Association of Tuberculosis Status with Neurologic Disease and Immune Response in HTLV-1 Infection

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Abstract

The human T cell lymphotropic virus type 1 (HTLV-1) is the etiologic agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1 infected individuals have increased susceptibility to *Mycobacterium tuberculosis* infection but the influence of tuberculosis (TB) on the course of HTLV-1 infection is unknown. The aim of this study was to evaluate the influence of TB on immunological, virologic, and neurologic features of HTLV-1 infection. This is a retrospective analysis of individuals enrolled in a cohort study from an HTLV-1 clinic who were evaluated for past or latent tuberculosis (LTB) and classified clinically as HTLV-1 carriers, probable HAM/TSP and definite HAM/TSP. Spontaneous cytokine production (interferon-gamma [IFN-γ], tumor necrosis factor [TNF], and interleukin[IL]-10), serum chemokines (CXCL9 and CXCL10) and HTLV-1 proviral load were evaluated. Of 172 participants, 64 did not have histories of TB (TB-group), 81 had LTB and 27 had TB in the past (TB+ group). In the TB+ group, there was a higher frequency of HAM/TSP patients (35%) than in HTLV-1 carriers (10%) (OR = 3.8, $p = .0001$). HAM/TSP patients with histories of TB had higher IFN-γ/IL-10 and TNF/IL-10 ratios when compared with HAM/TSP patients without histories of TB. There were no differences in serum chemokine production and proviral load across TB groups stratified on HTLV-1 clinical status. In conclusion, TB may influence the development of HAM/TSP, and patients with these two diseases have an impairment in the modulation of immune response.

Keywords: HTLV-1, tuberculosis, immune response

Introduction

THE HUMAN T CELL lymphotropic virus type 1 (HTLV-1) is the etiologic agent of adult T cell leukemia/lymphoma and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), but the majority of HTLV-1-infected individuals remain as HTLV-1 carriers.[1–3] There is a high prevalence of HTLV-1 infection in Brazil, especially in Salvador, Bahia where the seroprevalence in the general population is 1.83%.[4] Among blood donors, 9/1000 individuals in the state of Bahia are infected with HTLV-1.[5] HTLV-1 predominantly infects CD4+ T cells.[6] Other cells, however, can be infected with the virus, including CD8+ T cells, dendritic cells, macrophages, and B cells.[7–9]

HTLV-1 induces a spontaneous cell proliferation and high production of proinflammatory cytokines, such as interferon-gamma (IFN-γ) and tumor necrosis factor (TNF).[10] Unstimulated peripheral blood mononuclear cells (PBMC) from HAM/TSP patients produce more proinflammatory cytokines than cells from HTLV-1 carriers, but IL-10 and IL-5 are detected in cell cultures of both groups of patients.[10,11] Since IL-10 modulates inflammatory response, IL-10 production could be related to the maintenance of the clinical status of HTLV-1 carriers.[12] This hypothesis was supported by an *in vitro* study that showed that the addition of exogenous IL-10 in cell cultures from HTLV-1 carriers decreased the IFN-γ production, which was not observed in cell cultures from HAM/TSP patients.[13,14] Proinflammatory chemokines

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CXCL9 and CXCL10 are also spontaneously produced by HAM/TSP patients and HTLV-1 carriers. But higher levels of CXCL9 and CXCL10 are detected in serum and cerebrospinal fluid from HAM/TSP patients when compared with HTLV-1 carriers.[13,15,16]

The immunological abnormalities observed in HTLV-1-infected individuals may lead to increased susceptibility to acquiring other infectious diseases such as strongyloidiasis,[17,18] severe scabies,[19] and tuberculosis (TB).[20–22] According to the Ministry of Health from Brazil, the country has the eighteenth highest number of TB cases worldwide. Thus, Brazil is endemic area for both HTLV-1 and *Mycobacterium tuberculosis* infection.

Few studies have focused on HTLV-1 and TB coinfection. Pedral-Sampaio *et al.* demonstrated that there was a high prevalence (8.5%) of HTLV-1 infection in individuals diagnosed with TB; furthermore, HTLV-1 infection was associated with increased risk of *M. tuberculosis* infection and disease severity.[21] In Peru, it was reported that HTLV-1/TB-infected individuals have higher death rates due to TB than TB patients without HTLV-1.[22] Additionally, in a population where HTLV-1 and TB are prevalent, HTLV-1 infection may increase the susceptibility to active TB.[23] Recently, in a study performed in an infectious diseases hospital in Salvador, Bahia, 10% of TB patients were found to be coinfected with HTLV-1.[20] These studies show that there is an association between HTLV-1 and TB, and this association may lead to a worse prognosis in TB patients.[24] However, there are no studies evaluating the potential influence of TB on the clinical status of HTLV-1 infection. In this study, we evaluated the associations between TB status with immunological and virological features in HTLV-1 infected individuals with and without neurological symptoms. We hypothesized that TB influences the clinical status, immune response, and proviral load in HTLV-1 infection.

