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Immunological and Viral Features in Patients With Overactive Bladder Associated With Human T-Cell Lymphotropic Virus Type 1 Infection

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The majority of patients infected with human T-cell lymphotropic virus-type 1 (HTLV-1) are considered carriers, but a high frequency of urinary symptoms of overactive bladder, common in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) have been documented in these patients. The aim of this study was to determine if immunological and viral factors that are seen in HAM/TSP are also observed in these patients. Participants were classified as HTLV-1 carriers (n = 45), HTLV-1 patients suffering from overactive bladder (n = 45) and HAM/TSP (n = 45). Cells from HTLV-1 overactive bladder patients produced spontaneously more proinflammatory cytokines than carriers. TNF-α and IL-17 levels were similar in HAM/TSP and HTLV-1 overactive bladder patients. High proviral load was found in patients with overactive bladder and HAM/TSP and correlated with proinflammatory cytokines. In contrast with findings in patients with HAM/TSP, serum levels of Th1 chemokines were similar in HTLV-1 overactive bladder and carriers. Exogenous addition of regulatory cytokines decreased spontaneous IFN-γ production in cell cultures from HTLV-1 overactive bladder patients. The results show that HTLV-1 overactive bladder and HAM/TSP patients have in common some immunological features as well as similar proviral load profile. The data show that HTLV-1 overactive bladder patients are still able to down regulate their inflammatory immune response. In addition, these patients express levels of chemokines similar to carriers, which may explain why they have yet to develop the same degree of spinal cord damage as seen in patients with HAM/TSP. These patients present symptoms of overactive bladder, which may be an early sign of HAM/TSP. ***J. Med. Virol. 84:1809–1817, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: HTLV-1; immune response; cytokines; chemokines; proviral load

INTRODUCTION

Human T-cell lymphotropic virus type 1 (HTLV-1) is a complex retrovirus belonging to the *Deltaretrovirus* family. It is associated etiologically with adult T cell leukemia/lymphoma (ATLL) and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) [Uchiyama, 1997]. The HTLV-1 infection has been considered an infection with low morbidity. However, a cross-sectional study showed a higher frequency of neurological symptoms, erectile dysfunction, and urinary disturbances in HTLV-1 carriers than in uninfected healthy controls, suggesting that the spectrum of disease may be broader [Caskey et al., 2007].

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Urinary symptoms of overactive bladder can be documented in virtually all patients with HAM/TSP and in up to 25% of HTLV-1 carriers [Castro et al., 2007; Oliveira et al., 2010]. Urinary complaints may be the first symptoms of HAM/TSP, being documented up to 10 years prior to the appearance of the myelopathy [Araujo et al., 1998; Castro et al., 2007; Oliveira et al., 2007]. Urodynamic studies in HTLV-1 infected patients suffering from overactive bladder show similar abnormalities to those with HAM/TSP, including detrusor dyskinesia and detrusor overactivity, supporting the argument that signs and symptoms in patients with overactive bladder are due to HTLV-1 [Oliveira et al., 2010]. In addition, studies have shown that urinary symptoms in these patients are not associated with urinary infection [Rocha et al., 2007]. Indeed, patients who do not fulfill the criteria for HAM/TSP but complain of these urinary symptoms have been classified as probable HAM/TSP [De Castro-Costa et al., 2006]. Although in a population based study, overactive bladder was found to be a common disease [Tikkinen et al., 2007], the higher frequency of overactive bladder in HTLV-1 infected patients when compared to uninfected, healthy controls suggests that central nervous system (CNS) involvement by the virus may lead to overactive bladder [Caskey et al., 2007; Oliveira et al., 2010].

HTLV-1 infects predominantly T cells leading to lymphoproliferation and overproduction of proinflammatory cytokines [Hollsberg, 1999; Nagai and Osame, 2003]. Compared with HTLV-1 carriers, patients with HAM/TSP display high proviral load [Nagai et al., 1998; Olindo et al., 2005; Primo et al., 2009; Grassi et al., 2011] and an increased number of Tax specific CD8+ T cells [Nagai et al., 2001]. In addition, an elevated level of proinflammatory chemokines (CXCL9 and CXCL10) is found in the serum and cerebrospinal fluid (CSF) of these patients [Narikawa et al., 2005; Guerreiro et al., 2006]. HAM/TSP patients also display high levels of proinflammatory cytokines such as IFN-γ and TNF-α [Santos et al., 2004]. Furthermore, mononuclear cells extracted from these patients are incapable of being modulated by regulatory cytokines and anti-cytokines [Santos et al., 2006]. These immunological abnormalities are considered to play, in conjunction with proviral load, a pivotal role in CNS damage and the development of HAM/TSP. It is interesting that, at least in regards to the overproduction of proinflammatory cytokines, 40% of HTLV-1 carriers present a similar pattern of immunological responses to those observed in HAM/TSP [Santos et al., 2004]. Additionally, in a case series study, patients with overactive bladder associated with HTLV-1 infection had higher proviral load than HTLV-1 carriers [Silva et al., 2007].

