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Detection of HTLV-1 proviral DNA in BM mononuclear cells and cultured mesenchymal stromal cells isolated from patients with HTLV-1 infection☆

Evandra Strazza Rodrigues[a,b], Maria do Carmo Favarin[a], Mayra Dorigan de Macedo[a,b], Katia Kaori Otaguiri[a,b], Maristela Delgado Orellana[a,c], Osvaldo Massaiti Takayanagui[c], Patrícia Vianna Bonini Palma[a], Svetoslav Nanev Slavov[a,c], Dimas Tadeu Covas[a,c], Simone Kashima[a,b,*]

[a] Regional Blood Center of Ribeirão Preto, University of São Paulo, Brazil
[b] Department of Clinical, Toxicological and Bromatological Analyses, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil
[c] Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil

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ABSTRACT

The bone marrow (BM) biology during HTLV-1 infection is obscure. In this study, we investigated BM mononuclear cells and mesenchymal stromal cells (MSC) from HTLV-1 asymptomatic and symptomatic individuals. An infiltration of $CD4^+$ T-cell lymphocytes in the BM of HTLV-1-infected individuals was observed when compared to healthy controls. The provirus detection in the BM $CD4^+$ T cells confirmed the presence of integrated HTLV DNA. In regard to MSC, we observed that the number of fibroblast progenitor cells was lower in HTLV-1 infected individuals than in healthy controls. Isolated HTLV-1 infected BM-MSC demonstrated surface expression markers and *in vitro* differentiation potential similar to uninfected individuals. The presence of HTLV-1 proviral DNA in the BM-MSC of HTLV-1-infected patients was demonstrated but no p19 antigen was detected in supernatant from cultured MSC. We suppose that HTLV-1 infects human MSC probably by cell-to-cell contact from the infected $CD4^+$ T-lymphocytes infiltrated into the bone marrow.

1. Introduction

Human T lymphotropic virus type 1 (HTLV-1) infection reaches 5–10 million people worldwide and is associated to two major clinical manifestations known as adult T-cell leukemia/lymphoma (ATLL) and an inflammatory neurologic disease, the HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain and Cassar, 2012). Minor proportion of the HTLV-1-infected individuals (2–5%) develops clinically overt infection, while the majority remains as HTLV-1 asymptomatic carriers (Osame et al., 1997). ATLL is an aggressive T-cell malignancy with poor prognostic outcome. In this particular case, significant advances have been obtained using allogeneic hematopoietic stem cell transplantation (HSCT), achieving long-term remission in 30–40% of ATLL patients (Utsunomiya, 2016; Ishida et al., 2013). The effectiveness of the ATLL treatment using HSCT may be related to immunogenicity of HTLV-1-infected cells, since that Tax-specific $CD8^+$ CTLs are induced in patients who achieved complete remission. Post-transplant graft-*versus*-HTLV-1 may contribute to make the HTLV-1 proviral load undetectable in a significant proportion of ATLL individuals after allogeneic-HSCT (Akimoto et al., 2007; Tamai et al., 2013). However, in other ATLL patients submitted to allogeneic-HSCT complete disease remission is not observed and HTLV-1 proviral load becomes detectable again (Hishizawa et al., 2010; Shiratori et al., 2008). The reason why HTLV-1 infection remains undetectable in some patients with ATLL and not in others is still unknown. It is possible that factors related to the cells serving as HTLV-1 reservoirs may contribute to viral latency and the impossibility to achieve complete remission in ATLL.

According to previous *in vivo* studies, HTLV-1 is preferentially found in peripheral blood $CD4^+$ T cells, however the virus can also infect $CD8^+$ T cells, dendritic cells (DCs), mesenchymal stromal cells and numerous mammalian cells *in vitro* (Richardson et al., 1990; Koyanagi et al., 1993; Macatonia et al., 1992; Zacharopoulos et al., 1992; Rodrigues et al., 2014). Levin et al. (1997) investigated the bone marrow (BM) as a potential viral reservoir in HTLV-1-infected individuals. They evaluated the BM from three HTLV-1-infected patients

☆ Institution where processing and analysis were performed: Molecular Biology Laboratory, Regional Blood Center of Ribeirão Preto, São Paulo, Brazil. Rua Tenente Catão Roxo, 2501 - Ribeirão Preto, São Paulo, Brasil – ZIP Code: 14051-140

* Correspondence to: Regional Blood Center of Ribeirão Preto, Rua Tenente Catão Roxo, 2501 – Ribeirão Preto, São Paulo, Brazil.
E-mail address: skashima@hemocentro.fmrp.usp.br (S. Kashima).