Materials and Methods

Study design and population

This is a retrospective analysis of HTLV-1-infected individuals from the HTLV-1 Clinic at the Hospital Universitario Professor Edgard Santos, Federal University of Bahia, Salvador, Bahia, Brazil. The diagnosis of HTLV-1 was performed by enzyme-linked immunosorbent assay (ELISA) (Murex Biotech Limited, Dartford, United Kingdom) and confirmed by western blot (HTLV blot 2.4; Genelabs, Singapore). Since 2004, we have conducted a cohort study within our HTLV-1 Clinic. Determination of IFN-γ, TNF, IL-10, CXCL9, and CXCL10 along with HTLV-1 proviral load from HTLV-1-infected individuals is performed at the time of enrollment in the clinic as detailed below. Serum samples from these individuals are collected and stored at −20°C.

The inclusion criteria were male and female patients 18 to 70 years old who had available data on TB status. The exclusion criteria were patients coinfected with HIV, autoimmune diseases, other confounding chronic diseases, and use of corticosteroids.

The clinical status of HTLV-1 infection was classified as follows: (1) HTLV-1 carriers: infected individuals without neurological symptoms, Expanded Disability Status Scale (EDSS) = 0 (zero)[25], and Osame's motor disability score (OMDS) = 0 (zero)[26]; (2) Probable HAM/TSP: HTLV-1-infected individuals who did not fulfill the criteria for HAM/TSP (EDSS ≤2.5 and OMDS = 0) but presented with urgency in the absence of urinary tract infection along with detrusor overactivity in the urodynamic study[27]; (3) HAM/TSP: patients who fulfill criteria for HAM/TSP according to the World Health Organization criteria (EDSS >2.5 and OMDS >0).

For this study, HTLV-1 carriers, probable HAM/TSP and HAM/TSP patients were evaluated for history of *M. tuberculosis* infection. HTLV-1-infected individuals who did not have evidence of active or history of TB and had negative tuberculin skin tests (TST) were classified as TB negative. Patients who had positive TST but no history of TB were classified at the latent TB group (LTB). Most patients from the LTB group had a TST greater than or equal to 10 millimeters (mm), which generally suggests exposure to *M. tuberculosis*. Those patients who had a history of TB with documentation of acid fast bacilli in the sputum were classified as TB+. All TB patients received and were cured after multidrug chemotherapy for TB. Additionally, all of them had a positive TST, and radiologic features of TB were present in 23 (85.1%) of the cases.

Informed consent was obtained from all individuals and the Institutional Review Board of the Federal University of Bahia approved this project.

PBMC culture

Fresh PBMC were isolated from heparinized blood samples by density gradient centrifugation using Ficoll-Hypaque (GE Healthcare Bio-sciences, Uppsala, Sweden). A total of 3×10^6 cells/ml were cultured without stimulation in RPMI 1640 (Life Technologies Gibco BRL, Grand Island, NY), 10% serum fetal bovine (Sigma, St. Louis, MO), glutamine, HEPES, and antibiotics for 72 h at 37°C with 5% CO_2. Supernatant fluids were collected and stored at −20 °C for cytokine assays.

Measurement of cytokines and chemokines

ELISA was used to measure the spontaneous production of IFN-γ, TNF, and IL-10 from supernatant fluids. The serum chemokines CXCL9 and CXCL10 were measured by ELISA. The reagents were purchased from BD Bioscience Pharmingen (San Jose, CA). The results were expressed as pg/ml based on a standard curve.

Determination of HTLV-1 proviral load

DNA extraction was performed as previously described.[13] The HTLV-1 proviral load was quantified using a real-time *Taq*Man polymerase chain reaction method.[28] Amplification and data acquisition were carried out using the ABI Prism 7700 Sequence detector system (Applied Biosystems). The normalized value of the HTLV-1 proviral load was calculated as the ratio of (HTLV-1 DNA average copy number/albumin DNA average copy number) $\times 2 \times 10^6$.[13] The data were expressed as the number of HTLV-1 DNA copies/10^6 mononuclear cells.

Statistical analysis

The Chi-square test was used to compare the gender distribution between the three groups (TB−, LTB, and TB+), the

frequency of HTLV-1 carriers, probable HAM/TSP and HAM/TSP patients with history of TB (TB+ group).

The one-way analysis of variances was used to compare the age distribution. The Kruskal–Wallis test with post-test was used to compare cytokine and chemokine production and HTLV-1 proviral load.

To evaluate the association between TB status and HTLV-1 clinical status and to assess potential confounders, we performed ordinal logistic regression considering the dependent variable of clinical status of HTLV-1 infection (HTLV-1 carrier, probable HAM/TSP and HAM/TSP) as ordinal data. The odds ratios from these models approximated the risk of having a clinical state that is one category higher (i.e., more severe) as a result of the defined increase in a continuous independent variable or a change from ''no'' to ''yes'' in a dichotomous independent variable.

Analyses were done with GraphPad Prism 5 (San Diego, CA) and STATA version 10 (StataCorp, College Station, TX) and a p-value $< .05$ was considered to be statistically significant.

Results

A total of 172 HTLV-1-infected individuals were enrolled into this study. All of them were evaluated for history of TB. Sixty-four subjects were considered as TB negative (negative TST and no history of TB); 81 were LTB (positive TST, no history of TB, and negative chest radiography); and 27 had a history of TB (Table 1). The female/male frequency was 52/12 in the TB- group; 47/34 in the LTB group; and 14/13 in the TB+ group ($p = .004$). Comparing the age distribution of the three groups, the patients with history of TB were older than patients from TB- group (56 ± 10 and 50 ± 12.3 years; $p = .04$) (Table 1). The diagnosis of TB was made before the diagnosis of HAM/TSP, for all but one case. There was a gap of 5.8 ± 8.28 years (mean $\pm$ standard deviation) between the diagnosis of TB and HAM/TSP.