Although CNS damage is considered to be the cause of overactive bladder in these patients, very little is known about the pathogenesis of the disease. In the present study, the immune response and proviral load of HTLV-1 infected patients with overactive bladder were characterized. The results were compared with those observed in HTLV-1 carriers and HAM/TSP patients. Our data showed an overlap of the immunological abnormalities and proviral load in HTLV-1 infected patients with overactive bladder and patients with frank HAM/TSP.

MATERIALS AND METHODS

Study Design and Patients

The present study is a cross-sectional study comparing immunological response and proviral load in groups of patients infected with HTLV-1 that are enrolled in a cohort group. The study was initiated in 2004 in the HTLV-1 clinic of the Complexo Hospitalar Universitário Professor Edgard Santos, Salvador, Brazil. All HTLV-1 infected patients enrolled in the study were referred from two blood banks. All patients had serology for HIV, hepatitis B, hepatitis C viruses, and syphilis conducted by the blood bank prior to their enrolment in the study. The diagnosis of HTLV-1 was made as described previously [Santos et al., 2004]. At the time of enrolment and during follow-up at the clinic, patients underwent three stool sample examinations for parasites (*Strongyloides stercoralis* and *Schistosoma mansoni*), answered an interviewer-administered questionnaire with specific questions about urinary symptoms, and had a complete clinical and neurological examination. Neurological and motor dysfunctions were measured by two scales: Expanded Disability Status Scale [Kurtzke, 1983] and Osame's Motor Disability Score [Izumo et al., 1996]. The HTLV-1 cohort is followed yearly and clinical and immunological studies were carried out at the entry point and in years of follow-up visits. A total of 135 patients were recruited from the HTLV-1 cohort and included in the study. The clinical status (HTLV-1 carriers, HTLV-1 overactive bladder and HAM/TSP) of the patients within the cohort was determined by neurologists and urologists. From these clinical groups, the samples were randomly selected. Written informed consent was obtained from all participants, and the Ethics Committee of the hospital approved the study.

Case Definitions

HTLV-1 carriers. Asymptomatic patients with Expanded Disability Status Scale = 0 and Osame's Motor Disability Score = 0. *HTLV-1 infected patients with overactive bladder*: HTLV-1 infected patients with symptoms of overactive bladder who did not fulfill the criteria for HAM/STP (Expanded Disability Status Scale $\leq$2.5 and Osame's Motor Disability Score = 0). These patients also had urgency in the absence of urinary tract infection and urodynamic studies demonstrating detrusor overactivity. Overactive bladder was defined by the criteria set by the International Continence Society as urinary urgency, with or without urgency incontinence, usually with

urinary frequency and nocturia in the absence of urinary tract infection or other obvious pathology [Abrams et al., 2002; Abrams et al., 2009]. In addition to urgency, the majority of the patients had urge-incontinence and nocturia with a nighttime frequency greater than two times per night. *HAM/TSP*: Patients who fulfill the criteria of HAM/TSP according to the WHO guidelines (Expanded Disability Status Scale $\geq$2.5 and Osame's Motor Disability Score $\geq$1).

Cell Culture

Fresh peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples by density gradient centrifugation with Ficoll-Hypaque (GE Healthcare Bio-Sciences, Uppsala, Sweden). The cells were cultured in RPMI 1640 (Life Technologies Gibco BRL, Grand Island, NY), 10% human AB serum (Sigma, St. Louis, MO), glutamine, HEPES, and antibiotics (complete RPMI). A total of 3×10^6 cells were kept unstimulated and in some experiments exogenous recombinant interleukin 10 (IL-10, 100 ng/ml, DNAX Institute, Palo Alto, CA) or transforming growth factor beta (TGF-β, 50 μg/ml, R&D System, Minneapolis, MN) were added to the samples. Cell cultures were incubated at 37°C with 5% CO_2 and 95% air for 72 hr. Supernatant fluid was collected and stored at −20°C for cytokine assays.

Measurement of Cytokines by ELISA

ELISA used to measure IFN-γ and TNF-α was performed with reagents purchased from BD Biosciense Pharmingen, San Jose, CA and used according to the manufacturer's recommendations as previously detailed [Santos et al., 2004]. All samples were tested in duplicates. The detection limit was 30 pg/ml for all cytokines. The results were expressed as picograms per milliliter based on a standard curve generated with the GraphPrism software (GraphPrism Soft Inc., San Diego, CA).

