Author's response to reviews

Title: Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers

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PDF covering letter
Dear Dr. Emma Parkin,

Please find enclosed the manuscript entitled “Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers” with the corrections. All the suggestions made by the reviewers were considered. The answers to the specific questions are below.

Yours Sincerely,
Dr. Edgar M. Carvalho

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**Answer to the review #1**

1. We agree that even asymptomatic HTLV-I carriers may have increased spontaneous lymphocyte proliferation and IFN-\(\gamma\) production. However IFN-\(\gamma\) levels are quite variable in this population and they can be divided in high or low producers of IFN-\(\gamma\). Here we consider that IFN-\(\gamma\) levels in asymptomatic were similar to that observed in HAM/TSP when the levels of IFN-\(\gamma\) are higher than the mean minus one standard deviation (1,322 pg/mL) of that observed in HAM/TSP. This information was added in result section page 8 lines 19-21.

2. All the cytokine staining was performed after 20 hours of incubation based on preliminary results. A comment about that was added on page 6 lines 21-22 in methods section.

3. This is a very important point. In this cross-sectional study there was no difference regarding neurological findings in HTLV-I carriers with high or low IFN-\(\gamma\) production. A cohort is being currently performed with both groups of individuals to evaluate if there will be difference in progression from infection to disease or if sub-clinical manifestation of myelopathy may be documented in a higher proportion of carriers who have immunological abnormalities similar to that observed in HAM/TSP.

4. There was no statistical significant difference between the percentage of CD4 and CD8 TNF-\(\alpha\) producing cells in HAM/TSP and both CD4 and CD8 contribute equally to the difference seen in IFN-\(\gamma\) production. This information was added on page 10, lines 8-12.

5. The data in figure 3C is now clarified as suggested. While there was no difference regarding the frequency of CD4 and CD8 T cells producing IFN-\(\gamma\) in asymptomatic carriers (0.5 ± 0.4 vs 0.5 ± 0.3), there was a significant difference in the frequency of CD4 cells producing IFN-\(\gamma\) in HAM/TSP (1.4 ± 0.5) compared to the CD8 T cells (3.6 ± 0.5). A sentence with this information was included on page 10 lines 3-7. While in this small group CD4 and CD8 contribute equally for IFN-\(\gamma\) producing cells in asymptomatic carriers, previous data from other studies, including a study from our group, have shown that CD4 T cells are the major source for secreted IFN-\(\gamma\) in
asymptomatic carriers. Therefore we conclude that during the progressing from carrier to HAM/TSP a switch from CD4 to CD8 may occur.

Answers to the reviewers # 2

1. We agree that ideally all the immunological tests should have been performed in all patients. However it was not possible due to several factors: 1) The amount of blood drawn or obtention of blood more than once is dependent of the acceptance of the patients. Therefore the yield of cells is variable and not all experiments can be done in the same patients; 2) The high cost of the reagents and the fact that most of them are not available in Brazil is an important limiting factor; 3) Specifically in relation to the FACS analysis we did not have this equipment available at the beginning of the study.

2. An asymptomatic carrier was considered a high IFN-γ producer when the IFN-γ levels were above the mean minus one standard deviation of the levels of IFN-γ in HAM/TSP patient. This information is now on page 8 lines 19-21. There were no criteria for the choice of the 8 months interval. In fact the difference between the two evaluations was between 6 months to one year with a mean of 8 months. This information was added on page 9 lines 1-2.

3. Due to the rapid interaction of IL-4 with the IL-4 receptor, the levels of this cytokine in supernatants of lymphocyte cultures is not reliable, and sometimes very low even when there is other evidence of IL-4 production such as high expression of mRNA for this cytokine, or cell staining for IL-4. Therefore we do not measure IL-4 in lymphocyte supernatants. The cells were stained for IL-5, but due to technical problems, the preparations did not yield reliable results.

4. There is no standard therapy for HTLV-I at this moment. The ideal would be a combination of an immunomodulator able to down regulate the T cell response associated with an anti-retroviral agent. Currently we are performing a study using beta interferon and also in vitro studies with TNF-α inhibitors. These would be options to be used while we await an effective retroviral drug against HTLV-I.

5. Both HTLV-I and HAM/TSP were spelled out in the abstract.

6. Corrections were made on page 3, 4 and 7.