Relationship between Human T Lymphotropic Virus (HTLV) Type 1/2 Viral Burden and Clinical and Treatment Parameters among Patients with HIV Type 1 and HTLV-1/2 Coinfection

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Background. Human T lymphotropic virus types 1 (HTLV-1) and 2 (HTLV-2) are frequent copathogens among individuals infected with human immunodeficiency virus type 1 (HIV-1). The long-term effects of coinfection are unknown, and little information exists regarding how levels of HTLV-1/2 viral burden are affected by antiretroviral medications.

Methods. Factors associated with HTLV-1/2 viral burden were examined in patients with HIV–HTLV-1/2 coinfection. A total of 72 subjects were evaluated. The variables analyzed included HTLV-1/2 proviral load, HTLV-1/2 tax/rex mRNA expression, HIV load, HTLV-1/2 viral antigen detection in peripheral blood mononuclear cell (PBMC) cultures, T cell subsets, demographic variables (age, race, sex, and reported use of injection drugs), and administration of highly active antiretroviral therapy.

Results. An HTLV-1/2 proviral DNA copy number >20,000 copies/10^6 PBMCs was significantly associated with the following variables: (1) a positive HTLV-1 Western blot test result, (2) a positive HTLV-1/2 PBMC culture result, (3) a positive tax/rex mRNA result, (4) an HIV load <10,000 copies/mL, and (5) higher CD4 cell counts among subjects with HIV–HTLV-1 coinfection. There was no correlation between HTLV-1/2 proviral copy number or HTLV-1/2 tax/rex mRNA detection and administration of antiretroviral therapy.

Conclusions. HTLV-1/2 proviral burden was significantly higher among patients with HIV–HTLV-1 coinfection than among patients with HIV–HTLV-2 coinfection. Highly active antiretroviral therapy may be of limited value in controlling virus expression of HTLV-1/2 in patients with HIV–HTLV-1/2 coinfection.

HIV–human T lymphotropic virus (HTLV) type 1 (HTLV-1) and HIV–HTLV type 2 (HTLV-2) coinfections occur frequently in metropolitan areas where injection drug use is a common mode of viral transmission. Previous studies have reported HIV–HTLV-1–coinfected subjects with elevated CD4 cell counts and HIV–HTLV-2–coinfected subjects with elevated CD8 cell counts [1, 2]. Other studies have associated a higher HTLV-1 load with HTLV-associated disease [3] and with HIV-1 coinfection [4].

Limited data exist regarding the effects of antiretroviral therapy on HTLV-1/2 virus expression and disease progression. We initiated a prospective study to answer these questions at the Medical Center of Louisiana HIV Outpatient Clinic, which serves a largely indigent patient population with very high rates of HIV–HTLV-1/2 coinfection [2]. The current study has 3 principal objectives: (1) to assess the relationship between HTLV-1/2 viral burden and the administration of HAART among a group of patients with HIV–HTLV-1 and HIV–HTLV-2 coinfections, (2) to assess differences in HTLV-1 and HTLV-2 viral burden in cases of

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HIV-HTLV coinfection; and (3) to assess the relationship among CD4 and CD8 cell counts and HIV-1 load and HTLV-1/2 viral burden.

PATIENTS, MATERIALS, AND METHODS

All study participants received their evaluations at the Medical Center of Louisiana HIV Outpatient Clinic (New Orleans). HTLV-1/2 serologic testing by EIA was performed routinely for all patients at the time of clinic entry. Any patient with a positive HTLV-1/2 EIA result was invited to participate in the study. Informed consent was obtained from patients, and human experimentation guidelines of the US Department of Health and Human Services and of the Tulane University Health Sciences Center were observed. At enrollment, each study subject participated in a screening interview that was conducted by the research study nurse, who also performed chart reviews for each subject at the time of entry in the study. Study variables for this analysis included age, race, sex, self-reported injection drug use; CD4 and CD8 cell counts (within 1 month of enrollment); plasma HIV loads (within 1 month of enrollment); and administration of antiretroviral therapy. For purposes of this study, antiretroviral therapy was defined as administration of any 3-drug regimen for a period of at least 3 months prior to, and including the week of, enrollment in the study.

Blood samples were collected from each participant at baseline for Western blot analysis to confirm and differentiate HTLV-1 from HTLV-2 infections (Zeptometrix) and to obtain PBMCs for cultures, nucleic acid isolation, and PCR assays. PBMC cultures were performed, as previously described [4], for detection of HTLV-1/2 p19 viral antigens (Zeptometrix).

