Reviewer’s report

Title: Effects of IFN-γ coding plasmid supplementation in the immune response and protection elicited by Trypanosoma cruzi attenuated parasites.

Version: 0 Date: 09 Aug 2017

Reviewer: Amanda Francisco

Reviewer's report:

Thank you for the opportunity to review and advice regarding the manuscript: Effects of IFN-γ coding plasmid supplementation in the immune response and protection elicited by Trypanosoma cruzi attenuated parasites.

Comments, suggestions and questions are listed below.

Line 2 - If the conclusion is that IFN-gama coding plasmid supplementation it is not protective I would suggest to reformulate the title.

Line 60 - Explain the real cause (geographical movement) for that as we know that it is not by natural infection.

Line 63 - Fibrosis, stroke, heart failure, ventricular arrhythmia and sudden death are also involved in chronic chagasic cardiomyopathy. In the text it is not clear that around 30% of infected individuals could develop single forms of the disease (cardiac or digestive) or both. It also should be clear that around 70% of people are asymptomatic.

Line 67 - That is not the main reason. The majority of infected individuals will interrupt the treatment with the nitro compounds before finish the course due to the severity of adverse effects and length of treatment.

Line 119 - Brazil should be with z and not s.

Line 127 - Did the research group test the same vaccination protocol in the BALB/c mice model?

Line 196 - TCC+pVXVR-mIFN-γ had an increase in IgG levels after dose 3, one month after the last challenge. What would have happen after a longer period of time? How the levels of IgG would appear after 2-3 months?

Despite the increase of IgG levels after dose 2 in Figure 1A, in Figure B there is no increase in the different sub-types after dose 2.
Previous studies using benznidazole treatment to cure T. cruzi infection in mice demonstrated the development of central memory T cells in the cured mice that were capable of transferring a degree of early protection from T. cruzi infection to recipient mice. I was wondering if flow cytometry would be a better option to answer your question? Cellular response?

The analysis was done only in heart. I would suggest to remove target organs and replace with heart only. I would suggest to answer what is happening in terms of tissue specific response. What can be concluded in terms of fibrosis and inflammation on those mice hearts? What about the pathology results?

Samples were collect 30 days after infection. How would you explain the difference in the IgG subtypes between TCC and TCC+pVXVR-mIFN-γ?

How could the group show in another way the unchanged values for IL-10 production?

I would highly recommend to add this results in the supplementary data.

Wrong spelling.

The paper state that: 'infection with attenuated parasites per se is efficient in regulating parasite burden in target organs and peripheral blood'. My question is for how long that regulation will last?

Data not shown. It would be interesting to see that result on the supplementary data.

I would suggest to reformulate this sentence. Any other experiment to answer that question?

Did the research group performed an experiment to measure the NO levels?

Other comments:

1- Figure 3 is out of focus.

2- If the experiment were done in triplicate why not include all mice in the graphs? Why show only 4?

3- Add controls basal levels in the graphs as it was mentioned that they were included in the experiments.

4- Too much IFN-gama could be detrimental. Add controls to Figure 4.
5- Abstract should be rewritten. An abstract is a short summary of your completed research.

6- In all figures (when possible) add how many days of infection at the top of the graphs. That help not having to go to the legend.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Unable to assess

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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