Reviewer's report

Title: Resistance of L. amazonensis and L. braziliensis to nitric oxide correlates with disease state

Version: 1 Date: 18 July 2006

Reviewer: Marcelo Genestra

Reviewer's report:

General

-----------------------------------------------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) The Title is not appropriate, therefore it does not mention that the disease is the Leishmaniasis;

2) Leishmania must be in italic in all the text;

3) NaNO2 (pH 5.0) was used in some experiments. Why 5.0 pH? This acidic pH is appropriated for Promastigotes cultures? (page 7);

4) Page 5: "Putative NO-mediated leishmanicidal actions include inhibition of mitochondrial respiration, inactivation of glutathione peroxidase, increasing susceptibility to oxidant damage, etc". But Leishmania parasites lack some or all of the usual antioxidant defense mechanisms present in aerobic or other aerotolerant cells, such as catalase, superoxide dismutase, reduced glutathione, and the glutathione-recycling enzymes glutathione peroxidase and glutathione reductase. How to explain this? (see Mehlotra RK. Antioxidant defense mechanisms in parasitic protozoa. Crit Rev Microbiol. 1996;22(4):295-314).

5) Page 7: "Evaluation of NO susceptibility of Leishmania spp promastigotes by Thymidine incorporation. L. amazonensis and L. braziliensis promastigotes in log phase growth, etc..." Parasites in log phase are infective ones? How much metacyclic forms in this phase?

6) Page 7: "The supernatants were removed and replaced with 200 µl of complete Schneider medium and incubated for 20 hrs at 25oC." With this protocol, the growth curve was restarted, and then, there are no infective ones;

7) "Two isolates of L braziliensis and two isolates of L. amazonensis, one NO-resistant and one NO-susceptible of each specie, were randomly selected for the macrophage infection assays." The n for these experiments is very small;

8) Only 1 reagent was used for determination of resistance or susceptibility; it is necessary more experiments, using, for example, other (as sodium nitroprussiate);

9) Page 9: where is Table 1? The NO produced is from only the macrophages or also from Leishmania parasites? Leishmania parasites possesses a constitutive NOS and also produces NO, including amastigotes (see Basu et al., 1997 and Genestra et al., 2006a,b); how to warrant that NO produced is from only the activated macrophages?

10) Page 9: "The storage time in liquid nitrogen of the NO susceptible isolates (mean ± SD = 6.6 ± 3.2 years) was similar to the NO resistant isolates (7.8 ± 2.7 years), p =0.3."; which is the main of this information? Which is its relevance for the experiments?

11) Page 10: The ability of two of each L amazonensis and L. braziliensis isolates, one NO-resistant and one NO-susceptible of each specie to infect and proliferate within culture-derived human macrophages was evaluated in vitro. "The n for these observations/hypothesis is very small;

12) Discussion, page 11: "In the current study we demonstrate for the first time resistance of some isolates of both L. braziliensis and L. amazonensis promastigotes to nitric oxide." It is not true, because the MSci
thesis of Fonseca-Geigel (Oswaldo Cruz Foundation, 2000) verified these data;

13) Page 11/12: "Specifically, TNF-Î± and IFN-Î³ elaborated by macrophages or T cells synergize to up-regulate iNOS and the NADH oxidase, with the resultant production of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI), respectively, etc". Respectively??????iNOS related to ROI?????

14) Page 12: "While these above mentioned studies focus on the host response and the ability of host cells/cytokines to influence the outcome of Leishmania infection, in the current study, we focused instead on the innate susceptibility of the parasite to leishmanicidal molecules, and their ability to resist to a host microbicidal response." Leishmanicidal molecules include NO and others. The response depends also of trypanothione reductase activity from amastigotes. The resistance test would have also to be carried through with (lesion or axenic) amastigotes;

15) Page 13: "In conjunction with our data, this suggests that there are inter- and intraspecies variations in susceptibility to toxic nitrogen products. After the end of this study we observed that NO resistant isolates remained resistant despite long-term cultivation (data not shown), and we hypothesize that there might be an innate genetic basis for NO resistance of leishmania parasites." There are no basis for this hypothesis;

16) Page 13: "Although early reports suggest that NO plays no role in intracellular killing of microbes by human macrophages [17].". Is "no role"?

17) Page 14: "Furthermore, the LPG-associated kinetoplastid membrane protein 11 has been reported to suppress iNOS activity because it contains a structural analog of L-arginine, a known inhibitor of iNOS". How a glycoconjugated could be a structure analog of a aminoacid, L-arginine?

18) Figure 1: It is necessary to separate the graphs. L. amazonensis resistant of L. braziliensis resistant and L. amazonensis susceptible of L. braziliensis susceptible, for A and B;

Minor Essential Revisions
1) Abstract/Results: "Seventeen isolates from nitric oxide-susceptible L. amazonensis or etc";

2) Background, line 7: viannia or Viannia?
3) Delete "260 km Southwest of the capital Salvador."
4) Page 8: PBMC / macrophages
5) Page 14: "we previously", "we report", "we investigated", etc;

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests.