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ORIGINAL COMMUNICATION

Association between HLA alleles and HAM/TSP in individuals infected with HTLV-1

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Abstract HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) develops in less than 5 % of HTLV-1 carriers. It is unclear which factors trigger the development of disease. The aim of this study was to explore the influence of HLA alleles on the development of HAM/TSP. A total of 40 HTLV-1-infected individuals belonging to the Spanish HTLV-1 cohort were examined. HTLV-1 proviral load was measured by real-time polymerase chain reaction. HLA class I (A, B, C) and class II (DRB1, DQB1) alleles were genotyped using the bead array technology. Median HTLV-1 proviral load in 12 HAM/TSP patients was greater than in 28 asymptomatic carriers (637 vs. 71 copies per 10^4 peripheral blood mononuclear cells; $p = 0.006$). Moreover, HAM/TSP was significantly associated with HLA-B*07 and HLA-DRB1*01:01 ($p = 0.039$). Interestingly, individuals with these HLA alleles had greater HTLV-1 proviral load than asymptomatic carriers ($p = 0.036$). In summary, HLA testing should be considered in asymptomatic HTLV-1 individuals and close monitoring of HTLV-1 proviral load along with periodic neurological evaluations should be prioritized in HLA-DRB1*01:01 and HLA-B*07 carriers.

Keywords HTLV-1 · HLA · Proviral load · Paraparesis

On behalf of the HTLV Spanish Study Group.

Members of HTLV Spanish Study Group are listed in Appendix.

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Introduction

Human T-lymphotropic virus type 1 (HTLV-1) was discovered in 1980 [1]. It is estimated that 15–20 million people are infected worldwide [2–4]. Endemic foci of infection have been reported in Japan, Melanesia, Iran, Central and West Africa, the Caribbean, and South America [5]. HTLV-1 is mainly transmitted by cell-to-cell contact [6, 7], since extracellular release of viral particles is a rare phenomenon in vivo, if it exists at all. Infected mothers may transmit the virus to their newborns through breast-feeding. Sexual intercourse and parenteral exposure may also contribute to inter-individual spread of HTLV-1 [2, 8, 9].

Although most HTLV-1-infected persons remain lifelong asymptomatic, approximately 3 % develop adult T cell leukemia/lymphoma (ATLL) and another 3 % develop HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [10]. HAM/TSP is a chronic inflammatory disease of the central nervous system characterized by spastic paraparesis, sphincter dysfunction, and mild sensory disturbance in the lower extremities [11]. The reason why some HTLV-1-carriers develop HAM/TSP remains

largely unknown, although immune-mediated mechanisms seem to be involved [12]. Clinical outcomes in HTLV-1 carriers most likely depend on an interaction between viral and host genetic factors [7]. In this regard, a high proviral load, specific viral variants and early life infection, such as perinatally from infected mothers, have all been associated with the development of clinical complications in HTLV-1 carriers [13–17].

Studies conducted more than a decade ago highlighted that specific human leukocyte antigen (HLA) haplotypes could influence the susceptibility to develop either ATLL or HAM/TSP by virtue of differences in host immune responsiveness to HTLV-1 specific antigens [18]. The presence of HLA-A*02 and/or HLA-Cw*08 was associated with a low HTLV-1 proviral load and a reduced risk of HAM/TSP [19, 20]. In contrast, HLA-B*07, HLA-B*54:01 and/or HLA-DRB1*01:01 were associated with and increased risk of disease [21–24]. Differences in methodologies to monitor HTLV-1 proviral load, criteria use to define HAM/TSP and demographics could all account for discordant results comparing those studies [25]. Herein, we investigate the potential association of HLA class I and II alleles with the development of HAM/TSP in a well characterized cohort of HTLV-1 individuals.

Patients and methods

The Spanish HTLV register records all reported cases of HTLV-1 and HTLV-2 infections in Spain since 1989. Up to January 2012, a total of 199 individuals with HTLV-1 infection had been reported in Spain. Twenty-five (13 %) had been diagnosed with HAM/TSP using well established criteria [17]. For the purpose of this study, only patients who had frozen peripheral blood mononuclear cells (PBMC) were chosen.

HTLV-1 proviral load was measured using an in-house real-time PCR method with primers and conditions reported elsewhere [26]. Briefly, DNA was extracted from $1x10^6$ PBMC using QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). Amplification was carried out using Taqman Universal Master Mix II (Applied Biosystems, Foster City, CA) with specific primers and probes targeting the *pol* region of HTLV-1. The HTLV-1 DNA copy number was normalized to the amount of cellular DNA by the quantification in parallel of the human albumin gene. Results were expressed as HTLV-1 DNA copies per 10^4 PBMC.