Semi-Quantitative Reverse Transcriptase-Polymerase Chain Reaction (PCR) to IL-17

Total RNA isolation from ex vivo PBMCs was performed utilizing Trizol LS reagent (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using 3 μg of total RNA and reverse-transcribed by M-MLV reverse transcriptase. PCR was performed in the samples with a final volume of 50 μl containing 2.0 mM of MgCl2, 0.2 mM of deoxyribonucleoside triphosphate (dNTP) mix (dATP, dCTP, dTTP, dGTP), 10X PCR buffer, 2.5 units of the *Taq*DNA polymerase recombinant (Invitrogen, Carlsbad, CA) and specific primers at 25–50 pmol using the Veriti Thermal Cycler (Applied Biosystems, Foster City, CA). The human primer sequences were: HPRT forward: GCGTCGTGATTAGTGATGATGAAC and HPRT reverse: GGATTA TACTGCCTGACCAAGG; IL-17 forward: GGAACAGAGAGTTAGACTTGC TG; IL-17 reverse: CTCATCCTTCAAAGACAGCCTCA. Thermocycling conditions included 30 cycles of 1 min at 95°C for denaturation, 1 min 30 sec at 60°C for annealing and extension for 1 min 30 sec at 72°C, plus a final extension step of 10 min at 72°C. The amplification products of PCR were visualized in 1.3% agarose gel staining with 0.2% of ethidium bromide (Sigma). The band intensity was calculated using Quantity One Software 4.6.3 (Basic) (BioRad, Hercules, CA). Results were expressed as relative units (RU) corrected for hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression.

Measurement of Serum Chemokines

Reagents used to measure the chemokines CXCL9 and CXCL10 in serum by ELISA were purchased from BD Biosciences (BD OptEIA™human MIG and IP-10 ELISA Set,San Diego, CA) and used according to the manufacturer's recommendations and performed in duplicates. The detection limit was 30 pg/ml for both chemokines. Optical density was measured with a 450 nm filter and the concentration was determined using a standard curve developed with the GraphPrism 5 program software.

DNA Extraction and HTLV-1 Proviral Load

DNA was extracted from 10^6 cells using proteinase K and salting-out method. The HTLV-1 proviral load was quantified using a real-time *Taq*Man PCR method [Dehee et al., 2002]. Albumin DNA was quantified in parallel to determine the input cell number and was used as an endogenous reference. Amplification and data acquisition were carried out using the ABI Prism 7700 Sequence detector system (Applied Biosystems). Standard curves were generated using a 10-fold serial dilution of a double-stranded plasmid (pcHTLV-ALB). The HTLV-1-infected human lymphocyte line MT2 was used as a control for quantification. All standard dilutions and control and individual samples were run in duplicate for both HTLV-1 and albumin DNA quantification. 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) $\times 2 \times 10^6$ and expressed as the number of HTLV-1 copies per 10^6 PBMCs.

Statistical Analysis

Data was expressed as median and interquartile (IQ) range in all figures. Categorical variables were analyzed using parametric tests (Chi-square test). Nonparametric tests (Kruska–Wallis test with post-test and Wilcoxon matched-pairs signed-ranks test) were used to compare continuous variables. Correlations between two variables were examined by Spearman rank correlation analysis. GraphPad Prism 5 (San Diego, CA) carried out the statistical evaluation and P-values < 0.05 were considered to indicate a significant difference.

RESULTS

A total of 135 HTLV-1 infected patients recruited from the HTLV-1 cohort were included in this study. The sample consisted of 45 HTLV-1 carriers (range, 22–66 years old; median 49 year old); 45 HTLV-1 overactive bladder patients (range, 20–66 years old; median 51 year old); and 45 HAM/TSP patients (range, 28–70 years old; median 53 year old). The groups did not differ in regards to the frequency of female (57.8, 66.7 and 64.4%, respectively), $P = 0.66$ (Chi-square test). Helminthes co-infections (*Strongyloides stercoralis* and/or *Schistosoma mansoni*) were detected in 8.9% of HTLV-1 carriers, 11.1% of HTLV-1 overactive bladder, and 2.2% in HAM/TSP patients. All the patients include in this study were seronegative for HIV infection and one HTLV-1 carrier was co-infected with the hepatitis C virus. There was no co-infection with the hepatitis B virus in the study population (Table I).

Profile of Proinflammatory Cytokines Produced by PBMCs From HTLV-1 Carriers, HTLV-1 Associated Overactive Bladder and HAM/TSP Patients

Figure 1 shows the levels of spontaneous cytokines produced by PBMCs from HTLV-1 infected patients. IFN-γ levels produced by cells from HTLV-1 overactive bladder patients and HAM/TSP were determined in 45 patients in each study group. INF-γ levels in HAM/TSP, overactive bladder patients and carriers are shown in Figure 1A. The HAM/TSP group had higher IFN-γ levels (median 2,683, IQ range 1,859–3,355 pg/ml) than HTLV-1 overactive bladder patients (median 1,814, IQ range 772–2,593 pg/ml) and HTLV-1 carriers (median 384, IQ range 14–979 pg/ml), $P < 0.01$ and $P < 0.001$, respectively; Kruskal–Wallis test. HTLV-1 carriers have significantly lower IFN-γ levels than HTLV-1 overactive bladder patients ($P < 0.001$). The levels of TNF-α in HTLV-1 carriers (median 324, IQ range 128–664 pg/ml) were lower than that observed in HTLV-1 overactive bladder patients (median 947, IQ range 411–1,614 pg/ml; $P < 0.001$) and HAM/TSP (median 1,579, IQ range 727–2,778 pg/ml, $P < 0.001$). There was no difference in TNF-α levels between HTLV-1 overactive bladder and HAM/TSP patients ($P > 0.05$) (Fig. 1B).

