

Major Article

Low Frequency Of Regulatory B-Cells And Increased CD4⁺ and CD8⁺ Interferon- γ -producing cells in patients with tropical spastic paraparesis associated with human T-cell lymphotropic virus type

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Abstract

Introduction: Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) is a disease caused by human T-cell lymphotropic virus type 1 (HTLV-1) that mainly infects CD4 T cells—for example, those of the CD4⁺CD25^{hi}FOXP3⁺ [Treg] phenotype—where it inhibits forkhead box protein 3 (FOXP3) expression and promotes interferon- γ (IFN- γ) expression. However, the role it exerts on regulatory B cells (CD19⁺CD24^{hi}CD38^{hi}; Breg) is unknown. **Methods:** The frequencies of Treg and Breg cells was evaluated and the Th1 profiles were assessed in TSP/HAM patients and healthy control subjects. **Results:** Low percentages of Breg cells and high production of IFN- γ were observed in patients compared to those in healthy control subjects. **Conclusions:** The low percentage of Breg cells in patients and the increase in the frequency of Th1 cells suggest an imbalance in the control of the inflammatory response that contributes to the immunopathogenesis of TSP/HAM.

Keywords: Tropical Spastic Paraparesis. HTLV-1 virus. Regulatory T cells. Regulatory B cells. Interferon- γ .

INTRODUCTION

Human T-cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus with oncogenic properties to be described¹. It affects 5–10 million people worldwide, especially endemic regions such as Africa, Japan, the Caribbean, Iran, Australia, Peru, Chile, Brazil, and Colombia². The incubation period of the virus ranges from years to decades and its transmission occurs through sexual contact, blood transfusions, needle sharing, and from mother to child through breast milk³. The virus is considered the causative agent of two potentially deadly diseases; namely, adult T-cell leukemia/lymphoma (ATL

or ATLL) and tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM). However, not all infected people develop these disorders; while an estimated 2–5% develop ATL and 0.25–2% develop TSP/HAM, the rest remain as asymptomatic carriers for the rest of their lives⁴.

In Colombia, the virus was first described in 1981 in the region of Tumaco (Nariño), with an estimated prevalence of 7.5–10% in the Pacific Coastal Areas⁵. However, the virus is distributed to non-coastal areas such as Antioquia, Risaralda, Córdoba and Cundinamarca, among others. Between 2010 and 2014, seven cases of HTLV-1 were reported in the regions of Nariño, Putumayo, Valle del Cauca, Risaralda and the Caribbean coast⁶. Despite the data shown above, epidemiological studies of the virus have not been carried out, which has limited knowledge of its prevalence in Colombia.

TSP/HAM is characterized by an upper motor neuron lesion and a slow and progressive axon-myelin degeneration

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in the anterolateral cords of the thoracic and/or lumbar spinal cord. The associated clinical manifestations include decreased strength and spasticity in unilateral or bilateral lower limbs, back pain, and urinary symptoms (nocturia, increased in urinary frequency, incontinence, and erectile dysfunction). In addition, Patellar hyperreflexia with or without clonus and Babinski sign or substitutes may occur, with less sensory involvement and without affecting the upper limb or cognitive functions. This weakness increases progressively, and in most cases, there is a spastic or scissor gait, which can evolve to compromise the ability to walk³.

The events that trigger the development of TSP/HAM are not clearly known. Nevertheless, its development is associated with deficiencies in the immunoregulatory process. The virus infects CD4⁺CD25^{hi}FOXP3⁺ Treg cells, activates the Tax viral protein, inhibits forkhead box protein P3 (FOXP3) expression, and promotes interferon gamma (IFN- γ) expression⁷. Therefore, infected cells acquire a Th1 type phenotype, becoming IFN- γ producing cells able to cross the blood-brain barrier and enter the central nervous system (CNS)⁸. The inflammatory response in the CNS is represented by TCD4⁺ cells, high production of IFN- γ , and TCD8⁺ cell recruitment specifically against the virus⁹.

Regulatory B-cells represent a subtype of cells that, like Treg cells, can inhibit the inflammatory response during infections and inflammation; however, they are altered in number and function during autoimmune diseases. B-cells with regulatory capacity have been described, with CD19⁺CD24^{hi}CD38^{hi}, CD19⁺CD24^{hi}CD27⁺, and CD19⁺CD25^{hi}CD71^{hi} phenotypes found in blood and in sites of inflammation¹⁰. Breg cells with a CD19⁺CD24^{hi}CD38^{hi} phenotype inhibit Th1 differentiation *in vitro*¹¹. They also inhibit the inflammatory response through mechanisms dependent on interleukin (IL)-10, IL-35, and transforming growth factor beta (TGF- β), capable of controlling the induction and functioning of the Treg cells. In other autoimmune pathologies such as multiple sclerosis and rheumatoid arthritis, a deficiency in CD19⁺CD24^{hi}CD38^{hi} Breg cells has been observed, which leads to low production of IL-10 and deficiency in the control of Th1 differentiation¹². However, the contribution of regulatory B-cells (Breg-CD19⁺CD24^{hi}CD38^{hi}) in the pathogenesis of TSP/HAM has not yet been described.

