**IL28B** gene polymorphisms and Th1/Th2 cytokine levels might be associated with HTLV-associated arthropathy

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**A B S T R A C T**

The present study is the first investigation of the association between single nucleotide polymorphisms (SNPs – rs8099917, rs12979860 and rs8103142) of the IL28B gene and the development of human T-lymphotropic virus (HTLV)-associated arthropathy (HAA). Individuals with HAA exhibited low interleukin (IL) 6 (p < 0.05) and high IL-10 (p < 0.05) levels compared with asymptomatic patients. TNF-α/CD4 + T cell count, TNF-α/CD8 + T cell count and IFN-γ/proviral load positively correlated in asymptomatic patients. The allelic and genotypic frequencies did not differ between patients with HAA and asymptomatic patients. Seven haplotypes were detected in the investigated population, with haplotype CCT (p < 0.05) being the most frequent among the HTLV-infected individuals, while haplotype TTG (p < 0.05) was detected in the group with HAA only. Compared with asymptomatic patients, individuals with HAA and genotype TT (rs8099917) exhibited larger numbers of CD8 + T cells (p < 0.05) and higher proviral load levels (p < 0.05). Those patients with HAA and genotypes CC (rs12979860) and TT (rs8103142) exhibited high TNF-β (p < 0.05) and IFN-γ (p < 0.05) levels. Those patients with HAA and genotype CT/TT (rs12979860) exhibited high IL-10 levels (p < 0.05). These results suggest that haplotypes CCT and TTG might be associated with susceptibility to HTLV infection and progression to HAA, respectively. Genotype TT (rs8099917) might be a risk factor for elevation of the proviral load and CD8 + T cell count. In addition, genotypes CC (rs12979860) and TT (rs8103142) seem to be associated with increased TNF-β and IFN-γ levels.

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1. Introduction

Human T-lymphotropic virus 1 (HTLV-1) and Human T-lymphotropic virus 2 (HTLV-2) are members of family Retroviridae, genus Deltaretrovirus [1]. These viruses exhibit similar biological properties, tropism for T lymphocytes [2–5] and a worldwide geographic distribution [6].

The main diseases associated with HTLV-1 are adult T-cell leukemia/lymphoma (ATLL) [7] and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [8]. Several rheumatic diseases have also been associated with HTLV-1 infection, including rheumatoid arthritis [9], Sjögren’s syndrome [10] and systemic lupus erythematosus [11]. Those clinical conditions seem to result from interactions between the virus and certain host immune response factors, thus causing an imbalance in the immunomodulation of cell proliferation and inflammation [12].

Studies have shown that HTLV-1 exhibits tropism for synovial CD68+ cells, which, upon becoming infected with the virus, produce tumor necrosis factor alpha (TNF-α), which stimulates cell proliferation and the destruction of articular cartilage through erosion [13]. Aono et al. [14] showed that extracellular HTLV-1...
Tax protein can activate transcription factors such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) in human synovial cells. The products of the activation of such factors might play a significant role in the pathogenesis of HTLV-associated arthropathy (HAA) [14].

HTLV-1 has been described as being able to infect a wide variety of cell types both in vitro and in vivo. Nevertheless, CD8+ and, to a lesser degree, CD8+ T cells are considered to be the main targets of HTLV-1 [15,16]. Although infection is latent in most T cells and thus undetectable by the host immune system, productive infection by HTLV activates lymphocytes, whose response is mediated by multiple cell and humoral immune response mechanisms [17].

Cytokines play a central role in regulating the immune response against HTLV-1 [18] and represent the main defensive strategy presented by the host immune system to control viral replication, and the proliferation of infected cells is mediated by CD8+ T lymphocytes [19,20].

Infection with HTLV-1 induces the activation and intense proliferation of infected T cells. This phenomenon is mainly associated with the function of the virus Tax gene, which is able to transactivate the interleukin-2 (IL-2) and IL-2 receptor genes [21]. Indiscriminate cell proliferation might result in the expansion of autoreactive T cells and considerable secretion of proinflammatory cytokines such as TNF-α. These changes might be associated with skin and neurologic injury [22]. Thus, studying the role of pro- and anti-inflammatory cytokines and the polymorphisms of the corresponding genes might be highly relevant for elucidating the pathophysiology of diseases associated with HTLV-1.