For determination of HTLV-1/2 tax/rex mRNA by RT-PCR, RNA was extracted from cryopreserved PBMC aliquots (2×10^6 cells), using RNeasy column purification methodology (Qiagen), as described exactly per the manufacturer's instructions, along with QIAshredder columns to assure full lysis of PBMCs. Samples were treated to remove traces of DNA, using RNase-free DNaseI, as described in the protocol. RT-PCR was then performed as described elsewhere [4]. Bands were visualized on ethidium bromide stained gels. To confirm that RT-PCR amplified RNA, and not DNA, was being visualized on gels, paired samples were run on the thermocycler in the presence or absence of the reverse-transcriptase enzyme in the reaction mixture.

For the real-time PCR assay, cryopreserved PBMC aliquots (1×10^6 cells) were used to extract DNA. Consensus primers and probe used were as follows: HTV-F5(7359), 5′-CGG ATA CCC IGT CTA CGT GTT T-3′; HTV-R4 (7519), 5′CTG AGC IGA IAA CGC GTC CA-3′; and Probe P-HTV, 5′-Reporter dye-FAM-ATC ACC TGG GAC CCC ATC GAT GGA-3′-TAMRA-Quencher dye [5]. Each 25-μL reaction contained from 1×10^1 to 1×10^6 copies of viral standard or 200 ng of purified genomic PBMC DNA and the following in sterile water: 1X TaqMan buffer A; 200 μmol/L each of dATP, dCTP, dGTP; 400 μmol/L UTP; 3.5 mmol/L $MgC1_2$; and 300 μmol/L each of the 2 HTLV primers (HTV-F5, HTV-R4), 200 nmol/L HTLV probe (P-HTV), 0.625 U of AmpliTaq Gold, and 0.25 U of uracil N-glycosylase. The samples were amplified in duplicate in 96-well arrays of optical-grade PCR plates. Reactions were performed in the Applied Biosystems (ABI) Prism 7700 Sequence Detection System. After 2 min at 58°C for uracil N-glycosylase digestion as contamination control and 10 min at 95°C for initial denaturation, the final reaction of 95°C for 15 s and 60°C for 60 s was continued and repeated for 40 cycles. Real-time PCR of RNaseP was run in parallel on the same patient DNA samples (not shown). The RNase P gene is a single-copy gene with 2 copies per diploid cell and was used as an endogenous DNA reference to normalize sample variability and allow accurate quantitation of cell equivalents. RNase P primers, TaqMan probe, reagents, and standards were from a commercially available kit (ABI). Universal thermal cycling parameters were used and performed on standards (10^1–10^4 copies) and patient samples, as described above. Assay results were normalized by dividing viral copy number by cell number to calculate the value for viral copies per cell, which were then multiplied by 10^6 to provide a final value of viral copies per 10^6 PBMCs.

To establish interlaboratory accuracy of the real-time PCR assay, 10 clinical samples from patients previously tested in a blinded context from the HTLV Outcomes Study prospective cohort of HTLV-1– and HTLV-2–infected blood donors for comparative analysis [6, 7]. Cryopreserved PBMCs were processed to isolate total genomic DNA, using Qiagen kits, and 250 ng of isolated DNA was amplified with HTLV-1/2–specific primers and probe on the ABI 7700 Sequence Detector. DNA was amplified in parallel with RNase P to normalize for cell number, and the number of HTLV-2 proviral copies per cell were determined. Resultant values were transformed to $\log_{10}$, and a linear regression analysis was performed. The output showed a highly significant P value (<.001), and $R^2 = 0.85$.

For statistical analysis, the HTLV-1/2 load variable was categorized into 3 groups: undetectable, ⩽20,000 copies per 10^6 PBMCs, and >20,000 copies per 10^6 PBMCs. These cutoff points were used to place results in approximate tertials for analysis. Also, previous reports suggest a possible correlation between HTLV-1 loads >20,000 copies/10^6 PBMCs and expression of disease [8]. CD4 and CD8 cell counts were categorized into 3 ordinal categories, using well-established cutoff points (<200 cells/mm^3, 200–500 cells/mm^3, and >500 cells/mm^3). Frequencies were computed, and χ^2 statistics or Fisher's exact test were used to test for differences between groups. Hypothesis tests were 2-sided, and SAS software, version 8.2 (SAS Institute), was used to conduct statistical analysis. In addition, Cochran-Armitage tests of trend were used to test for

Table 1. Factors associated with human T lymphotropic virus (HTLV) types 1 and 2 proviral DNA load ($n = 72$).

