4	0.195	10	Stavudine	1, 2	10	8
5	0.176	10	No	1, 2	3	18
6	0.156	9	No	2	0	0
7	0.018	6	No	1, 2	2	1
8	0.541	6	No	1, 2	5	3
9	0.352	8	No	1, 2	>1	12
10	0.074	3	No	1, 2	0	0
11	0.529	11	No	1, 2	12	4
12	0.209	9	No	1, 2	0	0

*HIV indicates human immunodeficiency virus; HSV, herpes simplex virus.

Table 2.—Frequency of HIV-1 RNA Detection in the Enrollment Episode of Genital HSV-2*

Patient No.	Site	Duration of Lesion, d	Anti-HSV Therapy	No. of Days HSV-2 Isolated/ No. of Days Sampled (%)	No. of Days HIV-1 RNA Detected†/ No. of Days Sampled (%)	Maximum HIV-1 Lesional Titer‡	Maximum Lesion Size, mm ²	Plasma HIV-1 RNA at Start of Recurrence, $\times 10^3$ Copies/mL
1	Rectal area	23	Yes (day 6)	7/23 (39)	14/18 (78)	>5000	88	208
2	Rectal area	26	No	14/27 (52)	2/3 (66)	25	134	181
3	Buttock	18	No	5/19 (26)	0/4 (0)	NA	220	32
4	Buttock	14	Yes (day 10)	9/14 (64)	8/9 (89)	>5000	20	22
5	Buttock	17	No	9/17 (53)	11/12 (92)	>5000	56	47
6	Rectal area	31	No	7/31 (23)	12/14 (86)	>5000	9	100
7§	Face	120	No	4/4 (100)	2/2 (100)	>5000	200	1600
8	Penis	11	No	6/12 (50)	2/4 (50)	2500	25	35
9	Back	22	No	1/24 (4)	2/3 (67)	50	80	28
10	Penis	7	No	6/8 (75)	2/4 (50)	2500	18	66
11	Buttock	6	No	1/12 (8)	3/6 (50)	>5000	14	13
12	Buttock	13	No	3/14 (21)	3/7 (43)	2500	100	25

*HIV indicates human immunodeficiency virus; HSV, herpes simplex virus; and NA, not applicable because lesional HIV-1 RNA was not detected at any time point.

†HSV cultures were collected every day and HIV-1 RNA swabs were collected every other day from onset of lesion until healing.

‡Estimated copies per 200 µL of swab specimen.

§Acyclovir-resistant HSV was confirmed on day 14 and topical antiviral therapy initiated. Only samples detected prior to initiation of therapy are included.

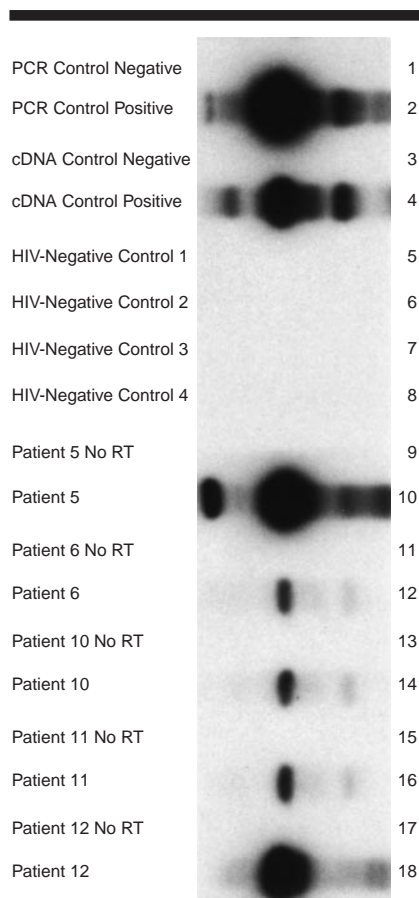


Figure 1.—Autoradiographs of genital herpes lesion swabs showing human immunodeficiency virus 1 (HIV-1) RNA detected in all lesions. The autoradiographs show the initial lesion swabs from patients 5, 6, 10, 11, and 12 with and without reverse transcriptase (RT). Lanes 5 through 8 show lesional swabs of herpes simplex virus 2 lesions for HIV-1–negative patients. Polymerase chain reaction (PCR) and complementary DNA (cDNA) controls demonstrate that the PCR and cDNA reactions are free from contamination.