Frequency of HTLV-1 clinical status in TB+ group

Table 1 summarizes the frequency of the clinical forms of HTLV-1 infection by TB status. Using ordinal logistic regres-

sion with HTLV-1 clinical status as an ordinal outcome, a history of TB was associated with increased odds of having HAM/TSP relative to those with no history of TB (OR 3.8; 95% CI 1.9–9.1, $p = .0001$). There was no difference in risk associated with having latent TB relative to no history of TB (OR 0.97; 95% CI 0.50–1.9, $p = .94$). Models incorporating age and gender as potential confounders, neither of which was statistically significant, yielded similar results (data not shown).

Spontaneous cytokine production

Among the HTLV-1 carriers, there was no difference in spontaneous cytokine production when we compared across the three study groups (TB−, LTB, and TB+). The same result was seen regarding probable HAM/TSP and HAM/TSP patients across the three TB groups.

Since IL-10 regulates proinflammatory cytokine production, we evaluated the ratio between IFN-γ and IL-10 (IFN-γ/IL-10) production and the TNF/IL-10 ratio.

Among the HTLV-1 carriers, the median IFN-γ/IL-10 ratio in the TB- group was 39.4 (interquartile range [IQR] 16–419), compared with 33.9 (5–447) in the LTB group and 84 (6–447) in the TB+ group, ($p = .8$) (Fig. 1A). There was no statistical difference between the three groups when we compared the TNF/IL-10 ratios: 8.6 (1–156) for TB−; 8.8 (1–106) for LTB; and, 25.6 (0.6–303) for TB+ group (Fig. 1B).

Among probable HAM/TSP patients, the IFN-γ/IL-10 ratios were 30.9 (13.9–52) in TB− group; 48.7 (1–1285) in LTB group; and 5.0 (1–2264) in TB+ group ($p = .9$) (Fig. 1C). Regarding TNF/IL-10 ratios, there was no difference between the three groups ($p = .2$): 1.0 (0.6–12.7) in TB− group; 26.9 (1–990) in LTB group; and 1.0 (1–1911) in TB+ group (Fig. 1D).

Among the HAM/TSP patients, the IFN-γ/IL-10 ratio tended to increase across the TB status groups: 34.3 (9–328) for TB−; 184 (15–2015) for LTB; and 1537 (53–2685) for TB+. The difference between TB− and TB+ groups was statistically significant ($p = .03$) (Fig. 1E). A similar result was seen at TNF/IL-10 ratio: 4.8 (1–50) for TB−; 92 (0.9–329) for LTB; and 136 (15–1583) for TB+ ($p = .02$ for the comparison between TB− and TB+ groups) (Fig. 1F).

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE HUMAN T CELL LYMPHOTROPIC VIRUS TYPE 1-INFECTED INDIVIDUALS WITHOUT HISTORY OF TUBERCULOSIS (TB−), WITH LATENT TUBERCULOSIS, AND WITH HISTORY OF TUBERCULOSIS (TB+)

	TB status			
	TB–(n = 64)	LTB(n = 81)	TB +(n = 27)	p
Gender (F/M)	52/12	47/34	14/13	.004*
Age [mean (range)]	50 (26–70)	53 (22–70)	56 (32–69)	.04**
HTLV-1 status:				
HTLV-1 carriers (n = 99) %	42	48	10	.0001***
Probable HAM/TSP (n = 36) %	19	70	11	
HAM/TSP (n = 37) %	40.5	24.5	35	