Therefore, this work aimed to evaluate the frequency of circulating regulatory B-cells and their relationship to the frequency of TCD4⁺ and TCD8⁺ IFN- γ -producing cells in patients with TSP/HAM to provide information on immunoregulation mechanisms mediated by Breg cells in the pathogenesis of this disease.

METHODS

Study population

Nineteen patients over 18 years of age from the Neurology Service at San José University Hospital in Popayán, Cauca-Colombia were studied. The included participants had neurological symptoms suspicious of TSP/HAM according to the treating neurologist, including spastic paraparesis, gait alterations, paresthesia, alterations in sensitivity, sensation

of muscular weakness, and urinary incontinence, as well as hyperreflexia in the lower limbs, muscular hypertonia/hypotonia and positive Babinski sign. These individuals underwent serology and western blot for HTLV-1, nine of which had positive findings and were selected for study inclusion. Additionally, nine healthy control subjects negative for HTLV-1 by serology, human immunodeficiency virus (HIV)-negative and without acute or chronic conditions were included and were matched to patients according to age and sex.

Ethical considerations

Each participant signed informed consent forms and completed a survey containing information on sociodemographic and neurological clinical data prior to providing a peripheral venous blood sample. All procedures were approved by the Ethics Committee of the University of Cauca.

Peripheral venous blood sampling

Peripheral blood sampling was performed by venous puncture in the forearm following biosafety protocols. This procedure was performed by trained personnel. Approximately 15 mL of peripheral blood was taken from each participant in tubes with and without additives (Vacutainer, Beckton-Dickinson, USA).

Detection of HTLV-1 virus infection

Enzyme-linked immunosorbent assays (ELISAs) were performed from the serum sample to detect the presence of anti-HTLV-1 antibodies, according to the manufacturer's recommendations (DIA.PRO, Milan, Italy). In positive samples, the diagnosis was confirmed by Western blot, according to the manufacturer's recommendations (Bio-Rad, Berkeley, CA).

Extraction of mononuclear cells from peripheral blood

Peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation with Ficoll-Hypaque (BioWhittaker, Walkersville, MD). Cell viability was evaluated using Trypan blue dye (Sigma Immunochemicals, St. Louis, MO, USA).

Phenotyping of Treg and Breg cell subpopulations

Approximately 1×10^6 PBMC were deposited into flow cytometry tubes (Falcon, Becton Dickinson, Oxnard, California). To identify Treg cells, surface labeling was performed with anti-CD4- fluorescein isothiocyanate (FITC) (clone: OKT4, eBioscience, San Diego, CA) and anti-CD25-PECy5 (clone: BC96 eBioscience) antibodies. The cells were fixed with 2% paraformaldehyde, permeabilized with the fixation/permeabilization buffer solution (eBioscience, San Diego, CA) according to the manufacturer's recommendations, and subsequently incubated with anti-FOXP3-PE antibody (clone: 259D BioLegend). Finally, the cells were washed and resuspended in phosphate-buffered saline (PBS) and read on an Accuri C6 flow cytometer (BD Biosciences). For the phenotyping of the Breg cells, anti-CD19-PE (Beckman Coulter, Brea, CA, USA), anti-CD24-Alexa-fluor-647 (clone: ML5 BioLegend) and anti-CD38-PECy5 (clone: HIT2 BioLegend) antibodies were added for 30 minutes at room temperature.

Subsequently, the cells were washed with PBS and fixed with 2% paraformaldehyde. The samples were read in a flow cytometer.

Evaluation of the intracellular production of IFN- γ

For the detection of intracellular IFN- γ , 1×10^6 PBMC were stimulated with phorbol myristate acetate (PMA) (40 ng/mL) (InvivoGen, San Diego, CA, USA) and ionomycin (1 μ g/mL) (Sigma, St. Louis, MO, USA) in the presence of 10 μ g/mL Brefeldin A (Sigma, St. Louis, MO, USA) for six hours at 37°C and 5% CO₂. At the end of the incubation period, the cells were labeled with anti-CD4-PECy5 (clone: RPA-T4) and anti-CD8-PECy5 (clone: RPA-T8, BioLegend) antibodies for 30 minutes at room temperature, then washed with PBS and fixed with 2% paraformaldehyde. The cells were permeabilized with fixation/permeabilization buffer solution (eBioscience, San Diego, CA) according to the manufacturer's recommendations and incubated with anti-IFN- γ -FITC antibody (clone 4S.B3, eBioscience) for 1.5 hours at 4°C under darkness. Finally, the cells were washed with PBS and analyzed on an Accuri C6 flow cytometer (BD Biosciences).

