JOURNAL OF VIROLOGY, Oct. 2000, p. 8946–8952 Vol. 74, No. 19
0022-538X/00/$04.00+0

Effects of Genital Tract Inflammation on Human Immunodeficiency Virus Type 1 V3 Populations in Blood and Semen

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Received 12 January 2000/Accepted 8 July 2000

We have examined cell-free viral populations in the blood plasma and seminal plasma compartments of men infected with subtype C human immunodeficiency virus type 1 (HIV-1) using the V3-specific heteroduplex tracking assay (V3-HTA). We studied two cohorts of subjects who had visited either a sexually transmitted disease (STD) clinic for genital tract inflammation in the form of urethritis ($n = 43$) or a dermatology clinic (controls, $n = 14$) in Malawi. We have previously shown that the presence of urethritis is associated with an eightfold increase in virus load in the seminal plasma compartment (M. S. Cohen et al., Lancet 349:1868–1873, 1997). The purpose of this study was to determine whether genital tract inflammation and its treatment caused genetic instability in cell-free HIV-1 populations. In a cross-sectional analysis at study entry, three-fourths of the STD and control subjects had multiple V3 populations in their blood while 60% of the STD subjects and 79% of the control subjects had multiple V3 populations in their semen. Overall, one-fourth of all of the subjects showed discordance between results with blood and semen specimens when samples were compared for the presence and absence of subpopulations. When differences in the relative levels of abundance of bands were also taken into account, two-fifths of all of the subjects showed discordance between the compartments. Among the subset of subjects in whom multiple virus populations could be detected, half showed discordance between the compartments. There were no differences between STD and control cohorts for these comparisons of the compartments in this cross-sectional analysis at study entry. Longitudinal analysis of the viral populations from two separate clinic visits over 1 to 4 weeks showed that the complexity of each V3 population as measured by Shannon entropy was different in blood and semen at the two time points, indicating that the blood and semen constitute different compartments for HIV-1. The seminal plasma compartment was more dynamic than the blood plasma compartment for the STD subjects who were treated for urethritis, with changes being noted in the presence or absence of V3-HTA bands in the semen of 29% of these subjects but in the blood of only 9% of these subjects. However, the changes were generally small. Overall, our results suggest that 40% of male subjects show discordance between seminal and blood viral populations and that the complexity of each V3 population was different between the two compartments. Both of these results point to the partial independence of the seminal compartment as a viral niche within the body.

Human immunodeficiency virus type 1 (HIV-1) is primarily transmitted by sexual intercourse (23). The efficiency of transmission of HIV-1 is likely to depend on the concentration of HIV-1 in genital secretions; increased heterosexual transmission has been associated with higher viral loads in blood (17, 21, 22). The genotypic and phenotypic characteristics of HIV-1 are also important for efficient transmission (12, 13, 31). The male genital tract represents a unique compartment, since mutations conferring resistance to antiviral drugs, changes in the viral envelope, and/or other characteristics can differ in HIV-1 sequences recovered from blood and semen (5, 7, 8, 11, 28, 32). The amount of HIV-1 (cell free and cell associated) in semen can vary widely between different men (4, 7, 25) and can vary over time in each man (16). While high HIV-1 levels in semen are correlated with advanced HIV-1 disease, the virus has been detected and/or cultured from semen collected during all phases of infection (1, 25, 26, 30).

Classical sexually transmitted infections that cause genital ulcers or mucosal inflammation facilitate transmission of HIV-1 by increasing the infectiousness of the index case, the susceptibility of the exposed sexual partner, or both (14). We have shown that men with urethritis have higher HIV-1 levels in their semen than does a control population and that appropriate antibacterial therapy reduces HIV-1 concentration in semen (6).

We have previously used a V3-specific heteroduplex tracking assay (V3-HTA) to examine populations of HIV-1 variants in blood plasma (18, 20). In the present study we used this assay to compare populations of HIV-1 in the cell-free plasma fractions of blood and semen in different patient cohorts over time and before and after antibacterial therapy for urethritis. We focused on the virus from cell-free plasma from blood and semen since cell-free virion pools contain the actively replicating virus and, in one documented case, the cell-free virus from semen from a transmitter contained the same variant as was in the recipient (32). We were particularly interested in deter-

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mining the relationship between HIV-1 variants in blood and semen because of previous work showing differences between the peripheral blood and seminal cell compartments in men with subtype B HIV-1 (8). In addition, our clinical study (6) provided samples appropriate for evaluating the effect(s) of local inflammation caused by urethritis on populations of HIV-1 in semen and blood.

MATERIALS AND METHODS

Source of patient samples. A subset of the cohort of HIV-1-infected men from Malawi originally described by Cohen et al. (6) was chosen for this study. Blood and semen were collected at 1- and 2-week intervals from two groups of men seen either at a sexually transmitted disease (STD) clinic or (as controls) at a dermatology clinic in Lilongwe, Malawi. A subset was chosen because reverse transcription (RT)-PCR products for V3-HTA could be obtained from matched blood and semen samples collected on the initial visits of the subjects to either the STD clinic or the dermatology clinic. Men from this subset were then selected for a second subset if RT-PCR products for V3-HTA could be obtained from a second set of matched blood and semen samples collected at a subsequent visit to one of the clinics. All participants in the study were given physical exams and were tested for STDs (6). Subjects were determined to have urethritis if discharge was found during the physical examination and there were at least five white blood cells per high-power field on a Gram-stained urethral smear. All of the STD subjects and none of the controls in the present analysis had urethritis. Two of the control subjects had syphilis, and three had asymptomatic trichomonas and were included because they did not have urethritis. All subjects with STDs were treated with appropriate antibiotics, and controls who did not have an STD were not treated with any antibiotics.

