Higher Human T Lymphotropic Virus (HTLV) Provirus Load Is Associated with HTLV-I versus HTLV-II, with HTLV-II Subtype A versus B, and with Male Sex and a History of Blood Transfusion

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Background. High human T lymphotropic virus (HTLV)–I provirus load (VL) has been associated with an increased risk of HTLV-associated myelopathy, but little is known about variation in HTLV-I or -II VLs by demographic characteristics and risk behaviors.

Methods. We measured HTLV-I and HTLV-II VLs in a large cohort of 127 HTLV-I–seropositive and 328 HTLV-II–seropositive former blood donors, by use of real-time polymerase chain reaction using tax primers. Multivariable linear regression was used to control for confounding by relevant covariates.

Results. The mean VLs were 3.28 log10 copies/106 peripheral blood mononuclear cells (PBMCs) (range, 0.5–5.3 log10 copies/106 PBMCs) for HTLV-I and 2.60 log10 copies/106 PBMCs (range, 0.05–5.95 log10 copies/106 PBMCs) for HTLV-II (P < .0001). HTLV-II VLs were higher in those subjects with subtype A infection (mean, 2.82 log10 copies/106 PBMCs) than in those with subtype B infection (mean, 2.29 log10 copies/106 PBMCs) (P = .005). Higher HTLV-I VL was associated with previous receipt of a blood transfusion (P = .04), and lower HTLV-II VL was associated with female sex (P = .007). These associations persisted in virus-specific multivariate linear regression models controlling for potential confounding variables.

Conclusions. VL was significantly higher in HTLV-I than in HTLV-II infection and was higher in HTLV-II subtype A than in HTLV-II subtype B infection. Chronic HTLV VLs may be related to the infectious dose acquired at the time of infection, with higher VLs following acquisition by blood transfusion and lower VLs following sexual acquisition.

Human T lymphotropic virus (HTLV) types I and II are human type C retroviruses derived most likely from simian-to-human transmission thousands of years ago [1, 2]. The 2 HTLV types share ∼60% nucleotide homology but differ in their epidemiology and disease associations. HTLV-I is prevalent in Africa, the Caribbean, Brazil, and southern Japan and causes adult T cell leukemia (ATL) and HTLV-associated myelopathy (HAM) [3]. HTLV-II is prevalent among Amerindians, African pygmies, and injection drug users (IDUs) in the Americas and Europe and has been linked to HAM, as well as to a higher occurrence of pneumonia and bronchitis [4, 5]. HTLV-II subtype A is more prevalent among North American and European IDUs and their sex partners, whereas subtype B is more prevalent among Native Americans [4].

HTLV-I and -II viral RNA is low to undetectable in human serum, and viral propagation is thought to occur via the clonal expansion of lymphocytes with integrated proviral DNA [6] or the transfer of viral RNA
via direct contact between infected and uninfected cells [7].
How the level of proviral DNA in lymphocytes is regulated is
unknown but may involve either the activity of the viral transac-
tivator protein Tax, which differs between HTLV-I and some
HTLV-II subtypes [8], or the degree of lymphocyte turnover
in the infected host [6]. High levels of HTLV-I proviral DNA,
HTLV-I Tax mRNA, and anti-HTLV antibody have been as-
associated with the occurrence of HAM [9, 10]. However, the
pathogenic mechanism is unresolved and may be due to either
direct viral neurotoxicity [11], an autoimmune response di-
rected at viral proteins homologous to neuronal proteins [12],
or “bystander” immunologic damage to neural tissue [13]. Data
are less clear with regard to the association between HTLV-I
provirus load (VL) and ATL, with one study showing signifi-
cantly higher prediagnostic VLs in patients with ATL than in
asymptomatic carriers [14] and another showing an association
between high HTLV-I VLs and the presence of abnormal lymph-
cytes thought to be indicative of preleukemia, although this
was not proven prospectively [15].

HTLV-II VL has received comparatively less study than HTLV-
I VL. One study found no difference in HTLV-II VLs between
IDUs and endemic African Pygmies and no correlation between
HTLV-II VLs and CD8+ lymphocyte counts or stage of AIDS,
in the IDUs who were coinfected with HIV [16]. Other studies
have found high HTLV-II VLs in patients with HIV/HTLV-II
coinfection [17, 18] and, notably, in coinfected patients with
sensory neuropathy [19]. HTLV-II VLs also increased after the
initiation of antiretroviral therapy for HIV in coinfected pa-
tients [20, 21].