Statistical analysis

The sociodemographic variables and clinical characteristics of the patients were analyzed by measures of central tendency and dispersion. The frequencies of Treg, Breg, and IFN- γ producing T-cells in the peripheral blood of TSP/HAM patients and control subjects were compared by Mann-Whitney U tests. The statistical analysis was performed in GraphPad Prism Software 5 (San Diego, CA, USA).

RESULTS

Socio-demographic and clinical characteristics

Among the nine confirmed patients with TSP/HAM, 55.6% were men and 44.4% were women, with ages ranging from 32–79 years (median: 58.8 years), mainly from the rural area of the Department of Cauca (55.6%). As an epidemiological antecedent of the risk of acquiring HTLV-1 infection, six patients (75%) had received blood transfusion at least once in their lifetimes and none had been intravenous drug users or had had homosexual relationships prior to the study. According to the neurological clinical examinations, the most frequent signs and symptoms were paraparesis of the lower limbs, paresthesia, gait alterations and hypotonia, which were present in 55.6% of the cases. Babinski sign and hyperreflexia in the lower limbs were present in 66.6% of the cases. Urological symptoms such as urinary incontinence and erectile dysfunction occurred in 88.8% of patients (Table 1).

Percentage of CD4⁺CD25^{hi} and CD4⁺CD25^{hi}FOXP3⁺ cells in TSP/HAM patients and control subjects

Cells with a regulatory function capable of suppressing the inflammatory response are altered in number and function in different chronic inflammatory and autoimmune diseases. These cells are identified by the high expression of CD25 in addition to the expression of the FOXP3 transcription factor¹³, which is considered the best lineage marker in this cellular subpopulation. In the present study, the frequencies of cells

with CD4⁺CD25^{hi} and CD4⁺CD25^{hi}FOXP3⁺ phenotypes in peripheral blood of TSP/HAM patients and control subjects were evaluated by flow cytometry. The analysis of the frequencies of these subpopulations showed no significant differences between TSP/HAM patients and control subjects, although an increasing tendency of these two subpopulations was observed in the patient group. The median percentages of CD4⁺CD25^{hi} cells in the patients and control groups were 2.3 (interquartile range [IQR] 1.25–5.1) and 1.58 (IQR 1.06–2.5), respectively. In contrast, the percentages of CD4⁺CD25^{hi}FOXP3⁺ cells were 3.38 (IQR 2.26–3.48) and 2.5 (1.52–3.44), respectively (Figure 1).

Percentages of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) in TSP/HAM patients and control subjects

Under normal conditions, Breg cells are characterized by their inhibition of the inflammatory response; however, the number of these cells is altered in autoimmune diseases. In this study, the median percentage of Breg cells with CD19⁺CD24^{hi}CD38^{hi} phenotype was significantly lower in patients with TSP/HAM compared to that in the control subjects (2.9 [IQR 1.9–2.4] vs. 8.5 [IQR 4.4–11.2], $p < 0.05$) (Figure 2). This result suggests that within this pathology there are alterations in the number of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) which contribute to the control deficiency of the inflammatory response of the disease.

Percentages of CD4⁺ and CD8⁺ IFN- γ producing cells in TSP/HAM patients and control subjects

Previous studies have shown an increase in the frequency of IFN- γ producing cells in TSP/HAM patients⁷. In the present study, quantification the percentages of CD4⁺ and CD8⁺ IFN- γ -producing cells showed an increased percentage in patients compared to that in the control subjects ($p < 0.01$). The median percentages of T CD4⁺IFN- γ ⁺ T cells in the patients and controls were 9.7% (IQR 2.9–23.7) and 0.78% (IQR 0.28–4.1), respectively. Similar results were observed for CD8⁺IFN- γ ⁺ T cells, with medians of 16.1% (IQR 4.9–47.3) and 1.6% (IQR 1.5–8.04), respectively ($p < 0.05$) (Figure 3).