Viral RNA isolation, RT-PCR, and V3-HTA. Cell-free plasma from blood and semen were separated as described previously (6) and stored frozen. Viral RNA was extracted from the blood plasma and seminal plasma using a QIAamp viral RNA extraction kit (Qiagen, Valencia, Calif.). RT-PCR was performed as described previously (20), with each RT-PCR being repeated at least once to verify representative sampling of the RNA templates. V3-HTA was performed with the subtype C probe as described previously (20). The consensus subtype B probe was constructed by amplifying the same 159-bp V3 sequence as that used for the subtype C probe from a JR-FL molecular subclone (pUC112-1 from Irvin Chen, University of California, Los Angeles [15]) by using the C+V3 and C−V3 primers (20). The PCR product was cloned using a pT7Blue Perfectly Blunt cloning kit (Novagen, Madison, Wis.). The subtype B probe was labeled by first digesting the plasmid with *Nhe*I, end-labeling one strand by a fill-in reaction of the *Nhe*I overhangs with α-^{35}S-dATP and the Klenow fragment of DNA polymerase I (New England Biolabs, Beverly, Mass.), and then inactivating the Klenow fragment and digesting with *Kpn*I. The labeled probe was then purified away from the unincorporated nucleotides using a MicroSpin G-50 column (Amersham Pharmacia, Arlington Heights, Ill.). V3-HTA with the subtype B probe was otherwise performed in the same manner as with the subtype C probe. For subjects with four samples (two blood plasma and two seminal plasma samples), multiple V3-HTA bands first were scored for the presence or absence of bands between samples. The relative abundance of each band within a sample was then visually estimated for each subject to generate a data set for statistical analysis.

Statistical methods. We used Fisher's exact test for hypotheses of independence between two factors with two levels when tables contained cells with results from fewer than five observations, and we used the chi-square test otherwise. To compare two groups with more than two levels, we used the Mantel-Haenszel test. We used the Wilcoxon rank sum test to compare medians between the STD and control groups and used the Student paired *t* test to examine subject-specific differences. To study the frequency distribution of bands in a viral population rather than the total number of bands, we computed the Shannon entropy, i.e.,

$$-\sum_i p(i)\log p(i)$$

where $p(i)$ is the relative frequency of band i ($i = 1,\ldots n$, where n is the total number of bands) (24). This quantity measures the amount of uncertainty in the distribution and was used in a similar manner by Delwart et al. (9). It is 0 when there is a single band (i.e., $n = 1$) and reaches its maximum value when the observed bands are equally frequent [i.e., $p(i) = 1/n$ for all i]. We monitored the changes in the distribution of bands in an individual via determining the entropy and looked for changes in this value. To relate the changes in RNA values to changes in the entropy, we fitted a generalized-estimating-equation model with an unstructured covariance matrix (for visits 1 and 2 in both STD and control groups) using PROC GENMOD in SAS version 7.0 (SAS Institute, Inc., Cary, N.C.).

TABLE 1. HIV-1 viral loads in blood and semen, the number of days between the two clinic visits, CD4$^+$ T-cell counts, diagnosed STDs, and ages for the 43 subjects enrolled at the STD clinic

Subject	Vital load[a] in:				No. of days between visits	CD4 count[b]	STD[c]	Age (yr)
	Blood		Semen					
	Visit 1	Visit 2	Visit 1	Visit 2				
S008	83	150	160	72	11	ND[d]	Gon, Tri	21
S017	610	380	25	6.2	13	178	NPI	28
S018	390	250	50	91	12	476	Gon, Tri, Ulc	42
S021	47	21	56	190	13	154	Gon	25
S029	170	170	51	26.4	21	496	Gon	35
S031	90	43	42	84	7	333	Gon, Ulc	39
S036	28	87	110	2.8	11	631	Gon	38
S047	590	920	84	37	19	469	Gon	27
S053	1,300	760	1,200	240	14	318	Tri, Ulc	27
S059	920	1,300	4,100	890	7	259	Gon	28
S067	80	59	110	5.2	14	ND	Gon	23
S073	39	32	40	17	8	275	Gon	35
S075	390	800	160	12	14	ND	NPI	27
S081	79	130	1,900	610	13	101	Gon	23
S083	ND	ND	3,600	318	8	166	Gon, Tri, Ulc	29
S088	33	130	1,500	1,000	7	569	Gon	34
S094	210	ND	340	22	14	101	Tri, Ulc	25
S099	370	160	660	270	14	ND	Gon, Tri	26
S100	160	150	3,900	1,600	14	210	Gon	27
S102	7.3	3.7	130	70	28	ND	NPI	29
S128	110	36	130	74	14	ND	Gon	30
S134	390	580	290	1	15	534	Gon	35
S137	480	750	110	160	15	69	Gon	44
S139	37	130	240	940	14	ND	Gon	26
S141	9.7	6.9	140	74	14	115	Gon	28
S148	120	66	960	1,200	7	344	Gon	40
S159	750	430	960	290	7	98	Tri	26
S171	260	180	190	71	8	441	Tri, Ulc	21
S172	63	19	150	42	14	344	Gon	24
S180	170	230	1,500	450	9	37	Gon, Tri, Ulc	31
S191	1,200	800	235	18	11	187	Ulc	50
S199	430	260	340	130	9	184	Tri, Ulc	27
S200	2,200	5,700	55	700	6	132	Ulc	25
S206	420	540	860	2,400	7	168	Gon, Ulc	36
S007	130		18			273	NPI	34
S019	15		300			712	Gon	26
S084	ND		260			248	Gon, Ulc	27
S101	670		770			235	Gon, Chl	32
S103	8.9		100			454	Gon	41
S107	120		5.6			806	NPI	24
S111	200		79			ND	Gon	50
S173	15		180			36	Gon	29
S194	67		1,500			322	Gon	22

[a] Times 10^3 copies per milliliter of HIV-1 RNA.
[b] CD4$^+$ T cells per microliter.
[c] Diagnosed STD. Gon, gonorrhea; Tri, trichomonas; Ulc, genital ulcers; Chl, chlamydia; NPI, no pathogen identified.
[d] ND, not determined.