The analysis of HLA class I (A, B, C) and II (DRB1, DQB1) alleles was performed by PCR-SSO using the bead array technology at the *Centro de Transfusiones de la Comunidad de Madrid* in Spain. For the purpose of this study, alleles HLA-A*02 and HLA-Cw*08 that had previously been associated to protection from HAM/TSP [19, 20], were classified as HLA protectors. In addition, alleles HLA-B*07, HLA-B*54:01 and HLA-DRB1*01:01 that have been associated with disease susceptibility [21–24], were labelled as HLA pathogenic.

Statistical analysis

Results are given as proportions and median values. Comparisons were made using the Chi square test, with Fisher's correction when appropriated. Differences were considered to be significant only when p values were lower than 0.05. All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL).

Results

A total of 40 individuals with HTLV-1 infection had frozen PBMC and were examined. Twelve had been diagnosed with HAM/TSP, with the remaining 28 subjects asymptomatic HTLV-1 carriers. The median age of the study population was 46 years-old, and 57.5 % were females. Although most were from Latin America (80 %), 12.5 % were native Spaniards and 7.5 % were Africans. The main route of infection was heterosexual contact (at least 55 % of cases), with 12.5 % attributed to vertical transmission and 10 % to parenteral exposure. As shown in Table 1, no significant differences in demographics were seen when comparing HAM/TSP patients and asymptomatic HTLV-1 carriers, except for the proportion of native Spaniards. Two of the Spanish HAM/TSP patients acquired HTLV-1 infection following contaminated solid organ transplantation.

Patients with HAM/TSP had a median HTLV-1 proviral load greater than asymptomatic carriers (637 vs. 71 copies per 10^4 PBMC; $p = 0.006$). The prevalence of the different HLA alleles in the study population is depicted in Table 2. The HLA-B*5401 allele was absent in this population. No association between the presence of protective HLA alleles (HLA-A*02 and/or HLA-Cw*08) and HAM/TSP was found. Furthermore, no significant differences in HTLV-1 proviral load were recognized when comparing individuals with and without these HLA alleles (157 vs. 363 copies/10^4 PBMC; $p = 0.3$). In contrast, an association between pathogenic HLA alleles (HLA-B*07 and HLA-DRB1*01:01) and HAM/TSP was found ($p = 0.039$). Moreover, the subset of patients harbouring these alleles exhibited a greater HTLV-1 proviral load than patients with other HLA alleles (575 vs. 157 copies/10^4 PBMC; $p = 0.027$).

The country of origin of HAM/TSP patients harbouring HLA-B*07 and DRB*0101 was Spain ($n = 2$), Brazil ($n = 1$), Ecuador ($n = 1$) and the Dominican Republic

Table 1 Main baseline characteristics of the study population

	All HTLV-1 patients ($n = 40$)	HAM/TSP patients ($n = 12$)	Asymptomatic HTLV-1 carriers ($n = 28$)	p
Median age (years, IQR)	46 (38–46)	47	44	0.2
Female gender (%)	23 (57.5)	8 (67)	15 (54)	0.5
Origin				
Latin America	32 (80 %)	8 (67 %)	24 (86 %)	0.2
Spain	5 (12.5 %)	4 (33 %)	1 (3 %)	0.02
Africa	3 (7.5 %)	0	3 (11 %)	0.5
Risk group				
Sexual	22 (55 %)	6 (50 %)	16 (57 %)	0.9
Vertical	5 (12.5 %)	0	5 (18 %)	0.3
Organ transplant	2 (5 %)	2 (17 %)	0	0.08
Transfusion	2 (5 %)	2 (17 %)	0	0.08
Unknown	9 (22.5 %)	2 (17 %)	7 (25 %)	0.7

Table 2 Comparison of HLA alleles and HTLV-1 proviral load in the study population

	HAM/TSP patients ($n = 12$)	Asymtomatic HTLV-1 carriers ($n = 28$)	p
HLA protective	5 (42 %)	12 (43 %)	1
A*02	5 (42 %)	12 (43 %)	1
Cw*08	0	3 (11 %)	0.5
HLA pathogenic	5 (42 %)	3 (11 %)	0.039
B*07	3 (25 %)	2 (7 %)	0.1
B*54:01	0	0	–
DRB1*01:01	2 (17 %)	1 (3.5 %)	0.2
Median HTLV-1 proviral load (DNA copies/10^4 PBMC)	637	71	0.006

($n = 1$). According to the Allele Frequency net (http://www.allelefrequencies.net) no differences between rates of HLA-B*07 and HLA-DRB1*01:01 alleles should be recognized comparing Spaniards, Latin Americans and African populations (0–10 %) [24].