Ratios of the percentages of CD4⁺IFN- γ ⁺/CD19⁺CD24^{hi}CD38^{hi} and CD8⁺IFN- γ ⁺/CD19⁺CD24^{hi}CD38^{hi} cells in TSP/HAM patients and control subjects

Considering the low percentages of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) and high percentages of CD4⁺ and CD8⁺ IFN- γ producing cells TSP/HAM patients, the coefficient of the ratio of CD4⁺IFN- γ ⁺/CD19⁺CD24^{hi}CD38^{hi} and CD8⁺IFN- γ ⁺/CD19⁺CD24^{hi}CD38^{hi} cells was calculated between patients and control subjects. The coefficient was higher in patients than that in control subjects for the ratio of CD4⁺IFN- γ ⁺/CD19⁺CD24^{hi}CD38^{hi}, (median: [IQR] 2.7 [0.9–11.2] in patients vs median: [IQR] 0.07 [0.03–1.1] in control subjects; $p < 0.05$) and for the ratio of CD8⁺IFN- γ ⁺/CD19⁺CD24^{hi}CD38^{hi}, (median: [IQR] 5.2 [1.4–18.7]) in patients vs median: [IQR] 0.28 [0.13–1.2] in control subjects; $p < 0.01$) (Figure 4). This inverse relationship suggests an expansion of IFN- γ producing cells and a reduction in the number of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) in TSP/HAM.

TABLE 1: Sociodemographic and clinical characteristics of patients with tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) and in control subjects.

		TSP/HAM	Control subjects
		(n=9) n (%)	(n=9) n (%)
Male		5 (55.6)	5 (55.6)
Female		4 (44.4)	4 (44.4)
Median age (range)		58.8 (32-79)	57.6(31-78)
Residence	Urban	4(44.4)	8(88.8)
	Rural	5 (55.6)	1 (11.1)
* BMI (kg/m ²)	Normal	6 (66.7)	4 (44.4)
	Overweight	3(33.3)	5(55.5)
Breastfeeding background		4(44.4)	8 (88.8)
Blood donation background		3(33.3)	4 (44.4)
Transfusion background		6 (75)	0(0)
Neurological symptoms	LL spastic paraparesia	5 (55.6)	
	Llparesthesia **	5 (55.6)	
	Gait alterations	5 (55.6)	
	Babinski sign	6 (66.6)	
	LL normotonia	2 (22.2)	
	LL hypertonia	1 (11.1)	
	LL hypotonia	5 (55.6)	
	LL hyporeflexia	1 (11.1)	
	LL hyperreflexia with or without clonus	6 (66.6)	
	Cerebellar signs	2 (22.2)	
Urological symptoms	Alterations of cranial pairs	1 (11.1)	
	Incontinence	5 (55.6)	
	Erectile dysfunction	3 (33.3)	

BMI**: body mass index; *LL**: lower limbs. Obesity and low weight were examined in patients and control subjects in cases in which no patient with these characteristics was found.

DISCUSSION

The present work evaluated the percentages of Treg (CD4⁺CD25^{hi} and CD4⁺CD25^{hi}FOXP3⁺), Breg (CD19⁺CD24^{hi}CD38^{hi}), and CD4 and CD8 IFN- γ -producing cells in nine patients with TSP/HAM, compared to those in healthy control subjects. Regarding sociodemographic data, the presentation of the disease at older ages (median age 58.8 years) could be associated with the natural evolution of the virus and the chronicity of infection that is consistent with what has been reported in other studies¹⁴⁻¹⁶. Intravenous drug use, homosexual relations, and blood transfusions have been described as epidemiological risk factors for acquiring HTLV-1 infection. From these, only a background history of blood transfusions was present among the patients and could have been the main route of transmission in these cases.

TSP/HAM patients with neurological characteristics typical of the disease associated with HTLV-1 infection initially present sensory/motor manifestations (hyperreflexia, hypertonia), urological manifestations (urinary incontinence, sexual dysfunction, neurogenic bladder), which evolve to progressive spastic paraparesis in the lower limbs, gait alterations, and generalized pyramidal signs (Babinski sign and substitutes, Hoffman's sign)^{17,18}. In our study, there was a greater tendency for patients to develop this type of symptomatology. These findings correlate with prior reports in the literature in which patellar hyperreflexia and Babinski sign may be present in 100% and 90–100% of patients infected with the virus^{18,19}. The other frequent symptoms in this group of patients included urological symptoms, reaffirming the importance of looking for this symptomatology in all patients in whom infection with