RESULTS

The STD and control groups are well matched. We have used a collection of samples previously obtained (6) to examine the effects of STDs on cell-free HIV-1 populations in blood and seminal plasma. Paired blood and semen samples had been collected during initial clinic visits by 43 HIV-1-infected men with urethritis from an STD clinic cohort (Table 1) and 14 HIV-1-infected men from a dermatology clinic (control) cohort (Table 2). Age, CD4$^+$ T-cell counts, and viral loads in blood were similar in STD and control subjects. Median ages were 28 years for the STD group and 29 years for the control group, respectively ($P = 0.94$). Median CD4$^+$-T-cell counts were 259/μl for the STD subjects and 268/μl for the control

TABLE 2. HIV-1 viral loads in blood and semen, the number of days between the two clinic visits, $CD4^+$ T cell counts, and ages for the 14 control subjects enrolled at the dermatology clinic

Subject	Viral load[a] in:				No. of days between visits	CD4 count[b]	Age (yr)
	Blood		Semen				
	Visit 1	Visit 2	Visit 1	Visit 2			
C010[d]	43	6.5	6,700	13	17	ND[c]	26
C011[d]	540	270	11	26	14	67	28
C012	380	270	2,500	5,600	14	863	48
C018	490	1,600	1,300	2,300	14	116	28
C030	580	2,300	10.8	45	14	322	31
C034	3,500	3,300	38	250	14	268	23
C047	140	130	860	210	14	291	40
C059[d]	5.2	4.6	1	40	15	465	25
C061	360	160	3,200	4,400	14	262	25
C071	320	680	68	13	14	ND	45
C124	54	47	7.3	8.7	10	ND	25
C128[d]	220	120	56	23	6	159	30
C111	69		890			210	31
C113[d]	360		38			279	30

[a] Times 10^3 copies per milliliter of HIV-1 RNA.
[b] $CD4^+$ T cells per microliter.
[c] ND, not determined.
[d] Subjects C010 and C011 had syphilis without urethritis; subjects C059, C128, and C113 had asymptomatic trichomonas without urethritis.

subjects ($P = 0.73$). Median viral loads in blood plasma were 1.6×10^5 and 3.4×10^5 copies/ml for the STD and control groups, respectively ($P = 0.52$). The median viral load in seminal plasma was higher in subjects with urethritis (1.8×10^5 copies/ml) than in control subjects (6.2×10^4 copies/ml), but the difference was not statistically significant ($P = 0.31$). Men in the larger STD cohort from the original study ($n = 86$) had significantly higher viral loads in semen than did men in the original control cohort ($n = 49$) (6); however, only subjects from whom V3 sequences could be reproducibly amplified from both blood and semen were studied here.

Gonorrhea was the most common cause of urethritis, being present in a majority of the members of the small STD cohort studied (Table 1); 10 subjects had trichomonas, 1 subject had chlamydia, and 6 subjects had more than one pathogen. Twelve subjects also had a genital ulcer, which we have previously shown is correlated with increased excretion of HIV-1 in semen (10). Two of the control subjects had syphilis and three had asymptomatic trichomonas, but none of these five subjects had urethritis.

Blood and seminal plasma samples from a second clinic visit by 34 of the STD subjects and 12 of the control subjects were also examined (Tables 1 and 2). The subjects seen at the STD clinic were treated for their STDs with antibiotics, so that the inflammation had cleared in most of the men by the second clinic visit. None of the subjects were under treatment with antiretroviral therapy. The median changes in blood plasma viral loads between the two visits were decreases of 3.6×10^3 copies/ml for the STD group and 2.3×10^4 copies/ml for the control group; neither of these median changes was statistically significant ($P = 0.78$, $P = 0.66$), and the medians were not significantly different from one another ($P = 0.67$). The median change in seminal plasma viral load for the control group was an increase of 2.5×10^4 copies/ml, which was also not statistically significant ($P = 0.47$). For the STD group, the median change in seminal plasma viral load after therapy was a decrease of 1.0×10^5 copies/ml, which was statistically significant ($P = 0.04$) and was significantly different from the median change in the control group ($P = 0.003$).

Cross-sectional comparison of the blood and seminal compartments at the first visit. HIV-1 populations in the cell-free fractions of blood and semen samples from the first clinic visit were analyzed using V3-HTA, and the patterns were scored for the presence of multiple bands, with each band representing a distinct genotype. All of the samples were first analyzed using a subtype C probe. However, because many of the subjects had only a single V3 population that was detected with the subtype C probe, we also used a subtype B probe for V3-HTA that was more sensitive to minor differences among subtype C sequences because of the clusters of mismatches between the subtype B probe and the subtype C sequences (Fig. 1A). By using both probes, a larger number of V3 variants could be identified. V3-HTA with the subtype B probe was done only on the RT-PCR products from those subjects for whom only single bands were seen in both the blood plasma and seminal plasma compartments using the subtype C probe. Figure 1B shows the V3-HTA results with the subtype B and subtype C probes on the same samples from two representative subjects. For both of these subjects, single bands with the subtype C probe were resolved as multiple bands using the subtype B probe. Overall, 31 of the 43 STD subjects (72%) and 10 of the 14 controls (71%) had multiple populations in their blood. Similar results were obtained for the seminal compartment with the two cohorts; 26 STD subjects (60%) and 11 control subjects (79%) had multiple genotypic populations in their semen ($P = 0.22$).