Variable	HTLV proviral DNA, copies/1×10^6 PBMCs			
	Undetectable ($n = 18$)	⩽20,000 ($n = 26$)	>20,000 ($n = 28$)	*P*
Race				
Black	16 (23.2)	26 (37.7)	27 (39.1)	.25[a]
White	2 (66.7)	0 (0.0)	1 (33.3)	
Sex				
Male	13 (22.0)	21 (35.6)	25 (42.4)	.34[a]
Female	5 (38.5)	5 (38.5)	3 (23.0)	
Age, years				
⩽45	8 (40.0)	6 (30.0)	6 (30.0)	.19[b]
>45	10 (19.2)	20 (38.5)	22 (42.3)	
Injection drug use				
Yes	6 (18.2)	14 (42.4)	13 (39.4)	.49[b]
No	11 (28.9)	12 (31.6)	15 (39.5)	
Antiretroviral therapy				
Yes	14 (29.2)	16 (33.3)	18 (37.5)	.50[b]
No	4 (16.7)	10 (41.7)	10 (41.7)	
CD4 cell count, cells/mm^3				
<200	6 (22.2)	10 (37.1)	11 (40.7)	.64[b]
200–499	9 (34.6)	9 (34.6)	8 (30.8)	
⩾500	3 (15.8)	7 (36.8)	9 (47.4)	
CD8 cell count, cells/mm^3				
<200	1 (11.1)	3 (33.3)	5 (55.6)	.55[a]
200–499	4 (44.4)	2 (22.2)	3 (33.3)	
⩾500	13 (24.1)	21 (38.9)	20 (37.0)	
HIV load, copies/mL[c]				
⩽10,000	9 (21.4)	12 (28.6)	21 (50.0)	.04[b]
>10,000	9 (31.0)	14 (48.3)	6 (20.7)	
HTLV status				
HTLV -1	0 (0.0)	5 (25.0)	15 (75.0)	<.001[b]
HTLV-2	18 (34.6)	21 (40.4)	13 (25.0)	
HTLV culture result				
Positive	2 (8.3)	6 (25.0)	16 (66.7)	.002[b]
Negative	16 (33.3)	20 (41.7)	12 (25.0)	

NOTE. Data are no. (%) of patients.

[a] Determined using Fisher's exact test.

[b] Determined using χ^2 test.

[c] HIV load data missing for 1 subject; 5 of 7 patients receiving HAART had undetectable HIV loads.

trend in CD4 and CD8 cell ordinal categories, with respect to the dichotomous variables of interest.

RESULTS

Of a total of 108 HTLV-1/2–infected patients with positive ELISA results who were enrolled in the study, 72 subjects had clinical and laboratory data for analysis (1 subject did not have an HIV-1 load result within the exact time frame but was included in the analysis). Thirty-six subjects were excluded for ⩾1 of the following reasons: (1) a negative or indeterminate Western blot result, (2) an inadequate blood sample to perform assays, or (3) missing clinical data. Six subjects with a Western blot result positive for both HTLV-1 and HTLV-2 were also excluded.

Among the subjects, 69 (96%) were black, 59 (76%) were male, 52 (72%) were >45 years of age, and 33 (47%) reported injection drug use. A total of 20 subjects (28%) were HTLV-1 seropositive by Western blot testing, and 52 subjects (72%) were HTLV-2 seropositive. A total of 24 subjects (33%) had positive HTLV-1/2 qualitative culture results. HTLV-1/2 loads