HTLV-II VL has not been correlated with age or sex, and
neither HTLV-I VL nor HTLV-II VL has been studied in relation
to the route of infection. We therefore measured HTLV-I and
HTLV-II VLs in a well-characterized cohort of former blood
donors and correlated these levels with data on demographic
characteristics, risk factors for infection, and HTLV-II subtype.

SUBJECTS AND METHODS

Study design and population. The present study was a cross-
sectional analysis of blood samples obtained at the baseline visit
of the HTLV Outcomes Study cohort, formerly known as the
Retrovirus Epidemiology Donor Study HTLV cohort. The study
protocol was approved by the Committee on Human Research
at the University of California, San Francisco, and by institu-
tional review boards at other participating institutions. En-
rollment criteria and methods have been reported in detail
elsewhere [22–24]. In brief, blood donors found to be HTLV
seropositive during predonation screening at 5 centers across
the United States in 1988–1992 had confirmation of seropos-
itivity performed by use of Western blot, followed by HTLV
typing by use of type-specific antibody or polymerase chain
reaction (PCR) assays [25]. All subjects were seronegative for
HIV at baseline. Demographic, risk factor, and health history
information was obtained by interview with trained study co-
ordinators. HTLV-II subtypes were determined previously by
use of restriction fragment–length polymorphism analysis [26].

Laboratory methods. Peripheral blood mononuclear cell
(PBMC) samples were stored at −70°C until used, and PBMCs
were digested in a PCR solution with proteinase K. Quantitation
of proviral DNA of HTLV-I and HTLV-II was performed by
use of real-time PCR. We used a single set of primers for both
HTLV-I and -II, from highly conserved sequences of the viral
tax regions, designated as HTLV-F5 (5′-CGG ATA CCC IGT
CTA CGT GTT T-3′) and HTLV-R4 (5′-CTG AGC IGA IAA
CGC GTC CA-3′). To quantitate the cellular input for each
reaction, HLA-DQ-α copy number was measured separately.
The primers used for HLA-DQ-α were GH26 (5′-GTG CTC
CAG GTG TAA ACT TGT ACC AG-3′) and GH27 (5′-CAC
GGA TCC GGT AGC AGC GGT AGT AGA GTT G-3′). For each
sample, 25 μL of DNA lysate was added to the PCR mixture
(5 μL for the HLA-DQ-α reactions). Real-time PCR was per-
formed by use of the GeneAmp 5700 machine (Applied Bio-
systems), with the following cycle conditions: 10 min at 95°C,
followed by 45 cycles of 30 s at 95°C, 30 s at 64°C, and 45 s at
72°C. Reactions were performed in triplicate [27].

Fluorescence intensity of Syber green incorporated into the
amplified product was measured at every PCR cycle, and the
threshold cycle number of each sample was recorded. For each
run, a standard curve was generated from 1:10 serial dilutions
of MT2 (HTLV-I; obtained from American Type Culture Col-
collection) or MoT (HTLV-II) cell lines, with a range of 10−1−104
copies/reaction. Mean HTLV copy numbers for these cell lines
were 2.1 HTLV-I copies/cell for MT2, as determined elsewhere
[28], and 11.7 HTLV-II copies/cell for MoT, determined ex-
perimentally using HTLV-II plasmids of known concentration
(provided by J. Kropp, Gladstone Institute of Virology and Im-
munology, San Francisco, CA, and E. Wattel, Centre Leon Ber-
nard, Lyon, France). The number of copies in the test sample
was then calculated by interpolation of the experimentally
determined threshold cycle number onto the control standard
regression curve. To determine the VL of each sample, the
number of copies of HTLV-I or HTLV-II was divided by the
HLA-DQ-α copy number. The lower limit of detection for the
assay was 1 copy/105 cells. Reproducibility of the assay was
measured by testing 40 specimens in duplicate and comparing
the results, by use of linear regression with R2 = 0.97.

