Human T Lymphotropic Virus Type 1 (HTLV-1) Proviral Load in Asymptomatic Carriers, HTLV-1–Associated Myelopathy/Tropical Spastic Paraparesis, and Other Neurological Abnormalities Associated with HTLV-1 Infection

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Recent reports have demonstrated that human T lymphotropic virus type 1 (HTLV-1) is associated with other neurological abnormalities in addition to HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). It has been well established that high HTLV-1 proviral loads are associated with the development of HAM/TSP. We now demonstrate, for the first time, to our knowledge, that HTLV-1 proviral loads in patients with other neurological abnormalities are also significantly higher than in asymptomatic HTLV-1 carriers.

Human T lymphotropic virus type 1 (HTLV-1) infection is associated with the development of HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the majority of HTLV-1–infected individuals have lifelong asymptomatic infection [1, 2], there are presently no specific biomarkers that can predict the risk of progression from an asymptomatic to a symptomatic state. A high HTLV-1 proviral load in cells seems to be a risk factor for the development of HAM/TSP, because the proviral load is significantly higher in symptomatic than in asymptomatic carriers [3].

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Other inflammatory diseases (e.g., uveitis, arthritis, and polymiositis) have also been associated with HTLV-1, demonstrating the broad, systemic inflammatory spectrum of HTLV-1 infection [4]. We recently also demonstrated that infection is associated with other neurological abnormalities (ONAs) [5] which include isolated mild cognitive deficits, isolated peripheral neuropathy, and amyotrophic lateral sclerosis syndrome (ALS) [6–8]. In the present study, we investigate whether these conditions are also associated with high proviral loads relative to the proviral loads in asymptomatic carriers.

Subjects and methods. During the period 1992–2005, a total of 606 HTLV-1–infected individuals in Rio de Janeiro, Brazil, were evaluated for the presence of neurological diseases. HTLV-1–infected individuals had cases that fulfilled international criteria for positive serologic test results (i.e., ELISA and Western blot tests), and all infected individuals were routinely examined at least by 1 of 3 neurologists (A.A., A.C.L., and M.T.T.S.) and subsequently classified as symptomatic or asymptomatic carriers. Symptomatic carriers included individuals with HAM/TSP (determined on the basis of World Health Organization criteria [9]) or with other neurological conditions of undetermined cause that were probably associated with HTLV-1 (i.e., ONAs). HTLV-1–infected individuals with isolated peripheral neuropathy, ALS, isolated mild cognitive deficits, and isolated neurogenic bladder dysfunction were considered to have an ONA. The criteria for each one of these conditions, with the exception of isolated neurogenic bladder dysfunction, have been published elsewhere [6–8]. Patients with urinary incontinence and/or retention, abnormal urodynamic study findings, and normal neurological examination findings were considered to have isolated neurogenic bladder dysfunction; alternative causes of bladder dysfunction were clinically excluded by an experienced urologist.

Ninety-three asymptomatic carriers and 197 patients with HAM/TSP were randomly selected for determination of the HTLV-1 proviral load. Of 21 patients with isolated peripheral neuropathy, 11 were evaluated for proviral load quantification. Likewise, of 77 individuals who previously underwent a full neuropsychological assessment, proviral loads were determined for 45 (18 of whom did not meet HAM/TSP criteria [i.e., nonmyelopathic individuals]). The HTLV-1 proviral load was determined for all patients with ALS ($n = 5$) and patients with isolated neurogenic bladder dysfunction ($n = 8$).

The HTLV-1 proviral load in peripheral blood leucocytes was measured by real-time PCR assay (SmartCycle; Cepheid) using the TaqMan system (Applied Biosystems). Standard curves were

generated by amplification of a β-globin gene fragment from HTLV-1–negative genomic DNA and the HTLV-1 p*X* region (*tax* gene) fragment from a cell line containing a single copy of HTLV-1 provirus (TARL-2) [10]. PCR was performed using 200 ng of DNA with 12.5 μL of TaqMan 2× universal PCR master mix (Applied Biosystems), 15 pmol of p*X* primers, 50 pmol of β-globin primers, and 2.5 pmol of the fluorescent probes, for a total volume of 25 μL. The HTLV-1 proviral load was calculated as follows: number of copies/100 cells $=$ [*tax* copies/(β-globin copies/2)] $\times$ 100. The lower limit of detection for the assay was 1 copy per 10^4 cells. A nonparametric test was used to assess differences in the HTLV-1 proviral load among groups. Informed consent was obtained from every enrolled patient.