*Chi-square test.
**Difference between TB− and TB+ (ANOVA).
***Nonparametric test for trend (of HTLV-1 status) across TB groups
HAM/TSP = HTLV-1-associated myelopathy/tropical spastic paraparesis. Odds ratio = 3.8; 95% confidence interval 1.9, 9.1 by ordinal logistic regression with HTLV-1 clinical status as the outcome.
ANOVA, analysis of variance; HAM, HTLV-1-associated myelopathy; HTLV-1, human T cell lymphotropic virus type 1; TB, tuberculosis; TSP, tropical spastic paraparesis.
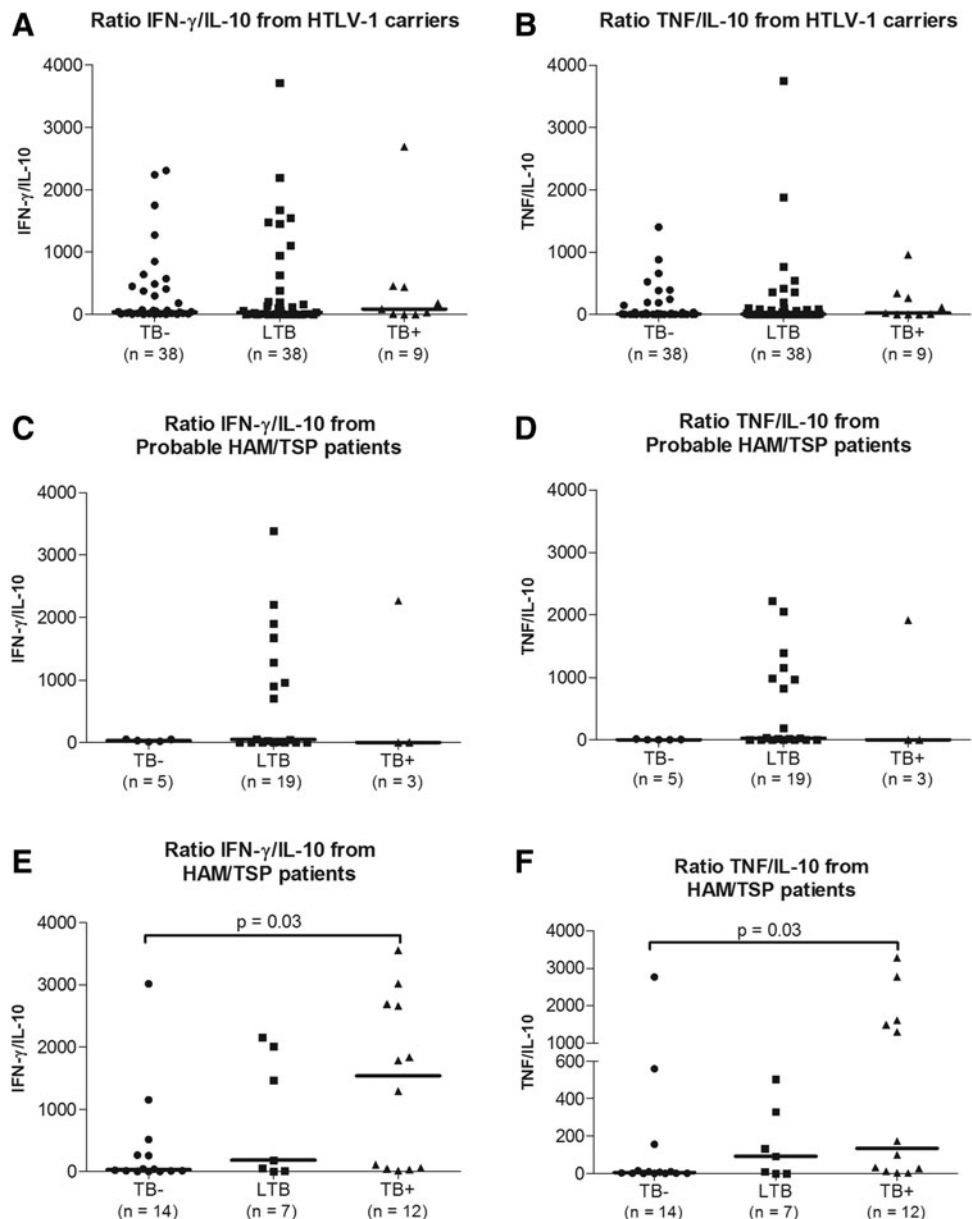
FIG. 1. IFN-γ/IL-10 and TNF/IL-10 ratios from HTLV-1 carriers, probable HAM/TSP and HAM/TSP patients. The cytokine ratios are calculated with values of spontaneous cytokine production from HTLV-1 carriers (**A** and **B**), probable HAM/TSP patients (**C** and **D**) and HAM/TSP patients (**E** and **F**) stratified by TB status: no history of TB (TB− group), LTB, and history of TB (TB+). Statistical analysis is by Kruskal–Wallis test with post-test. HAM, HTLV-1-associated myelopathy; HTLV-1, human T cell lymphotropic virus type 1; IFN-γ, interferon-gamma; LTB, latent TB group; TB, tuberculosis; TNF, tumor necrosis factor-alpha; TSP, tropical spastic paraparesis.

Serum chemokine production

Serum CXCL9 levels (Fig. 2A) and CXCL10 levels (Fig. 2B) from HTLV-1 carriers did not differ across the three groups (TB−, LTB, and TB+). Similar patterns of serum CXCL9 and CXCL10 levels were seen in subjects with HAM/TSP (Fig. 2E, F) stratified by TB status. Among probable HAM/TSP patients, there was no difference in CXCL9 production in those three groups (Fig. 2C). However, probable HAM/TSP patients from the TB− group produced more CXCL10 (499 pg/ml [271–1880]) than the LTB group (225 pg/ml [129–336]) (*p* = .04) (Fig. 2D).

HTLV-1 proviral load

Proviral load from HTLV-1 carriers (Fig. 3A), probable HAM/TSP (Fig. 3B) and HAM/TSP subjects (Fig. 3C) did not differ by TB status.

Discussion

Patients with HAM/TSP have a higher proviral load and higher production of proinflammatory cytokines than HTLV-1 carriers, and proviral load is considered a marker of HAM/TSP. In this study we found a strong association between prior history of TB and the presence of HAM/TSP. Furthermore, our exploratory analysis of spontaneous cytokine production by PBMC showed that HAM/TSP patients with histories of TB had higher TNF/IL-10 and IFN-γ/IL-10 ratios than HAM/TSP patients without TB. This indicates an imbalance between proinflammatory and regulatory cytokines, which may contribute to the development of severe CNS disease in patients with TB coinfected with HTLV-1.