Interferon lambda 3 (INF-λ3), also known as interleukin-28B (IL-28B), is among the cytokines with a major role in immunomodulation and in the antiviral response [23–25] and has demonstrated the potential to increase CD8+ T cell counts in the peripheral blood of monkeys [26]. Three single nucleotide polymorphisms (SNPs) have been described for this gene: rs12979860 (C>T) and rs8099917 (T>G), which are located 3 kb and 8 kb upstream of the IL28B gene, respectively, and rs8103142 (T>C), which is located in exon 2.

As a function of the immunologic aspects of HTLV-1 infections and the pathophysiology of rheumatic diseases, the present study investigated for the very first time the association between SNPs of the IL28B gene and the development of HAA.

2. Materials and methods

2.1. Study population

The present study was conducted with 96 HTLV-1-infected individuals, with 64 being asymptomatic (19 men and 45 women) and 32 (six men and 26 women) exhibiting the signs and symptoms of rheumatic disease. All patients received care at the Outpatient Clinic for Infectious Diseases, Tropical Medicine Unit, Federal University of Pará Universidade Federal do Pará (Ambulatório de Infectologia do Núcleo de Medicina Tropical da Universidade Federal do Pará – UFPA). Patient ages varied from 21 to 79 years old. All patients were assessed by a rheumatologist, who verified the presence of symptoms meeting all of the diagnostic criteria for rheumatic disease. Thus, the patients were clinically evaluated as to the presence of tenosynovitis, joint pain and arthritis in addition to myalgia and proximal muscle weakness, the latter being assessed by means of the Mingazzini test. The individuals presenting with a predominance of pain and signs of inflammation in large joints with a mono-, oligo- or polycyclic pattern were selected for the present study. The control group was composed of 300 HTLV-1 seronegative individuals from both genders (150 men and 150 women) aged 19 to 68 years old without symptoms of joint disease.

2.2. Sample collection

Blood samples (5 mL) were collected in EDTA-coated tubes from all of the participants. The samples were sent to the Laboratory of Virology, Institute of Biological Sciences, UFPA, where they were tested for anti-HTLV-1/2 antibodies via enzyme-linked immunosorbent assay (ELISA); infection was confirmed via nested polymerase chain reaction (PCR) as described previously [27].

2.3. Ethical issues

The study was approved by the research ethics committee of João de Barros Barreto University Hospital (process No. 2061/2005) in compliance with Health National Council resolution 196/96, which addresses the guidelines and regulatory norms for human subjects research.

2.4. DNA extraction

Total DNA was extracted from peripheral blood leukocytes using the phenol–chloroform method. The procedure included cell lysis, protein precipitation and DNA purification steps, as previously described [27].

2.5. Polymorphism determination

The extracted DNA was subjected to real-time PCR (qPCR) using a Step One PLUS Sequence Detector (Life Technologies, Foster City, CA, USA). The assays used for each polymorphism included one pair of primers and one pair of probes; VIC® and FAM® labeling was applied to each allele of the corresponding polymorphisms. The target sequence of polymorphism rs12979860 was sent to Applied Biosystems, which designed the following primer and probe sequences: 5’-GCC TGT CTT GAT TAA CAC A-3’ (forward primer), 5’-GCC CGG AGT TAC TAA AC3’- (reverse primer), 5’-TGG TTC C-3’ (allele T probe). The same was performed in the case of polymorphism rs8099917, resulting in the following primer and probe sequences: 5’-GCC TCA GGT CCC AGG TC-3’ (forward primer), 5’-GCC TTG CCT GTC TAG GAA GAG T-3’ (reverse primer), 5’-GCC GAC CTT GAC TC VIC3’- (probe) and 5’-CGG CAC TGG CAG TC FAM-3’ (probe).

For polymorphism rs8099917, pre-designed assay part number C_11170009.10 (Life Technologies, Foster City, CA, USA) was used. A total of 5.0 µL of TaqMan® Universal PCR Master Mix [2X], 0.5 µL of TaqMan® Assay [20X], 3.5 µL of H2O and 1 µL of DNA were used in each reaction, with a final reaction volume of 10 µL. The following parameters were used for the amplification and detection of alleles: 60 °C for 30 s, followed by 95 °C for 10 min, 50 cycles at 92 °C for 30 s and 60 °C for 90 s.