Results. The HTLV-1 proviral load was determined in 321 individuals infected with HTLV-1; 93 were asymptomatic carriers, and 228 were symptomatic carriers (197 individuals with HAM/TSP, 11 with isolated peripheral neuropathy, 8 with isolated neurogenic bladder dysfunction, 7 with isolated mild cognitive deficits, and 5 with ALS). The median proviral load was significantly higher in symptomatic carriers than in asymptomatic carriers (table 1). As previously reported [3], there was considerable variance in the proviral loads both in patients with HAM/TSP and in asymptomatic carriers, and the proviral load was much higher in the former group. Patient age had no influence on proviral load.

The proviral loads in patients with ALS and patients with isolated neurogenic bladder dysfunction were markedly elevated (17.7 and 7.9 copies/100 cells, respectively). There was no significant difference between proviral loads observed in

Table 1. Demographic data for persons with symptomatic and asymptomatic human T lymphotropic virus type 1 (HTLV-1) carriage.

Neurological status	No. of subjects	Age, mean years[a]	No. (%) of female subjects	HTLV-1 proviral load, median copies/100 cells (range)	*P*
HTLV-1 positive					
All subjects	606[b]	…	…	…	
Symptomatic carriers	228	52.9	155 (67.9)	6.3 (0–66.4)	<.0001
Asymptomatic carriers	93	50	53 (56)	1.0 (0–13.8)	
HAM/TSP					
Patients with HAM/TSP	197	53	141	6.3 (0–44.6)	<.0001
Asymptomatic HTLV-1 carriers	93	…	…	1.0 (0–13.8)	
ALS					
Patients with ALS	5	58.2	2	17.7 (0.6–66.4)	.005
Asymptomatic HTLV-1 carriers	93	…	…	1 (0–13.8)	
INBD					
Patients with INBD	8	45.2	5	7.9 (0.2–10.4)	.001
Asymptomatic HTLV-1 carriers	93	…	…	1.0 (0–13.8)	
IPN					
Patients with IPN	11	53.7	5	5.1 (0–13.9)	.11
Asymptomatic HTLV-1 carriers	93	…	…	1.0 (0–13.8)	
IMCD					
Patients with IMCD[c]	7	52.3	2	1.0 (0–7)	.122
Asymptomatic HTLV-1 carriers[c]	11	…	…	0.08 (0–2)	
ONA					
Patients with ONA[d]	26	50.9	12	5.3 (0–13.9)	.02
Asymptomatic HTLV-1 carriers	93	…	…	1.0 (0–13.8)	
Condition					
ONA	26	…	…	5.3 (0–13.9)	.048
HAM/TSP	197	…	…	6.3 (0–44.6)	

NOTE. ALS, amyotrophic lateral sclerosis; HAM/TSP, associated myelopathy/tropical spastic paraparesis; IMCD, isolated mild cognitive deficit; INBD, isolated neurogenic bladder dysfunction; IPN, isolated peripheral neuropathy; ONA, other neurological abnormalities (this asymptomatic carrier group is distinct from the other asymptomatic carrier group described in this article).

[a] There were no differences in ages between groups.

[b] Three hundred twenty-one of 606 HTLV-1– infected individuals had HTLV-1 proviral load quantified.

[c] Eighteen of 45 subjects with full neuropsychological assessment and HTLV-1 proviral load quantification (all without HAM/TSP criteria— nonmyelopathic individuals).

[d] Patients with ALS excluded.

patients with HAM/TSP and in patients with ALS ($P = .9$) or between patients with HAM/TSP and those with isolated neurogenic bladder dysfunction ($P = .54$).

Of 77 individuals who had previously undergone a full neuropsychological assessment, 45 had their proviral loads quantified (27 patients with HAM/TSP and 18 nonmyelopathic individuals). As we expected, the median proviral load in patients with HAM/TSP was higher than in nonmyelopathic individuals (4 vs. 0.17 copies/100 cells; $P < .0001$). Among the patients with HAM/TSP, there was no difference in proviral loads between those with and those without cognitive dysfunction. Of the nonmyelopathic group, 7 had abnormal cognitive assessment findings (isolated mild cognitive deficit), and 11 had normal neuropsychological results (true asymptomatic carriers). The proviral load was slightly higher for patients with isolated cognitive deficits than for true asymptomatic carriers (1 vs. 0.08 copies/100 cells, respectively), but this difference was not statistically significant (table 1).