The V3-HTA patterns in the blood and seminal compartments of each subject were next compared for differences in the number of bands. V3-HTA results illustrating examples of the V3 population patterns are shown in Fig. 2. Subjects S159 and C011 had the same V3-HTA pattern in their blood and semen (Fig. 2A). In contrast, subject S171 had two V3 populations in his blood but only one of them was detected in his semen, while subject C047 had two V3 populations in his semen and only one was detected in his blood (Fig. 2B). In all cases, the test was reproduced at least once with each sample to document sufficient template sampling to justify the comparisons.

The results of the compartment comparisons are shown in Table 3. Nearly every subject (in both the STD and control groups) with a single viral population in his blood had that same population in his semen. In the STD and control subjects with multiple viral populations in their blood, most had the same populations in their semen. Overall, less than one-fourth of the subjects showed differences between the two compartments. The STD and control groups had similar percentages of subjects in each category in Table 3 ($P = 0.78$), indicating that the higher HIV-1 RNA load in the semen of men with an STD did not change the likelihood that their seminal virus would be different from the virus in blood, at least as assessed by a determination of discrete V3 populations.

As a more sensitive measure of the similarity or difference between the blood and seminal compartments, we rescored the V3-HTA patterns for the subjects with multiple bands in their blood for differences in the relative levels of abundance of the populations. Examples of V3-HTA patterns showing this type of difference are shown in Fig. 2C. More subjects showed differences between the compartments by this analysis, with half of the subjects with multiple V3 populations having different V3-HTA patterns between blood and semen (16 of 31 STD subjects and 5 of 10 control subjects), and again there was no difference between the STD and control subjects ($P = 0.75$). When relative-abundance differences are taken into account, two-fifths of all of the subjects showed discordance between the compartments.

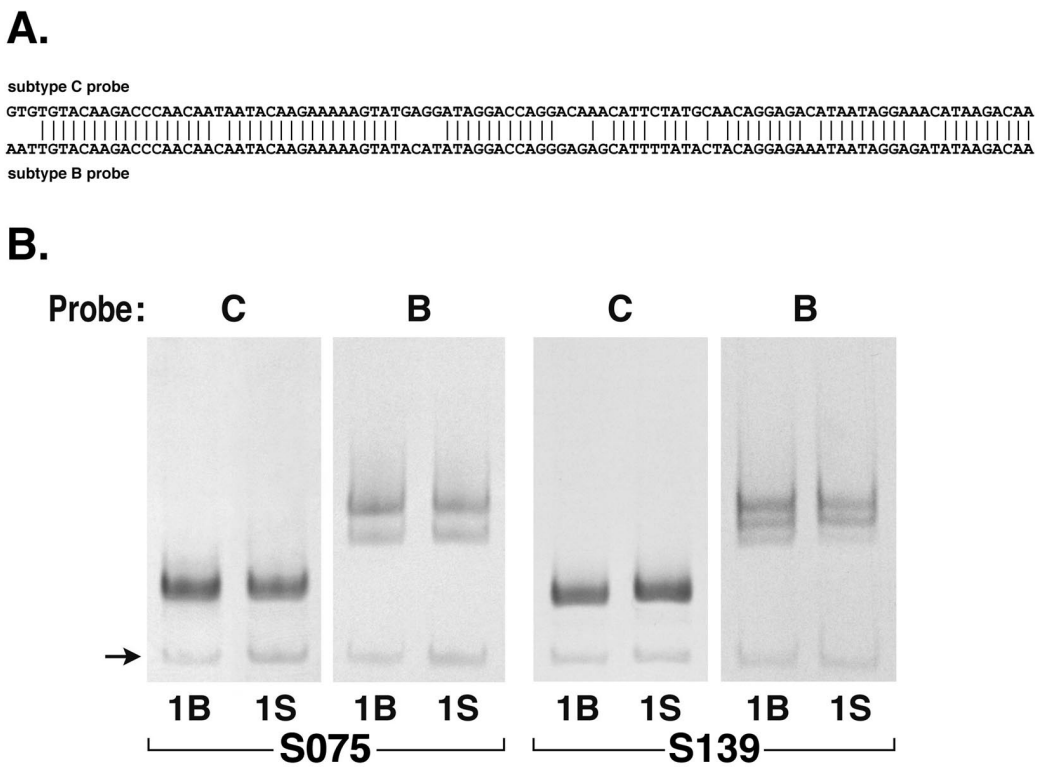

FIG. 1. (A) Comparison of the probes used for V3-HTA. The subtype C probe was cloned from subject C128 as described previously (20), while the subtype B probe was derived from the JR-FL molecular clone (15). (B) Examples of V3-HTA patterns for two subjects that showed different numbers of bands with the subtype C and subtype B probes. Only the bottom portions of the gels are shown. The arrow indicates the position of the probe homoduplex. Lanes are labeled by sample type at the first visit (blood [1B] or semen [1S]).

Temporal changes in virus populations in blood and semen. Blood and seminal plasma samples from two separate clinic visits were analyzed by V3-HTA for 34 STD and 12 control subjects. The median time between visits was 13 days for the STD group and 14 days for the control group. Examples of the V3-HTA patterns seen in the comparison of the two sets of blood and semen samples are shown in Fig. 3. Subject S094 had the same pattern in all four samples, while subject S029 had the