Prior studies suggest that HTLV-1-infected individuals are at high risk of acquiring *M. tuberculosis* infection.[20,22,23,29] Initially, studies in the United States and Japan showed that HTLV-1 carriers had low delayed type hypersensitivity
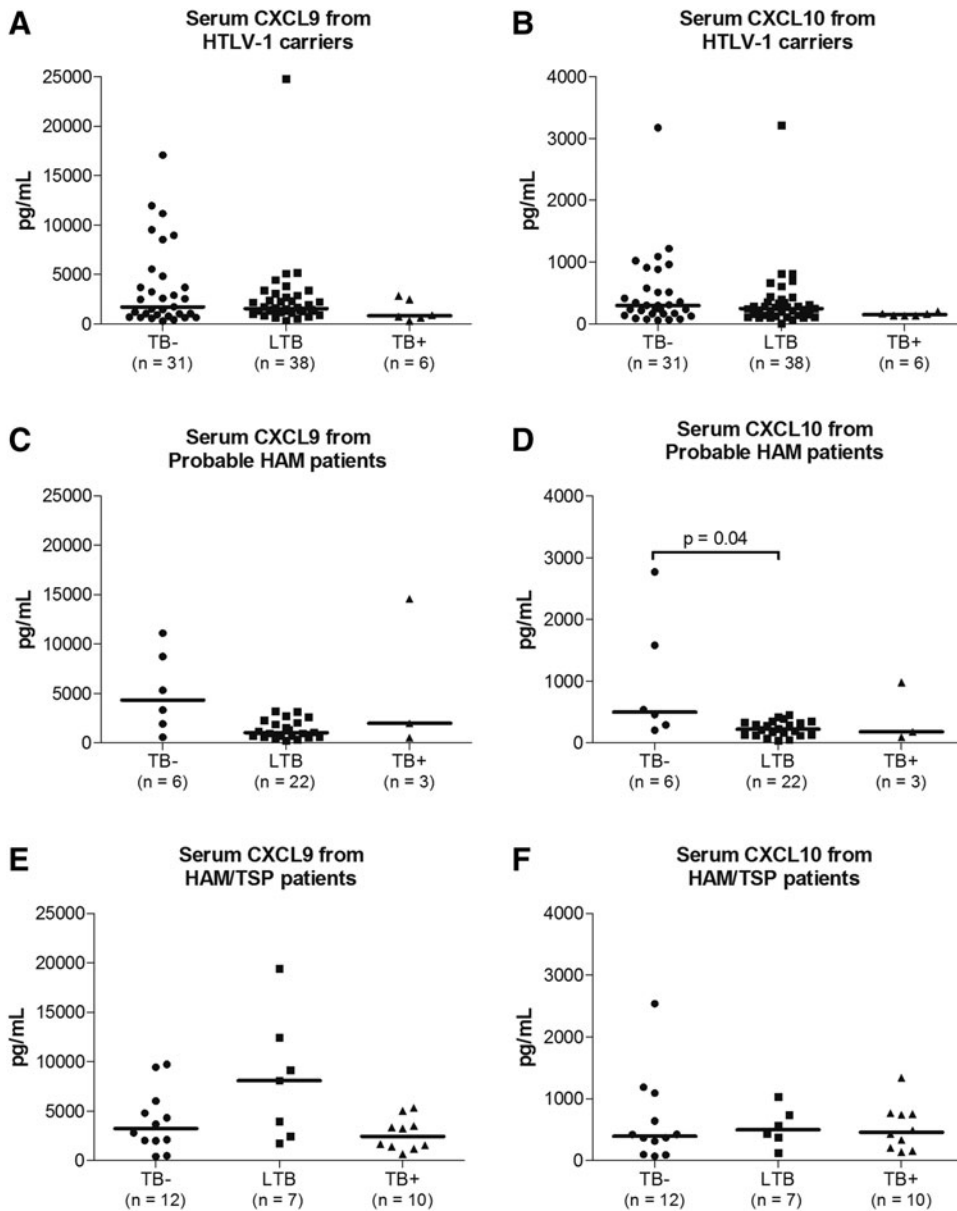
FIG. 2. Serum chemokine production from HTLV-1 carriers, probable HAM/TSP and HAM/TSP patients. The serum CXCL9 and CXCL10 levels were evaluated in HTLV-1 carriers (**A** and **B**), probable HAM/TSP patients (**C** and **D**) and HAM/TSP patients (**E** and **F**) stratified by TB status: TB negative; LTB; and TB+ group. Statistical analysis is by Kruskal–Wallis test with post-test.

response to PPD (purified protein derivative from *M. tuberculosis*) compared with seronegative individuals.[30,31] In addition, an *in vitro* study conducted by Mascarenhas *et al.* demonstrated that mononuclear cells from HTLV-1-infected individuals did not proliferate when stimulated with PPD.[32] These observations raised the possibility that HTLV-1 carriers are at higher risk of acquiring TB. Moreover, coinfection with HTLV-1 and *M. tuberculosis* was associated with higher mortality compared with patients with only TB.[21] Although we have documented the association between TB and HTLV-1 infection in two previous studies, we have not detected any impairment in the response to TST among HTLV-1-infected patients in either study.[20,33] Herein the TST was positive in all patients with histories of TB and also in a large percentage of HTLV-1 without histories of TB. Additionally, we have previously shown that TST is better than interferon-gamma release assay (IGRA) in detecting exposure to *M. tuberculosis* in HTLV-1-infected individuals (unpublished data).

