Persistent Paradox of Natural History of Human T Lymphotropic Virus Type I: Parallel Analyses of Japanese and Jamaican Carriers

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Human T lymphotropic virus type I (HTLV-I) is endemic in southern Japan and the Caribbean, but the incidence of HTLV-I–associated diseases varies across geographic areas. We compared markers of disease pathogenesis among 51 age- and sex-matched HTLV-I carrier pairs from Japan and Jamaica. The mean antibody titer ($P = .03$) and detection of anti-Tax antibody ($P = .002$) were higher in Jamaican subjects than in Japanese subjects, but provirus load was similar between the 2 groups ($P = .26$). The correlation between antibody titer and provirus load was more prominent among Jamaican subjects than among Japanese subjects ($P = .06$). These findings underscore the differences in host immune response to HTLV-I infection in 2 populations.

Human T lymphotropic virus type I (HTLV-I) is endemic in southern Japan, the Caribbean, parts of West Africa, the Middle East, South America, and Melanesia. HTLV-I is associated with adult T cell leukemia (ATL) and HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) in a small fraction of infected persons [1, 2]. The pathogenic potential of HTLV-I is largely orchestrated by the Tax protein, a product of the viral regulatory gene *tax*, which promotes genetic mutations, inhibits cell cycle, and drives viral and host gene expression [3]. Because HTLV-I does not encode oncogenes and lacks common integration sites, Tax likely plays a pivotal role in oncogenesis.

ATL develops after a long latency period, primarily among persons who acquired infection during infancy, whereas HAM/TSP occurs after a shorter latency period, frequently among persons who acquired infection later in life. Patients with ATL typically have high HTLV-I provirus loads (i.e., the amount of integrated HTLV-I genome in peripheral blood mononuclear cells) and high antibody titers against HTLV-I structural proteins but lack antibodies against the Tax regulatory protein [4]. In contrast, antibody titers against HTLV-I structural proteins and the Tax regulatory protein normally correlate with provirus load in asymptomatic carriers [5]. ATL cells may also show low levels of *tax* mRNA. HTLV-I–infected cells are thought to expand in vivo predominantly by mitotic, clonal expansion [6]. Such clones must survive the host's immune surveillance [7]. Because the cytotoxic T lymphocyte (CTL)–mediated response is primarily directed against Tax, cells that do not express Tax and that lack anti-Tax antibody are more likely to be protected from CTL response. In contrast, patients with HAM/TSP have high provirus loads, high antibody titers against the HTLV-I structural and Tax proteins [8], and high CTL responses against Tax [9].

The epidemiologic profile of HTLV-I–associated diseases differs between Japan and the Caribbean. In Japan, the annual incidence of ATL is 86 cases/100,000 population and is 3 times higher in men than in women, with a peak around age 60 years [10]. In the Caribbean, the annual incidence is ~20 cases/100,000 population, with little evidence of male predominance and with a peak around age 40 years. In contrast, the annual incidence of HAM/TSP is higher among Jamaican subjects than among Japanese subjects (20 vs. 3 cases/100,000 population), with a slightly higher risk for women in both populations.

Because viral sequence variations between HTLV-I strains isolated from geographically diverse populations are minimal, the differences in morbidity are likely to be due to host factors [3]. The distribution of age, sex, route and dose of infection, comorbidity, nutrition, social environment, and host genetics each may also play a role. To examine whether the differences in morbidity in populations in which HTLV-I is endemic may be reflected by markers of disease pathogenesis, we compared

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HTLV-I viral markers in Japanese and Jamaican carriers by use of standardized laboratory procedures.

Methods. In Japan, the Miyazaki Cohort Study enrolled adult residents of 2 rural villages in Miyazaki, Japan, in the context of government-sponsored annual health examinations [5]. Between 1984 and 2000, participants were monitored by use of a questionnaire, a physical examination, and a phlebotomy. The baseline prevalence of HTLV-I infection in this population was 27%. Specimens were stored at −80°C until use.

In Jamaica, the Food Handlers Study was established in 1985 by enrolling food licensure applicants from all Jamaican parishes [11]. The baseline prevalence of HTLV-I infection in this population was 6%. Subsequently, 201 HTLV-I–seropositive subjects and 225 age- and sex-matched seronegative control subjects were entered in a case-control study and provided blood samples and questionnaire data. In 1993–1994, 666 subjects from the initial cohort were recalled for additional follow-up. Specimens were stored at −80°C in a central repository.