RNA Expression to IL-17 in Cells From HTLV-1 Infected Patients

Expression of IL-17 was evaluated in ex vivo PBMCs of 12 HTLV-1 carriers, 16 HTLV-1 overactive bladder patients, and in 14 HAM/TSP patients (Fig. 2). The cells from HTLV-1 infected patients showed an increased expression of IL-17 messenger RNA (mRNA) in HTLV-1 overactive bladder (median 40, IQ range 18–53 RU) and HAM/TSP patients (median 49, IQ range 19–65 RU) when compared with HTLV-1 carriers (median 10, IQ range 5–18 RU). While a significant difference in the IL-17 mRNA expression was observed between HTLV-1 carriers and HTLV-1 overactive bladder ($P < 0.001$, Kruskal–Wallis test), no difference was observed between HTLV-1 overactive bladder and HAM/TSP patients ($P > 0.05$).

HTLV-1 Proviral Load in PBMCs From HTLV-1 Infected Patients

The proviral load was determined from cells of 37 HTLV-1 carriers, 39 patients with overactive bladder and 36 HAM/TSP patients. The proviral load from HTLV-1 carriers differed significantly from that observed in HTLV-1 overactive bladder and HAM/TSP patients (Fig. 3). The proviral load in HTLV-1 carriers (median 12,472, IQ range 1,161–54,160 copies/10^6 cells) was lower than that observed in HTLV-1 overactive bladder (median 50,439, IQ range 15,975–148,089 copies/10^6 cells, $P < 0.01$, Kruskal–Wallis test) and in HAM/TSP patients (median 117,594, IQ range 33,201–215,620 copies/10^6 cells, $P < 0.001$). No difference between proviral load in cells from HTLV-1 overactive bladder and HAM/TSP ($P > 0.05$) was observed.

Correlation of HTLV-1 Proviral Load and Proinflammatory Cytokines in HTLV-1 Infected Patients

To evaluate the relationship between proviral load and the modifications in immunological parameters,

TABLE I. Clinical and Demographic Characteristics of HTLV-1 Infected Patients (HTLV-1 Carriers, HTLV-1 Associated Overactive Bladder and HAM/TSP Patients)

	HTLV-1 infected patients			
	HTLV-1 Carriers (n = 45)	HTLV-1 Overactive Bladder (n = 45)	HAM/TSP (n = 45)	*P*-value
Age, median (range)	49 (22–66)	51 (20–66)	53 (28–70)	0.47[a]
Female, n (%)	26 (57.8)	30 (66.7)	29 (64.4)	0.66[b]
Helminthes infection[c], n (%)	4 (8.9)	5 (11.1)	1 (2.2)	—
Hepatitis infection[d], n (%)	1 (2.2)	0	0	—

[a]Kruskal–Wallis test with the Dunn multiple comparisons. The levels of significance was set at $P < 0.05$.
[b]Chi-square test.
[c]Helminthes infection refers to co-infection with *Strongyloides stercoralis* and/or *Schistosoma mansoni*.
[d]Hepatitis infection refers to co-infection with C virus.