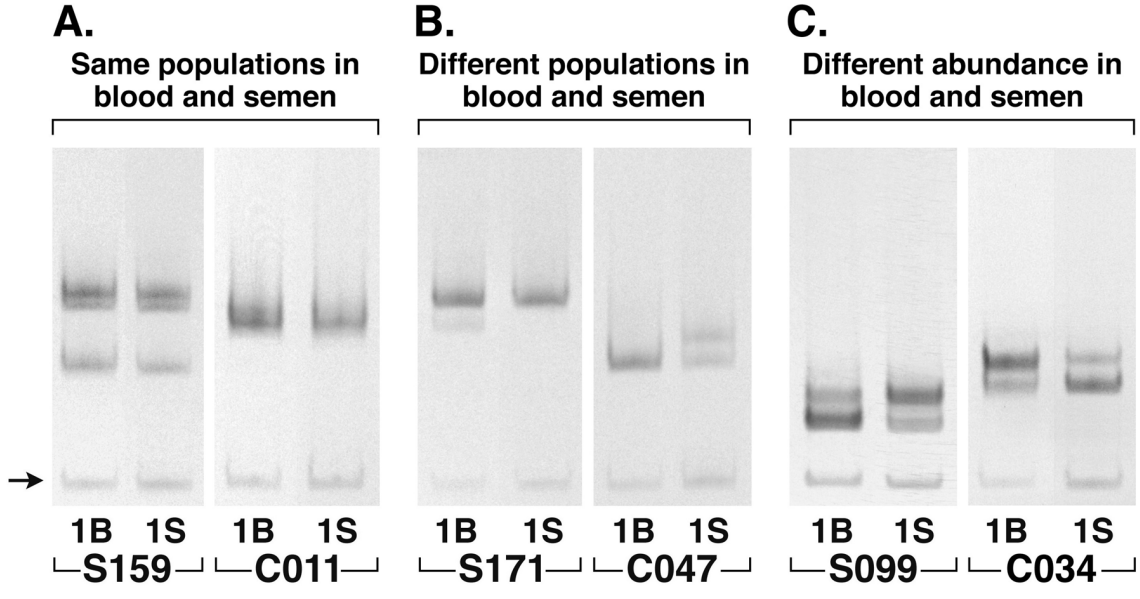

FIG. 2. Examples of the V3-HTA patterns of subjects with the same bands in blood and semen (A), subjects with different bands in blood and semen (B), and subjects with different relative levels of abundance of the same bands in blood and semen (C). The lanes are labeled as in Fig. 1, and the arrow indicates the position of the probe homoduplex. The V3-HTA patterns with the subtype C probe are shown for subjects S099 and C034; the V3-HTA patterns with the subtype B probe are shown for the other four subjects.

TABLE 3. Comparison of the bands in blood and seminal compartments at the first visit

Subjects (*n*)	No. of subjects (%) whose blood specimen had:			
	A single viral population that was:		Multiple viral populations that were:	
	Same as that in semen	Different from that in semen	Same as those in semen	Different from those in semen
With STD (43)	11 (26)	1 (2)	22 (51)	9 (21)
Control (14)	3 (21)	1 (7)	8 (57)	2 (14)

same pattern in his blood over time but a change in his seminal pattern over time. Subject S036 had the same pattern in his blood and semen at both visits, but the pattern changed over time.

The V3-HTA patterns were scored for each of the four samples from the 46 subjects first for changes in which bands were present and then for changes in the relative abundance of each band. Changes in the blood and the seminal compartments were analyzed in parallel for each subject for both analyses (Table 4). When only the presence or absence of specific bands was taken into account, the virus populations were stable in both compartments, since only one control subject (8%) showed changes in the seminal plasma and none showed changes in the blood plasma. For the STD subjects, the seminal compartment was more dynamic while their genital inflammation was being treated because 10 of the STD subjects (29%) showed changes in their semen but only three of these subjects (9%) showed changes in their blood ($P = 0.02$).

In the second analysis in which the relative abundance of each band was also considered, similar results were obtained (Table 4). For the control group, three subjects (25%) showed changes in the viral populations in their blood and three other subjects showed changes in their semen, which were not statistically different between the compartments ($P = 0.51$). In the STD group, 3 subjects (9%) showed changes in their blood while a total of 15 subjects (44%) showed changes in their semen, although in this case the difference between the compartments did not reach statistical significance ($P = 0.08$). It should be noted that the subjects included in these groups were selected based on our ability to reproducibly amplify the V3 regions from all four samples. The ability to amplify the V3 region from extracted RNA tended to correlate with higher viral loads; thus, this subset of subjects is, by necessity, a biased sampling of the original cohort (6).

The complexity of the V3 populations in both the numbers of bands and abundance of each band was calculated as the Shannon entropy. This entropy was calculated for the four samples from each subject. Statistical modeling was done using the entropy values and the viral loads (at the first visit) from each compartment for the STD and control subjects together. Higher viral loads in the blood at the first visit were significant predictors of higher V3 complexity at both visits ($P < 0.0001$), but the seminal viral loads at the first visit were not predictive ($P = 0.57$). The complexity of the V3 populations in the blood plasma was different from that in the seminal plasma at each time point (first blood sample versus first semen sample and second blood sample versus second semen sample) and across time points (first blood sample versus second semen sample and second blood sample versus first semen sample), indicating that these are separate compartments for HIV-1 ($P = 0.01$ for all comparisons). The complexity of each V3 population was the same, however, between the two visits within each compartment ($P = 0.65$ for blood plasma and $P = 0.95$ for seminal plasma). All of these comparisons held true for the STD group alone, but the control group sample size was too small for reliable parameter estimation.