Statistical analysis. VLs, expressed as HTLV-I or -II copies
per 106 PBMCs, were log10 transformed for all analyses, to
approximate the normal distribution. Undetectable VLs were
assigned the value of 1 copy/105 cells, which is below the lower
limit of detection of the VL assay, before log10 transformation.
Means, SEs, and ranges were calculated for each HTLV type
and in subsets defined by demographic and risk factor variables.
Table 1. Demographic and risk factor profile of the study population from the baseline visit of the HTLV Outcomes Study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HTLV-I</th>
<th>HTLV-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>127 (100)</td>
<td>328 (100)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>2 (2)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>20–29</td>
<td>9 (7)</td>
<td>19 (6)</td>
</tr>
<tr>
<td>30–39</td>
<td>25 (20)</td>
<td>144 (44)</td>
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<td>40–49</td>
<td>55 (43)</td>
<td>110 (34)</td>
</tr>
<tr>
<td>50–59</td>
<td>16 (13)</td>
<td>35 (11)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>20 (16)</td>
<td>18 (5)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>93 (73)</td>
<td>243 (74)</td>
</tr>
<tr>
<td>Male</td>
<td>34 (27)</td>
<td>85 (26)</td>
</tr>
<tr>
<td>Race/ethnicity&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>46 (36)</td>
<td>112 (34)</td>
</tr>
<tr>
<td>Black</td>
<td>54 (43)</td>
<td>107 (33)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>6 (5)</td>
<td>86 (26)</td>
</tr>
<tr>
<td>Asian</td>
<td>15 (12)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Other/unspecified</td>
<td>4 (3)</td>
<td>14 (4)</td>
</tr>
<tr>
<td>Lifetime no. of sex partners&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>42 (33)</td>
<td>73 (22)</td>
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<td>5–8</td>
<td>31 (24)</td>
<td>81 (25)</td>
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<td>38 (30)</td>
<td>89 (27)</td>
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<tr>
<td>&gt;21</td>
<td>14 (11)</td>
<td>78 (24)</td>
</tr>
<tr>
<td>History of IDU&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>125 (98)</td>
<td>243 (74)</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (1)</td>
<td>84 (26)</td>
</tr>
<tr>
<td>History of blood transfusion&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No/unlikely</td>
<td>104 (82)</td>
<td>136 (42)</td>
</tr>
<tr>
<td>Yes/likely</td>
<td>10 (8)</td>
<td>182 (55)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of subjects. IDU, injection drug user.

<sup>a</sup> Nos. may not add to totals, because of missing data (≤10% per variable).

RESULTS

VLs and epidemiologic data were available for 127 subjects with HTLV-I and 328 subjects with HTLV-II (~85% of the HTLV cohort). Demographic and risk factor characteristics of the subjects are presented in table 1. Most subjects were middle-aged and women, and there was a substantial proportion of black persons in both HTLV groups and of Hispanic persons in the HTLV-II group. A greater proportion of subjects with HTLV-I than subjects with HTLV-II had <4 lifetime sex partners, whereas a greater proportion of subjects with HTLV-II admitted to having >20 partners. A history of IDU was reported by 25% of the subjects with HTLV-II (48 men and 36 women), although only ~1% said they currently injected drugs. More than half of the subjects with HTLV-II (37 men and 145 women) reported (yes or likely) sexual intercourse with an IDU. One-third of the HTLV-I group and one-fifth of the HTLV-II group had a history of blood transfusion; in comparison, <10% of HTLV-seronegative blood donors enrolled in the cohort reported such a history.

VLs for both viral types ranged from 0.05 to 5.95 log<sub>10</sub> copies/10<sup>6</sup> PBMCs. HTLV-I VLs (mean [SE], 3.28 [0.12] log<sub>10</sub> copies/10<sup>6</sup> PBMCs) were significantly higher than HTLV-II VLs (mean [SE], 2.60 [0.09] log<sub>10</sub> copies/10<sup>6</sup> PBMCs; <i>P</i> < .0001; figure 1A and 1B). Among subjects with HTLV-II, HTLV-II VLs were significantly higher in the 190 subjects with subtype A (mean [SE], 2.82 [0.11] log<sub>10</sub> copies/10<sup>6</sup> PBMCs), compared with the 138 subjects with subtype B (mean [SE], 2.29 [0.15] log<sub>10</sub> copies/10<sup>6</sup> PBMCs; <i>P</i> = .005; figure 2D). All analyses by demographic and risk factor variables were performed separately for HTLV-I and for HTLV-II.

HTLV-I VLs were lower in white (mean [SE], 3.03 [0.22] log<sub>10</sub> copies/10<sup>6</sup> PBMCs) than in nonwhite (mean [SE], 3.48 [0.14] log<sub>10</sub> copies/10<sup>6</sup> PBMCs) subjects, although this difference was not statistically significant (<i>P</i> = .08; figure 2A). HTLV-I VLs were higher in those with a history of blood transfusion (mean [SE], 3.62 [0.14] log<sub>10</sub> copies/10<sup>6</sup> PBMCs) than in those without a history of blood transfusion (mean [SE], 3.1 [0.16] log<sub>10</sub> copies/10<sup>6</sup> PBMCs; <i>P</i> = .04; figure 2B). HTLV-I VLs did not differ significantly by age, sex, number of sex partners,
Figure 2. Human T lymphotropic virus (HTLV) provirus load (VL) presented as median (central line), 25th and 75th percentiles (box boundaries), and 10th and 90th percentiles (error bars). A, HTLV-II VL by HTLV-II subtype A vs. B (P = .005). B, HTLV-II VL by sex (P = .007). C, HTLV-I VL by nonwhite vs. white race/ethnicity (P = .08). D, HTLV-I VL by history of blood transfusion (P = .04). PBMCs, peripheral blood mononuclear cells.

