

Toll-Like Receptor 2 (TLR2) May Be Involved in the Immunopathogenesis of HTLV-I Associated Myelopathy / Tropical Spastic Paraparesis (HAM/TSP)

Hugo Marcelo Barbosa¹, Karina Carvalho¹, Helena Tomiyama¹, Walter Kleine Neto²,

Ioko Nukui², Ester C. Sabino², Marco Antônio Chieia¹, and Esper G. Kallas*¹

¹Federal University of São Paulo, São Paulo, Brazil; ²São Paulo Blood Bank, São Paulo, Brazil.

Introduction

The HTLV-I is an exogenous human retrovirus that infects 10–20 million people worldwide. The majority of infected individuals remain lifelong asymptomatic carriers while 0.25–3% develop an inflammatory disease of the central nervous system termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Around 10-20 million are contaminated worldwide by HTLV-I, approximately 1-2 million living in Brazil, the country with the highest absolute number of infected inhabitants in the world.

In vivo, the most important target of HTLV-I infection is the CD4⁺ T cell, although additional evidence has accumulated over the past several years demonstrating that HTLV-I can infect several additional cellular compartments, including CD8⁺ T cells. T cells are involved in the immunological control of viral infection and in the pathogenesis of HAM/TSP and others HTLV-I-related diseases, but the importance of viral replication in different T cell compartments is not clear. Moreover, even though high proviral load (pVL) and T cell hyperactivation had been associated with the development of HTLV-I-related neuroinflammatory diseases, the immunopathogenesis of HAM/TSP is not completely understood.

Recently, it has been described a modulation of T cell function by innate receptors belonging to "Toll-like receptor family", with emphasis on TLR2, which resembles a costimulatory receptor for antigen-specific T cells. Activated T cells express TLR2, produce IFN- γ , and have markedly enhanced proliferation in response to TLR2 ligand. In addition, TLR2 engagement on CD8⁺ T cells lowers the threshold for antigen-induced T cell activation.

We hypothesize that pVL in different T cell compartments and the expression of TLR2 are related to development of HAM/TSP. Our data demonstrated a different outcome of HTLV-I infection in CD4⁺ and CD8⁺ T cell compartments and suggest the involvement of TLR2 pathogen signaling in the immunopathogenesis of HTLV-I-associated neuroinflammatory disease.

Methods

- Samples were obtained from 9 health volunteers (Control), 7 HTLV-I infected asymptomatic volunteers (HTLV-I+), and 9 patients with HAM/TSP (HAM/TSP). Blood was collected in heparin-coated tubes and PBMC isolated by centrifugation over a Ficoll-Histopaque cushion for cryopreservation.
- PBMC were fractionated in CD4⁺ and CD8⁺ T cells by MACS columns.
- HTLV-I pVL was quantified by RT-PCR in total PBMC, CD4 and CD8 enriched conditions.
- PBMC were surfaced stained with fluorochrome-labeled antibodies to TLR2, CD3, CD4 and CD8. Fluoresce minus one (FMO) was used for gating strategy (Figure 1).
- Nonparametric analyzes were performed, significance threshold was defined at p<0.05.

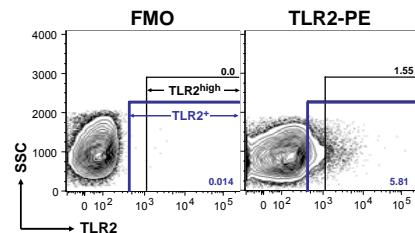


FIGURE 1. Gate strategy to determine the percentage of TLR2 positive (TLR2⁺) T cells and those highly expressing TLR2 (TLR2^{high}), using the fluorescence minus one (FMO) approach in a Control sample.

Results

HAM/TSP had elevated pVL in whole PBMC, CD4⁺ and CD8⁺ T cell enriched conditions when compared to HTLV+ as depicted in Figure 2. However, the CD4-pVL was greatly higher than CD8-pVL.

We observed that the proportion of TLR2^{high} cells was significantly increased in different T cell subpopulations in HAM/TSP, including total T cells, CD8⁺ T cells and CD4-CD8⁺ T cells (Figure 3).

Nevertheless, the mean fluorescence intensity (MFI) of TLR2 in CD4-CD8⁺ T cell subpopulation was inversely correlated with CD4-pVL in HAM/TSP as depicted in Figure 4 ($r=-0.785714$, $p<0.05$).

FIGURE 2. Proviral load in whole PBMC, CD4⁺ and CD8⁺ enriched conditions, determined in samples obtained from HTLV-I+ and HAM/TSP.

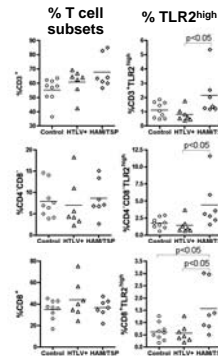
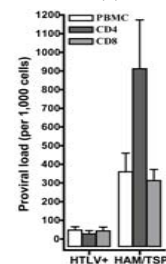


FIGURE 3. Characterization of expression of TLR2 in T cell subtypes (TLR2⁺) and those highly expressing TLR2 (TLR2^{high}).

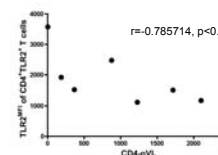


FIGURE 4. Correlation between expression of TLR2 in TLR2⁺CD4⁺ T cell subpopulation with CD4-pVL.

Discussion

The immunopathogenesis of HAM/TSP is far of been completely understood. Most HTLV-I infected subjects are asymptomatic carries and only a small fraction will develop some HTLV-I-related disease. Therefore, virus-host interactions are important in its pathogenesis. One of the most important regulators of such relationship is the modulation of immune response by innate receptors.

TLR2 has been implicated in the pathogenesis of neurologic diseases, such as multiple sclerosis and Alzheimer disease. Although the exact mechanism is not known, TLR2-activation by neuronal necrotic cells and other auto-antigens, including extracellular matrix degradation components, may be involved. Moreover, glial cells, important elements of CNS innate immunology, express TLR2. These data motivated us to study the role of TLR2 in development of HAM/TSP.

We could verify higher expression of TLR2 in different T cell subtypes from HAM/TSP patients. Our data support the notion that TLR2 may participate in T cell hyperactivation, through TLR2-engagement by autoantigens, which are very frequent in chronic inflammation like the one observed in CNS of such patients.

Moreover, we observed elevated CD4-pVL in HAM/TSP, previously described. Curiously, There was not significant difference in the proportion of CD4-CD8⁺ T cells between studied groups, although there was an inverse correlation between MFI of TLR2 in CD4-CD8⁺ T cell subpopulation and CD4-pVL only in HAM/TSP patients. This data suggest that TLR2 may have a role in viral replication control, but more studies are necessary to understand the importance of TLR2 in immunopathogenesis of HAM/TSP.

Conclusions

For the first time we described a marked upregulation of TLR2 on several T cell subtypes from HAM/TSP patients, constituting a candidate marker for either progression or severity of such disease. TLR2 expression on CD4⁺ T cells may be involved in viral replication control. One should explore the TLR2 pathway to better understand the immunopathogenesis of HAM/TSP, with potential therapeutic implications.