In only one of the analyses of these data was there a statistically significant difference in the frequency of changes between the two compartments, which was for the STD group when changes were scored by the presence or absence of bands. When relative abundance was included, the differences between changes in the blood and changes in the semen were no longer statistically significant; also, when the magnitude of the differences was included in the entropy calculation, the compartments were the same. Therefore, there are differences

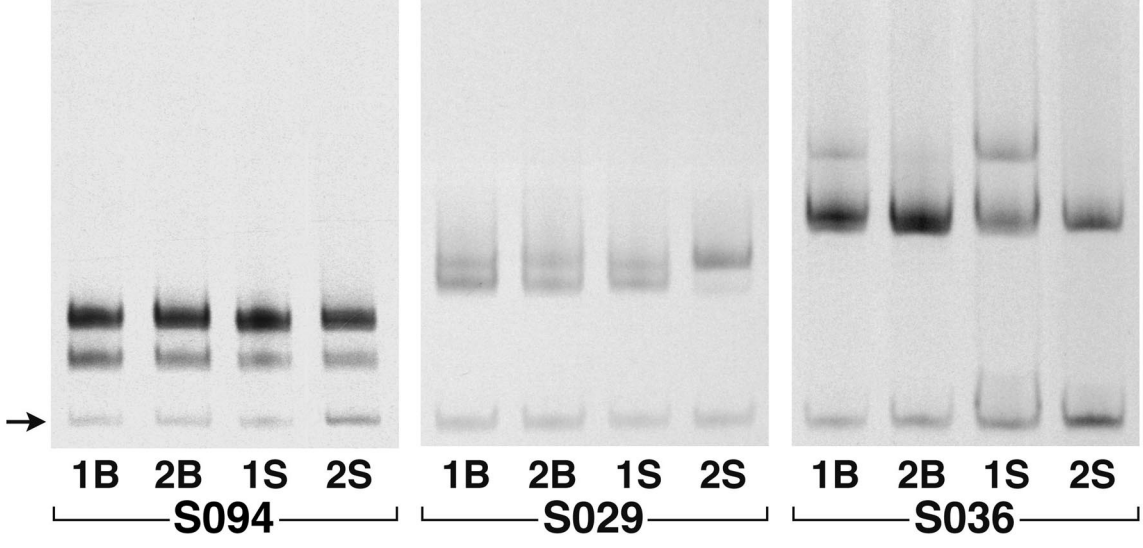

FIG. 3. Examples of V3-HTA patterns for subjects from whom specimens were collected at two time points for each compartment. Lanes are labeled by visit number (1 or 2) as well as sample type (blood [B] or semen [S]). The subtype C probe was used for subject S094, and the subtype B probe was used for subjects S029 and S036. The arrow indicates the position of the probe homoduplex.

TABLE 4. Comparison of the blood and seminal compartments over time

Subjects	No. (%) of subjects			
	Presence of bands compared		Relative abundance of bands compared	
	Same blood patterns	Different blood patterns	Same blood patterns	Different blood patterns
With STD				
Same semen patterns	24 (71)	0 (0)	19 (56)	0 (0)
Different semen patterns	7 (20)	3 (9)	12 (35)	3 (9)
Control				
Same semen patterns	11 (92)	0 (0)	6 (50)	3 (25)
Different semen patterns	1 (8)	0 (0)	3 (25)	0 (0)

between the seminal samples and the blood samples at each time point, but the differences are small.

DISCUSSION

We examined the V3 populations in the cell-free fractions of matched blood and semen specimens collected from men who were concomitantly infected with HIV-1 and one or more STD pathogens causing urethritis. Virions in the cell-free fractions of both blood and semen were studied because they represent the viral variants being produced at the time of sample collection. At the initial visit, multiple V3 populations were present in the blood and semen from both the STD subjects and a control population. About two-fifths of all of the subjects had detectable differences in viral populations between the blood and semen and the complexity of each V3 population was significantly different between the compartments, as might be predicted based on earlier work (5, 7, 8, 11, 28, 32). There were no differences in these analyses between the STD and control groups at the initial visit, indicating that the presence of urethritis did not result in an increased likelihood of having multiple V3 populations and did not change the relationship between the blood and seminal compartments.

We also compared the blood and seminal compartments over time. Our results demonstrated that HIV-1 populations in blood remained largely stable over the full 28 days of the study. However, the viral populations in semen specimens from 29% of the treated STD subjects showed changes in the presence or absence of V3-HTA bands over this time, although the changes were generally small and this difference in the frequency of changes in the two compartments was not significant when it was calculated using values for the relative abundance of the bands or entropy measures. This observation suggests that HIV-1 populations in the seminal compartment are more dynamic than in the blood compartment, specifically while the viral load is decreasing during treatment and resolution of genital inflammation.

Inflammation of the genital tract caused by sexually transmitted diseases causes a significant increase in excretion of HIV-1 (reviewed in reference 29) and likely facilitates transmission of HIV-1 (21). However, it is not clear whether the increase in viral burden in the genital tract results from HIV-1 replication in cells already present in the genital tract, HIV-1-infected cells from blood directed to the genital tract by inflammatory cytokines and chemokines (2), transudation of cell-free virus from the blood plasma to the seminal plasma, or combinations of the above. However, increased recovery of HIV-1 in semen has been associated with increased numbers of leukocytes in semen (1).

These results do not allow final conclusions about the source of HIV-1 in semen. However, if the influx of cell-free HIV-1 or HIV-1-infected cells from blood was primarily responsible for the increased level of HIV-1 in semen from men with urethritis, we would expect a stronger relationship between HIV-1 in blood and semen from the STD subjects at the initial visit. The continued change in HIV-1 in semen after appropriate antibacterial therapy supports the idea of local genital tract HIV-1 replication, likely in response to continued exposure of HIV-1 to key inflammatory cytokines (2, 25).

We examined the viral populations in these compartments using V3-HTA in order to understand better the relationship between HIV-1 in blood and semen. We adapted the V3-HTA to detect viral populations based on other features of V3 sequence variability because subtype C HIV-1, unlike other subtypes, tends to remain R5 throughout the disease (3, 19, 20, 27). By using a probe for V3-HTA from a different subtype as well as the homologous subtype C probe, we were able to increase the sensitivity of the assay for other features of genetic variation (Fig. 1).