DISCUSSION

The present study of HTLV VLs at the baseline visit of a large cohort of generally asymptomatic former blood donors found that HTLV-I VLs were significantly higher than HTLV-II VLs and that HTLV-II VLs were significantly higher in subtype A than in subtype B infections. Analyses by demographic and behavioral characteristics revealed higher HTLV-I VLs in those subjects with a history of blood transfusion and borderline lower HTLV-I VLs in white subjects. HTLV-II VLs were significantly lower in women than in men. There was a borderline association of lower VLs with increasing numbers of sex partners, but this did not persist after adjusting for covariates.

HTLV-I VLs have been reported in numerous studies, ranging from $<1$ copy/10,000 lymphocytes to $>1$ copy/10 lymphocytes [6, 9, 10, 14]. The results of the present study are consistent with those of previous reports, with a mean HTLV-I VL of $\approx 1000$ copies/10$^6$ PBMCs and a range of 1–100,000 copies/10$^6$ PBMCs. By use of an end-point dilution PCR assay, a previous study revealed VLs of 20–200,000 copies/10$^6$ PBMCs in 49 subjects with HTLV-II, with and without HIV coinfection [17]. A competitive PCR assay found 50–162,390 copies/10$^6$ PBMCs in Italian IDUs [16], and those with the highest VLs were found to have clonal expansion of HTLV-II–infected lymphocytes [29]. These VL results are also similar to those of the present study.

In the only other comparative study of HTLV-I and HTLV-
II VLs that we were able to find, Kaplan et al. reported that HTLV-I VLs were significantly higher than HTLV-II VLs [27]. Their study included a subset of individuals from the present study but used a different assay to measure VLs. The present report is the only published in vivo study of HTLV-II VLs according to HTLV-II subtype. Differences in VLs between HTLV-I and HTLV-II, and between HTLV-II subtype A and subtype B, may be related to differences in tax gene structure and protein expression among the HTLV types and subtypes. HTLV-II Tax has been shown to have a lower transforming activity than HTLV-I Tax [30], which may account for the lack of leukemogenesis by HTLV-II. Compared with HTLV-II subtype B, HTLV-II subtype A has less ability to inhibit p53 function in T lymphocytes [8, 31] and to induce CREB- and NF-κB–mediated transactivation [32]. These in vitro data would suggest lower VLs in HTLV-II subtype A infection, which is the opposite of our finding. One explanation may be that tax genes from wild-type HTLV-II subtype A isolates may have different biologic activity than the HTLV-II prototypes commonly used for in vitro experiments [31–33]. It is also possible that differences in VLs between HTLV types and subtypes are due to differences in modes of transmission or to host factors, and not to biological characteristics of each virus.

The association of higher HTLV-I VLs with a history of blood transfusion is the most suggestive evidence we found that the route of infection affects HTLV VLs. Fully 34% of our HTLV-I cohort had received a blood transfusion, compared with 10% of our HTLV-seronegative comparison group, which is consistent with our previous report of blood transfusion as a risk factor for infection (adjusted odds ratio, 4.5; 95% confidence interval, 2.6–7.8) [34]. These same subjects with HTLV-I and a history of blood transfusion have now been shown to have higher VLs than subjects with HTLV-I without this risk factor. Blood transfusion is associated with a higher dose of HTLV-I–infected lymphocytes, so it is biologically plausible that VLs are higher. Since VLs have been shown to remain relatively constant over time [35], one may infer that a higher infecting dose from blood transfusion translates to a higher persistent VL, such as those detected in our subjects. Alternatively, altered immune function from comorbid illness or severe trauma at the time of blood transfusion could have affected the VL set point. Our data are consistent with those of a previous report of median HTLV-I VLs of 100–200 copies/10^5 PBMCs in the first year after blood transfusion–transmitted infection in a Jamaican study [14]. However, in the same study, asymptomatic carriers had higher HTLV-I VLs (median, ~1000 copies/10^5 PBMCs) than did asymptomatic carriers in the present study, perhaps because of a higher proportion of mother-to-child infection.