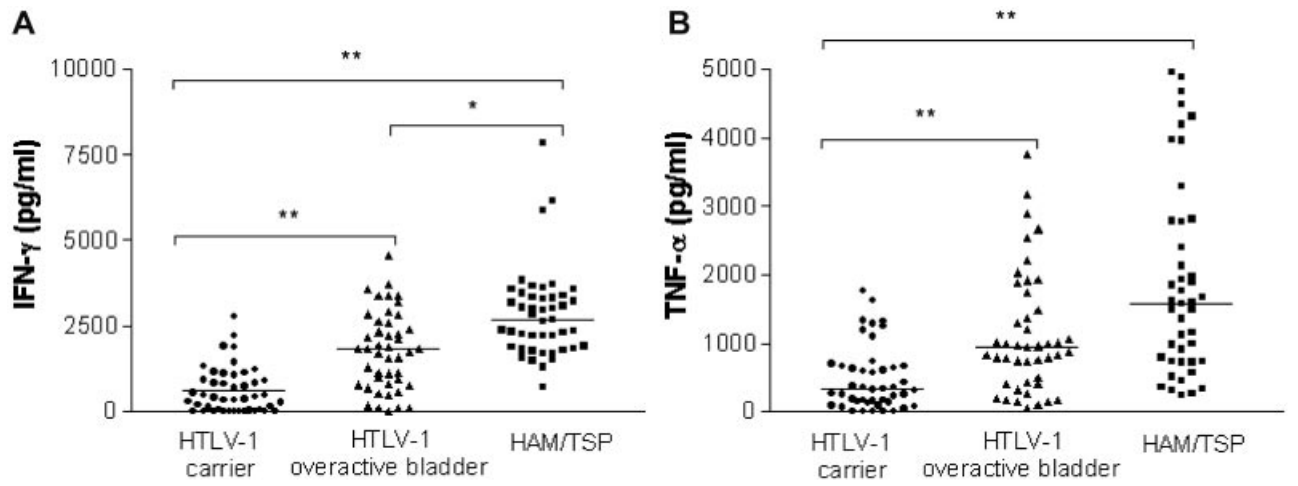

Fig. 1. Levels of spontaneous IFN-γ (**A**) and TNF-α (**B**) produced by PBMCs from HTLV-1 carrier (n = 45), HTLV-1 associated overactive bladder, n = 45, and HAM/TSP (n = 45). Cytokines data represent the values in unstimulated cultures. The levels of cytokines were measured by ELISA and expressed as pg/ml. Statistical analysis was performed by Kruskal–Wallis test ($^{*}P < 0.01$; $^{**}P < 0.001$).

the values of proviral load were correlated with the levels of proinflammatory cytokines in an analysis combining the three groups of HTLV-1 infected patients. Figure 4A shows a weak, although significant, positive correlation between HTLV-1 proviral load and spontaneous IFN-γ production (r = 0.43, 95% confidence interval: 0.257 to 0.5712, $P < 0.0001$, Spearman's rank correlation). The same weak, but significant positive correlation (r = 0.37, 95% confidence interval: 0.19 to 0.52, $P < 0.0001$) was observed between proviral load and spontaneous TNF-α production (Fig. 4B).

Levels of Th1 Chemokines in Serum of Different Groups of HTLV-1 Infected Patients

Levels of serum chemokines CXCL9 and CXCL-10 were determined in 43 HTLV-1 carriers, 39 HTLV-1 infected patients with overactive bladder, and in 32 patients with HAM/TSP. CXCL9 levels were significantly higher in HAM/TSP (median 2,386, IQ range 1,813–3,478 pg/ml) than in both HTLV-1 overactive bladder patients (median 816, IQ range 579–1,414 pg/ml) and HTLV-1 carriers (median 868, IQ range 620–1,398 pg/ml, $P < 0.001$, Kruskal–Wallis test). There

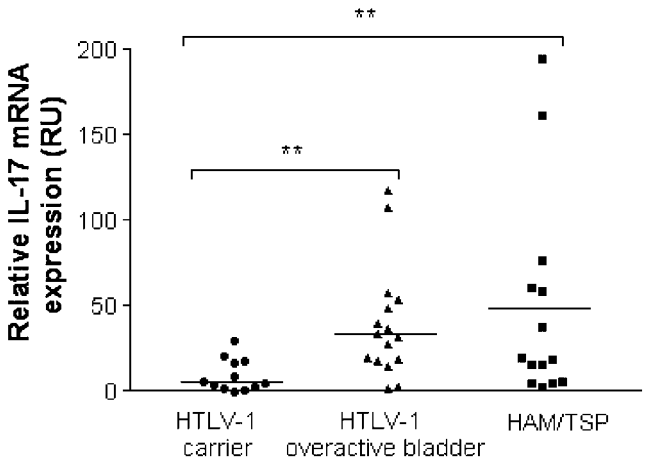

Fig. 2. Semi-quantitative reverse transcriptase polymerase chain reaction (PCR) to detect IL-17 in ex vivo cells from HTLV-1 carrier (n = 12), HTLV-1 associated overactive bladder (n = 16), and HAM/TSP (n = 14). Relative quantification of mRNA IL-17 was performed using the comparative threshold cycle method using HPRT as an endogenous control and the results were expressed as relative units (RU). Statistical analysis was performed by Kruskal–Wallis test ($^{**}P < 0.001$).

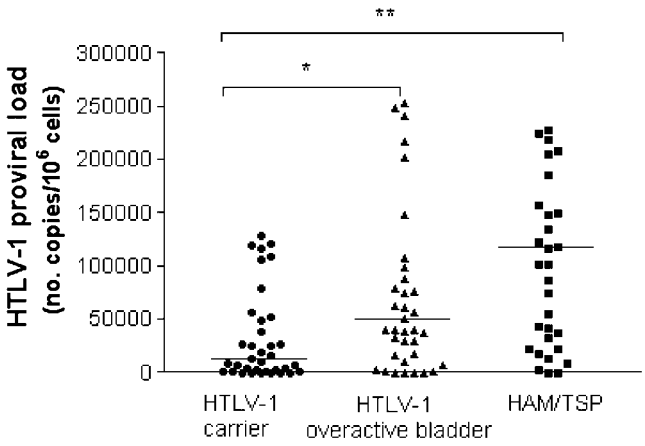

Fig. 3. HTLV-1 proviral load in PBMCs from HTLV-1 carriers (n = 37), HTLV-1 associated overactive bladder (n = 39), and HAM/TSP (n = 36). Proviral load was quantified by real-time TaqMan PCR method and the normalized value of the proviral load was calculated as the ratio of (HTLV-1 DNA average copy number/albumin DNA average copy number) × 2 × 10^6 and expressed as the number of HTLV-1 copies per 10^6 PBMCs. Statistical analysis was performed by Kruskal–Wallis test ($^{*}P < 0.01$;$^{**}P < 0.001$).