2.6. CD4+ and CD8+ T cell count

The blood samples from the individuals diagnosed with HTLV infection were processed within four hours of collection. The T cell count was performed via flow cytometry (BD FACScalibur 4 colors) using a BD Multitest immunomonitoring kit according to the standards recommended by the manufacturer (BD Biosciences, San Jose, CA, USA).
2.7. HTLV proviral load

The proviral load was measured via qPCR using the three-target-sequel TaqMan system formulated by Life Technologies (Life Technologies, Foster City, CA, USA) according to a previously described protocol [28].

2.8. Serum cytokines measurement

The serum concentrations of cytokines TNF-α, TNF-β, IFN-γ, IL-6, IL-8 and IL-10 were measured using ELISA (Human ELISAReady-SET-Go, EBioscience, Inc. California, San Diego, USA). This method uses specific monoclonal antibodies to detect the aforementioned cytokines and was performed according to the manufacturer’s instructions.

2.9. Statistical analysis

The allelic and genotypic frequencies were calculated by direct counting. Comparative analysis of the allelic and genotypic frequencies and the calculation of the Hardy–Weinberg equilibrium were performed using the chi-square (χ²) test. Simple logistic regression analysis was performed to investigate the association of the genetic models (dominant and recessive) with the risk of developing HAA. The linkage disequilibrium and haplotype analyses were performed using Haploview software v4.2 [29]. The haplotypes were constructed using the Haploview 4.2 Expectation Maximization algorithm [29], which provides highly precise estimates of population frequencies. D² confidence interval 0.7–0.98 was considered as indicative of high linkage disequilibrium.

CD4⁺ and CD8⁺ T cells count, proviral load and serum cytokine levels were submitted to the Shapiro–Wilk test for assessing the existence of a normal distribution. The variables which were in a normal distribution were compared using Student's t-test. In the presence or absence of normal distribution comparative analyses were performed using the Mann–Whitney test. In the presence of absence of normal distribution the correlation analyzes were performed by Pearson's and Spearman's tests, respectively, with two-tailed p and alpha < 0.05. The tests were performed using BioEstat 5.0 [30] and GraphPad Prism 5.0 [31] software; the significance level was set to 5% (p < 0.05).

3. Results

3.1. Allelic and genotypic frequencies

The allelic and genotypic frequencies of polymorphisms rs8103142, rs12979860 and rs8099917 among (symptomatic and asymptomatic) HTLV carriers and controls are described in Table 1. Hardy–Weinberg analysis showed that all the populations were at equilibrium (p > 0.05).

Analysis of polymorphism rs8103142 showed that allele T and heterozygous genotype TC were the most frequent among the investigated groups. Genotype TC occurred in 49% of the controls and 48.4% of the patients, being more prevalent among asymptomatic individuals (52.4%) compared to those with HAA (46.0%). Analysis of polymorphism rs8099917 showed that homozygous genotype TT was the most frequent in all of the groups, its frequency varying from 45.3% among the asymptomatic patients to 47% in the control group. Relative to polymorphism rs12979860, heterozygous genotype CT was the most prevalent in all of the groups, its frequency varying from 43.8% to 52.3%. Allele C was the most frequent, varying from 51.7% in the control group to 51.6% among the patients. This high frequency persisted in the asymptomatic patient group (53.8%), falling to 46.9% among patients with HAA. None of the differences in the allelic and genotypic frequencies was statistically significant, and none of the suggested genetic models were associated with the risk of developing HAA (Table 2).