We also quantified the proviral loads of 11 of 21 patients with isolated peripheral neuropathy. Although the median load for this group tended to be higher than for the group of asymptomatic carriers (5.1 copies/100 cells vs. 1 copy/100 cells), the difference was not statistically significant (table 1).

Because the elevated proviral loads observed in patients with ONAs could be merely a result of the high proviral loads observed in the group with ALS (which, in fact, is a myelopathy associated with spinal cord anterior horn cell lesions), we excluded the individuals with ALS in an additional analysis. The analysis confirmed that the difference in HTLV-1 proviral loads between ONA and asymptomatic carriers remained significant (figure 1); the median proviral load for this modified group was significantly higher than that for asymptomatic carriers ($P = .02$) but lower than the median proviral load for patients with HAM/TSP (5 vs. 6.3 copies/100 cells; $P = .048$).

Discussion. In a previous evaluation of a large cohort of HTLV-1–infected individuals, we showed that other neurological abnormalities besides HAM/TSP are associated with infection [5–8]. We now report that the proviral loads for these cases (ONAs) differ from the proviral loads observed for asymptomatic carriers. For patients with ALS and for patients with isolated neurogenic bladder dysfunction, the proviral loads were higher than for asymptomatic carriers and were similar to the proviral loads observed for patients with HAM/TSP. For patients with isolated peripheral neuropathy and patients with isolated mild cognitive deficits, the proviral loads had a tendency to be higher than for asymptomatic carriers, but the variance was not statistically significant (table 1). The possibility remains to be established that individuals with isolated cognitive deficits and individuals with isolated peripheral neuropathy have only a mild, restricted inflammatory response elicited by a proviral load, which is lower than that required for the development of myelopathic abnormalities (i.e., HAM/TSP, ALS, and isolated neurogenic bladder dysfunction).

The observed elevations of HTLV-1 proviral load and the clinical presentations described by us [6] and by Matzusaki et al. [6, 11] suggest that ALS is a neurological manifestation associated with HTLV-1 myelopathy. In relation to isolated neurogenic bladder dysfunction, we hypothesize that an inflammatory reaction restricted to the sacral segments of the spinal cord could be responsible for bladder dysfunction without the pyramidal syndrome typically observed in individuals with HAM/TSP [12]. The high proviral loads observed in patients with isolated neurogenic bladder dysfunction (7.9 copies/100 cells) confirm our impression that this urologic condition is associated with HTLV-1 infection, even in the absence of myelopathy. The possibility that isolated neurogenic bladder dysfunction might progress to a full HAM/TSP presentation suggests that close follow-up of such patients is of the utmost importance.

The factors that determine which cases of HTLV-1 infection will progress from an asymptomatic to a symptomatic state are still unclear. Host genetics, together with viral factors, have been clearly implicated [13]. The former has been shown to be related to cytotoxic T cell responses against a number of viral proteins [14]. Cytotoxic T cells could limit HTLV-1 replication in vivo, and this control seems to be more efficient for asymptomatic carriers, whose results show lower proviral loads than those of patients with HAM/TSP [15]. Recently, a complex equation to calculate the risk of development of HAM/TSP was reported, and it identified 88% of cases of myelopathy in a cohort of Japanese patients [16]. Age, proviral load, infection

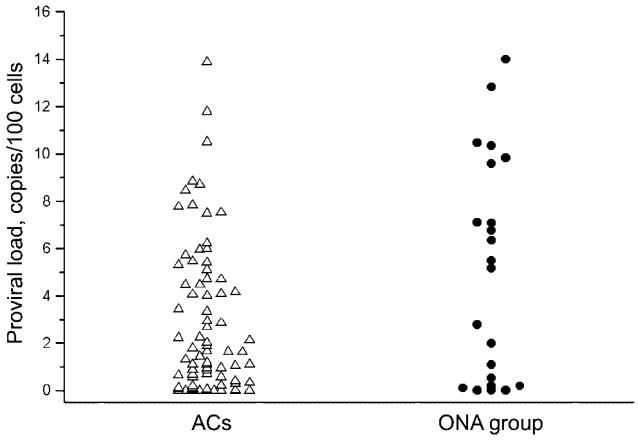

Figure 1. Human T lymphotropic virus type 1 (HTLV-1) proviral load in asymptomatic carriers (ACs) and patients with other neurological abnormalities associated with HTLV-1 (ONAs) ($P = .02$). ONAs include isolated peripheral neuropathy, isolated mild cognitive deficit, and isolated bladder dysfunction.

with HTLV-1 subgroup B, and the presence of certain genetic polymorphisms were considered to be risk factors. The external validity of this mathematical model is weak, however, as a result of the specific genetic background of the Japanese population. Thus, a universal biomarker to predict the risk for the development of HAM/TSP and ONA remains to be identified.