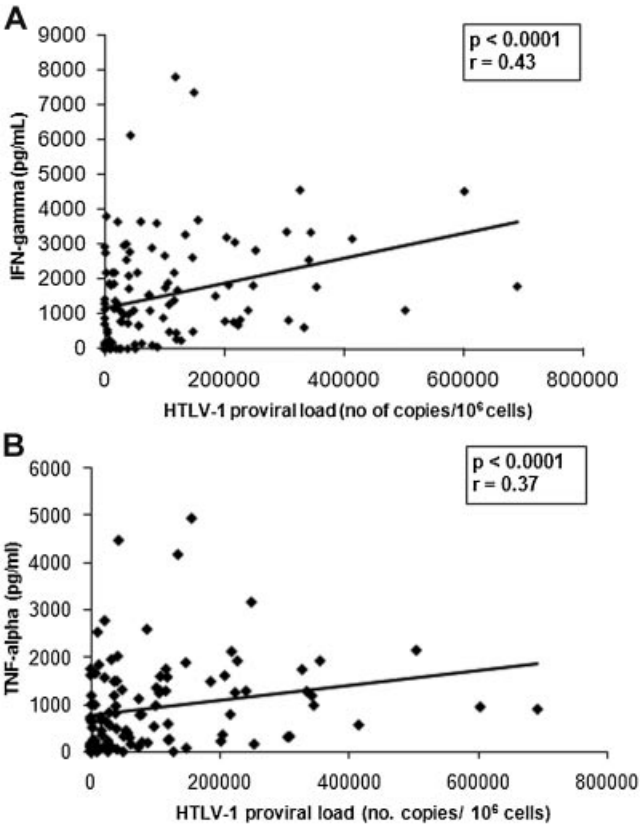

Fig. 4. Correlation between HTLV-1 proviral load and proinflammatory cytokines in groups of HTLV-1 infected patients. **A**: Correlation between HTLV-1 proviral load and spontaneous IFN-γ produced by cells from HTLV-1 carriers (n = 37), HTLV-1 associated overactive bladder, n = 39, and HAM/TSP (n = 36). **B**: Correlation between HTLV-1 proviral load and spontaneous TNF-α produced by cells from all combined groups (n = 112). Correlation was analyzed by Spearman's rank correlation.

was no difference in the levels of CXCL9 between HTLV-1 carriers and HTLV-1 overactive bladder patients ($P > 0.05$, Fig. 5A). Serum levels of CXCL10 (Fig. 5B) followed the same pattern observed with CXCL9 with no difference in the levels of CXCL10 between HTLV-1 carriers (median 183, IQ range 100–417 pg/ml) and HTLV-1 overactive bladder patients (median 242, IQ range 123–427 pg/ml, $P > 0.05$). A significant difference between HTLV-1 carriers and HAM/TSP (median 640, IQ range 340–1,141 pg/ml, $P < 0.001$) and HTLV-1 overactive bladder and HAM/TSP patients ($P < 0.001$) was observed.

Down-Regulation of IFN-γ Production by Regulatory Cytokines (IL-10 and TGF-β) in Different Groups of HTLV-1 Infected Patients

Table II shows the ability of regulatory cytokines to regulate IFN-γ production in cells from HTLV-1 infected patients (n = 15, in each group). While the addition of IL-10 decreased spontaneous IFN-γ production in cell cultures from HTLV-1 carriers by 59% ($P = 0.02$, Wilcoxon matched-pairs signed-ranks test), and in HTLV-1 overactive bladder by 65% ($P = 0.002$), there was hardly any suppression in cell cultures of HAM/TSP patients (15%, $P = 0.17$). When TGF-β was added to cell cultures of HTLV-1 carriers and HTLV-1 infected patients with overactive bladder, there was a suppression of IFN-γ production by 89 and 70% ($P = 0.31$ and $P = 0.006$, respectively) when compared to baseline levels in each group.

DISCUSSION

HTLV-1 infection has been neglected by health authorities and to a certain degree the scientific community mainly due to the traditionally held concept