Considerable linkage disequilibrium among SNPs rs8103142/ rs12979860 (D²: 0.934; LOD: 30.42; r²: 0.854; confidence interval D²: 0.86–0.98), rs8103142/rs8099917 (D²: 0.918; LOD: 11.46; r²: 0.405; confidence interval D²: 0.76–0.98) and rs12979860/rs8099917 (D²: 1.0; LOD: 16.12; r²: 0.491; confidence interval D²: 0.89–1.00) was found between HAA patients and asymptomatic individuals (Fig. 1A). Imbalance was also found relative to SNPs rs8103142/rs12979860 (D²: 0.934; LOD: 30.42; r²: 0.854; confidence interval D²: 0.86–0.98), rs8103142/rs8099917 (D²: 0.918; LOD: 11.46; r²: 0.405; confidence interval D²: 0.76–0.98) and rs12979860/rs8099917 (D²: 1.0; LOD: 16.12; r²: 0.491; confidence interval D²: 0.89–1.00) between the non-infected control group and the HTLV-infected individuals (Fig. 1B). Analysis of asymptomatic and HAA patients (Fig. 1A) and of non-infected controls and HTLV-infected patients (Fig. 1B) showed significant linkage disequilibrium (LOD > 2).

### Table 1

Allelic and genotypic frequencies of IL28B gene polymorphisms.

<table>
<thead>
<tr>
<th>Genetic profile</th>
<th>Control n (%)</th>
<th>HTLV-1 n (%)</th>
<th>χ²</th>
<th>p</th>
<th>HAA n (%)</th>
<th>Asymptomatic n (%)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs8103142</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>81 (27.0)</td>
<td>25 (26.3)</td>
<td>10</td>
<td>0.055</td>
<td>19 (48.4)</td>
<td>9 (31.3)</td>
<td>15</td>
<td>0.055</td>
</tr>
<tr>
<td>CC</td>
<td>72 (24.0)</td>
<td>24 (25.3)</td>
<td>0.065</td>
<td>0.9681</td>
<td>33 (51.6)</td>
<td>15 (23.8)</td>
<td>1.029</td>
<td>0.5465</td>
</tr>
<tr>
<td>T</td>
<td>309 (51.5)</td>
<td>94 (49.5)</td>
<td>0.055</td>
<td>0.8802</td>
<td>19 (50.0)</td>
<td>63 (50.0)</td>
<td>2.685</td>
<td>0.1407</td>
</tr>
<tr>
<td>C</td>
<td>291 (48.5)</td>
<td>94 (49.5)</td>
<td>0.055</td>
<td>0.8802</td>
<td>19 (50.0)</td>
<td>63 (50.0)</td>
<td>2.685</td>
<td>0.1407</td>
</tr>
<tr>
<td>rs8099917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>141 (47.0)</td>
<td>44 (45.8)</td>
<td>15</td>
<td>0.061</td>
<td>68 (67.2)</td>
<td>86 (67.2)</td>
<td>2.048</td>
<td>0.1407</td>
</tr>
<tr>
<td>TG</td>
<td>132 (44.0)</td>
<td>42 (43.8)</td>
<td>0.017</td>
<td>0.9146</td>
<td>3 (10.9)</td>
<td>7 (10.9)</td>
<td>0.061</td>
<td>0.9698</td>
</tr>
<tr>
<td>GG</td>
<td>27 (9.0)</td>
<td>10 (10.4)</td>
<td>0.178</td>
<td>0.9146</td>
<td>3 (9.6)</td>
<td>7 (10.9)</td>
<td>0.061</td>
<td>0.9698</td>
</tr>
<tr>
<td>T</td>
<td>414 (69.0)</td>
<td>130 (67.7)</td>
<td>0.444</td>
<td>0.8053</td>
<td>20 (31.3)</td>
<td>42 (32.8)</td>
<td>0.048</td>
<td>0.9565</td>
</tr>
<tr>
<td>G</td>
<td>186 (31.0)</td>
<td>62 (32.3)</td>
<td>0.113</td>
<td>0.8053</td>
<td>20 (31.3)</td>
<td>42 (32.8)</td>
<td>0.048</td>
<td>0.9565</td>
</tr>
</tbody>
</table>

rs12979860 |        |              |    |   |           |                    |    |   |
| CC              | 80 (26.7)     | 26 (26.8)    | 8 | 0.001 | 95 (31.2)  | 34 (53.1)         | 60 | 0.834 |
| CT              | 150 (50.0)    | 48 (49.5)    | 14 | 0.9573 | 34 (53.1)  | 60 (46.2)         | 0.834 | 0.4468 |

HTLV-1: Human T lymphotropic virus type 1. HAA: HTLV-1-associated arthropathy.
Haploview software identified seven haplotypes (Table 3): Haplotypes 1 (TCT), 2 (CTG), 3 (CTT), 4 (CCT), 5 (TCG) and 6 (TTT) occurred in both controls and HTLV-infected individuals (Table 3), whereas haplotype 7 (TTG) was found only among HTLV-infected patients (p = 0.0119), specifically in the subgroup with HAA (p = 0.0510). Haplotype 4 (CCT) was more frequent among HTLV-infected individuals compared to the controls (p = 0.0138).