Discussion

HTLV-1 has spread worldwide but is endemic in some parts of Japan, Central and South America, and Sub-Saharan Africa [25]. More interestingly is the recognition of familial clusters of either ATLL or HAM/TSP, suggesting that host factors may influence the susceptibility to develop disease in HTLV-1 carriers. For this reason, the HLA allelic background has been considered by many investigators as a potential determinant of HTLV-1 disease outcome. Whereas HLA-B*07, HLA-B*54:01 and HLA-DRB*01:01 have been associated with increased susceptibility to HAM/TSP in Japan [21–23, 25], it has not been confirmed in France [26]. In our study, HLA-B*07 and/or HLA-DRB*01:01 were significantly more frequent in HAM/TSP patients than in asymptomatic HTLV-1 carriers. It must be noted that most of our HTLV-1 individuals (80 %), including those with HAM/TSP, came from Latin America. In contrast with others, we did not find any significant association between HAM/TSP and either older age or female gender. However, the relatively small size of our study population might have precluded recognition of any influence of demographics on HTLV-1-associated neurological disease. In contrast, the median HTLV-1 proviral load was significantly greater ($\sim$10-fold higher) in HAM/TSP patients than in asymptomatic carriers, as found in other studies [15, 17].

We did not find any evidence of a protective effect of HLA-A*02 or HLA-Cw*08 on HAM/TSP, as it was originally reported in Japan [25]. This is in agreement with more recent results obtained by others, especially in studies conducted in Europe [26, 28, 29]. More interesting was the recognition of a significant association between pathogenic HLA-B*07 and/or HLA-DRB*01:01 and high HTLV-1 proviral load, which hypothetically could account for the greater proportion of HAM/TSP in this subset of patients compared to HTLV-1 individuals carrying other HLA alleles.

This is the first study that has examined the distribution of HLA alleles in HTLV-1 individuals living in Spain. We acknowledge that our findings should be interpreted cautiously given the relative small size of our study population that reflects the low HTLV-1 prevalence in the country. It ranges from 0.01 % in pregnant women [30] to 0.1 % in hospital outpatient surveys [31]. A total of 199 individuals with HTLV-1 infection had been reported in Spain up to January 2012. Nearly 80 % were foreigners, mainly immigrants from regions in Latin America (57 %) and Africa (15 %), where HTLV-1 is endemic. Additionally, a total of 25 cases of TSP/HAM and 14 of ATLL had been diagnosed. Both HAM/TSP patients and asymptomatic HTLV-1 carriers were chosen from the national Spanish registry, with only the subset of individuals with available frozen PBMC included in the study. It should be highlighted that no significant differences were found in demographics when comparing the study population and the rest of patients, which might account for any unrecognized bias, with the only exception of the Spanish origin. This is probably because two out of the four Spanish HAM/TSP patients acquired HTLV-1 infection following a contaminated solid organ transplantation, which might have involved a large HTLV-1 inoculum [32]. In addition, the

association of HLA DRB1*01:01 and HLA-B*07 alleles with a high HTLV-1 proviral load indirectly further supports our findings.

In summary, HLA alleles DRB1*01:01 and HLA-B*07 influence the susceptibility to develop HAM/TSP in HTLV-1 carriers. This effect seems to be largely mediated by an increased HTLV-1 proviral load. Thus, HLA testing in all HTLV-1 carriers and close monitoring of proviral load and neurological clinical manifestations in those with pathogenic HLA alleles are warranted.

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Conflicts of interest No conflicts for all authors.

Ethical standard This study was approved by the ethics committee of the hospital and all patients gave written informed consent.

Appendix

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References

1. Poiesz B, Ruscetti F, Gazdar A et al (1980) 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 USA 77:7415–7419
2. de The G, Bomford R (1993) An HTLV-I vaccine: why, how, for whom? AIDS Res Hum Retroviruses 9:381–386
3. Hlela C, Shepperd S, Khumalo N, Taylor G (2009) The prevalence of human T-cell lymphotropic virus type 1 in the general population is unknown. AIDS Rev 11:205–214
4. Watanabe T (2011) Current status of HTLV-1 infection. Int J Hematol 94:430–434
5. Proietti F, Carneiro-Proietti A, Catalan-Soares B et al (2005) Global epidemiology of HTLV-I infection and associated diseases. Oncogene 24:6058–6068
6. Igakura T, Stinchcombe J, Goon P et al (2003) Spread of HTLV-1 between lymphocytes by virus-induced polarization of the cytoskeleton. Science 299:1713–1716
7. Bangham C (2003) The immune control and cell-to-cell spread of human T-lymphotropic virus type 1. J Gen Virol 84:3177–3189
8. Manns A, Hisada M, La Grenade L (1999) Human T-lymphotropic virus type 1 infection. Lancet 353:1951–1958
9. Goncalves D, Proietti F, Ribas J et al (2010) Epidemiology, treatment and prevention of HTLV-1-associated diseases. Clin Microbiol Rev 23:577–589
10. Verdonck K, González E, Van Dooren S et al (2007) Human T-lymphotropic virus 1: recent knowledge about an ancient infection. Lancet Infect Dis 7:266–281
11. Nakagawa M, Nakahara K, Maruyama Y et al (1996) Therapeutic trials in 200 patients with HTLV-1-associated myelopathy/tropical spastic paraparesis. J Neuro Virol 2:345–355
12. Oliere S, Douville R, Sze A, Belnaoui S, Hiscott J (2011) Modulation of innate immune responses during HTLV-1 pathogenesis. Cytokine Growth Factor Rev 22:197–210
13. Furukawa Y, Yamashita M, Usuku K et al (2000) Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type 1 in the tax gene and their association with different risks for

