Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:52:e20180481: 2019
doi: 10.1590/0037-8682-0481-2018

Short Communication

Polymorphism in the interleukin-10 gene is associated with overactive bladder phenotype associated with HTLV-1 infection

***Marcos Braz*[1],[2], *Joyce Moura Oliveira*[2], *Jamile Leão Rêgo*[1],[2], *Edgar Marcelino de Carvalho*[1],[2],[3],[5], *Silvane Maria Braga Santos*[2],[4],[5] *and Léa Cristina Castellucci*[1],[2],[5]**

[1]. Programa de Pós-graduação em Ciências da Saúde, Universidade Federal da Bahia, Salvador, BA, Brasil.
[2]. Serviço de Imunologia, Universidade Federal da Bahia, Salvador, BA, Brasil.
[3]. LAPEC, Instituto Gonçalo Moniz, Fundação Osvaldo Cruz, Salvador, BA, Brasil.
[4]. Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brasil.
[5]. Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais, Brasília, DF, Brasil.

Abstract

Introduction: Human T-cell lymphotropic virus type 1 (HTLV-1)-associated inflammatory diseases are not well understood; however, their clinical manifestations may be influenced by the host genetic background. **Methods**: We genotyped 298 individuals with HTLV-1 and 380 controls for interleukin-10 (IL10) gene variants—rs3024496, rs1800871, rs1800896—and used logistic regression analysis to determine their association with clinical phenotypes. **Results**: No association with HTLV-1 infection was observed. However, allele A of rs1800896 (1082bp upstream) was associated with protection against neurological impairment, specifically overactive bladder (OR=0.447, 95% CI 0.28–0.70, p=0.001). **Conclusions**: Our data suggests that IL10 regulation ameliorates neurological damage in HTLV-1 infections.

Keywords: HTLV-1. SNP rs1800896 (-1082bp A/G). Overactive bladder.

Human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus with two main clinical manifestations: Adult T-cell leukemia/lymphoma (ATLL) and HTLV-1 associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP), a demyelinating disease of the spinal cord, in addition to other inflammatory diseases that impact the morbidity of people infected with this virus[1]. It is estimated that about 10 million people are infected with HTLV-1 globally[2]. The virus is endemic to Japan, the Caribbean Basin, and in some Latin American countries. In Brazil, HTLV-1 infection is most prevalent in the Northeast region of the country[2]. The vast majority of people infected with HTLV-1 remain asymptomatic, with approximately 5–10% developing clinical symptoms[2]. Host factors such as genetics, proviral load, and immune response may contribute to the development of ATLL, HAM/TSP, or other clinical manifestations such as urinary complaints in HTLV-1 positive individuals who develop an overactive bladder[3,4]. Overactive bladder is considered an early sign of HAM/TSP and therefore these patients are identified as probable HAM/TSP cases according to the criteria proposed by De Castro-Costa[5]. The HTLV-1 virus has a tropism for $CD4^+$ and $CD8^+$ T cells, but other cells can also be infected[6]. Cultures of peripheral blood mononuclear cells (PBMC) from HAM/TSP patients show increased spontaneous production of pro-inflammatory cytokines, such as IFN-γ and TNF, lower production of IL-10, and a high proviral load compared to asymptomatic carriers[6]. Nevertheless, despite this knowledge of HTLV-1 immunopathogenesis, there are few studies describing the role of host genetic factors in HTLV-1 infection. Of the documented genes, *IL28B*, *IL10*, and *IL6*, are used as biomarkers across different populations[7-10]. In this context, the study of immune

Corresponding author: Léa Cristina Castellucci.
e-mail: leacastel@hotmail.com
Orcid: 0000-0002-9625-2469
Received 18 December 2018
Accepted 29 March 2019

response genes may reveal new markers of susceptibility or resistance to infection and disease. Here, we investigate whether polymorphisms in the *IL10* gene are associated with genetic susceptibility to HTLV-1 infection in a population from the city of Salvador, Northeast Brazil.