### 3.2. CD4+ and CD8+ T cell count and proviral load

Comparative analysis of the CD4+ T cell count between HAA patients and the asymptomatic individuals did not reveal any significant differences (Fig. 2A), as was the case relative to polymorphisms rs8103142, rs8099917 and rs12979860 (data not shown).

No difference was found in the CD8+ T cell count between patients with HAA and the asymptomatic patients (Fig. 2B). Nevertheless, when the presence of polymorphism rs8099917 was considered (Fig. 3J), the CD8+ T cell count was higher among patients with both HAA and genotype TT compared to the asymptomatic patients (p < 0.05). Polymorphisms rs8103142 and rs12979860 were not associated with significant differences (data not shown).

The proviral load for asymptomatic patients and HAA patients is depicted in Fig. 2C. No significant difference was found in proviral load, regardless of the presence of polymorphisms rs8103142 and rs12979860 (data not shown). However, when the presence of polymorphism rs8099917 was considered (Fig. 3K), a difference was found between patients with genotypes TT or TG/GG in the HAA group (p < 0.05) as well as between the groups of asymptomatic and symptomatic individuals with genotype TT (p < 0.05).

### 3.3. Serum cytokine concentrations

The concentration of TNF-α did not significantly differ between asymptomatic individuals and HAA patients (Fig. 2D), regardless of whether the presence of polymorphisms rs8099917, rs8103142 and rs12979860 was considered (data not shown).

The concentration of TNF-β did not significantly differ between patients with HAA and asymptomatic patients (Fig. 2E), whether the presence polymorphism rs8099917 was considered (data not shown). However, when the presence of polymorphism rs8103142 was considered, the TNF-β levels were higher in patients with both HAA and genotype TT compared to those with genotype TC/CC (Fig. 3F; p < 0.05). Considering the presence of polymorphism rs12979860 (Fig. 3A), the TNF-β levels were higher in symptomatic patients with genotype CC compared to those with genotype CT/TT (p < 0.05). IFN-γ levels were also higher among patients with both HAA and genotype CC compared to patients with genotype TT (p < 0.05).

### Table 2

Genetic models and the risk of developing HAA in HTLV-patients.

<table>
<thead>
<tr>
<th>Genetic model</th>
<th>SNP Genotype</th>
<th>Frequencies</th>
<th>OR</th>
<th>95% CI</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asymptomatic</td>
<td>HAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>rs8103142</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>15 (23.8)</td>
<td>10 (31.3)</td>
<td>1</td>
<td>0.27–1.77</td>
<td>0.4376</td>
</tr>
<tr>
<td>TC + CC</td>
<td>48 (76.2)</td>
<td>22 (68.7)</td>
<td>0.6875</td>
<td>0.27–1.77</td>
<td>0.4376</td>
</tr>
<tr>
<td>Recessive</td>
<td>rs8099917</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TC</td>
<td>48 (76.2)</td>
<td>23 (71.9)</td>
<td>1</td>
<td>0.48–3.28</td>
<td>0.6476</td>
</tr>
<tr>
<td>CC</td>
<td>15 (23.8)</td>
<td>09 (28.1)</td>
<td>1.2522</td>
<td>0.48–3.28</td>
<td>0.6476</td>
</tr>
<tr>
<td>Dominant</td>
<td>rs12979860</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>29 (45.3)</td>
<td>15 (46.9)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG + GG</td>
<td>35 (54.7)</td>
<td>17 (53.1)</td>
<td>0.9390</td>
<td>0.40–2.20</td>
<td>0.8848</td>
</tr>
<tr>
<td>Recessive</td>
<td>rs12979860</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TG</td>
<td>57 (89.1)</td>
<td>29 (90.6)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>07 (10.5)</td>
<td>03 (9.4)</td>
<td>0.8424</td>
<td>0.20–3.50</td>
<td>0.8134</td>
</tr>
</tbody>
</table>

HAA: HTLV-1-associated arthropathy.