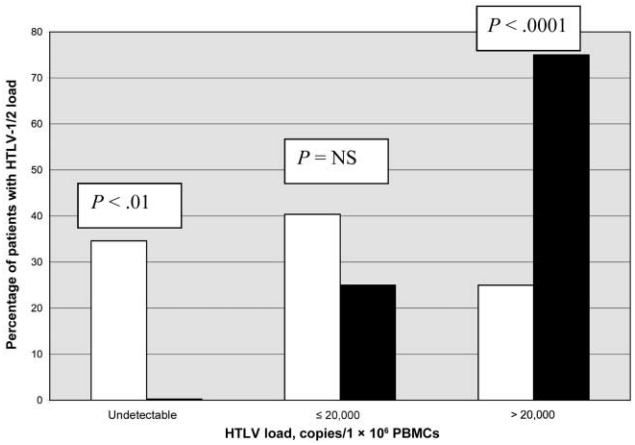

Figure 1. Human T lymphotropic virus (HTLV) types 1 and 2 status, as determined by Western blot findings, by HTLV load ($n = 72$). The x axis shows the 3 strata of HTLV-1/2 proviral DNA load (undetectable, ⩽20,000 proviral copies/10^6 PBMCs, and >20,000 proviral copies). The y axis depicts percentages of subjects falling into each stratum (HTLV-1 is shown in black, and HTLV-2 is shown in white). The figure illustrates the observation that patients with HIV–HTLV-1 coinfection have significantly higher HTLV-1/2 proviral DNA levels than do those with HIV–HTLV-2 coinfection.

were found to be undetectable in 18 subjects (25%), were ⩽20,000 copies/10^6 PBMCs in 26 subjects (36%), and were >20,000 copies/10^6 PBMCS in 28 subjects (39%).

Table 1 shows factors associated with each HTLV-1/2 load stratum. The following variables were significantly associated with an HTLV-1/2 load >20,000 copies/10^6 PBMCs: (1) HTLV-1 Western blot status ($P < .0002$), (2) positive HTLV-1/2 p19 qualitative culture result ($P < .002$), and (3) plasma HIV-1 load <10,000 copies/mL ($P < .04$). Factors not associated with higher HTLV-1/2 load included demographic variables of age, race, and sex; use of injection drugs; CD4 or CD8 cell count; and administration of HAART. However, when subjects were analyzed on the basis of HTLV-1 versus HTLV-2 serostatus, subjects with HIV–HTLV-1 coinfection were found to have a significant trend towards having a higher CD4 cell count ($P < .04$). There were no observable differences with respect to HIV load and HTLV-1 versus HTLV-2 status (data not shown).

Figure 1 depicts HTLV-1/2 loads on the basis of HTLV-1/2 Western blot results. All subjects with HIV–HTLV-1 coinfection had detectable HTLV-1/2 proviral DNA loads (100%), compared with only 34 (65%) of 50 subjects with HIV–HTLV-2 coinfection ($P < .01$). Furthermore, among 28 subjects with an HTLV-1/2 load >20,000 copies/10^6 PBMCs, 75% had HIV–HTLV-1 coinfection, and 25% had HIV–HTLV-2 coinfection ($P < .0001$).

Table 2 shows the analysis with measurements of HTLV-1/2 tax/rex mRNA. For this analysis, PBMC aliquots were available for RNA extraction on a total of 51 subjects. Inadequate baseline PBMC aliquots remained for the other 21 subjects. Among those samples tested, 15 (29%) of 51 samples had detectable bands of RT-PCR products on ethidium bromide stained gels. As we anticipated, there was a strong correlation between positive RT-PCR result and HTLV-1/2 proviral load ($P \leqslant .008$); however, no correlation existed between detection of HTLV-1/2 tax/rex mRNA and administration of HAART.

DISCUSSION

This study highlights important characteristics of HTLV-1/2 viral burden among HIV-1–infected patients. We analyzed subjects with regard to HTLV-1/2 load cutoff points of undetectable, ⩽20,000 copies/10^6 PBMCs, and >20,000 copies/10^6 PBMCs. These cutoff points were selected as benchmarks on the basis of prior studies that correlated these values with expression of clinical disease among HTLV-1–infected individuals [8]. No benchmark exists for HTLV-2 load and disease; therefore, these cutoff points roughly divided the cohort in tertials for purposes of statistical analysis. We examined the groups separately (HIV–HTLV-1–coinfected subjects and HIV–HTLV-2–coinfected subjects) and combined them to account for possible differences in these viruses, but we did not find significant differences between the 2 analyses (data not shown).

HTLV-1 serostatus was strongly associated with high proviral

Table 2. Human T lymphotropic virus (HTLV) types 1 and 2 tax/rex mRNA detection by RT-PCR.