As HTLV-1 infection is associated with upregulation of Th1-type immune responses,[10,34,35] it would be logical that this response would be protective against TB. Nevertheless, the decreased PPD-specific TNF production and high levels of proinflammatory cytokines could lead to greater severity of TB in coinfected patients.[33] In the present study, we evaluated the potential influence of TB on HTLV-1 infection. Since the exacerbated proinflammatory cytokine/chemokine production is also related to the immune response in HAM/TSP patients, *M. tuberculosis* infection might influence the clinical and immunological features of HTLV-1 infection. We observed no difference in spontaneous TNF, IFN-γ, and IL-10 production between the groups of subjects categorized by TB status. If we compare the extreme ends of clinical status of HTLV-1 infection (carriers and HAM/TSP), we observed that, even with high IFN-γ and TNF production in HAM/TSP patients, there was a low IL-10 production in these patients. Among HTLV-1 carriers and probable HAM/TSP patients, IFN-γ/IL-10 and
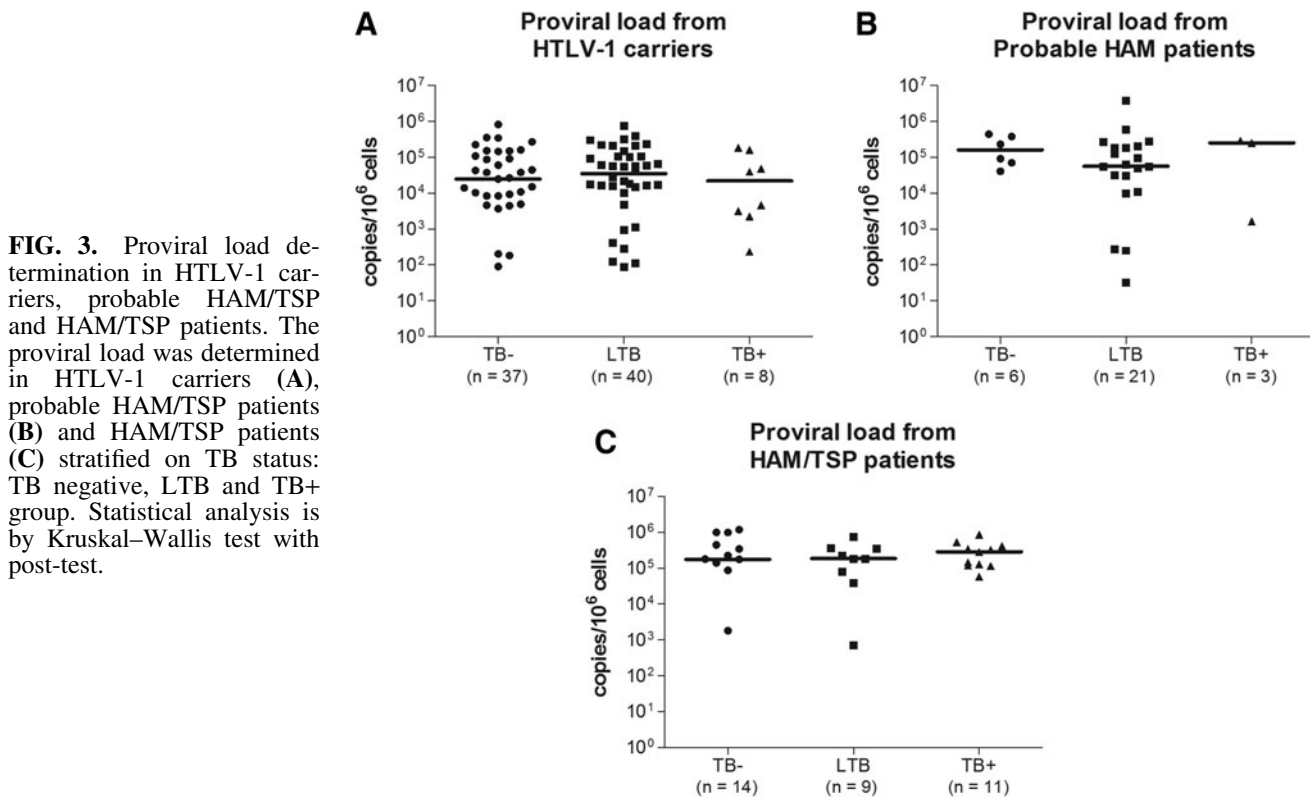
FIG. 3. Proviral load determination in HTLV-1 carriers, probable HAM/TSP and HAM/TSP patients. The proviral load was determined in HTLV-1 carriers **(A)**, probable HAM/TSP patients **(B)** and HAM/TSP patients **(C)** stratified on TB status: TB negative, LTB and TB+ group. Statistical analysis is by Kruskal–Wallis test with post-test.

TNF/IL-10 ratios did not differ by TB status. However, among HAM/TSP patients, these ratios were higher in patients with histories of TB compared with the TB negative group. These results suggest that HAM/TSP patients with histories of TB may have an impairment in the down-modulation of the inflammatory response, which may contribute to tissue damage in the CNS and consequently myelopathy.[2] This lack of modulation has been previously documented, since the exogenous addition of IL-10 in mononuclear cell culture from HAM/TSP patients did not decrease the IFN-γ production.[13,14]

It is known that the chemokines CXCL9 and CXCL10 are found in high levels in cerebrospinal fluid and serum from HAM/TSP patients, and they may be potential biomarkers for the neurological injury seen in these patients.[13,16] In the present study, we evaluated serum concentrations of CXCL9 and CXCL10 in the three groups based on TB status, but we did not find any difference between them with the exception of CXCL10 production in probable HAM/TSP, where individuals with no history of TB produced more CXCL10 than probable HAM/TSP patients with LTB. This latter difference may be due to the outliers observed in the TB-negative group. Thus, TB may have no influence on serum chemokine production in HTLV-1 patients.