HTLV-II VLs were significantly lower in women than in men. Two previous studies of HTLV-I VLs found no differences by sex among asymptomatic carriers [9, 36], but we were unable to find other published reports of differences in HTLV-II VLs by sex. Other reports found that women with HAM have either higher or lower HTLV-I VLs than do men with HAM [9, 10]. Women are clearly overrepresented among both patients with HTLV-I HAM and patients with HTLV-II HAM [37]. Previous epidemiological data suggest that sexual acquisition of HTLV-I is associated with development of HAM, whereas mother-to-child acquisition is associated with ATL [38, 39].

We therefore hypothesize that women are more likely to acquire HTLV-II sexually, with a smaller infectious dose, and, hence, to have lower VLs than men, most of whom acquire the virus by parenteral (IDU or blood transfusion) or mother-to-child routes. Against this hypothesis are data from the present study, which showed no association between HTLV-II VLs and the lifetime number of sex partners or history of sex with an IDU, for both sexes. However, in an analysis limited to women, we did find a nonsignificant trend toward lower HTLV-II VLs with increasing number of lifetime sex partners. Retrospective interview data may not be the most accurate in assigning the actual mode of transmission. In support of the hypothesis are data showing lower mean HTLV-I VLs in 11 cases of sexually acquired HTLV-I, compared with the 11 transmitting persons [40].

That infectious dose may predict the chronic set point for VLs is consistent with the observation that expansion of HTLV within an infected human is predominantly due to clonal expansion of lymphocytes with integrated HTLV provirus [6]. Thus, the number of initially infected lymphocytes would influence the number of lymphocyte clones, although the size of each clone may depend on factors that cause multiplication of lymphocytes. Recent data from the Wattel laboratory support this hypothesis, in that humans with presumed breast-milk acquisition of HTLV-II as infants had increased clonality and VLs, compared with those with risk factors suggesting infection acquired later in life [41]. An alternative explanation is that infection during infancy or childhood produces higher VLs because of a weaker initial immune response.

The borderline association we found between lower HTLV-I VLs and white race may be explained either by genetic background or by route of infection associated with a particular racial group. Bangham et al. have previously reported that HTLV-I VLs may be correlated with specific HLA subtypes [42] and that these subtypes are also associated with an increased risk of developing HAM. Since HLA phenotypes differ by racial origin, it is conceivable that the race/ethnicity association we observed is due to genetic background. On the other hand, other host characteristics, such as route of HTLV infection, lymphocyte activation, and concomitant infections, may also differ by race. It has been recognized that the incidence of HTLV-associated diseases, such as ATL, may differ between endemic Japanese and Jamaican populations, perhaps because
of differences in biologic response to infection [23]. Furthermore, comparative studies across race and ethnicity populations using similar methodologies to measure VL and controlling for route of infection and HLA type should help resolve this issue.

Strengths of the present study include the use of a well-defined, generally asymptomatic group of human subjects and a sensitive, reproducible, and high-throughput assay to measure VLs. Potential weaknesses include the relatively large variability of VLs and the fact that the differences in VLs, although statistically significant, were of relatively small magnitude, compared with the interpersonal range of observed values. Determining causality for the associations we observed is also problematic in this cross-sectional study, since different characteristics of the subjects (i.e., race/ethnicity and mode of acquisition) may be confounded. That associations persisted after multivariable analysis is reassuring but not definitive.

In conclusion, we have demonstrated associations between higher HTLV-I VLs and history of blood transfusion and, perhaps, nonwhite race and between lower HTLV-II VLs and female sex. Discovering the biological determinants of VL is important because the incidence of HTLV-associated diseases seems to be related to VL. Although reasons for the race/ethnicity association remain speculative, we hypothesize that associations with sex and history of blood transfusion result from a biological relationship between infectious dose of HTLV-I and HTLV-II and the chronic VL measured in an individual. Further studies in humans with well-defined time and mode of infection or experimental studies in animals are required to investigate this hypothesis.

HTLV OUTCOMES STUDY (HOST) MEMBERS

The HOST is presently the responsibility of the following persons.

**Study headquarters.** University of California, San Francisco (San Francisco, CA): E. L. Murphy (Principal Investigator) and J. Engstrom.


**Central laboratory.** Blood Centers of the Pacific (San Francisco, CA): M. P. Busch and L. H. Tobler.

**Diagnostic review panel.** E. L. Murphy, R. Sacher, and J. Friday.

References