Our findings that ONAs exist in HTLV-1 infection and that both are associated with high proviral loads reinforce their causal relationship. Thus, the speculation of neurological disease in HTLV-1 infection is wider than previously described, and HAM/TSP can be considered to be no more than the tip of the iceberg.

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References

1. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA **1980**; 77:7415–9.
2. Gessain A, Barin F, Vernant JC, et al. Antibodies to human T-lymphotropic virus type-1 in patients with tropical spastic paraparesis. Lancet **1985**; 2:407–10.
3. Nagai M, Usuku K, Matsumoto W, et al. Analysis of HTLV-1 proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. J Neurovirol **1998**; 4:586–93.
4. Osame M, Nakagawa M, Umehara F, et al. Recent studies on the epidemiology, clinical features and pathogenic mechanisms of HTLV-1 associated myelopathy (HAM/TSP) and other diseases associated to HTLV. J Neurovirol **1997**; 3(Suppl 1):S50–1.
5. Leite AC, Mendonca GA, Serpa MJ, Nascimento OJ, Araujo AQ. Neurological manifestations in HTLV-1-infected blood donors. J Neurol Sci **2003**; 214:49–56.
6. Silva MT, Leite AC, Alamy AH, Chimelli L, Andrada-Serpa MJ, Araujo AQ. ALS syndrome in HTLV-1 infection. Neurology **2005**; 65:1332–3.
7. Leite AC, Silva MT, Alamy AH, et al. Peripheral neuropathy in HTLV-1 infected individuals without tropical spastic paraparesis/HTLV-I-associated myelopathy. J Neurol **2004**; 251:877–81.
8. Silva MT, Mattos P, Alfano A, Araujo AQ. Neuropsychological assessment in HTLV-1 infection: a comparative study among TSP/HAM, asymptomatic carriers, and healthy controls. J Neurol Neurosurg Psychiatry **2003**; 74:1085–9.
9. Osame M. Review of WHO Kagoshima meeting and diagnostic guidelines for HAM/TSP. In: Blattner W, ed. Human retrovirology: HTLV. New York: Raven Press, **1990**:191–7.
10. Tateno M, Kondo N, Itoh T, Chubachi T, Togashi T, Yoshiki T. Rat lymphoid cell lines with human T cell leukemia virus production: biological and serological characterization. J Exp Med **1984**; 159: 1105–16.
11. Matsuzaki T, Nakagawa M, Nagai M, et al. HTLV-1-associated myelopathy (HAM)/tropical spastic paraparesis (TSP) with amyotrophic lateral sclerosis-like manifestations. J Neurovirol **2000**; 6:544–8.
12. Araujo A, Silva MT, Leite AC, Coutinho F, Andrada-Serpa MJ. Isolated bladder dysfunction in HTLV-1 infection. Neurology **2006**; 66(Suppl 2):A171.
13. Furukawa Y, Yamashita M, Usuku K, Izumo S, Nakagawa M, Osame M. Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type 1 in the tax gene and their association with different risks for HTLV-1-associated myelopathy/tropical spastic paraparesis. J Infect Dis **2000**; 182:1343–9.
14. Jeffery KJ, Usuku K, Hall SE, et al. HLA alleles determine human T-lymphotropic virus-1 (HTLV-1) proviral load and the risk of HTLV-1-associated myelopathy. Proc Natl Acad Sci USA **1999**; 96:3848–53.
15. Bangham CR. The immune response to HTLV-1. Curr Opin Immunol **2000**; 12:397–402.
16. Vine AM, Witkover AD, Lloyd AL, et al. Polygenic control of human T lymphotropic virus type 1 (HTLV-1) provirus load and the risk of HTLV-1-associated myelopathy/tropical spastic paraparesis. J Infect Dis **2002**; 186:932–9.