The high HTLV-1 proviral load has been considered the main risk factor for developing HAM/TSP in HTLV-1-infected subjects.[36–38] We found no relationship between TB status and proviral load among both HTLV-1 carriers and patients with HAM/TSP. A limitation of our study was not knowing the exact time that each HTLV-1-infected subject acquired HTLV-1 infection. Since most of our patients did not receive blood transfusions, however, we believe that the majority of these patients acquired HTLV-1 through breast-feeding or sexual contact. An additional limitation is that cultures for *M. tuberculosis* were performed in only a few patients. We clearly showed, however, that the diagnosis of TB preceded the diagnosis of HAM/TSP, and there was an association between TB and HAM/TSP. Additional studies that include clinical and radiological evaluations for TB should be done in HTLV-1-infected patients to confirm the results presented here. Moreover, prospective, longitudinal evaluation of HTLV-1-infected subjects with TB would clarify the potential relationship between this exposure and the course of HTLV-1 infection.

In conclusion, our data suggest that history of TB may be a risk factor for development of HAM/TSP. Our observation of a higher IFNγ/IL-10 and TNF/IL-10 ratios in HAM/TSP patients with history of TB suggests that an impairment in the modulation of the inflammatory responses is one of the factors that may contribute to development of HAM/TSP in patients coinfected with TB and HTLV-1.

Acknowledgments

Author Disclosure Statement

The authors declare no conflicts of interest.

References

1. Gessain A, Barin F, Vernant JC, *et al.*: Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985;2:407–410.

2. Osame M, Usuku K, Izumo S, *et al.*: HTLV-I associated myelopathy, a new clinical entity. Lancet 1986;1:1031–1032.

3. 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 U S A 1980;77:7415–7419.

4. Dourado I, Alcantara LC, Barreto ML, da Gloria Teixeira M, Galvao-Castro B: HTLV-I in the general population of Salvador, Brazil: A city with African ethnic and socio-demographic characteristics. J Acquir Immune Defic Syndr 2003;34:527–531.

5. Catalan-Soares B, Carneiro-Proietti AB, Proietti FA: Heterogeneous geographic distribution of human T-cell lymphotropic viruses I and II (HTLV-I/II): Serological screening prevalence rates in blood donors from large urban areas in Brazil. Cad Saude Publica 2005;21:926–931.

6. Richardson JH, Edwards AJ, Cruickshank JK, Rudge P, Dalgleish AG: In vivo cellular tropism of human T-cell leukemia virus type 1. J Virol 1990;64:5682–5687.

7. de Revel T, Mabondzo A, Gras G, *et al.*: In vitro infection of human macrophages with human T-cell leukemia virus type 1. Blood 1993;81:1598–1606.

8. Knight SC, Macatonia SE, Cruickshank K, Rudge P, Patterson S: Dendritic cells in HIV-1 and HTLV-1 infection. Adv Exp Med Biol 1993;329:545–549.

9. Yamano Y, Cohen CJ, Takenouchi N, *et al.*: Increased expression of human T lymphocyte virus type I (HTLV-I) Tax11-19 peptide-human histocompatibility leukocyte antigen A*201 complexes on CD4+ CD25+ T Cells detected by peptide-specific, major histocompatibility complex-restricted antibodies in patients with HTLV-I-associated neurologic disease. J Exp Med 2004;199:1367–1377.

10. Carvalho EM, Bacellar O, Porto AF, Braga S, Galvao-Castro B, Neva F: Cytokine profile and immunomodulation in asymptomatic human T-lymphotropic virus type 1-infected blood donors. J Acquir Immune Defic Syndr 2001;27:1–6.

11. Santos SB, Porto AF, Muniz AL, *et al.*: Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers. BMC Infect Dis 2004;4:7.

12. Brito-Melo GE, Peruhype-Magalhaes V, Teixeira-Carvalho A, *et al.*: IL-10 produced by CD4+ and CD8+ T cells emerge as a putative immunoregulatory mechanism to counterbalance the monocyte-derived TNF-alpha and guarantee asymptomatic clinical status during chronic HTLV-I infection. Clin Exp Immunol 2007;147:35–44.

13. Santos SB, Oliveira P, Luna T, *et al.*: Immunological and viral features in patients with overactive bladder associated with human T-cell lymphotropic virus type 1 infection. J Med Virol 2012;84:1809–1817.

14. Santos SB, Porto AF, Muniz AL, *et al.*: Modulation of T cell responses in HTLV-1 carriers and in patients with myelopathy associated with HTLV-1. Neuroimmunomodulation 2006;13:145–151.

15. Guerreiro JB, Santos SB, Morgan DJ, *et al.*: Levels of serum chemokines discriminate clinical myelopathy associated with human T lymphotropic virus type 1 (HTLV-1)/tropical

spastic paraparesis (HAM/TSP) disease from HTLV-1 carrier state. Clin Exp Immunol 2006;145:296–301.

