BRAZ J INFECT DIS 2018;xxx(xx):xxx–xxx

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

In vitro basal T-cell proliferation among asymptomatic HTLV-1 patients co-infected with hepatitis C and/or HIV-1

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ARTICLE INFO

Article history:
Received 13 November 2017
Accepted 2 February 2018
Available online xxx

Keywords:
HCV
HTLV-1
HIV-1
T-cell proliferation

ABSTRACT

Background: Infection with HTLV-1 can be associated with myelopathy/tropical spastic paraparesis (HAM/TSP) and other inflammatory diseases. Lymphocytes from about half of HTLV-1-infected subjects spontaneously proliferate in vitro, and how this phenomenon relates to symptomatic disease and viral burden is poorly understood.
Objective: To evaluate T-cell proliferation in vitro among patients co-infected with HTLV-1/HCV/HIV-1.
Material and methods: From 610 HTLV-infected patients of the HTLV outpatient clinic from Institute of Infectious Diseases "Emilio Ribas" in São Paulo, 273 agreed to participate: 72 had HAM/TSP (excluded from this analysis) and 201 were asymptomatic, a classification performed during a regular neurological appointment. We selected the subgroup made up only by the 201 asymptomatic subjects to avoid bias by the clinical status as a confounder effect, who had laboratory results of HTLV-1 proviral load and T-cell proliferation assay in our database. They were further grouped according to their serological status in four categories: 121 HTLV-1 asymptomatic mono-infected carriers; 32 HTLV-1/HCV, 29 HTLV-1/HIV-1, and 19 HTLV-1/HIV-1/HCV co-infected patients. Clinical data were obtained from medical records and interviews. DNA HTLV-1 proviral load (PVL) and T-cell proliferation (LPA) assay were performed for all samples.
Results: From a total of 273 subjects with HTLV-1, 80 presented co-infections: 29 had HIV-1, 32 had HCV, and 19 had HIV-1 and HCV. Comparing the groups based on their serological status, independently of being asymptomatic carriers, we observed a significant increase of

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https://doi.org/10.1016/j.bjid.2018.02.002

PVL ($p < 0.001$) and LPA ($p = 0.001$). However, when groups were stratified according to their clinical and serological status, there was no significant increase in HTLV-1 PVL and LPA.
Conclusion: No significant increase of basal T-cell proliferation among HTLV-1 co-infected was observed. This interaction may be implicated in liver damage, worsening the prognosis of co-infected patients or, on the contrary, inducing a higher spontaneous clearance of HCV infection in HTLV-1 co-infected patients.

Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus etiologically linked to adult T-cell leukemia/lymphoma,[1,2] HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and other inflammatory diseases.[3] This virus is endemic in Japan, Caribbean Basin, and some countries in Latin America,[4,5] with 5–10 million people infected worldwide.[6,7] In Brazil, the highest prevalence of HTLV-1 is found in the North and Northeast, particularly in the cities of Belem, São Luiz, and Salvador.[8]

In areas endemic for retroviruses, the higher probability of occurrence of co-infections (HIV and HTLV-1, for example), with hepatitis C virus (HCV) may change some characteristics of the disease, such as an altered response to treatment,[9] and especially in the pathogenesis of liver disease.[9] Indeed, cell mediated immunity involved in the development and progression of liver disease associated with the interaction between HCV and HTLV-1 may contribute to changes in the natural history of the disease caused by these viruses, such as the development of hepatocellular carcinoma in co-infected subjects.[10]

In previous findings, HAM/TSP progression was associated with T-cell activation in the spinal cord, leading to an inflammatory process and demyelinization.[11] A possible cause for higher immune activation could be the presence of higher DNA proviral loads (PVL) among HAM/TSP patients.[12] Alternatively, those findings could be due to duration of disease, since we studied a broad range of TSP/HAM cases.[13] In case of HTLV-1/HIV-1co-infection, down-regulation of T-cell proliferation, usually present with HIV infection, may not occur, a finding that could be related to the lower survival rate of such patients.[14] Based on the consequences of co-infection on HAM/TSP development, we examined the possibility of an association among asymptomatic HTLV-1-co-infected subjects, increase of HTLV-1 DNA proviral load, and T-cell proliferation in a large cohort of HTLV-1-infected subjects in Sao Paulo city, Brazil.

Material and methods

The HTLV outpatient clinic from Institute of Infectious Diseases "Emilio Ribas" (IIER) has been following a cohort of 610 HTLV-1-infected patients for 19 years, starting in July 1997. For the purpose of this study, we recruited a total of 201 HTLV-1-infected subjects who were older than 18 years and remained in active follow-up from June 2011 to May 2012, and were clinically asymptomatic. The Ethical Review Board of the IIER approved the protocol (Number 13/2011), and a signed informed consent was obtained from all participants prior to study inclusion.

All 201 volunteers were asymptomatic according to neurological evaluation and were selected if laboratory results of HTLV-1 proviral load and T-cell proliferation were available and retrievable in the patient's record. Eligible patients were classified according to their serological status in four categories: 121 HTLV-1 asymptomatic monoinfected carriers; 32 HTLV-1/HCV, 29 HTLV-1/HIV-1, and 19 HTLV-1/HIV-1/HCV co-infected patients.

Blood samples were collected in acid-citrate-dextrose solution, and PBMC were separated by Ficoll density gradient centrifugation (Pharmacia, Uppsala, Sweden). Cells were washed with saline solution; cell number was adjusted to 2×10^6 cells and then stored at $-80\,^{\circ}C$. DNA was extracted using a commercial kit (Illustra Tissue and Cells GenomicPrep Mini Spin kit, Easton Turnpike, Fairfield, CA) according to manufacturer's instructions. After this procedure the DNA was stored at $-80\,^{\circ}C$ for later analysis.

Quantification of HTLV-1 proviral load

The HTLV-1 proviral load was quantified by real-time PCR using primers and probes targeting the *pol* gene: SK110 (5′-CCCTACAATCCAACCAGCTCAG-3′), and SK111 (5′-GTGGTGAAGCTGCCATCGGGTTTT-3′). The internal HTLV-1 TaqMan probe (5′-FAMCTTTACTGACAAACCCGACCTACCCATGGATAMRA-3′) was selected using the Oligo (version 4, National Biosciences, Plymouth, MI, USA). For quantification of the human albumin gene, the primers Alb-S (5′-GCTGTCATCTCTTGTGGGCTGT-3′) and Alb-AS (5′-AAACTCATGGGAGCTGCTGGTT-3′) and albumin TaqMan probe (5′-FAMCCTGTCATGCCCACACAAATCTCTCCTAMRA-3′) were used as described previously.[15,16] Based on the median of asymptomatic individuals, 200 copies/10^4 PBMCs of PVL was the value used as a cut off to discriminate from HAM/TSP subjects.

T-cell proliferation (LPA) assay using peripheral blood mononuclear cell cultures (PBMC)

T-cell proliferation assay was performed as described in detail elsewhere.[17] Briefly, 10 mL of peripheral heparinized blood

Please cite this article in press as: Assone T, et al. In vitro basal T-cell proliferation among asymptomatic HTLV-1 patients co-infected with hepatitis C and/or HIV-1. Braz J Infect Dis. 2018. https://doi.org/10.1016/j.bjid.2018.02.002