Reviewer's report

Title: Benznidazole-treatment of late chronic Chagas' disease leads to an overall cytokine down-regulation that upon Trypanosoma cruzi antigen stimulation can be shifted towards a Type-1 modulated profile by monocytes, NK and CD8+ T-cells

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Reviewer: Manuel Fresno

Reviewer's report:

The article by Sathler-Avelar et al describes the action of benznidazol (Bz) on indeterminate chagasic patients that present overall cytokine down-regulation. However after in vitro stimulation with T. cruzi antigen cytokine secretion shifts towards a Th1-modulated immune response. This is an interesting work that agrees with some observations in the mouse model regarding the strengthen of memory CD8+ T cell after Bz, and points to the benefits of treating chagasic indeterminate patients with benznidazol.

However, some specific points need to be addressed

My major concern is with the way the manuscript is written and the use of some words, not related to the english usage, that can be improved, but with the meaning transmitted to the reader:

1. The title should be changed to a more appropriate one according to the results presented. It sounds like a conclusion of the paper, which is not entirely correct. Basically what the manuscript shows is that blood cultures from Bz- treated indeterminate (not late chronic) when stimulated in vitro have a Th1 profile.

2. Conclusion of the abstract. The data presented does not support that NKs CD8 and monocytes are " the" major source of cytokines. Other non-immune sources have been not addressed. Moreover the data presented dose not reflect actual circulating cytokines. A better wording will be are “a” major source. Moreover, the beneficial effect of Bz is not demonstrated in this paper. Thus eliminate beneficial. Conclusions reflecting more accurately the work will be related to this sentence “ Bz treatment of indeterminate patients shifts the response of NK, CD8 an monocyte towards a type 1 response” This will be also the main line of the title.

3. Why they call the patients late chronic. Is there a new tool for classification of patients? Moreover, in methods only the word indeterminate is used. So, unless is well explained and demonstrated in the paper, please change "late chronic indeterminate patients" for “indeterminate patients” all over the manuscript

4. In line 267 of the manuscript is stated, “ The small decrease of Il-12+ cells contributes to.” The word contributes is not correct. The data shown are only correlations no cause/effect relationship. So add the conditional “may “or “can contribute” or better “ could be responsible for” or "could explain”
5. In line 356 “findings demonstrated that increased frequency of inflammatory-like monocytes and NK-cells, together with high levels of regulatory-like monocytes and modulated adaptive immunity could be important to the establishment/maintenance of asymptomatic chronic Chagas disease.” More correct wording “findings demonstrated an increased frequency of inflammatory-like monocytes and NK-cells, together with high levels of regulatory-like monocytes and modulated adaptive immunity that could be important to the establishment/maintenance of asymptomatic chronic Chagas disease.

6. The use of the word “demonstrating or demonstrate” or related words in many sentences is excessive and not exactly related to the content of the article in many places. The manuscript does not demonstrate many points stated as such. A better word will be “show” “indicate” or related ones.

Other points:
1. In the previous publication of Vitelli-Avelar DM, et al. Scand J Immunol. 2008 68:516-525. there is preliminary data of IL-10+ CD4 and Monocytes on Bz-treated Ind and Card patients and Bz on monocytes of cardiac patient seem to increase and in this manuscript they show that decrease in Card. Please comments or discuss the apparent discrepancy.

2. In the methods section the patient groups analyzed differ in number and gender. Is this because patients are not the same before and after treatment? If yes, please justify the results.

3. In vitro cultures were done with whole blood. Did the authors get rid of erythrocytes before cultures or FACS analysis? Please clarify.

4. In page 12 line 254: instead of “type1-modulated immune profile”, it would be best say “type1-regulated immune profile”, since IL-10 is a regulatory cytokine, and exclude IL-4 (Th2 cytokine) because variation is very small.

5. The results suggest that overall cytokine down-regulation is dependent on IL-10, since this cytokine is up-regulated in innate and adaptive immune cells, being up-regulated after antigenic stimulation only in monocytes.

6. The last results section should be explained in more detail because of the complexity of the results analysis shown in figure 5.

7. Finally, there is no mention about the mode of action of Bz on the parasite. It is supposed to be effective because the nitro group are reduced by parasite enzymes to produce free radicals, superoxide anions, hydrogen peroxide.

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

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

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