HTLV-I carriers from the Miyazaki Cohort were matched with those from the Food Handlers Cohort by age (±2 years), sex, and year of sample collection (±2 years). Hepatitis C virus (HCV) infection was found to be highly endemic (27%) in 1 of the study villages in the Japanese cohort but not in the other Japanese village or in the Jamaican cohort. To avoid the potential confounding effect of HCV on HTLV-I provirus load [12], we excluded subjects from either village known to be seropositive for HCV. Analysis included 102 HTLV-I carriers, 51 from each study cohort (18 men and 84 women).

The study protocol followed the human-experimentation guidelines of the US Department of Health and Human Services and those of the institutional review boards of the National Cancer Institute, the University of the West Indies, Miyazaki Medical College, and the Harvard School of Public Health. Informed consent was obtained from all participants.

Serum samples from the Japanese cohort were screened for antibodies to HTLV-I by use of a particle agglutination assay (Serodia-HTLV; Fujirebio) and were confirmed by use of an immunoblot assay (Problot HTLV-I; Fujirebio). Serum samples from the Jamaican cohort were screened by use of ELISAs (CAMB WV 21E, Biotech Research Labs; Dupont ELISA, Dupont; and Genetic Systems HTLV, Bio-Rad Laboratories) and were confirmed by use of a Western blot (HTLV Blot 2.4; Genelabs Diagnostics).

Antibody titers were measured by use of ELISAs (Cambridge-Biotech and Organon Teknika) on serial 5-fold dilutions at the National Cancer Institute's Viral Epidemiology Section laboratory (Frederick, MD). Anti-Tax antibody was detected by use of an in-house ELISA at Eisai (Tokyo, Japan). Provirus load was quantified in triplicate by use of a real-time polymerase chain reaction using an ABI PRISM 7700 Sequence Detector (Perkin Elmer/Applied Biosystems). HTLV-I provirus copy number/10^5 cells was normalized by the number of β-actin copies [8]. Each viral marker assay was performed with matched pairs placed on the same plate, to minimize plate-to-plate variability.

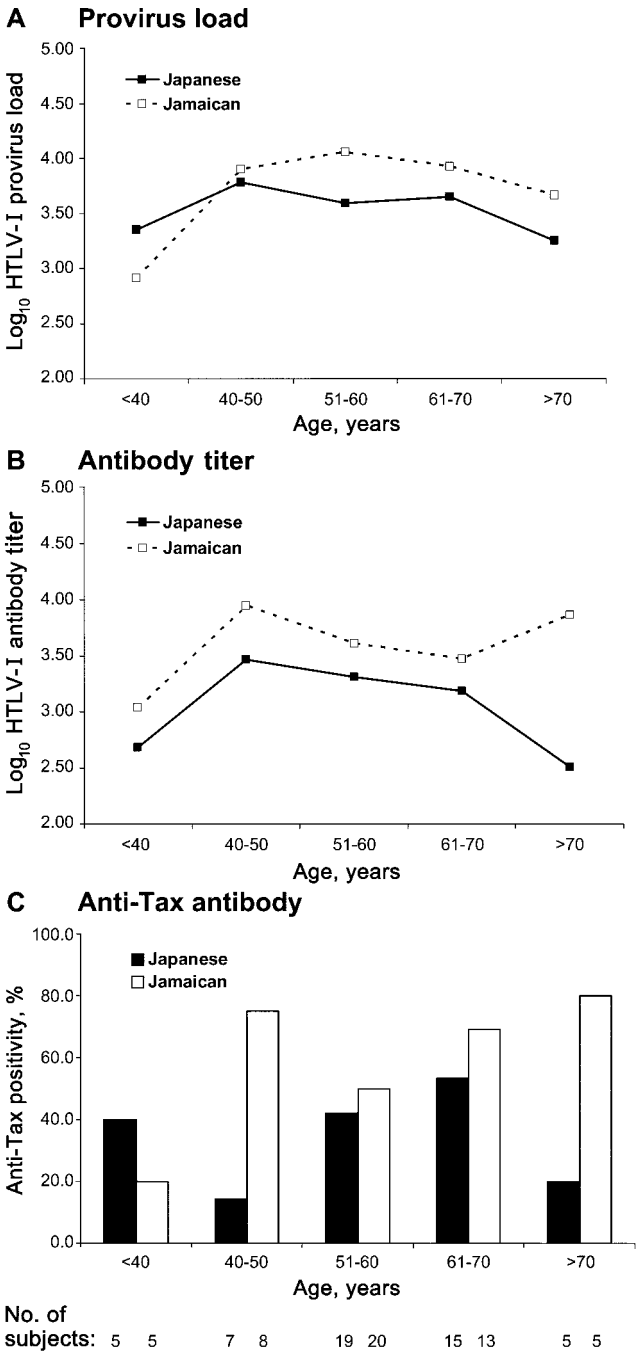

Figure 1. Human T lymphotropic virus type I (HTLV-I) provirus load *(A),* antibody titer *(B),* and antibody to Tax *(C),* by age group and by study population. The nos. of subjects for each age category are common for all panels, as shown at the bottom.