<sup>a</sup> Simple logistic regression.
The IL-8 levels did not significantly differ between asymptomatic patients and patients with HAA (Fig. 2H), whether the presence of polymorphisms rs8099917 and rs8103142 was considered (data not shown). However, when polymorphism rs12979860 (Fig. 3D) was considered, the IL-8 levels were lower among symptomatic patients with genotype CC compared to patients with genotype CT/TT (*p < 0.05) and asymptomatic patients (*p < 0.05).

IL-6 levels were lower among patients with HAA (*p < 0.05; Fig. 2G). When the presence of polymorphism rs8103142 was considered, IL-6 levels were lower among asymptomatic patients with genotype CC compared to those with genotype TT (Fig. 3H; *p < 0.05). As for polymorphism rs12979860, IL-6 levels were higher among asymptomatic patients with genotypes CT and TT (Fig. 3C) compared to patients with HAA (p < 0.05). Similar results were obtained by performing intragroup analyses comparing asymptomatic patients with genotype CC (*p < 0.05).

The levels of IL-10 were higher among patients with HAA compared to asymptomatic individuals (Fig. 2I; *p < 0.05). When the presence of polymorphism rs8099917 was considered (Fig. 3M), IL-10 levels were higher among symptomatic patients with genotypes TG and GG (*p < 0.05) compared to asymptomatic patients. When considering polymorphisms rs8103142 (Fig. 3I; *p < 0.05) and rs12979860 (Fig. 3E; *p < 0.05), IL-10 levels were higher among patients with both HAA and genotypes TC or CC and TT or TT, respectively, compared to asymptomatic patients. Similar results were found among HAA patients with polymorphism rs8103142 genotypes TC and CC (*p < 0.05).

3.4. Cytokine balance comparison

A comparison of the serum cytokine balance between HAA patients and asymptomatic individuals (Fig. 2J–N) revealed signif-
significant difference in TNF-α/IL-10 (Fig. 2J; \(p < 0.05\)), TNF-β/IL-10 (Fig. 2K; \(p < 0.05\)) and IFN-γ/IL-10 ratios (Fig. 2L; \(p < 0.05\)), with lower values observed in symptomatic patients. The IL-6/IL-10 (Fig. 2M) and IL-8/IL-10 ratios (Fig. 2N) did not exhibit significant differences.

3.5. Correlation of CD4+/CD8+ T cell count and proviral load with cytokine concentrations

Correlation analyses revealed significant differences between TNF-α levels and CD4+ (Fig. 4A; \(p = 0.0212\); \(r = 0.3995\)) and CD8+ T cell counts (Fig. 4B; \(p = 0.0484\); \(r = 0.3461\)). Similar results were obtained with respect to the correlation between IFN-γ levels (Fig. 4C; \(p = 0.0549\); \(r = 0.3322\)) and proviral load among asymptomatic patients. Analyzing the correlation of cytokines TNF-β, IL-6, IL-8 and IL-10 with proviral load and CD4+ and CD8+ T cell count between asymptomatic and HAA patients did not reveal any significant differences (data not shown).

4. Discussion

Few studies have sought to explain the development of HAA. Yakova et al. (2005) found that the HTLV proviral load was higher in a group of 12 patients with rheumatoid arthritis compared with asymptomatic infected controls and similar to that exhibited by patients with HAM/TSP. These findings indicate that just as in HAM/TSP, viral replication also plays a relevant role in the development of HAA [32]. Our results disagree with those of Yakova et al. (2005), as the proviral load in the peripheral blood of the 32 individuals with HAA was similar to that detected in asymptomatic patients. Our results thus suggest that the mechanism underlying HAA might differ from the one attributed to HAM/TSP. Our results also did not demonstrate any difference in CD4+ and CD8+ T cell counts between symptomatic and asymptomatic patients, which suggests that the number of these cells is not a limiting factor for the development of HAA.

