Quantification of Human T-Lymphotrophic Virus Type I (HTLV-I) Provirus Load in a Rural West African Population: No Enhancement of Human Immunodeficiency Virus Type 2 Pathogenesis, but HTLV-I Provirus Load Relates to Mortality

Koya Ariyoshi,1,4 Neil Berry,1,5 Fatim Cham,1 Shabbar Jaffar,4 Maarten Schim van der Loeff,1 Ousman Jobe,1 Pa Tamba N’Gom,1 Olav Larsen,1 Sören Andersson,2 Peter Aaby,1 and Hilton Whittle1

1Medical Research Council Laboratories, Fajara, The Gambia; 2Bandim Health Project, Danish Epidemiology Science Centre, Bissau, Guinea-Bissau; 3Department and 4Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom (M.S.v.d.L.).

Both human immunodeficiency virus (HIV) and human T-lymphotropic virus type I (HTLV-I) are retroviruses that are transmitted horizontally through either sexual contact or contaminated blood or vertically from mother to child, and both establish persistent infection. Risk factors for infection are shared by the 2 retroviruses; consequently, individuals who harbor 1 virus may often be coinfected with the other. In west Africa, both HIV-2 and HTLV-I are endemic [1, 2], but very little is known about the interaction between the 2 persistent viral infections.

Although HIV-2–infected individuals, in general, progress to disease more slowly and survive longer than do HIV-1–infected individuals [3], we have observed clinical diversity in HIV-2 infection and have demonstrated that outcomes are significantly associated with both HIV-2 provirus DNA load in peripheral blood mononuclear cells (PBMCs) and HIV-2 RNA load in plasma [1, 4, 5]. Previous studies have investigated HIV-2 provirus DNA load in infected individuals in a rural village of Guinea-Bissau, where there is high prevalence of both HIV-2 and HTLV-I infections [1]. The previous study found no difference in the geometric mean of HIV-2 provirus DNA load between those with and those without HIV-2/HTLV-I coinfection but did not examine the interaction with HIV-2 RNA load in plasma nor did it examine the association between HTLV-I infection and survival.

Several observations, which might explain the stimulation of HIV-1 and/or HIV-2 replication by HTLV-I, have been tested in in vitro experiments. These include the findings that the HIV-2 rev-responsive element is responsive to the HTLV-I rex gene product [6, 7] and that HTLV-I tax and rex products interact indirectly with the long-terminal repeat region by upregulating the expression of interleukin (IL)-2 or the IL-2 receptor [8]. Regardless of which mechanisms are involved, the expression of HTLV-I genes is crucial for the interaction with HIV; hence, the effect of HTLV-I on HIV replication in vivo is thought to relate to HTLV-I provirus load, which varies considerably, being up to 100,000-fold different among infected individuals [9]. In the present study, we have examined HTLV-I provirus DNA load in relation to HIV-2 provirus DNA load in PBMCs and HIV-2 RNA load in plasma of coinfected subjects, and we have studied the effects of dual infection on mortality. The results lead us to conclude that HIV-2/HTLV-I coinfection does not enhance HIV-2 infection, but increased HTLV-I provirus load may contribute to excess mortality.

Subjects, materials, and methods. In a previous study, 133 HIV-2–infected subjects and 160 HIV-uninfected subjects were recruited in a rural village in Guinea-Bissau between January 1991 and May 1991; the epidemiological methods, serological
diagnosis, and the method of CD4 cell counting have been described elsewhere [10]. PBMCs and plasma samples cryopreserved in liquid nitrogen were available from 126 HIV-2–infected subjects and 159 HIV-uninfected subjects. HIV-2 provirus loads and HIV-2 RNA loads were determined by in-house quantitative polymerase chain reaction (PCR) assays, as described elsewhere [1, 4, 5].

DNA extracted from cryopreserved PBMC samples was first screened for HTLV infection by a nested PCR with HTLV tax/rev–specific primers, following a published method [11]. Plasma samples from all subjects were screened by use of the Vironostica HTLV-ELISA (Organon Teknika), and samples that had discrepancies between ELISA and PCR results were subjected to HTLV Western blot analysis (HTLV-blot 2.4; Diagnostic Biotechnology) for confirmation. HTLV-I infection was defined by either repeated detection of HTLV provirus by nested PCR or seropositivity fulfilling the Western blot criteria for HTLV-I infection (reactivity against at least 2 Env proteins and 1 core protein). All HTLV proviruses detected by PCR were thought to be of HTLV type I by restriction-enzyme analysis using endonucleases Sau3A and TaqI [11].