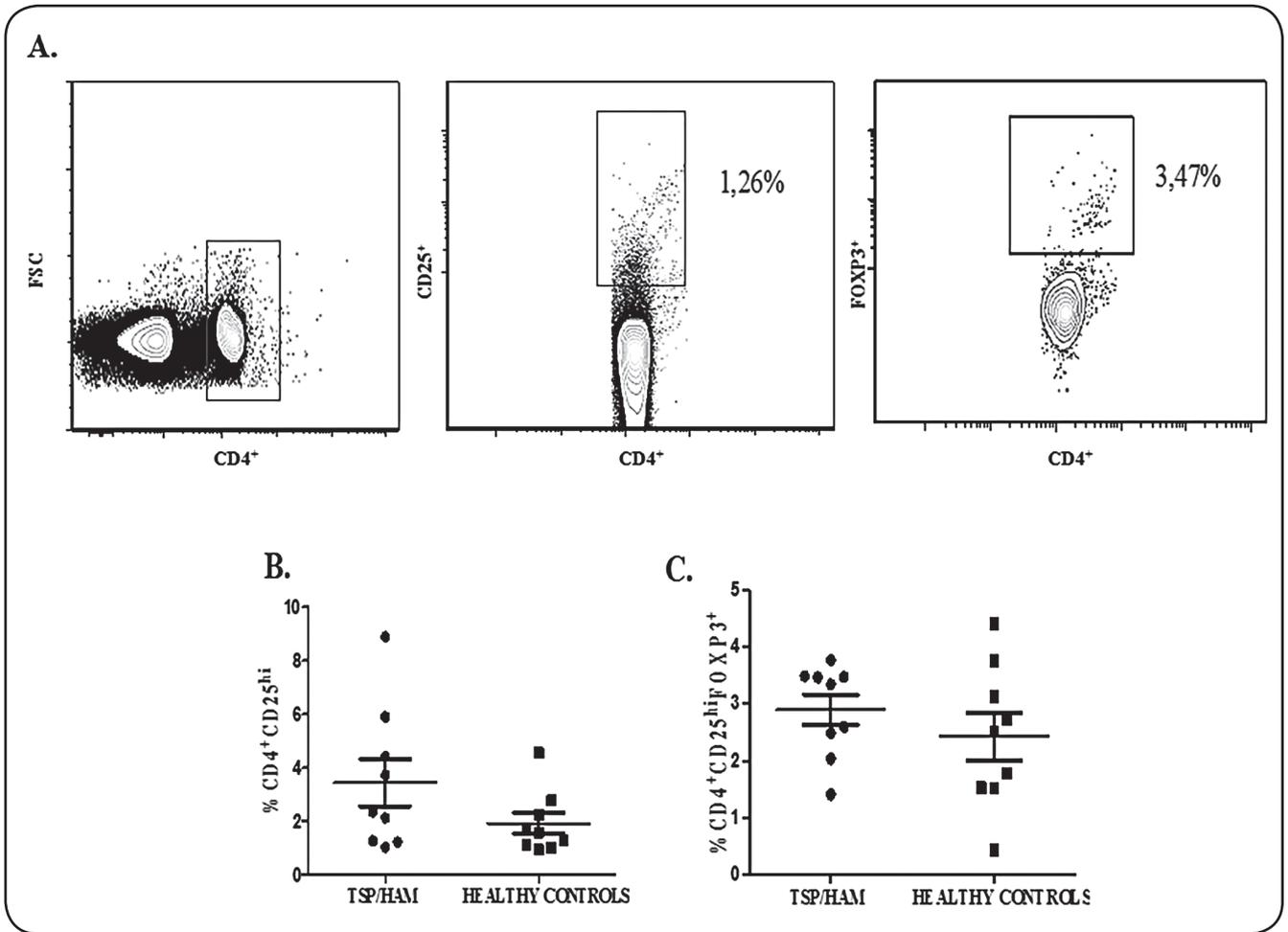


FIGURE 1: Quantification of the frequency of circulating Treg cells. Representative graph showing the flow cytometry analysis strategy for CD4⁺CD25^{hi} and CD4⁺CD25^{hi}FOXP3⁺ cellular subpopulations (A). Dot graphs showing the frequency of CD4⁺CD25^{hi} cells (B) and CD4⁺CD25^{hi}FOXP3⁺ cells (C) in nine TSP/HAM patients and nine healthy control subjects. Each dot represents individual values in each group, while the horizontal lines indicate the median and standard errors. The data were analyzed using Mann–Whitney U tests. p = ns.

