Reviewer’s report

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

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

General comments

In this manuscript, the authors investigated the specific immune response in patients with cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) before and after treatment (called here “cured” individuals). The investigation is interesting and shows some valuable data that should be published. However, many points should be clarified, revised and investigated in order to strengthen the results.

Specific comments: Major Compulsory Revisions

1. The markers used by the authors to characterize central and effector CD4+ memory T cells seem to be not enough for that. They would have included at least CCR7 in their panel, based on published papers, including reference 6 cited in the MS. It could explain the high % of cells producing IFN-gamma in presence of SLA, that it is more characteristic of effector memory T cells.

2. Central memory CD4+ T cells can produce high levels of IL-2, however the authors did not show any data of IL-2 production by these cells. Have it been measured in the culture supernatants of PBMC stimulated with SLA by ELISA or by intracellular staining? Also the proliferation of CD4+ T cell was not evaluated.

3. How was done the gate strategy for CD45RA? The gate strategy including CD45RA should be shown in the MS. How was the strategy to define CD62L high and CD62L low shown in Fig2A? Was it based in the negative control, isotype control, fluorescence minus one (FMO)? The figure 2A should show also the percentage of CD62L high and low subsets in healthy controls based on these markers.

4. The authors say that the positivity observed in the cutaneous test done in the patients after the treatment suggests that this test may be more efficient to show the presence of Leishmania-specific T cells on those “cured” individuals than in vitro assay. However, the way that the experiments were done, concerned to the memory markers and the cytokine pattern, it is not convincing that the in vitro
experiments are less efficient than cutaneous test to show the presence of memory after treatment. It should be changed in the text.

5. The title should be revised, since the data is not convincing that the cells based on the authors’ criteria are really effector cells. Also it is mainly based on the IFN-gamma production, and it should be stressed out in the title.

6. The results show a discrepancy of the in vivo test and the in vitro test in cured individuals. However, the in vitro test is mainly based in the IFN-gamma production. It can exclude that other cytokines, such as IL-2, could be produced by those cells. T should be discussed.

7. Figure 2 B is comparing the % of effector memory and central memory in PBMC of CL and MC patients and it shows that there is no difference between the groups. But, how is the percentage of these markers in healthy controls? It could clarify if the % of effector and central memory in MC and CL according to these markers are the same as the ones observed in healthy. In this case there is no variation of it in human leishmaniasis independent of the cure of patients after the treatment.

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'