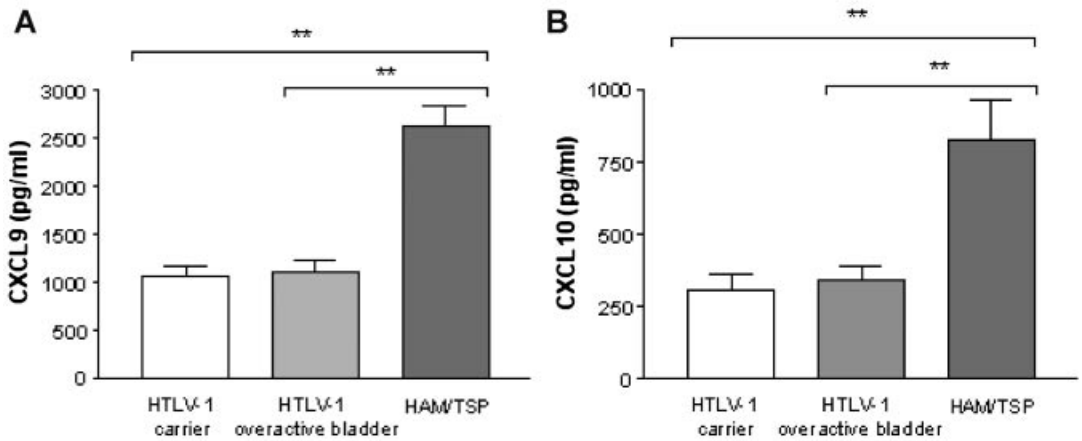

Fig. 5. Levels of type 1 chemokines, CXCL9 (**A**) and CXCL10 (**B**) in serum of HTLV-1 carriers (n = 43), HTLV-1 associated overactive bladder, n = 39, and HAM/TSP (n = 32). Enzyme immunoassay (OptEIA™human MIG and IP-10 ELISA Set) was used to measure the chemokines MIG (CXCL9) and IP-10 (CXCL10). Kruskal–Wallis test was used for statistical analysis (**$P < 0.001$).

TABLE II. Down Regulation of Spontaneous IFN-γ Production by Regulatory Cytokines (IL-10 and TGF-β) in Cell Cultures of HTLV-1 Infected Patients (HTLV-1 Carriers, HTLV-1 Associated Overactive Bladder and HAM/TSP Patients)

Regulatory cytokine added	HTLV-1 carrier			HTLV-1 Overactive bladder			HAM/TSP		
	IFN-γ[a] (pg/ml)	Suppression[b] (%)	*P*-value[c]	IFN-γ (pg/ml)	Suppression (%)	*P*-value	IFN-γ (pg/ml)	Suppression (%)	*P*-value
None	157 (0–905)	—	—	1,462 (0–3,672)	—	—	2,713 (1,256–3,323)		—
IL-10	64 (0–469)	59	0.02	511 (0–3,557)	65	0.002	2,297 (729–2,813)	15	0.17
TGF-β	17 (0–839)	89	0.31	426 (0–3,672)	70	0.006	2,825 (931–3,117)	0	0.19

[a]Data of spontaneous IFN-γ synthesis were expressed as median and interquartile range for each group (N = 15).
[b]Suppression of spontaneous production of IFN-γ in the presence of IL-10 and TGF-β was calculated: [(spontaneous IFN-γ—treated IFN-γ/spontaneous IFN-γ) × 100]. The results were expressed as percent of inhibition and the ability to suppress about 30% of IFN-γ synthesis were considered efficient.
[c]*P*-value compares spontaneous production of IFN-γ before and after the addition of IL-10 and TGF-β to cell cultures of each group of patients (Wilcoxon matched-pairs signed-ranks test).

that it is a low morbidity infection. However, recent studies have pointed out that the majority of HTLV-1 infected patients considered as healthy carriers have signs and symptoms of inflammatory diseases such as periodontal disease, sicca syndrome and arthropathy [Caskey et al., 2007; Giozza et al., 2008; Garlet et al., 2010]. Additionally, a large proportion of HTLV-1 infected patients have symptoms of overactive bladder, which is observed in up to 100% of HAM/TSP patients [Caskey et al., 2007; Oliveira et al., 2010]. These HTLV-1 infected patients who do not fulfill the criteria for HAM/TSP may also present neurological signs and symptoms such as foot numbness, leg weakness, and difficulty walking, as well as hyperreflexia and Babinski sign [Caskey et al., 2007]. Our results demonstrate that HTLV-1 infected patients suffering from overactive bladder produce more IFN-γ, TNF-α, IL-17 and show a higher proviral load than HTLV-1 carriers. The similarities between viral and immunological features in HTLV-1 overactive bladder and HAM/TSP patients may suggest that overactive bladder is an early stage of HAM/TSP.

By the International Continence Society criteria, overactive bladder is defined by the presence of urgency for voiding [Abrams et al., 2002; Abrams et al., 2009]. The majority of HTLV-1 infected patients with overactive bladder had urge-incontinence as well as other urinary symptoms such as nocturia and frequency. Urodynamic studies in these patients showed similar alterations to those found in patients with HAM/TSP [Castro et al., 2007]. Studies have shown that in HTLV-1 infection, symptoms of overactive bladder were related to the infection itself rather than to urinary tract infection [Castro et al., 2007; Rocha et al., 2007]. Additionally, treatment of urinary infection, when present, does not change the symptoms of overactive bladder [Rocha et al., 2007]. Therefore, it is likely that urinary symptoms in HTLV-1 infected patients are due to CNS involvement in the course of viral infection. This assertion is in agreement with the concept that HTLV-1 infected patients with urinary symptoms who do not fulfill the criteria for HAM/TSP are classified as probable HAM/TSP [De Castro-Costa et al., 2006]. However, the relationship between urinary symptoms of overactive bladder and CNS involvement due to HTLV-1 infection needs to be clarified. It is known that the immune response plays a role in the pathogenesis of CNS damage in HTLV-1 infection [Nagai and Osame, 2003; Santos et al., 2004; Nakamura et al., 2009]. This study evaluated whether immunological alterations found in HAM/TSP were also present in patients with overactive bladder associated to HTLV-1.