Limitations of this study should be emphasized. First, while V3-HTA can identify subpopulations of HIV-1, such populations must represent at least 3 to 5% of the whole (W. Resch, N. Parkin, E. L. Stuelke, and R. Swanstrom, submitted for publication), and this method of population characterization was restricted to the small region encoding V3. Therefore, we would not be able to detect variations in minor subpopulations or subpopulations defined by variability in other regions of the viral genome. Second, our questions would be best served if we were able to study subjects before, during, and after urethral inflammation. However, we were able to examine each subject's HIV-1 populations only at the time of inflammation and briefly thereafter. Our conclusions about the effect of genital inflammation on the relationship between the blood and seminal compartments were drawn from cross-sectional comparisons of the STD and control cohorts. Third, our study cohorts were small subgroups of the original cohorts and were biased toward higher viral loads in both compartments, since higher RNA levels are generally correlated with better template sampling during PCR amplification. Finally, antibiotic treatment was a variable in this study, since most of the control subjects did not receive antibiotics and each subject who was treated was given different antibiotics appropriate for his infection.

These limitations notwithstanding, the results of this study offer further insight into issues critical to our understanding of the transmission and the prevention of transmission of HIV-1 from men to their partners. Reduction of viral burden in the semen of men with STDs is not likely to reduce heterogeneity in viral populations. In addition, biological interventions to prevent sexual transmission of HIV-1 from men to their partners, whether through vaccines, antiretroviral agents, or other means, must include an examination of HIV-1 in the genital tract, from which the virus is sexually transmitted. Populations of HIV-1 in blood cannot be expected to represent fully the populations in the semen, especially since HIV-1 populations in semen can change without similar changes occurring in blood.

ACKNOWLEDGMENTS

We thank the AIDSCAP Malawi Research Group (E. Nkata, E. Kachenje, T. Banda, G. Mughogho, C. Koller, J. Schock, G. Dallabetta, and B. Gilliam); Jerry Russell, Peter Killick, F. L. Musisi, and Terrie Taylor for administrative and logistical support; John Schmitz, Michelle Fiodisi, Elaine Doherty-Leach, and Lance Nkana for techni-

cal and laboratory support; and Carol Porter for assistance with data management.

This work was supported by grants R01-AI44667 (R.S.), R01-DK49381 (M.S.C.), P30-HD37260 (UNC Center for AIDS Research), U01-AI31496 (UNC Center for STD Research), N01-AI75329 (STD-CTU), and M01-RR00046 (UNC General Clinical Research Center) from the National Institutes of Health. This work was also supported by the National Institutes of Health Office of AIDS Research, the U.S. Agency for International Development (USAID) as part of Family Health International's AIDS Control and Prevention Project (623-02380A-00-4031-00), the World Health Organization (SD1/94/009), and Pfizer Inc. P.V. was supported by the Swiss National Science Foundation (3233-048902.96/1).