Age was treated as a categorical variable (≤50, 51–60, 61–70, and >70 years old). Reciprocal antibody titer and provirus load were $\log_{10}$ transformed, with undetectable loads coded as 0. De-

Table 1. Estimates of the differences in provirus load (log_{10} copies/10^5 cells) and reciprocal antibody titer (log_{10}) in 102 carriers of human T lymphotropic virus type I, by study population.

Parameter, variable	All subjects	Japanese subjects	Jamaican subjects
Provirus load, log_{10} copies/10^5 cells			
Age group			
51–60 years	0.43 (−0.16 to 1.02)	0.14 (−0.60 to 0.89)	0.84 (−0.16 to 1.85)
61–70 years	0.37 (−0.24 to 1.00)	0.15 (−0.63 to 0.93)	0.74 (−0.33 to 1.81)
>70 years	0.07 (−0.69 to 0.82)	−0.21 (−1.19 to 0.76)	0.46 (−0.77 to 1.69)
Male	0.04 (−0.53 to 0.61)	−0.02 (−0.77 to 0.74)	0.18 (−0.74 to 1.09)
Japanese	−0.19 (−0.60 to 0.22)	…	…
Antibody titer, log_{10}			
Age group			
51–60 years	0.12 (−0.42 to 0.66)	0.18 (−0.44 to 0.80)	0.10 (−0.86 to 1.06)
61–70 years	0.11 (−0.46 to 0.68)	0.10 (−0.55 to 0.75)	0.14 (−0.88 to 1.15)
>70 years	−0.28 (−0.96 to 0.40)	−0.60 (−1.41 to −0.22)	0.06 (−1.12 to 1.23)
Male	0.13 (−0.38 to 0.65)	0.05 (−0.57 to 0.68)	0.22 (−0.65 to 1.09)
Japanese	−0.43 (−0.80 to −0.06)	…	…

NOTE. Data are incremental change (95% confidence interval). The respective reference groups are ≤50-year-old subjects, women, and Jamaicans. Models were adjusted for all variables shown.

tection of anti-Tax antibody was dichotomous. The prevalence of anti-Tax antibody was compared across study sites by use of McNemar's test. The means of age, antibody titer, and provirus load were compared across groups by use of the nonparametric Wilcoxon's signed rank test. Correlations between antibody titer and provirus load were examined by use of Spearman's correlation coefficient. Multiple linear regression analysis was used to associate log_{10} HTLV-I antibody titer and provirus load with age, sex, and study population. Wald-type 95% confidence intervals (CIs) are reported. All statistical tests were 2-sided.

Results. The mean age of the 102 subjects was 56.3 years (range, 26–78 years) and was higher in women than in men (54.6 vs. 46.8 years; $P = .03$). The mean antibody titer was 1:28,800 (range, 1:6–1:390,625) for the Jamaican subjects and 1:7290 (range, 1:18–1:108,030) for the Japanese subjects. The reciprocal antibody titer was significantly higher in Jamaican subjects than in Japanese subjects (3.6 vs. 3.2 log_{10}; $P = .03$). The mean titer did not vary by sex ($P = .99$) or by age group ($P = .97$).

Overall, 50 (49%) of the 102 subjects had anti-Tax antibody. Detection of anti-Tax antibody did not differ by sex (39% in men vs. 51% in women; $P = .35$) or by age group ($P = .13$, test for trend). Detection of anti-Tax antibody was more common in Jamaican subjects than in Japanese subjects in each age group except among the youngest (figure 1). Overall, 30 (59%) of the 51 Jamaican HTLV-I carriers had anti-Tax antibody, compared with 20 (39%) of the 51 Japanese HTLV-I carriers ($P = .002$).

HTLV-I provirus was detected in 96 (94%) of the 102 subjects. Provirus detection did not differ by age group ($P = .87$, test for trend), sex ($P = .95$), or study site ($P = .40$). The provirus load was <10–51,700 copies/10^5 cells (0–4.7 log_{10} copies/10^5 cells) among the Jamaican subjects and <10–20,200 copies/10^5 cells (0–4.3 log_{10} copies/10^5 cells) among the Japanese subjects. Among subjects with detectable provirus, the mean provirus load did not vary by sex ($P = .50$), by age group ($P = .28$, test for trend), or by study site (3.1 vs. 3.0 log_{10} copies/10^5 cells; $P = .26$).