One explanation put forward to account for the development of HAA asserts that HTLV displays tropism for synovial cells [13], which, once infected, are stimulated to proliferate by viral protein Tax [14]. The Tax protein might further activate transcription factors such as NF-κB, consequently stimulating the production of several proinflammatory cytokines, including TNF-α, IFN-γ, IL-6 and TNF-β [14,33,34], resulting in cartilage destruction and joint erosion. Our results did not indicate any significant difference in serum TNF-α, TNF-β, IFN-γ and IL-8 concentrations between patients with HAA and asymptomatic individuals. However, IL-10 levels were higher and IL-6 levels were lower among the former
compared with the latter, which might be related to the use of antirheumatic drugs, since it has been reported that these drugs decrease IL-6 dosages [35], but without effect on the dosages of IL-10 [36,37]. Furthermore, we measured the serum levels of both cytokines, which might differ from their synovial concentrations, which characterize local inflammatory reactions. These results are consistent with previous report that described high levels of IL-10 among patients with arthritis [37].

Such high IL-10 and low IL-6 levels in the peripheral blood might be indicative of good prognosis, as high IL-6 expression has been associated with the occurrence of HAA [38], the reason being that IL-6 is associated with the stimulation of bone resorption [39]. However, high IL-10 levels were also found in patients with ATLL [40], which might be because the viral Tax protein is able to stimulate IL-10 production [41]. Depending on the activation status of CD8+ T cells, IL-10 might stimulate their proliferation [42] and even increase their intracellular expression of the Th1 cytokine profile [43]. When such cytokines are secreted in the synovia, they might play a role in the development of joint disease; this hypothesis still requires further confirmatory studies.

The balance between proinflammatory and regulatory cytokines affords a better demonstration of the mechanisms involved in the immune response of patients. In the present study, the average TNF-α/IL-10, TNF-β/IL-10 and IFN-γ/IL-10 ratios were lower among HAA patients compared with asymptomatic patients, which might be related to the use of antirheumatic drugs, as mentioned above.

Correlation analysis showed that in the group of asymptomatic patients, TNF-α levels increased in parallel with the number of CD4+ and CD8+ T cells. Similarly, a positive correlation was also found between INF-γ levels and the proviral load, as described previously [44]. However, no such correlations were detected relative to patients with HAA, indicating that these phenomena are not required for HAA to occur. That same lack of correlation was also found by other authors relative to HAM/TSP [44,45].

IL-28 exhibits antiviral and immunomodulatory activity; it is secreted by macrophages and plasmacytoid dendritic cells – pDCs [23-25,46,47] and has been shown to increase the number of CD8+ T cells in the peripheral blood of mice and monkeys and to reduce the number of regulatory T cells in mice [26,48].

Studies have shown that polymorphisms of the IL28 gene are associated with the progression of HTLV-1 infection [49] and that these polymorphisms play an important role in the development and control of HAM/TSP pathogenesis [50,51]. In the present study, the allelic and genotypic frequencies of the investigated polymorphisms and the analyzed genetic models suggest that these polymorphisms by themselves do not determine the susceptibility to infection and the development of HAA. Our results further indicate that the number of CD4+ T cells in the peripheral blood is not associated with the genetic variability observed in the IL28 gene.

Analyzing polymorphism rs8099917 revealed that the proviral load and CD8+ T cell count were higher in patients with HAA and genotype TT compared with asymptomatic patients. These findings suggest that genotype TT is associated with poor prognosis of HAA, as the increase in the number of CD8+ T cells was associated with greater proviral load; these phenomena might make such patients a high-risk group for HAM/TSP. Studies have shown that genotype TT is associated with lower IL-28 expression, with consequently lower stimulation of the interferon stimulated genes – ISGs [52,53]. However, in patients treated with IFN-α, genotype TT is associated with increased stimulation of ISGs, resulting in better responses to treatment [54], which makes it protective against infection with the hepatitis C virus – HCV [55–58]. Patients with HAA are often treated with antirheumatic drugs that suppress the immune system, which in fact might be a determinant for the progression of infection in patients with genotype TT and low ISG expression.

SNP rs8099917 allele G has been associated with high ISG expression [53], which accounts for the high IL-6 levels found among the asymptomatic patients. Nevertheless, this cytokine profile might make these patients a high-risk group for HAA [40]. Recently, it was demonstrated the genotype GG (rs8099917) associated with HAM/TSP [51].