HTLV-1 infected patients (n =298) of both sexes, aged 18 to 78 years, were enrolled at Complexo Hospitalar Universitário Professor Edgard Santos (ComHUPES), an outpatient referral center for the treatment of HTLV-1 in the city of Salvador-Bahia, Brazil. The participants were divided into three groups according to their clinical form: Group 1 – 164 asymptomatic HTLV-1 carriers; Group 2 – 63 HTLV-1 infected individuals with symptoms of overactive bladder; and Group 3 – 71 HTLV-1 infected patients diagnosed with HAM/TSP. A group of 380 volunteer blood donors recruited at Fundação de Hematologia e Hemoterapia da Bahia (HEMOBA) were used as healthy controls for comparison with the HTLV-1 infected group. This control group comprised 264 males and 116 females, with a mean age of 34.80 ± 10.24 years. This study was approved by the ethical committee of the Maternidade Climério de Oliveira – Universidade Federal da Bahia (N° 35/2013). Demographic data (age, sex) by phenotype are provided in **Table 1**. Three single nucleotide variants (SNVs) of the *IL10* gene, rs3024496 (+4976 A/G), rs1800871 (-819bp A/G), and rs1800896 (-1082bp A/G), were genotyped by TaqMan RT-PCR (Thermo Fisher®) using previously designed genotyping assays. **Table 2** shows the allele frequencies for the SNVs in HTLV-1 infected cases and blood bank controls. Tests to check for Hardy-Weinberg equilibrium, and an unconditional logistic regression analysis to determine allele-wise (1 *df* test) and genotype-wise (2 *df* test) associations between *IL10* SNPs and HTLV-1 clinical phenotypes, were carried out in STATA 8.2 using the GenAssoc package. For stratified analyses, we corrected for multiple testing by multiplying our p-values by 9 to take the 3 SNVs x 3 clinical phenotypes into account. When comparing HTLV-1 infected individuals with blood bank controls, we found no associations between the SNVs at *IL10* +4976 A/G, -819bp A/G, or -1082bp A/G and *HTLV-1 per se,* as shown in **Table 3**. However, when we stratified patients according to neurological impairment (i.e. HTLV-1 overactive bladder or HAM/TSP) and compared these groups to infected individuals without neurological disease symptoms (i.e. HTLV-1 carriers) we observed an allele-wise association between the *A* allele of the -1082bp A/G polymorphism (rs1800896) and protection against overactive bladder (OR = 0.447, CI 0.28–0.70, p = 0.001, $p_{corrected}$ = 0.009). In the genotype-wise analysis, we observed significant protection against overactive bladder by both A/A homozygotes (OR = 0.17; CI: 0.06-0.45; p = 0.0001, $p_{corrected}$ = 0.0009) and A/G heterozygotes (OR = 0.30, CI: 0.12–0.74, p = 0.0001, $p_{corrected}$ = 0.0009) compared to G/G homozygotes. There were no associations with the HAM/TSP group. No significant associations were observed for the other *IL10* SNVs. The association between the A allele and HTLV-1 overactive bladder protection is robust to multiple testing correction for 3 variants x 3 clinical phenotypes ($p_{corrected}$ = p multiplied by 9).

HTLV-1 infection is a public health problem as it impacts the quality of life of HTLV-1 infected individuals. Although asymptomatic in most carriers, a significant proportion of HTLV-1 infected individuals will develop virus-related syndromes, culminating in HAM/TSP. In this study we identified an association between the allele A at the SNV rs1800896 located 1082bp upstream of the *IL10* gene and protection against the development of overactive bladder associated with HTLV-1 infection, which is considered to be a precursor to HAM/TSP[5]. Interestingly, we did not find an association with HAM/TSP. Others have looked for associations between *IL10* promoter region variants and clinical manifestations of HTLV-1 infection[11,12]. In a study undertaken in Japan,[11] protection against HAM/TSP was associated with the A allele for the SNV at position -592bp when compared to HTLV-1 carriers. Similar to our study, these studies did not identify an association between promoter variants at -819bp and -1082bp, or at -2763bp, -2849bp or -3575bp, and HAM/TSP. Using reporter gene studies. these researchers demonstrated that the allele A at -592bp was

TABLE 1: Distribution of sex and age in the groups.

Characteristics	Carriers		Overactive bladder		HAM/TSP		Total	
	N	%	N	%	N	%	N	%
	164	**55.03**	**63**	**21.14**	**71**	**23.83**	**298**	**100**
Male*	63	38.4	15	23.8	22	30.9	100	33.5
Age>50 years*	47	74.6	74	46.6	10	45.4	64	64
Age≤50 years*	16	25.4	8	53.3	12	54.5	36	36
Female*	101	61.5	48	76.2	49	69	198	66.4
Age>50 years*	57	56.4	30	62.5	32	65.3	119	60.1
Age≤50 years*	44	43.5	18	37.5	17	34.7	79	39.9
Average age (min-max)	51 (23-76)		54	(29-70)	53	(27-78)	53	(23-78)

*no statistically significant differences were found between the sex and age of the groups p>0,005.