REFERENCES

1. **Anderson, D. J., T. R. O'Brien, J. A. Politch, A. Martinez, G. R. I. Seage, N. Padian, C. R. Horsburgh, Jr., and K. H. Mayer.** 1992. Effects of disease stage and zidovudine therapy on the detection of human immunodeficiency virus type 1 in semen. JAMA **267:**2769–2774.
2. **Anderson, D. J., J. A. Politch, L. D. Tucker, R. Fichorova, F. Haimovici, R. E. Tuomala, and K. H. Mayer.** 1998. Quantitation of mediators of inflammation and immunity in genital tract secretions and their relevance to HIV type 1 transmission. AIDS Res. Hum. Retroviruses **14:**S43–S49.
3. **Bjorndal, A., A. Sonnerborg, C. Tscherning, J. Albert, and E. M. Fenyo.** 1999. Phenotypic characteristics of human immunodeficiency virus type 1 subtype C isolates of Ethiopian AIDS patients. AIDS Res. Hum. Retroviruses **15:**647–653.
4. **Byrn, R. A., and A. A. Kiessling.** 1998. Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir. J. Reprod. Immunol. **41:**161–176.
5. **Byrn, R. A., D. Zhang, R. Eyre, K. McGowan, and A. A. Kiessling.** 1997. HIV-1 in semen: an isolated virus reservoir. Lancet **350:**1141.
6. **Cohen, M. S., I. F. Hoffman, R. A. Royce, P. Kazembe, J. R. Dyer, C. C. Daly, D. Zimba, P. L. Vernazza, M. Maida, S. A. Fiscus, and J. J. Eron, Jr.** 1997. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. Lancet **349:** 1868–1873.
7. **Coombs, R. W., C. E. Speck, J. P. Hughes, W. Lee, R. Sampoleo, S. O. Ross, J. Dragavon, G. Peterson, T. M. Hooton, A. C. Collier, L. Corey, L. Koutsky, and J. N. Krieger.** 1998. Association between culturable human immunodeficiency virus type 1 (HIV-1) in semen and HIV-1 RNA levels in semen and blood: evidence for compartmentalization of HIV-1 between semen and blood. J. Infect. Dis. **177:**320–330.
8. **Delwart, E. L., J. I. Mullins, P. Gupta, G. H. Learn, Jr., M. Holodniy, D. Katzenstein, B. D. Walker, and M. K. Singh.** 1998. Human immunodeficiency virus type 1 populations in blood and semen. J. Virol. **72:**617–623.
9. **Delwart, E. L., H. Pan, H. W. Sheppard, D. Wolpert, A. U. Neumann, B. Korber, and J. I. Mullins.** 1997. Slower evolution of human immunodeficiency virus type 1 quasispecies during progression to AIDS. J. Virol. **71:** 7498–7508.
10. **Dyer, J. R., J. J. Eron, I. F. Hoffman, P. Kazembe, P. L. Vernazza, E. Nkata, C. Costello Daly, S. A. Fiscus, and M. S. Cohen.** 1998. Association of CD4 cell depletion and elevated blood and seminal plasma human immunodeficiency virus type 1 (HIV-1) RNA concentrations with genital ulcer disease in HIV-1-infected men in Malawi. J. Infect. Dis. **177:**224–227.
11. **Eron, J. J., P. L. Vernazza, D. M. Johnston, F. Seillier-Moiseiwitsch, T. M. Alcorn, S. A. Fiscus, and M. S. Cohen.** 1998. Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission. AIDS **12:**F181–F189.
12. **Fiore, J. R., A. Bjorndal, K. A. Peipke, M. Di Stefano, G. Angarano, G. Pastore, H. Gaines, E. M. Fenyo, and J. Albert.** 1994. The biological phenotype of HIV-1 is usually retained during and after sexual transmission. Virology **204:**297–303.
13. **Fiore, J. R., Y. J. Zhang, A. Bjorndal, M. Di Stefano, G. Angarano, G. Pastore, and E. M. Fenyo.** 1997. Biological correlates of HIV-1 heterosexual transmission. AIDS **11:**1089–1094.
14. **Fleming, D. T., and J. N. Wasserheit.** 1999. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex. Transm. Infect. **75:**3–17.
15. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236:**819–822.
16. **Krieger, J. N., R. W. Coombs, A. C. Collier, D. D. Ho, S. O. Ross, J. E. Zeh, and L. Corey.** 1995. Intermittent shedding of human immunodeficiency virus in semen: implications for sexual transmission. J. Urol. **154:**1035–1040.
17. **Lee, T. H., N. Sakahara, E. Fiebig, M. P. Busch, T. R. O'Brien, and S. A. Herman.** 1996. Correlation of HIV-1 RNA levels in plasma and heterosexual transmission of HIV-1 from infected transfusion recipients. J. Acquir. Immune Defic. Syndr. **12:**427–428.
18. **Nelson, J. A. E., S. A. Fiscus, and R. Swanstrom.** 1997. Evolutionary variants of the human immunodeficiency virus type 1 V3 region characterized by using a heteroduplex tracking assay. J. Virol. **71:**8750–8758.
19. **Peeters, M., R. Vincent, J. L. Perret, M. Lasky, D. Patrel, F. Liegeois, V. Courgnaud, R. Seng, T. Matton, S. Molinier, and E. Delaporte.** 1999. Evidence for differences in MT2 cell tropism according to genetic subtypes of HIV-1: syncytium-inducing variants seem rare among subtype C HIV-1 viruses. J. Acquir. Immune Defic. Syndr. **20:**115–121.
20. **Ping, L.-H., J. A. E. Nelson, I. F. Hoffman, J. Schock, S. L. Lamers, M. Goodman, P. Vernazza, P. Kazembe, M. Maida, D. Zimba, M. M. Goodenow, J. J. Eron, Jr., S. A. Fiscus, M. S. Cohen, and R. Swanstrom.** 1999. Characterization of V3 sequence heterogeneity in subtype C HIV-1 isolates from Malawi: underrepresentation of X4 variants. J. Virol. **73:**6271–6281.
21. **Quinn, T. C., M. J. Wawer, N. Sewankambo, D. Serwadda, C. Li, F. Wabwire-Mangen, M. O. Meehan, T. Lutalo, and R. H. Gray.** 2000. Viral load and heterosexual transmission of human immunodeficiency virus type 1. N. Engl. J. Med. **342:**921–929.
22. **Ragni, M. V., H. Faruki, and L. A. Kingsley.** 1998. Heterosexual HIV-1 transmission and viral load in hemophilic patients. J. Acquir. Immune Defic. Syndr. **17:**42–45.
23. **Royce, R. A., A. Sena, W. Cates, Jr., and M. S. Cohen.** 1997. Sexual transmission of HIV. N. Engl. J. Med. **336:**1072–1078.
24. **Shannon, C. E., and W. Weaver.** 1949. The mathematical theory of communication. University of Illinois Press, Champaign, Ill.
25. **Speck, C. E., R. W. Coombs, L. A. Koutsky, J. Zeh, S. O. Ross, T. M. Hooton, A. C. Collier, L. Corey, A. Cent, J. Dragavon, W. Lee, E. J. Johnson, R. R. Sampoleo, and J. N. Krieger.** 1999. Risk factors for HIV-1 shedding in semen. Am. J. Epidemiol. **150:**622–631.
26. **Tindall, B., L. Evans, P. Cunningham, P. McQueen, L. Hurren, E. Vasak, J. Mooney, and D. A. Cooper.** 1992. Identification of HIV-1 in semen following primary HIV-1 infection. AIDS **6:**949–952.
27. **Tscherning, C., A. Alaeus, R. Fredriksson, A. Bjorndal, H. Deng, D. R. Littman, E. M. Fenyo, and J. Albert.** 1998. Differences in chemokine coreceptor usage between genetic subtypes of HIV-1. Virology **241:**181–188.
28. **Vernazza, P. L., J. J. Eron, M. S. Cohen, C. M. van der Horst, L. Troiani, and S. A. Fiscus.** 1994. Detection and biologic characterization of infectious HIV-1 in semen of seropositive men. AIDS **8:**1325–1329.
29. **Vernazza, P. L., J. J. Eron, S. A. Fiscus, and M. S. Cohen.** 1999. Sexual transmission of HIV: infectiousness and prevention. AIDS **13:**155–166.
30. **Vernazza, P. L., B. L. Gilliam, J. Dyer, S. A. Fiscus, J. J. Eron, A. C. Frank, and M. S. Cohen.** 1997. Quantification of HIV in semen: correlation with antiviral treatment and immune status. AIDS **11:**987–993.
31. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science **261:**1179–1181.
32. **Zhu, T., N. Wang, A. Carr, D. S. Nam, R. Moor-Jankowski, D. A. Cooper, and D. D. Ho.** 1996. Genetic characterization of human immunodeficiency virus type 1 in blood and genital secretions: evidence for viral compartmentalization and selection during sexual transmission. J. Virol. **70:**3098–3107.