Author's response to reviews

Title: Role of CD8+ T cells in protection against Leishmania donovani infection in healed Visceral Leishmaniasis individuals

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Author's response to reviews: see over
To,

The Editor,

“BMC Infectious Diseases”

Subject: Submission of revised manuscript to “BMC Infectious Diseases”

Dear Sir,

We are thankful to the reviewers for providing useful comments which have helped us to improve the manuscript entitled “Role of CD8+ T cells in protection against Leishmania donovani infection in healed Visceral Leishmaniasis individuals”. We are providing the responses to the comments raised and the revised manuscript.

Sincerely,

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Reviewer's report:

Major Compulsory Revisions

We thank the referee for valuable comments. We have addressed all the queries raised by the reviewer and the manuscript has been revised accordingly.

Comment: The data demonstrating that >99% of the cells were viable after 120 hours of re-stimulation needs to be shown or this needs to be re-evaluated. This is a remarkably high number for a long-term (120 hr.) in-vitro re-stimulation assay.

Response: We did cell viability test only on gated lymphocytes population (R1, as shown in SSC Vs FSC plot) and not on total cells. We found >99% cells viable in R1 region which was true for both HVL and naive groups. We did immunophenotyping on this R1 population. For your perusal we have added graph figure 4 C illustrating cell viability test from one of the HVL individuals.

Comment: The authors need to confirm that their statistical tests were two-tailed.

Response: The statistical tests were two-tailed and this has been added in “Statistical analysis” section of the revised manuscript.

Comment: I would like to see the IFN-g versus Granzyme B correlation.

Response: Our data suggest no correlation between granzyme B and IFN-γ level ($r_s = 0.434, p = 0.158$). We have added this point in the revised manuscript.

Comment: The authors reference two reviews (References 5 and 6) to support the statement: "Previous studies have shown that majority of individuals who had VL or asymptomatic infection acquired strong immunity against re-infection with the same subspecies [5, 6]." Both of these references deal with cutaneous Leishmaniasis not visceral Leishmaniasis. The authors need to either acknowledge that prior infection MAY protect against re-infection with VL as is seen with cutaneous infection or find references that show it is also true for VL.
Response: As per reviewer’s suggestion, we have included two references (Manson-Bahr PE. *Trans R Soc Trop Med Hyg* 1961; Haldar JP et al., *Infect Immun* 1983) that show patients recovered from VL are usually immune to re-infection.

**Minor Essential Revisions**

Comment: The authors report the % of CD4 cells that are CD69+IFN-g+ in 4A but the dot plots in 4B report the frequency of IFN-g+ cells within the CD69+ (Gated on activated T cells) population. This is inconsistent and does not convey how the authors determined that cells were both CD69+ AND IFN-g+. Can the authors please plot CD69 versus IFN-g within the CD3+CD4+ population or some how convey how they determined the frequencies reported in 4A.

Response: We have modified the figure 4 and would like to present the data without intracellular IFN-g, keeping the theme of the manuscript intact. Please see the figure 4. The manuscript has been modified accordingly.

**Discretionary Revisions**

Comment: The axis and frequency text in Figure 4B are difficult to read.

Response: This has been improved.

**Quality of written English:** Needs some language corrections before being published

Response: Appropriate corrections have been made in the revised manuscript.

**Reviewer:** Sujit Kumar Bhattacharya

Reviewer's report: Good article

Response: We thank the referee for comment.

**Quality of written English:** Needs some language corrections before being published.

Response: Appropriate corrections have been made in the revised manuscript.

**Reviewer:** Anuradha Dube

Reviewer's report: The authors have attended all the queries and have made the corrections/modifications in the manuscript entitled “Role of CD8+ T cells in protection against *Leishmania donovani* infection in
healed Visceral Leishmaniasis individuals" which is up to my satisfaction. The Ms is now suitable for publication.

**Response:** We thank the referee for approving the manuscript for publication.