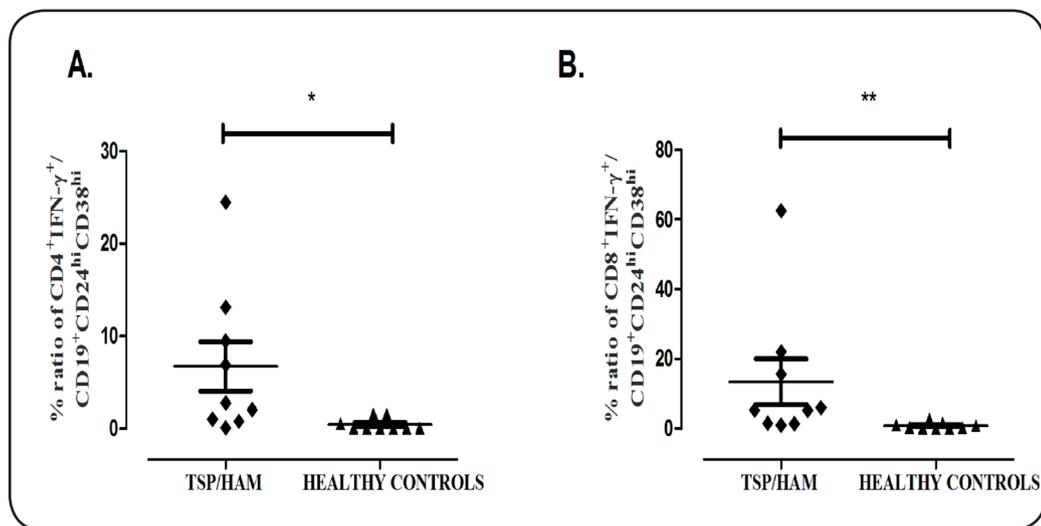


FIGURE 2: Quantification and percentage of Breg cells with CD19⁺CD24^{hi}CD38^{hi} phenotype in peripheral blood mononuclear cells. A representative flow cytometry graph of B cells (CD19⁺) corresponding to the CD19⁺CD24^{hi}CD38^{hi} subpopulations (A). The dot chart shows the frequency of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) in nine TSP/HAM patients and control subjects. Each dot represents individual values in each group, while the horizontal lines indicate the median and standard errors. The data were analyzed using Mann–Whitney U tests. ** = p < 0.01.