TABLE 2: Allelic and genotypic frequencies for polymorphisms in the IL10 gene.

IL10 rs1800896 (-1082 bp)	CASES		CONTROLS		TOTAL	
	N	%	N	%	N	%
A	379	67,2	398	63	777	65,0
G	185	32,8	234	37	419	35,0
AA	130	46,1	129	40,8	259	43,3
AG	119	42,2	140	44,3	259	43,3
GG	33	11,7	47	14,9	80	13,4
IL10 rs1800871 (-819 bp)						
A	196	37,3	231	37,9	427	37,6
G	330	62,7	379	62,1	709	62,4
AA	42	16	43	14,1	85	15,0
AG	112	42,6	145	47,5	257	45,2
GG	109	41,4	117	38,4	226	39,8
IL10 rs3024496 (+4976 bp)						
A	321	62,2	372	60,8	693	61,4
G	195	37,8	240	39,2	435	38,6
AA	109	42,2	111	36,3	220	39,0
AG	w103	39,9	150	49,0	253	44,9
GG	46	17,8	45	14,7	91	16,1

associated with lower transcriptional activation compared to the allele C in HTLV-1 Tax-induced Jurkat T cells[11]. A second study carried out in Iran[12] focused on comparisons of HTLV-1 carriers, or HAM/TSP positive individuals, with healthy controls. In this case they demonstrated that the allele T at -819bp, and the allele A at -592bp, were associated with HTLV-1 carriage and with HAM/TSP, respectively, as was a haplotype that included the -1082bp allele A. They concluded that the A/T/A haplotype was a risk factor for HTLV-1 infection *per se*, but did not confer additional risk of developing HAM/TSP. Neither the Japanese nor the Iranian study looked at the phenotype of overactive bladder. The different results obtained in these studies may be due to differences in genetic background, as well as due to the demographic and environmental variations and sample size. This could include the presence of haplotypes carrying different variations of the functional promoter region upstream of the *IL10* gene, and/or to differences in functional interactions between variants. Further work is required to determine whether the -1082bp variant, which was associated with overactive bladder in our study, is the functional etiological variant or is in linkage disequilibrium with alternative functional variants affecting HTLV-1 disease. Additional research is required to determine whether there are differences in the pathogenesis of overactive bladder and HAM/TSP that could account for the association with the *IL10* -1082bp variant with the overactive bladder phenotype but not with HAM/TSP. Others have shown[13] that while patients with HTLV-1 overactive bladder and HAM/TSP phenotypes share some immunological features and similar proviral loads, patients with the overactive bladder phenotype are better able to modulate their inflammatory immune response. Further analysis of the association between clinical phenotypes and genetic variants influencing the balance between pro- and anti-inflammatory responses could help us develop prognostic biomarkers for use in HTLV-1 infected individuals.

ACKNOWLEDGEMENTS

We thank the staff of Magalhães Neto HTLV-1 Outpatient, and HEMOBA for logistical support in sample collection. We also thank the INCT-DT (N°573839/2008-5) for the financial support. We thank Jenefer Blackwell for her helpful review of the manuscript and editorial suggestions.

TABLE 3: Results of logistic regression analyses for the genotyped *IL-10* polymorphisms.

Cases *Vs.* Controls				
*IL-10*_rs1800896	OR	CI (95%)	*P*	
A	1,194	0,94-1,50	0,136	
G	0,837	0,66-1,05	0,136	
A/G X G/G	1,210	0,72-2,01	0,461	
A/A X G/G	1,435	0,86-2,38	0,163	
*IL-10*_rs1800871	OR	CI (95%)	*P*	
A	0,975	0,76-1,23	0,836	
G	1,025	0,80-1,29	0,836	
A/G X G/G	0,829	0,57-1,18	0,306	
A/A X G/G	1,048	0,63-1,72	0,853	
*IL-10*_rs3024496	OR	CI (95%)	*P*	
A	1,058	0,83-1,33	0,633	
G	0,944	0,74-1,19	0,633	
A/G X G/G	0,671	0,41-1,08	0,105	
A/A X G/G	0,960	0,58-1,56	0,872	
Stratification of individuals by clinical form				
(A)				
*IL-10*_rs1800896	OR	CI (95%)	*P*	*P corrected*
A	0,447	0,28-0,70	**0,001**	**0,009**
G	2,235	1,40-3,54	**0,001**	**0,009**
A/G X G/G	0,300	0,12-0,74	**0,0001**	**0,0009**
A/A X G/G	0,175	0,06-0,45	**0,0001**	**0,0009**
(B)				
*IL-10*_rs1800896	OR	CI (95%)	*P*	***P corrected***
A	0,987	0,63-1,53	**0,956**	**0,36**
G	1,012	0,65-1,57	**0,956**	**0,36**
A/G X G/G	0,505	0,18-1,40	**0,376**	**0,16**
A/A X G/G	0,669	0,24-1,80	**0,376**	**0,16**