HIV-1 RNA titers ≥ 5000 copies/200- μ L sample, and 4 were associated with titers of between 500–5000 copies/200- μ L sample).

Relation of Lesional Titers of HIV-1 RNA to Plasma Titers of HIV-1 RNA

HIV-1 RNA was detected in lesions from persons with relatively low titers of HIV-1 RNA in plasma (patients 4 and 11), those with high HIV-1 RNA titers (patients 1, 2, and 6), and from persons taking and not taking antiretroviral therapy (Table 2).

Association Between Lesional HIV-1 RNA and HSV-2 by Culture

Detection of HIV-1 RNA in lesional swabs was significantly associated with isolation of HSV-2 by culture. HIV-1 RNA was present in 63 (80%) of the 79 swabs taken on days from which HSV-2 was also isolated vs 45 (50%) of the 90

lesional swab samples on days from which HSV-2 was not isolated (odds ratio [OR], 4.6; 95% confidence interval [CI], 1.8–8.7; χ^2 ; $P < .01$). Figure 3 illustrates the HSV-2 and HIV-1 RNA shedding in genital lesion swabs during the course of a genital lesion. High titers of HSV DNA and HIV-1 RNA were detected in lesional swabs early in the episode. By day 6, within 2 days after the initiation of acyclovir (400 mg orally every 8 hours), HSV-2 was no longer isolated from the lesion, and by day 11 HSV DNA as detected by PCR was no longer detected. The HIV-1 RNA titer in genital lesions persisted at high levels until HSV replication as detected by PCR fell to low levels and lesion healing occurred. HIV-1 RNA became undetectable once the genital lesion reepithelialized.

Comparison of HIV-1 Quasi Species in Lesions and Plasma

We also evaluated the genetic relationship of the HIV-1 RNA detected in lesional swabs with plasma RNA. For these analyses we took samples from patient 4 and used HTA to compare the viral quasi species in the genital lesion with those in plasma samples collected at the time of reactivation and shortly thereafter. Initially, we cloned HIV-1 envelope sequences from the lesional swab sample on the initial day of HSV reactivation. For these studies we used the ES-7 and ES-8 probes, which span a 700–base pair region of the HIV-1 envelope between regions C2–V5, including the V3 loop region. Two variants were identified in the lesional swab: a predominant variant and a minor variant labeled variant 2. Variant 2 was cloned, radiolabeled, and used in an HTA assay of sequential serum taken after the HSV reactivation. Figure 4 shows increasing concentrations of variant 2 between March 10, the initial onset of the lesion, and May 2, which was 52 days later, 45 days after healing of the lesions. Using Image Analysis software, we found that variant 2 increased 2.5-fold in plasma over the 7-week follow-up period. These data were corroborated by cloning of variant 2 from plasma on March 10 and May 2. Of 18 clones derived from the plasma obtained, on March 10, the day of onset of the lesion, 2 of 18 were variant 2. On May 2, five of the 18 clones derived from plasma were variant 2. Thus, by both methods a 2.5-fold increase in variant 2 was seen. These data suggest that variant 2 found in genital lesions is a replication competent clone of HIV-1.

COMMENT

We consistently detected HIV-1 RNA by both *gag* and envelope primers in HSV-2 lesions. The HIV-1 nucleic acid

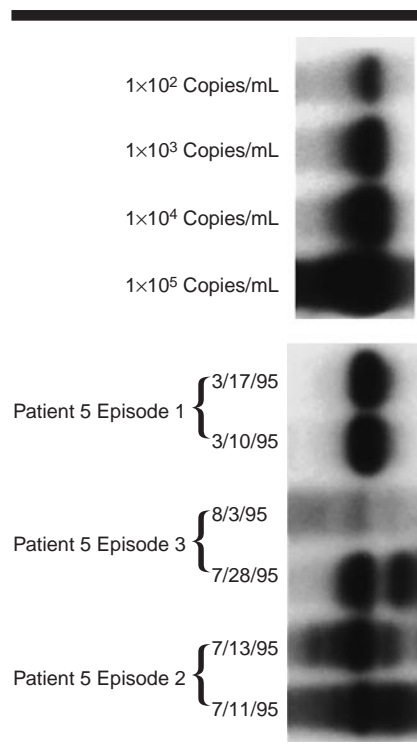


Figure 2.—Autoradiographs of polyacrylamide gel electrophoresis–run liquid hybridizations of human immunodeficiency virus 1 (HIV-1) polymerase chain reaction showing HIV-1 recovered on different days and from different episodes of genital herpes simplex virus 2 from patient 5. The control lanes show serial 10-fold dilutions of HIV-1 complementary DNA.