HTLV provirus DNA load was analyzed by limiting-dilution analysis as follows: sample DNA was diluted in PCR-compatible DNA lysis buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl [pH 8.3], 2.5 mmol/L MgCl2, 0.45% NP40, and 0.45% Tween-20) in 5-fold steps by use of a Gilson positive displacement pipette. Each time the diluted DNA was transferred, a new piston and a capillary were used. At each dilution, nested PCR was performed. To determine the end point dilution, aliquots of diluted samples around the end point were tested in quadruplicate. If ≥2 of 4 test results were positive, then the dilution was regarded as positive and the reciprocal value of the end point dilution was expressed as titer of HTLV-I provirus (copies/μg of DNA).

Comparisons between normally distributed variables were made by t test, whereas nonnormally distributed data were compared by the Wilcoxon test. Correlation with HTLV-I provirus titer was assessed by Spearman’s correlation coefficient. Discrete data were compared by the χ2 test or Fisher’s exact test, as appropriate. Survival analysis was conducted by Cox regression. The follow-up time was calculated from the 1991 clinical study to September 1998, when the study closed. The present study was approved by the Gambian government/Medical Research Council Joint Ethics Committee. Subjects were counseled before and after HIV testing and provided informed consent before participation.

Results. PCR results are shown in table 1. The diagnosis of HTLV-I infection by PCR or serologic testing was concordant in 265 (92.7%) of 285 samples tested. The discordant results were thought to be mainly due to unspecific ELISA reactivity. Three of 48 HTLV-PCR–positive subjects were found to be negative by the initial ELISA. Further Western blot tests showed that 1 subject was positive and 2 subjects were indeterminate; these 2 subjects were repeatedly positive by PCR, although the titers of HTLV-I provirus were low in both subjects (≤5 copies/μg of DNA). All 3 were regarded as being HTLV-I infected. Eighteen of the 237 HTLV-PCR–negative subjects were found to be positive by the initial ELISA. Further Western blot tests revealed that 7 subjects were negative and 9 subjects were indeterminate. Western blot tests were not performed for 2 subjects, since no serum was available. All 18 subjects were regarded as being HTLV-I uninfected.

The mean age, proportion female, and mean CD4 cell percentage of subjects with or without HTLV-I infection are shown in table 1. In HIV-2–infected subjects, HTLV-I infection was significantly associated with a higher CD4 cell percentage (P = .042), with older age (P = .01), and with being female (P < .0001). However, among HIV-uninfected subjects, HTLV-I infection was not associated with either CD4 cell percentage, age, or sex (P = 1.0, P = .2, and P = 4, respectively).

The median HTLV-I provirus load in the HIV-2–infected subjects was 625 copies/μg of DNA (interquartile range [IR],
625–3125 copies/μg of DNA), which was the same as for the HIV-uninfected subjects: 625 copies/μg of DNA (IR, 25–15,625 copies/μg DNA; \( P = .8 \), Wilcoxon test). In the 31 coinfected subjects for whom valid CD4 cell percentage data was available, HTLV-I provirus load did not significantly relate to CD4 cell percentage (\( r = -0.016; P = .9 \), Spearman’s test), nor did HTLV-I provirus load relate to age in those subjects (\( r = -0.11; P = .6 \), Spearman’s test).

In the present study, we examined the relationship between HTLV-I status and HIV-2 RNA load. The results are shown in figure 1. Interestingly, the geometric mean of HIV-2 RNA copies per milliliter of plasma was significantly lower among the 32 HIV-2/HTLV-I–coinfected subjects than among the 92 subjects infected with HIV-2 alone (212 copies/mL [95% confidence interval (CI), 97–465 copies/mL] and 724 copies/mL [95% CI, 420–1248 copies/mL], respectively; \( P = .02 \)). Furthermore, HIV-2 was not detectable at <100 copies/mL in 18 (56%) of 32 of subjects with HIV-2/HTLV-I coinfection, compared with 27 (29%) of 92 subjects without HIV-2/HTLV-I coinfection (\( P = .006 \)). The difference in the proportion remained significant after adjusting for sex and age (adjusted odds ratio, 2.60 [95% CI, 1.04–6.52]; \( P = .04 \)).