There is consensus that CNS tissue damage is the result of an exaggerated immune response with increased production of nitric oxide, metalloproteinases, and proinflammatory cytokines [Goto et al., 1997; Carvalho et al., 2001; Elkington et al., 2005]. In this study, it is shown that patients with overactive bladder associated to HTLV-1 had higher spontaneous secretion of IFN-γ and TNF-α than HTLV-1 carriers. TNF-α was similarly elevated in HAM/TSP and HTLV-1 overactive bladder patients. Increased levels of proinflammatory cytokines, such as IFN-γ, TNF-α, IL-1, IL-6, and GM-CSF have been detected in CSF and cell cultures from HAM/TSP patients when compared to HTLV-1 carriers [Nishiura et al., 1996; Santos et al., 2004].

IL-17 plays a pivotal role in the development of autoimmune diseases [Dong, 2008; Ouyang et al., 2008]. IL-17 mRNA was highly expressed in T lymphocytes from a HAM/TSP patient and in a Tax-expressing T cell line, whereas it was not detectable in HTLV-1-negative T cell lines [Dodon et al., 2004]. The present study shows high expression of IL-17 in HTLV-1 overactive bladder and HAM/TSP patients. These data suggest that IL-17 may participate in the pathogenesis of neurological disease related to HTLV-1 infection.

It is well known that high HTLV-1 proviral load is the most important risk factor in the development of HAM/TSP [Nagai et al., 1998; Jeffery et al., 1999; Matsuzaki et al., 2001; Furtado Mdos et al., 2012]. Various studies have shown that it is higher in HAM/TSP than in HTLV-1 carriers [Nagai et al., 1998]. Here, it is shown that proviral load is significantly

higher in HTLV-1 overactive bladder patients than in HTLV-1 carriers, and that no difference was observed between HTLV-1 overactive bladder and HAM/TSP patients. Although weak, a significant correlation between HTLV-1 proviral load and proinflammatory cytokines was also found.

It is known that CD8+ T cells have a decreased ability to kill virus-infected cells in patients with HAM/TSP [Bangham, 2009]. However, these cells, as well as CD4+ T cell are activated and produce high levels of proinflammatory cytokines [Biddison et al., 1997; Santos et al., 2004]. Therefore, a correlation between TNF-α, IFN-γ, and proviral load is in agreement with the concept that increased proviral load may occur despite a strong activation of CD4+ and CD8+ T cells. Since high proviral load is related to the pathogenesis of HAM/TSP, overactive bladder may reflect a progression from a HTLV-1 carrier status toward the HAM/TSP spectrum of disease. Longitudinal data are needed to support this contention.

Immunological and virological data from HTLV-1 infected patients with symptoms of overactive bladder fall somewhere between HTLV-1 carriers and HAM/TSP patients. In some cases, overactive bladder parameters are similar to those observed in HAM/TSP patients.

Since enhanced levels of chemokines that attract cells into the CNS contribute to the pathogenesis of HAM/TSP [Guerreiro et al., 2006], the production of Th1 serum chemokines was evaluated. In addition, it is known that the failure in the regulatory mechanisms to down regulate the immune response also contribute to the pathogenesis of HAM/TSP [Santos et al., 2006], so that the ability of regulatory cytokines to modulate the immune response was assessed in cells of HTLV-1 overactive bladder patients. The serum levels of proinflammatory chemokines in these patients were similar to the levels of HTLV-1 carriers. The results showed that while IL-10 and TGF-β were not able to down regulate cytokine production in HAM/TSP, these regulatory cytokines decreased IFN-γ production in both HTLV-1 carriers and HTLV-1 overactive bladder patients. Limitations in this study include the absence of immunological parameters analysis in the CNS of HTLV-1 overactive bladder patients. Experiments that examine CSF levels of markers of CNS involvement with HTLV-1 should be considered.

Collectively, the data suggest that HTLV-1 infected patients with overactive bladder have similar proviral load and some immunological parameters to those observed in patients with HAM/TSP. However, because HTLV-1 overactive bladder patients' immune system is still able to down regulate the inflammatory immune response, the recruitment of activated T cells to the CNS, presumably, is not enhanced by proinflammatory chemokines. Therefore, these patients are still protected from progression to HAM/TSP, presenting instead with overactive bladder, a less severe form of disease. The immunological and viral data in this article associated with previous clinical findings, coupled with the observation that urinary symptoms may precede the development of HAM/TSP by years [Araujo et al., 1998; Caskey et al., 2007; Oliveira et al., 2007] suggesting that overactive bladder may be an early stage of HAM/TSP.

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