Reviewer’s report

Title: Loss of antigen-specific circulating effector memory CD4+ T cell populations in individuals cured of leishmaniasis

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Reviewer: Alda Maria Da-Cruz

Reviewer's report:

In this manuscript cutaneous (CL) and mucosal leishmaniasis (ML) patients were long term followed-up since the active disease until two up to fifteen years after the end of therapy. The authors aimed to evaluate long lasting memory response to leishmanial antigens in terms of cytokine production, cell phenotype and in vivo DTH response (Leishmanin skyn test – LST). They observed that around 50% of both CL or ML patients have lost the ability to produced IFN-# and TNF after cure, although have remained LST positive. These data are quite important for the comprehension of a memory responses related to an efficient control of Leishmania infection. The following points were raised for the author’s analysis.

Major points:

1. The authors based their differential cell phenotype on CD62L cell surface expression to distinguish effector and central memory CD4CD45RA- cells. However, according to Human Immunology Project (Nature Reviews 12:2012) the differentiation of naïve, central and effector cells (only effector or effector memory profile) should be based on CCR7 and CD45RA. The authors have to stress this limitation in the discussion section. Considering this, they have to be careful when discriminating memory cells maybe preferably using the cell phenotype markers.

2. The title stress that a loss of antigen-specific CD4 effector memory cells in cured patients has occurred. Considering that this phenomenon was shown in around half of CL and ML patients and also the arguments above, the title should be reevaluated to better express the results obtained in this manuscript.

3. The differences between patients that maintain or nor IFN-# productions and also the possible explanation to this bias behavior have to be included in the discussion section.

4. Was the cytokine quantification obtained from active and cured patients performed in similar conditions or the authors used results obtained in the past? And cell phenotype of active patients was done from frozen cells?

4. Was the expression of CD62L on T CD4+ evaluated after SLA stimuli? In other word, did the leishmanial stimuli induced the proliferation or and enrichment of effector cells? Did the percentage of CDRAlowCD62Lhigh cells after antigen stimuli predominate over other cell types?

5. In discussion section it was initially mentioned that … “usually evidence of T
cells response have been documented after cure [18, 19, 20]. However, it these three references patients were evaluated during active disease and after cure. The main contribution of the present manuscript is that the same patients were evaluated.

6.

Minor points
1. Inform whether cytokine values are median or mean.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.