HTLV-1-associated myelopathy/tropical spastic paraparesis. J Infect Dis 182:1343–1349
14. Manns A, Cleghorn F, Falk R et al (1993) Role of HTLV-1 in development of non-Hodgkin lymphoma in Jamaica and Trinidad and Tobago. Lancet 342:1447–1450
15. Nagai M, Usuku K, Matsumoto W et al (1998) Analysis of HTLV-1 proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-1 carriers: high proviral load strongly predisposes to HAM/TSP. J Neurovirol 4:586–593
16. Iwanaga M, Watanabe T, Utsunomiya A et al (2010) Human T-cell leukemia virus type 1 (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. Blood 116:1211–1219
17. Grassi MF, Olavarria VN, Kruschewsky A et al (2011) Human T cell lymphotropic virus type 1 (HTLV-1) proviral load of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients according to new diagnostic criteria of HAM/TSP. J Med Virol 83:1269–1274
18. Usuku K, Sonoda S, Osame M et al (1988) HLA haplotype-linked high immune responsiveness against HTLV-I in HTLV-1-associated myelopathy: comparison with adult T-cell leukemia/lymphoma. Ann Neurol 23(suppl):143–150
19. Jeffery K, Usuku K, Hall S et al (1999) HLA alleles determine human T-lymphotropic virus 1 (HTLV-1) proviral load and the risk of HTLV-1-associated myelopathy. Proc Natl Acad Sci USA 96:3848–3853
20. Jeffery K, Siddiqui A, Bunce M et al (2000) The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. J Immunol 165: 7278–7284
21. Yamano Y, Kitze B, Yashiki S et al (1997) Preferential recognition of synthetic peptides from HTLV-1 gp21 envelope protein by HLA-DRB1 alleles associated with HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis). J Neuroimmunol 76:50–60
22. Rafatpanah H, Pravica V, Faridhosseini R et al (2007) Association between HLA-DRB1*01 and HLA-Cw*08 and outcome following HTLV-I infection. Iran J Immunol 4:94–100
23. Catalan-Soares B, Carneiro-Proietti A, da Fonseca F et al (2009) HLA class I alleles in HTLV-1-associated myelopathy and asymptomatic carriers from the Brazilian cohort GIPH. Med Microbiol Immunol 198:1–3
24. Gonzalez-Galarza F, Christmas S, Middleton D, Jones A (2011) Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. Nucleic Acids Res 39:D913–D919
25. Sonoda S, Chuan H, Tajima K (2011) Ethnoepidemiology of HTLV-1 related diseases: ethnic determinants of HTLV-1 susceptibility and its worldwide dispersal. Cancer Sci 102:295–301
26. Deschamps R, Béra O, Belrose G et al (2010) Absence of consistent association between human leukocyte antigen-I and -II alleles and human T-lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis risk in an HTLV-1 French Afro-Caribbean population. Int J Infect Dis 14:e986–e990
27. Treviño A, Parra P, Bar-Magen T et al (2012) Antiviral effect of raltegravir on HTLV-1 carriers. J Antimicrob Chemother 67: 218–221
28. Maloney E, Cleghorn F, Morgan O et al (1998) Incidence of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in Jamaica and Trinidad. J Acquir Immune Defic Syndr Hum Retrovirol 17:167–170
29. Goedert J, Li H, Gao X et al (2007) Risk of human T-lymphotropic virus type I-associated diseases in Jamaica with common HLA types. Int J Cancer 121:1092–1097
30. Treviño A, Aguilera A, Caballero E et al (2009) Seroprevalence of HTLV-1/2 infection among native and immigrant pregnant women in Spain. AIDS Res Hum Retroviruses 25:551–554
31. Treviño A, García J, de Mendoza C et al (2010) Prevalence of HTLV-1/2 infections in Spain: a cross-sectional hospital-based survey. AIDS Res Hum Retroviruses 26:861–864
32. Toro C, Rodés B, Poveda E, Soriano V (2003) Rapid development of subacute myelopathy in three organ transplant recipients after transmission of human T-cell lymphotropic virus type I from a single donor. Transplantation 75:102–104