16. Narikawa K, Fujihara K, Misu T, *et al.*: CSF-chemokines in HTLV-I-associated myelopathy: CXCL10 up-regulation and therapeutic effect of interferon-alpha. J Neuroimmunol 2005;159:177–182.

17. Gotuzzo E, Terashima A, Alvarez H, *et al.*: Strongyloides stercoralis hyperinfection associated with human T cell lymphotropic virus type-1 infection in Peru. Am J Trop Med Hyg 1999;60:146–149.

18. Porto MA, Alcantara LM, Leal M, Castro N, Carvalho EM: Atypical clinical presentation of strongyloidiasis in a patient co-infected with human T cell lymphotrophic virus type I. Am J Trop Med Hyg 2005;72:124–125.

19. Brites C, Weyll M, Pedroso C, Badaro R: Severe and Norwegian scabies are strongly associated with retroviral (HIV-1/HTLV-1) infection in Bahia, Brazil. Aids 2002;16:1292–1293.

20. de Lourdes Bastos M, Osterbauer B, Mesquita DL, *et al.*: Prevalence of human T-cell lymphotropic virus type 1 infection in hospitalized patients with tuberculosis. Int J Tuberc Lung Dis 2009;13:1519–1523.

21. Pedral-Sampaio DB, Martins Netto E, Pedrosa C, Brites C, Duarte M, Harrington W Jr.: Co-infection of tuberculosis and HIV/HTLV retroviruses: Frequency and prognosis among patients admitted in a Brazilian hospital. Braz J Infect Dis 1997;1:31–35.

22. Verdonck K, Gonzalez E, Henostroza G, *et al.*: HTLV-1 infection is frequent among out-patients with pulmonary tuberculosis in northern Lima, Peru. Int J Tuberc Lung Dis 2007;11:1066–1072.

23. Verdonck K, Gonzalez E, Schrooten W, Vanham G, Gotuzzo E: HTLV-1 infection is associated with a history of active tuberculosis among family members of HTLV-1-infected patients in Peru. Epidemiol Infect 2008;136:1076–1083.

24. Norrgren H, Bamba S, Da Silva ZJ, Koivula T, Andersson S: Higher mortality in HIV-2/HTLV-1 co-infected patients with pulmonary tuberculosis in Guinea-Bissau, West Africa, compared to HIV-2-positive HTLV-1-negative patients. Int J Infect Dis 2010;14 Suppl 3:e142–e147.

25. Kurtzke JF: Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology 1983;33:1444–1452.

26. Nakagawa M, Nakahara K, Maruyama Y, *et al.*: Therapeutic trials in 200 patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. J Neurovirol 1996;2:345–355.

27. De Castro-Costa CM, Araujo AQ, Barreto MM, *et al.*: Proposal for diagnostic criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). AIDS Res Hum Retroviruses 2006;22:931–935.

28. Dehee A, Cesaire R, Desire N, *et al.*: Quantitation of HTLV-I proviral load by a TaqMan real-time PCR assay. J Virol Methods 2002;102:37–51.

29. Marinho J, Galvao-Castro B, Rodrigues LC, Barreto ML: Increased risk of tuberculosis with human T-lymphotropic virus-1 infection: A case-control study. J Acquir Immune Defic Syndr 2005;40:625–628.

30. Tachibana N, Okayama A, Ishizaki J, *et al.*: Suppression of tuberculin skin reaction in healthy HTLV-I carriers from Japan. Int J Cancer 1988;42:829–831.

31. Welles SL, Tachibana N, Okayama A, *et al.*: Decreased reactivity to PPD among HTLV-I carriers in relation to virus and hematologic status. Int J Cancer 1994;56:337–340.

32. Mascarenhas RE, Brodskyn C, Barbosa G, *et al.*: Peripheral blood mononuclear cells from individuals infected with human T-cell lymphotropic virus type 1 have a reduced capacity to respond to recall antigens. Clin Vaccine Immunol 2006;13:547–552.

33. Bastos MD, Santos S, Souza A, *et al.*: Influence of HTLV-1 on the clinical, microbiologic and immunologic presentation of tuberculosis. BMC Infect Dis 2012;12:199.

34. Jacobson S: Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. J Infect Dis 2002;186 Suppl 2:S187–S192.

35. Jacobson S, Zaninovic V, Mora C, *et al.*: Immunological findings in neurological diseases associated with antibodies to HTLV-I: Activated lymphocytes in tropical spastic paraparesis. Ann Neurol 1988;23 Suppl:S196–S200.

36. Furtado Mdos S, Andrade RG, Romanelli LC, *et al.*: Monitoring the HTLV-1 proviral load in the peripheral blood of asymptomatic carriers and patients with HTLV-associated myelopathy/tropical spastic paraparesis from a Brazilian cohort: ROC curve analysis to establish the threshold for risk disease. J Med Virol 2012;84:664–671.

37. Matsuzaki T, Nakagawa M, Nagai M, *et al.*: HTLV-I proviral load correlates with progression of motor disability in HAM/TSP: Analysis of 239 HAM/TSP patients including 64 patients followed up for 10 years. J Neurovirol 2001;7:228–234.

38. Nagai M, Usuku K, Matsumoto W, *et al.*: Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: High proviral load strongly predisposes to HAM/TSP. J Neurovirol 1998;4:586–593.

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