Abbreviations: OR: odds ratio; **CI:** confidence interval; Stratified HTLV-1 groups: Neurological impairment causing overactive bladder (N=63); neurological impairment causing HAM/TSP (N=71); Asymptomatic carriers (N=.164). (A) Overactive bladder (cases) *X* Asymptomatic (control carriers); (B) HAM/TSP (cases) *X* Asymptomatic (control carriers).

Conflict of Interest

The authors declare that they have no commercial or financial conflicts of interest.

Financial Support

Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT N˚ 573839/2008-5).

REFERENCES

1. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A. 1980;77(12):7415-9.
2. Gessain A, Cassar O. Epidemiological Aspects and World Distribution of HTLV-1 Infection. Front Microbiol. 2012;15(3):388.

3. Araujo AQ, Silva MT. The HTLV-1 neurological complex. Lancet Neurol. 2006;5(12):1068-76.

4. Castro NM, Freitas DM, Rodrigues W Jr, Muniz A, Oliveira P, Carvalho EM. Urodynamic features of the voiding dysfunction in HTLV-1 infected individuals. Int Braz J Urol. 2007;33(2):238-44.

5. De Castro-Costa CM, Araújo AQ, Barreto MM, Takayanagui OM, Sohler MP, da Silva EL, et al. Proposal for diagnostic criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). AIDS Res Hum Retroviruses. 2006;22(10):931-5.

6. Santos SB, Porto AF, Muniz AL, de Jesus AR, Magalhães E, Melo A, et al. Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers. BMC Infect Dis. 2004;2;4:7.

7. Assone T, de Souza FV, Gaester KO, Fonseca LA, Luiz Odo C, Malta F, et al. IL28B gene polymorphism SNV rs8099917 genotype GG is associated with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in HTLV-1 carriers. PLoS Negl Trop Dis. 2014;18:8(9).

8. GadelhaSR,JuniorAlcântaraLC,CostaGC,AcostaAX,RiosD,Kashima S, et al. Correlation between polymorphisms at interleukin-6 but not at interleukin-10 promoter and the risk of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis in Brazilian individuals. J Med Virol. 2008;80(12):2141-6.

9. Treviño A, Lopez M, Vispo E, Aguilera A, Ramos JM, Benito R, et al. HTLV Spanish Study Group. Development of tropical spastic paraparesis in human T-lymphotropic virus type 1 carriers is influenced by interleukin 28B gene polymorphisms. Clin Infect Dis. 2012;55(1):e1-4.

10. Vallinoto AC., Santana BB, Sá KS, Ferreira TC, Sousa RC, Azevedo VN, et al. HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis Is Not Associated with SNP rs12979860 of the IL-28B Gene. Mediators Inflamm. 2015;2015:804167.

11. Sabouri AH, Saito M, Lloyd AL, Vine AM, Witkover AW, Furukawa Y, et al. Polymorphism in the interleukin-10 promoter affects both provirus load and the risk of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis. J Infect Dis. 2004;190(7):1279-85.

12. Shirdel A, Azarpazhooh MR, Sahebari M, Ghanbari M, Mirfeizi SZ, Hutchinson I, et al. Association of IL-10 Gene Polymorphisms and Human T Lymphotropic Virus Type I-Associated Myelopathy/tropical Spastic Paraparesis in North-East of Iran (Mashhad). Iran J Basic Med Sci. 2013;16(3):258-63.

13. Santos SB, Oliveira P, Luna T, Souza A, Nascimento M, Siqueira I, Tanajura, D, Muniz AL, Glesby MJ, Carvalho EM: Immunological and Viral Features in Patients With Overactive Bladder Associated With Human T-Cell Lymphotropic Virus Type 1 Infection . J Med Virol. 2012; 4:1809-17.