Patients with HAA and the wild-type genotype (CC) of polymorphism rs12979860 exhibited high TNF-β and IFN-γ production, which might increase the synovial expression of major histocompatibility complex (MHC) molecules and thus contribute to the pathogenesis of infection [14,59] in addition to stimulating bone resorption and inhibiting the synthesis of collagen [60]. Polymorphism rs12979860 was also associated with reduced IL-8 levels in the peripheral blood, which may be a protective factor against the development of HAA, as IL-8 is related to the development of rheumatoid arthritis [61].

Patients with HAA and genotype CT/TT (rs12979860) exhibited reduced TNF-β and IFN-γ production, which might be related to the possible suppression of ISG expression [55]. Several authors characterized genotype CC as having a good immune response [62] and genotype CT/TT as having a suppressed immune response [62–64], which might account for the results of the present study. However, such good immune responses are associated with the increased production of proinflammatory cytokines, which is counterbalanced by the aforementioned reduction in IL-8 levels. As a result, this polymorphism might be associated with poor prognosis in the development of HAA. The differences in IL-6 and IL-10 levels might be explained by the large linkage imbalance between polymorphisms rs8099917 and rs12979860 [65,66]. As such, the difference is attributed to polymorphism s12979860 and the use of antirheumatic drugs by those patients. Our results agree with Sanabani et al. (2012), showing a lack of correlation between the rs12979860 polymorphism and HTLV-1 proviral load [67].
The lack of a correlation between SNP rs8103142 and CD8+ T cell count, proviral load, and TNF-α and IL-8 levels suggests that this polymorphism has no relationship with these markers. Nevertheless, patients with HAA and the wild-type genotype (TT) of SNP rs8103142 exhibited higher TNF-β and IFN-γ levels, which might be an aggravating factor for the development of the disease. High TNF-β levels have been shown to stimulate the bone resorption and inhibit collagen synthesis in vitro [60], while IFN-γ stimulates the expression of MHC molecules, leading to increased antigen presentation in the synovial membrane [14,59]. These differences, however, might be related to the large linkage imbalance between polymorphisms rs8103142 and rs12979860 [65]. Those patients with HAA and genotypes TC and TT exhibited higher IL-10 levels compared with asymptomatic individuals, which might have a protective effect by regulating the high TNF-β and IFN-γ levels of the wild-type genotype. Nevertheless, asymptomatic individuals with the same genotype exhibited higher IL-6 levels.

Analysis of the set of haplotypes constituted by the investigated polymorphisms showed that haplotype CTT might be associated with susceptibility to HTLV infection. Studies have suggested that polymorphism rs8103142 might induce a less effective, albeit more sustained, antiviral response [66]. In addition, alleles C (rs12979860) and T (rs8099917) have been associated with good immune responses [56,67,68] and reduced II-28 and ISG expression [52,53], respectively. In combination, these characteristics might influence the susceptibility to HTLV infection and the high frequency of this haplotype among asymptomatic patients.

Haplotype TTG might be associated with the progression to HAA, as alleles T (rs12979860) and C (rs8099917) are related to aberrant induction of immune system pathways [62] and increased ISG expression [53], respectively, which combined might influence the progression of infection.

5. Conclusion

We conclude that the IL28B gene and its polymorphisms might play an important role in the development of HAA and influence the progression of disease. The reason is that genotype TT (rs8099917) seems to behave as a risk factor for having high cytotoxic T cell counts and proviral load levels, while the presence of genotypes TT (rs8103142) and CC (rs12979860) seems to be an aggravating factor that is associated with elevated serum TNF-β and IFN-γ levels. In contrast, genotypes TC and CC of polymorphism rs8103142 most likely behave as protective factors, as they are associated with high IL-10 levels. In addition, the results suggest that haplotype CCT is associated with susceptibility to HTLV infection and haplotype TCTG with progression to HAA.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

ACRV led the study design, data analysis and writing. KSGS performed all molecular analysis and writing. BBS and TCSF contributed substantially to the data analysis and writing. RCMS and CAMC examined the patients. VNA and RNMF assisted with cytokine analysis. LFAM, MOGI and RI assisted with study design and writing.

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