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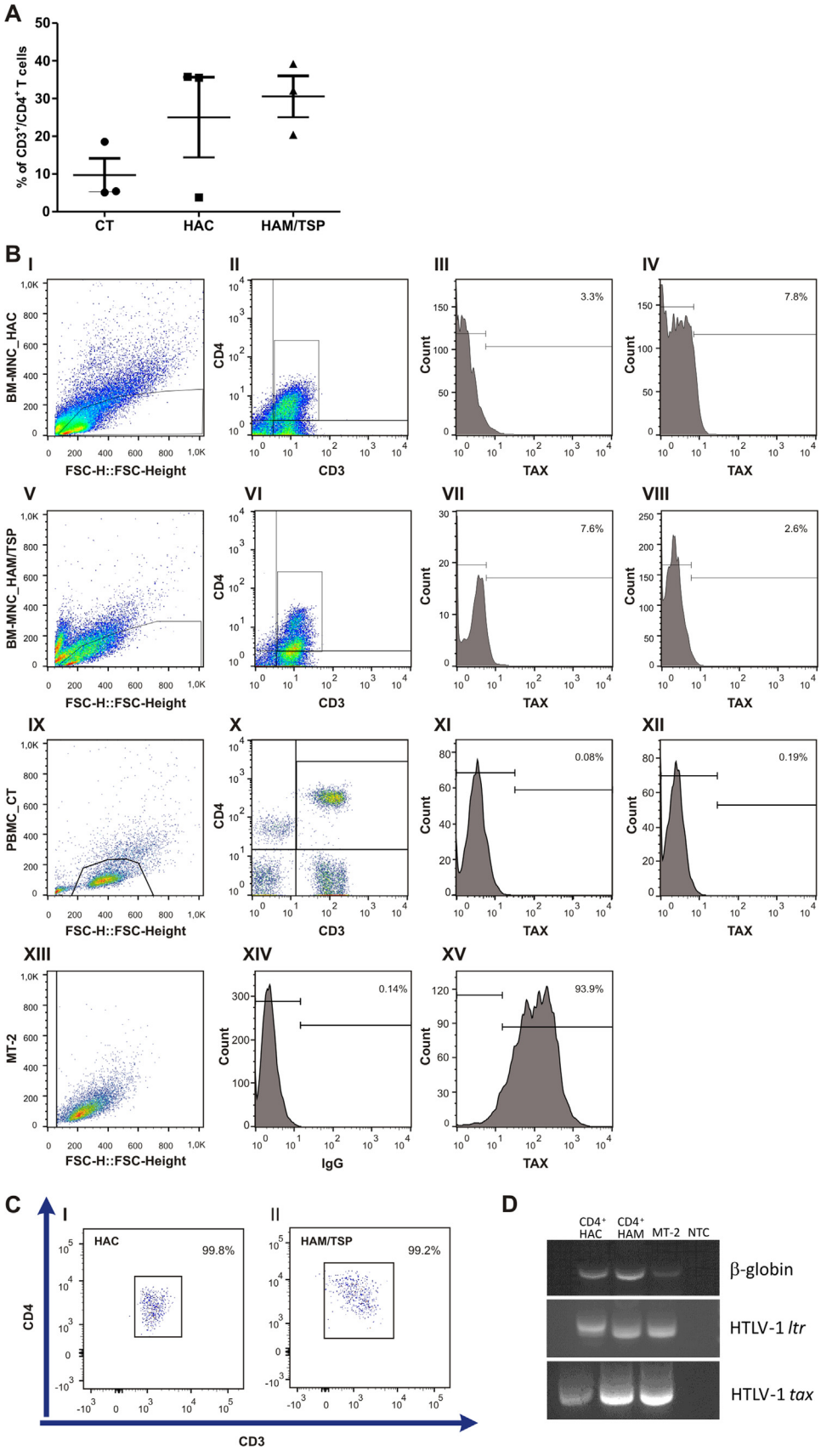

Fig. 1. Presence of HTLV-1 in BM $CD4^+$ T cells. **A)** Analysis of mononuclear bone marrow cells (BM-MNC) isolated from subjects infected with HTLV-1 and control individuals. The data are presented as mean ± SEM percentage of cells stained with anti-CD3 and anti-CD4 and evaluated by flow cytometry. Results were obtained from three independent experiments. T-test was used with Mann-Whitney test. *p = 0.02 compared to individuals HAM/TSP. **B)** Cell percentage of HTLV-1 Tax expression in HTLV-1 carriers (I–IV), HAM/TSP (V–VIII) individuals, healthy blood donors (IX–XII) and MT-2 (XIII–XV). I, V, IX and XIII, representative flow cytometry dot plots showing the complexity and internal granularity of mononuclear cells from BM, PBMC and MT-2, respectively; II, VI and X the dots in the upper right quadrant of the flow plot represent a gate in CD4-PerCP (peridinin chlorophyll protein complex) and CD3-APC (allophycocyanin) cells; III, IV, VII, VIII, XI, XII and XV the histograms represent the percentage of $CD4^+/CD3^+$ T cells expressing HTLV-1 Tax protein. **C)** Analysis by flow cytometry after cell sorting indicating the purity of BM $CD4^+$ T cells sorted from (I) HTLV-1 carriers and (II) HAM/TSP individual. No specific signal was evidenced in the isotype control. **D)** Amplification by nested PCR of the (I) TAX (444 pb) and LTR (253 bp) HTLV-1 genes from sorted BM $CD4^+$ T cells (II) the gene of the human β-globin gene (659 bp) was used as internal control of the PCR reaction. These procedures were performed only once.