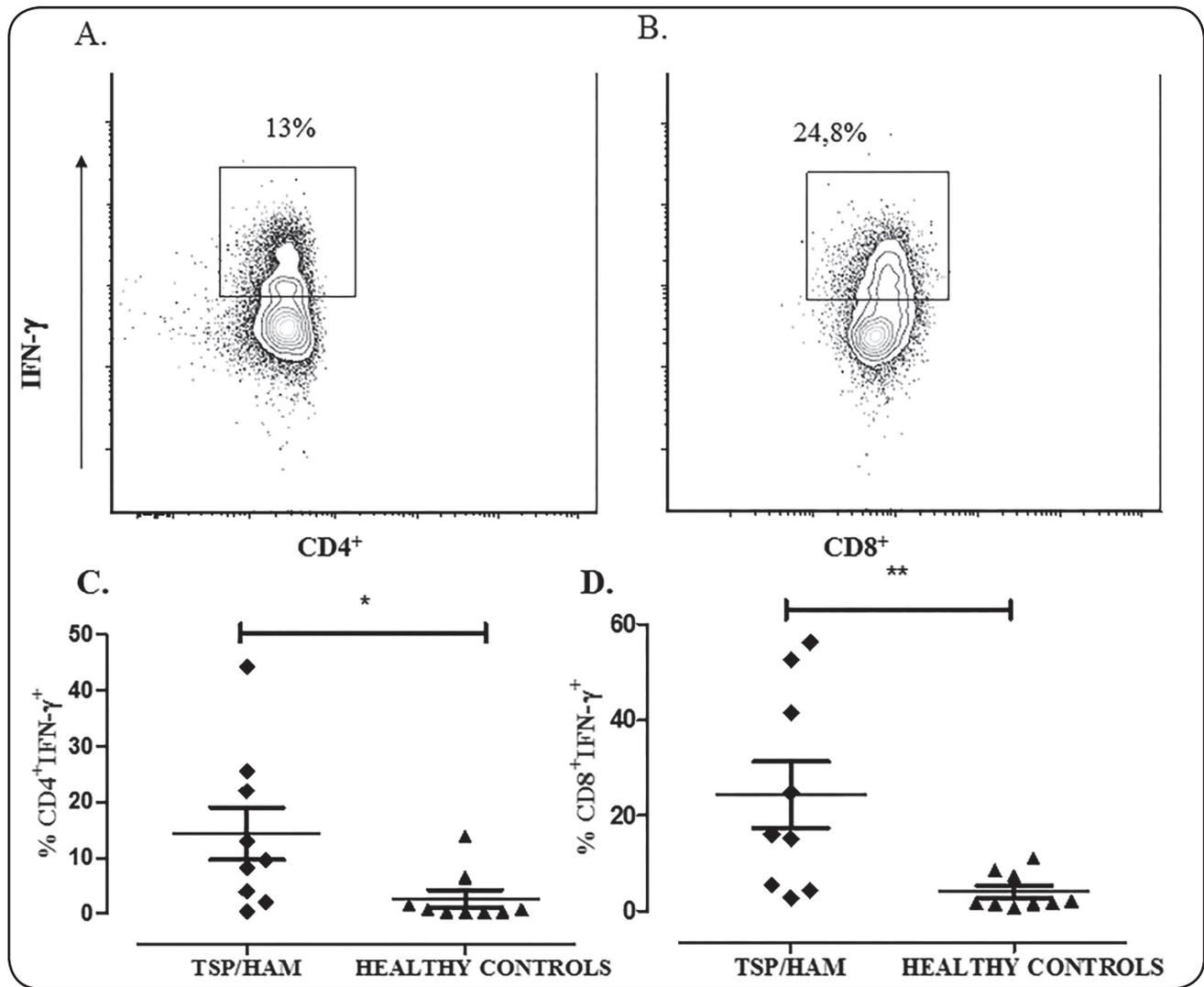


FIGURE 3: Quantification of the frequency of CD4⁺ and CD8⁺ lymphocytes producing interferon-gamma (IFN-γ). Representative flow cytometry graph of T CD4⁺ (A) and CD8⁺ (B) subpopulations of IFN-γ producing cells. The dot charts show the frequency of CD4⁺ (C) and CD8⁺ (D) IFN-γ producing cells in nine TSP/HAM patients and nine healthy control subjects. Each dot represents an individual value in each group, while the horizontal lines indicate the median and standard errors. The data were analyzed using Mann-Whitney U tests. ** = p < 0.01, * = p < 0.05.

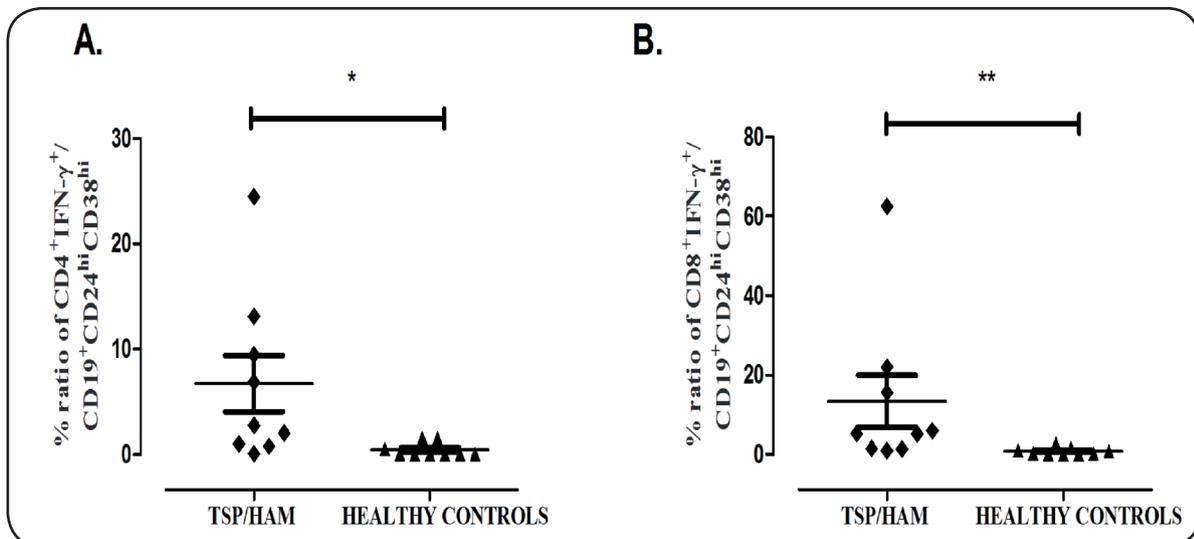


FIGURE 4: Percentage ratios of CD4⁺IFN-γ⁺/CD19⁺CD24^{hi}CD38^{hi} (A) and CD8⁺IFN-γ⁺/CD19⁺CD24^{hi}CD38^{hi} (B) cells in nine patients with TSP/HAM and nine healthy control subjects. Each dot represents an individual value in each group, while the horizontal lines indicate the median and standard errors. The data were analyzed using Mann-Whitney U tests with p < 0.05 (*) and p < 0.01 (**).