we detected in lesional swabs was virion associated, underscoring the infectious potential of these lesions. Heteroduplex tracking assay demonstrated that an HIV-1 variant present in the lesional swab specimen was present in increasing amounts in plasma over the course of follow-up, suggesting that replication competent virions of HIV-1 can persist in herpetic lesions.

Most genital herpes episodes were associated with at least 1 specimen with an HIV-1 RNA titer of more than 5000 copies/200 μ L of lesional swab specimen. In some specimens, end point dilution analysis revealed titers of more than 2×10^5 copies/mL of lesional swab sample. The titer of HIV-1 that is associated with transmission of HIV-1 during sexual activity is unknown. The presence of HIV-1 in semen is also an important component in the transmission of HIV-1 infection, and it is clear that HIV-1 transmission can occur without genital lesions. Thus, the relative contribution of HIV-1 shedding from genital lesions vs that from semen cannot be determined from our study. However, epidemiologic data support the intermittent nature of transmission of HIV-1 among stable sexual partners.²⁸ The “bursts” of HIV-1 virions that accom-

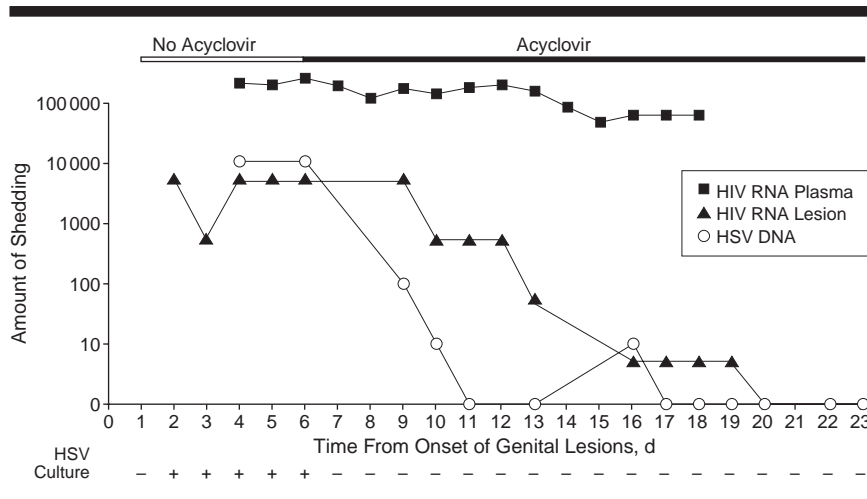


Figure 3.—Sequential detection of human immunodeficiency virus 1 (HIV-1) and herpes simplex virus (HSV) in genital lesion swabs during an outbreak of genital herpes. The plus sign denotes days in which HSV-2 was isolated in tissue culture; the minus sign indicates no HSV isolated. The HIV-1 RNA levels are shown as RNA copies per 200 μ L of lesional swab specimen. The HSV DNA levels are copies of HSV DNA per 50 μ L of lesional swab specimen. Acyclovir was given at a dose of 400 mg orally every 8 hours.

pany genital lesions may help explain the variable transmission rates of HIV-1 among couples.²⁸ The “clusters” of rapidly spreading HIV-1 that occur in association with source contacts with reported genital lesions suggest that shedding of HIV-1 in such lesions may be an underappreciated factor in the overall efficiency of sexual transmission of HIV-1.¹⁻⁷