To determine whether there was any further interaction between the 2 infections, we analyzed HTLV-I provirus DNA loads in relation to HIV-2 RNA and DNA loads, in the coinfected subjects. There was no significant correlation with either HIV-2 RNA load (\( n = 32; r = 0.13; P = .5 \), Spearman’s test) or HIV-2 provirus DNA load (\( n = 31; r = -0.32; P = .12 \), Spearman’s test).

After a median follow-up of 7.0 years, 9 (27%) of 33 HIV-2/HTLV-I–coinfected subjects, 19 (20%) of 93 subjects infected with HIV-2 alone, 4 (27%) of 15 subjects infected with HTLV-I alone, and 20 (14%) of 144 uninfected subjects had died. In HIV-2 infected subjects, after adjusting for sex, age, provirus DNA load, and RNA load, the mortality hazard ratio of the HTLV-I infected subjects relative to HTLV-I–uninfected subjects was 1.10 (95% CI, 0.34–3.54; \( P = .9 \)). In HTLV-I–infected subjects, after adjusting for sex, age, and HIV status, the mortality hazard ratio was 0.98 (95% CI, 0.5–2.0; \( P = 1.0 \)). However, when the 48 HTLV-I–infected subjects were categorized according to HTLV-I provirus load, there was clear indication that mortality was associated with increased HTLV-I load: 1 (10%) of 10 subjects with a low virus load (<100 copies/μg), 12 (26%) of 27 subjects with a medium virus load (100–9,999 copies/μg), and 5 (46%) of 11 subjects with a high virus load (>10,000 copies/μg) had died. Thus, we further examined the effect of HTLV-I load on a continuum with uninfected subjects, because the number of subjects was so small. Mortality adjusted for sex, age, and HIV status increased significantly with HTLV-I provirus load; mortality hazard ratio was 1.59 for each log 10 increase in HTLV-I (95% CI, 1.0–2.52; \( P = .038 \)).

**Discussion.** The dynamics of retroviral coinfections and their effect on survival is an important topic. This is the first study that has quantitatively assessed the effect of HTLV-I infection on HIV-2 load. We initially thought that HIV-2/HTLV-I coinfection would enhance the level of HIV-2 replication, on the basis of in vitro findings [6–8]. However, we have found no positive correlation between HTLV-I provirus load and either HIV-2 provirus DNA load or HIV-2 RNA load. Therefore we conclude that HTLV-I infection does not have a marked enhancing effect on HIV-2 replication in vivo and, therefore, does not affect mortality due to HIV-2 disease. These results were compatible with the epidemiological findings, which showed that mortality among HIV-2–infected subjects with HIV-2/HTLV-I coinfection was not significantly different from that for those without HIV-2/HTLV-I coinfection.

A study in Brazil reported no significant difference in HIV-1 load between the HIV-1/HTLV-I coinfected group and the group infected with HIV-1 alone [12]. Similar results have been obtained in a macaque model [13]. Intriguingly, our analysis of HIV-2 RNA load has suggested that suppression of replication of HIV-2 occurs as a consequence of HIV-2/HTLV-I coinfection; thus, the suppression phenomenon may be specific to the less-pathogenic virus of HIV-2. We, however, have to be cautious about concluding this, since the number of subjects in our study was rather small and the effect was less obvious after adjusting for sex and age.
HTLV-I provirus load was found to vary by >100,000-fold among subjects. Accumulating evidence indicates that HTLV-I provirus load may have some influence on pathogenesis [9, 14]. It is, therefore, important to know whether HIV infection per se and/or HIV-induced immunodeficiency influence HTLV-I provirus DNA loads. We did not find any in vivo evidence to indicate that HIV-2 infection was associated with a higher HTLV-I provirus load. Nor were CD4 cell levels correlated with HTLV-I provirus loads. Therefore, our data indicate that HIV-2 infection does not have a significant effect on HTLV provirus load.

Excess morbidity caused by other illnesses, in HTLV-I carriers, has been described in a large-scale cohort [15], but, to our knowledge, very few studies have investigated the relationship between HTLV-I load and survival, in a cohort of asymptomatic HTLV-I carriers [14]. The present study was limited by difficulties in investigating the actual cause of death among the recruited subjects, since most died at home without an attending health worker, as well as by the relatively small number of subjects with HTLV-I infection. However, our data on 7 years of survival follow-up suggest that high HTLV-I provirus loads may contribute to excess deaths. Further studies with a larger number of infected subjects are necessary to confirm the results.

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References