The correlation between provirus load and titer was marginally stronger in the Jamaican subjects ($R = 0.64$) than in the Japanese subjects ($R = 0.44$; $P = .06$), as was the correlation between antibody titer and anti-Tax positivity ($P = .02$). Age-associated changes in provirus load were more prominent in the Jamaican subjects than in the Japanese subjects (table 1). In both study populations, sex was not strongly correlated with provirus load. After adjustment for age and sex, the study population itself (either Jamaican or Japanese) was not significantly associated with provirus load (−0.19 log_{10} copies/10^5 cells). A 1-log_{10} increase in antibody titer and the presence of anti-Tax antibody were associated with a statistically significant 0.26- and 0.84-log_{10} increase in provirus load, respectively (data not shown); however, the associations of provirus load with age, sex, and study population remained essentially the same after adjustment for these additional viral markers.

In an adjusted model, antibody titer was significantly lower (−0.43 log_{10}) in Japanese subjects than in Jamaican subjects (table 1). In addition, titers were significantly lower after age 70 years among Japanese subjects, a trend that was not evident among the Jamaican subjects. Male sex was associated with a more substantial increase in HTLV-I titer among Jamaican subjects (0.22 log_{10}) than among Japanese subjects (0.05 log_{10}). The results did not change markedly by additional adjustment for provirus load and for the presence of anti-Tax antibody, although the difference in titer by study population was slightly attenuated (−0.25 log_{10}; 95% CI, −0.58 to 0.07 log_{10}).

Discussion. The present study represents the first effort to compare HTLV-I viral markers across population-based studies by use of standardized assays. As would be expected from a higher

incidence of HAM/TSP in Jamaica, both antibody titers and detection of anti-Tax antibody, which are markers of a strong immune response and persistent viral replication, were higher among Jamaican subjects than among Japanese subjects. Although provirus load is a predictor of HAM/TSP and a correlate of antibody responses among asymptomatic carriers [5], it did not differ between Jamaican and Japanese subjects. Our findings, combined with an observed strong in vitro expression of *tax* mRNA in patients with HAM/TSP [8], support the hypothesis that heightened viral replication and immune response characterize the Caribbean population's high risk for HAM/TSP.

In contrast, high provirus load in the absence of strong antibody responses to the structural and regulatory genes in the Japanese population suggest that clonal expansion of HTLV-I–infected cells, rather than viral replication, is the primary determinant of provirus load. High provirus load, combined with modest levels of antibody titer and a lack of anti-Tax antibody, is a viral marker profile that characterizes a high risk for ATL [12, 13]. Thus, an exceptionally low prevalence of anti-Tax antibody among Jamaicans <40 years old (figure 1) is of interest, because it may mirror the much more common occurrence of ATL in the Caribbean than in Japan among people <40 years old. Our interpretation requires caution, however, because the low prevalence of anti-Tax antibody may have also resulted from the small number of subjects in this age group.

Our analysis indicates that there may be an age-related decrease in antibody titer among Japanese subjects. The reason for this observation is unknown. This pattern was not evident among Jamaican subjects (table 1). Of note, the reported higher antibody titers among female than male Japanese HTLV-I carriers [5] did not hold in the present analysis, which was adjusted for other covariates simultaneously. Similarly, we had previously found HTLV-I provirus load to be higher among men than among women [5], but, in the present analysis, the sex difference in provirus load among the Japanese subjects was relatively small and not statistically significant after adjustment for other correlates.

Potential interactions of HTLV-I with other infections were considered. Our analysis excluded individuals known to be infected with HCV, which was highly endemic in 1 study site. Although there was no HIV infection in either study cohort, parasitic infections, including *Strongyloides stercoralis,* are likely more common in the tropical climate of the Caribbean than in the temperate climate of Japan. We were unable to examine this possibility, because data on parasitic infection were not readily available from either population. *S. stercoralis* has been shown to increase HTLV-I provirus load and accelerate clonal expansion [14].

Age at infection is an important factor that could not be accounted for in the present analysis. In addition, host genetics, time-dependent gene and protein expression, diversity and number of HTLV-I–infected clones, quantity of mRNA for *tax,* and other external influences associated with social environment will be critically important to further characterize the differences in disease risks among HTLV-I carriers. Only through multidisciplinary collaborations that reach across affected populations, combined with careful evaluation guided by epidemiologic methods, can this goal be achieved.

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