Tetanus toxoid, influenza, and pneumococcal immunizations transiently increase plasma HIV-1 RNA concentrations via in vivo activation of T cells from antigenic stimulation.²⁹⁻³² Our data support the hypothesis that antigenic stimulation on mucosal sites by reactivation of HSV, an unrelated infectious pathogen, can potentially increase HIV-1 replication on mucosal surfaces. This concept is underscored by the consistency of our findings, irrespective of the anatomic site of the HSV-2 lesions we sampled. While herpetic lesions often have serosanguinous fluid in them, raising the possibility that HIV-1 RNA detected was “contamination” from blood, we found no correlation between frequency of detection of HIV-1 in genital lesions and HIV-1 plasma RNA titers. As shown in Table 2, high titers of HIV-1 in lesional swabs were not related to levels in plasma, and subsequent reduction of HIV-1 RNA in genital lesions was not related to plasma RNA levels (Figure 3). The correlation between the detection of HIV-1 RNA in lesional swabs and the ability to isolate HSV-2 in the lesion along with the prompt reduction in HIV-1 RNA and HSV-2 titer in mucosal swabs associated with acyclovir therapy support the hypothesis that HSV-2 reactivation may play an important role in the titer of HIV-1 on mucosal surfaces.

The HSV-infected lesions are associated with an influx of CD4-bearing lymphocytes that carry activation markers.^{33,34} Activation of latently infected CD4 cells has been shown to markedly up-regulate HIV-1 replication,³⁵ and several HSV regulatory proteins up-regulate HIV-1 replication through their interaction with the HIV-1 long terminal repeat region.³⁶⁻⁴⁰ Coinfection of HSV and HIV-1 in lymphocytes has been described both in vitro and in vivo.^{41,42} Thus, the high titers of virion HIV-1 that we detected in genital herpetic lesions may be the result of an influx of activated, HIV-infected CD4-bearing cells into the mucosal ulceration, as well as a potential in vivo interaction between the 2 viral agents. Additional evidence of this biologic interaction of the 2 agents is that similar results were seen irrespective of the anatomic site of the HSV-2 reactivation. We were unable to demonstrate presence of proviral DNA in the lesion. However, our extraction methods were designed to maximize recovery of RNA from lesional swabs, and it is possible that some provirally infected cells were present in genital lesions, but escaped detection.

The duration, extent, and clinical severity of the HSV reactivations in the patients we studied did not differ from persons with similar CD4 cell counts in other studies of the natural history of genital herpes in HIV-1-infected persons.^{12,13} If anything, our population was skewed to persons with milder disease who did not feel antiviral therapy of their lesions was necessary or whose health care providers did not routinely prescribe antiviral therapy. Thus, we feel it likely our results would apply to most

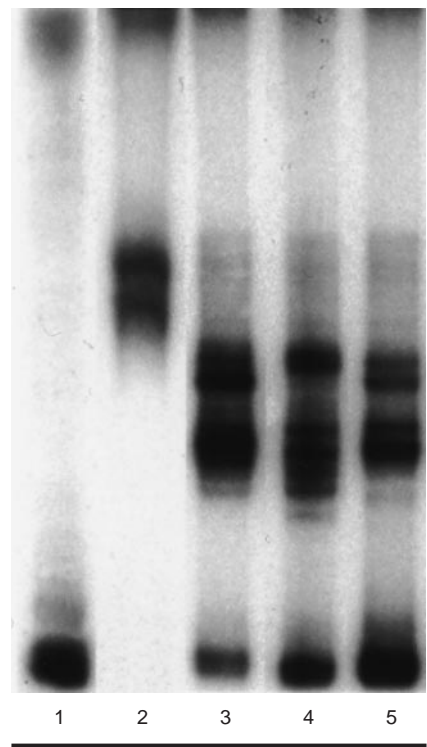


Figure 4.—Heteroduplex tracking assay showing increasing concentration in plasma of variant 2, which was cloned from a herpes simplex virus 2–positive genital lesion on March 7, 1995. Lane 1, showing the reaction between variant 2 and the polymerase chain reaction product of this variant, illustrates a single homoduplex band (positive control). Lane 2, showing the reaction between variant 2 and plasma from another study patient, has no homoduplex band but only heterogenous bands (negative control). Lanes 3 through 5 show variant 2 as it was probed against human immunodeficiency virus 1 RNA derived from plasma on March 10, March 28, and May 21. Increasing amounts of homoduplex bands to variant 2 are noted over time. By densitometry analysis variant 2 increased 2.5-fold in the May 7 plasma (lane 5) compared with the March 10 plasma (lane 3).

episodes of HSV-2 reactivation among HIV-1-infected persons. The association that we saw between treatment of the HSV episode with acyclovir and reduction of HIV-1 expression in the lesion warrants further study. For, if the findings are consistent, they suggest more attention is warranted to the treatment of genital ulceration due to HSV-2, especially among persons with early HIV infection.

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