HTLV-1 is suspected, or in those patients who consult for urinary symptoms with an unclear origin. This is important given that these types of alterations can become a silent problem and occur many years before the manifestation of the spastic paraparesis associated with HTLV-1²⁰. It is noteworthy that of the 19 patients suspected of TSP/HAM, only nine received confirmation of the disease, reaffirming the similarity between the clinical presentation of TSP/HAM and a variety of neurological diseases, especially those associated with motor neuron disorders. Therefore, according to each patient's clinical context, a call is made to consider HTLV-1 infection as an alternative diagnosis when other, more common diseases have been ruled out since this diagnosis could alter the patient prognosis and management.

Evaluation of the cells with regulatory function in the nine patients with TSP/HAM showed no significant difference in Treg cell populations with CD4⁺CD25^{hi} and CD4⁺CD25^{hi}FOXP3⁺ phenotypes from those in the control subjects. These results were discordant with those reported in the literature showing decreased expression of FOXP3-positive cells in patients with TSP/HAM^{7,21,22}. This difference could be due to the fact that CD4⁺CD25^{hi}FOXP3⁺ cells were quantified in this study from the total number of mononuclear cells isolated from patients and control subjects, without discriminating if they were infected, which leads us to suspect that the virus affects the expression of FOXP3 directly in infected cells, without directly or indirectly altering uninfected cells of the same phenotype.

Alterations in the number and function of Breg cells have been described in other chronic inflammatory and autoimmune diseases. To our knowledge, the present study is the first to describe the frequency of circulating Breg cells with CD19⁺CD24^{hi}CD38^{hi} phenotype in TSP/HAM pathology. The reduced frequency of these cells in TSP/HAM patients compared to those in healthy control subjects is similar to the results reported for this cell lineage in other autoimmune inflammatory pathologies such as systemic lupus erythematosus¹¹ and multiple sclerosis²³. The reduction of Breg cells in patients with TSP/HAM could be associated with defects in the regulation of Th1 type cells, which has been demonstrated to trigger a physiological process which results in tissue damage caused mainly by an increase in proinflammatory cytokines such as IFN- γ ⁷.

Evaluation of the production of IFN- γ in TCD4⁺ and TCD8⁺ lymphocytes in patients and healthy control subjects showed a significant increase in patients; consistent with that reported in the literature showing IFN- γ -producing cells proliferation by impairment of Treg cells to regulate Th1 cell differentiation in TSP/HAM²¹. Other phenomena that explain Th1 expansion include a cellular plasticity phenomenon described in 2017 by Yamano and Coler-Reilly, who demonstrated reduced expression of regulatory T-cells with CD4⁺CD25^{hi}FOXP3⁺ phenotype infected by HTLV-1 as a consequence of viral Tax protein activation. This protein activates the expression of the transcription factor T-bet, whose main function is to decrease the expression of FOXP3 and to increase IFN- γ production, resulting in cellular plasticity in which the cells are converted to a Th1-like phenotype^{23,24}.

Since a high production of IFN- γ in CD4⁺ and CD8⁺ cells and a low percentage of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) were observed in patients with TSP/HAM, we compared the percentage ratio of CD4⁺IFN- γ and CD8⁺IFN- γ cells to the percentage of Breg cells. The results showed a significant difference in both cases. Thus, the coefficient of the relationship between IFN- γ -producing and Breg cells was higher in patients with TSP/HAM compared to that in healthy control subjects. Therefore, patients with TSP/HAM had a greater number of IFN- γ -producing cells while the number of Breg cells decreased, which could be associated with defects in the regulation of Th1-type cells and may be related to inflammatory processes mediated by IFN- γ , which in turn mediates tissue damage.

To our knowledge, the present study is the first to demonstrate the role of Breg cells (CD19⁺CD24^{hi}CD38^{hi}) in the pathophysiology of TSP/HAM. The decrease of this cellular phenotype in the pathology is related to the deficiency of the regulatory processes of the immune system, which, combined with the effect exerted by the virus on Treg cells, triggers a predominance of Th1 cells, which is associated with characteristic tissue damage. As there are currently no accurate treatment for HTLV-1 infection nor TSP/HAM, the present results offer another perspective in the search for new targets; more specifically, for the application of innovative treatments focused on the phenotypes described to improve the immune regulatory process and prevent tissue damage associated with TSP/HAM.

One of the limitations of the study was the small number of patients evaluated; despite this, considering the low prevalence of the disease and the difficulty in clearly identifying patients infected with the virus, the results presented here contribute significantly to the study of the immunopathogenesis of the disease. Additionally, future studies that directly evaluate the function of regulatory cells in infected cells would complement the data presented here.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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