	Tax/rex mRNA		
Variable	Negative ($n = 36$)	Positive ($n = 15$)	P[a]
Receiving HAART			
Yes	23 (74.2)	8 (25.8)	.48
No	13 (65.0)	7 (35.0)	
HTLV status			
HIV–HTLV-1 coinfection	14 (73.7)	05 (26.3)	.70
HIV–HTLV-2 coinfection	22 (68.7)	10 (31.3)	
CD4 cell count, cells/mm^3			
<200	16 (80.0)	4 (20.0)	.43
200–499	11 (68.8)	5 (31.2)	
⩾500	9 (60.0)	6 (40.0)	
HIV load, copies/mL			
⩽10,000	19 (63.3)	11 (36.7)	.21
>10,000	16 (80.0)	4 (20.0)	
HTLV proviral load, copies/mL			
⩽20,000	22 (88.0)	03 (12.0)	.008
>20,000	14 (53.9)	12 (46.1)	
HTLV p19 culture result			
Positive	14 (66.7)	07 (33.3)	.60
Negative	22 (73.3)	08 (26.7)	

NOTE. Data are no. (%) of patients. Baseline PBMC aliquots available for 51 subjects.

[a] Determined using χ^2 test.

burden, compared with HTLV-2, and was also significantly associated with a positive p19 qualitative culture result (data not shown). Similar results have been shown among a study population of subjects with HTLV-1/2 monoinfection [7]. We also found a trend toward higher CD4 cell counts among subjects coinfected with HIV and HTLV-1; however, the small number of subjects ($n = 20$) limits the power of this finding. Of additional interest was the suggestion that an HIV-1 load <10,000 copies/mL correlated with higher levels of HTLV-1/2 proviral burden. In contrast, we could not obtain a significant association between administration of antiretroviral therapy and HTLV-1/2 viral burden. This may again relate to the relatively small number of subjects in the study and variation in the duration of administration of HAART.

It might be expected that HTLV-1/2 proviral burden would increase during immune reconstitution with HAART, because the reservoir of CD4 cells containing the HTLV-1/2 provirus could potentially expand. This was shown in a small number of patients by other investigators [9, 10]. Our finding that subjects with HIV–HTLV-1 coinfection with higher CD4 cell counts also had higher HTLV-1 proviral loads supports this contention.

We reasoned that even if HAART did not affect HTLV-1/2 proviral burden, it might stop downstream transcription events. This possibility was explored using RT-PCR for detection of HTLV-1/2 tax/rex mRNA. The tax/rex mRNA transcript was selected in this study, because expression of the tax/rex protein is considered central to many of the events associated with the immunopathogenesis of HTLV-1–associated diseases, and because our earlier work demonstrated upregulated expression of this transcript in patients with HIV-HTLV coinfections [4]. Our results failed to show any effects of HAART on HTLV-1/2 mRNA expression. Nonetheless, we acknowledge that this study is limited because of the small number of samples tested and because we only analyzed 1 region of the virus (i.e., tax/rex) at 1 point in time. Also, our assay was of a qualitative nature. Work is underway to perform serial analysis on samples collected from our patient cohort over time, which should enable us to more confidently assess the relationship between HAART and HTLV-1/2 RNA expression.

Despite limitations in this study, we acknowledge that the current study is the largest of its kind in the United States. The overall seroprevalence of HTLV-1/2 infection in the New Orleans HIV Outpatient Clinic approaches 8%, which illustrates the importance of understanding the role of these retroviruses as frequent copathogens during HIV-1 infection. HTLV-1/2 serologic testing has not normally been a routine part of testing in most HIV clinics. As additional information is obtained through studies, such as the current one reported here, it may be necessary for other HIV clinics in metropolitan areas to reevaluate the need to include HTLV-1/2 testing as a routine evaluation.

Several investigations suggest a correlation between levels of HTLV-1/2 viral burden and risk for disease development [3, 4, 8]. Therefore, it is of concern that HAART does not appear to provide a virologic benefit against HTLV-1 or HTLV-2. As patients survive longer in the HAART era, long-term complications of HTLV infection might emerge. We have begun to prospectively monitor our subjects to determine rates of long-term neurologic complications (such as peripheral neuropathy and tropical spastic paraparesis/HTLV-1–associated myelopathy) and hematologic malignancies. Thus far, we have identified 2 patients in our study population with cutaneous T cell lymphoma and 5 subjects with tropical spastic paraparesis/HTLV-1–associated myelopathy [11]. The long-term effects of HTLV-2 infection among HIV-1 infected patients are unknown, but our group and others have recently reported a potential survival benefit associated with HIV–HTLV-2 coinfection [2, 12].

In conclusion, our results indicate that HTLV-1 and HTLV-2 infections should be considered as important viral cofactors for HIV-1 infection, and additional work will be needed to determine tailored approaches to therapy of coinfected individuals.

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Potential conflicts of interest. All authors: no conflicts.

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