Author's response to reviews

Title: Zinc/Copper Imbalance Reflects Immune Dysfunction in Human Leishmaniasis: an ex vivo and in vitro study

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Dear Madam,

You may find included a revised version of the manuscript, changes made according to the suggestions by both referees are shown in red in the .doc file.

Referee 1:

1. Abstract section: the suggested changes have been made.
2. Background section: misprint has been corrected.
3. Discussion section: the suggested corrections have been made.

We have modified the sentence in the second paragraph into: We observed a reciprocal association between Cu/Zn levels and humoral and cellular immune response between the three patient groups (Table I and Fig. 1), as well as a complete reversal of increased Cu/Zn ratios after treatment in LCL patients."

As for the mechanism(s) involved in Cu increase and Zn decrease in LCL and ML in the same endemic area, we have added the following (and included a new reference): "In addition, (epi)genetic factors related to copper homeostasis might render normal individuals more susceptible to copper toxicity (17). Thus, increased Cu levels and decreased Zn levels might be a cause, rather than a consequence of LCL. On the other hand, absence of Cu increase, linked to uncontrolled IFN-g production, might underly evolution towards ML."

4. Reference section: we have changed reference 1 and added reference 17.

Referee 2:

1. Background section: same as ref. 1
2. Methods section: we refer to all experiments with whole blood or plasma as ex vivo (all patients and controls from Table I, Fig. 1 and 2), whereas buffy coats from normal donors were only used for the in vitro experiments described in Fig. 3. For the sake of clarity, we have modified the following sentence: Buffy coats from normal blood donors were used to obtain large quantities of cells required for in vitro experiments to examine the effect of exogeneous trace metals upon cytokine production."

3. Discussion section:

Our group (Bacellar et al., 1996; 2000; 2002; Brodskyn et al., 2000, Pompeu et al., 2001) and several others (Trujillo et al., 2002, Da Cruz et al., 2002) have not been able to detect IL-4 levels above detection limit (5-10 pg/ml) in PBMC supernatants from patients with LCL, ML, VL or healthy controls stimulated with Leishmania in vitro. In contrast, all these authors document IL-5 production as a reliable and reproducible measure of Th2 response. From the literature, it seems that IL-4 production in human leishmaniasis is limited to Old World LCL and VL (Mahmoodi et al., 2003; Milano et al.), which might reflect genetic differences in host and/or parasite.

We have added: "Absence of correlation between TNF-__ and trace metal levels underscores the specificity of the inhibitory effect of Cu upon IFN-g production, which might in fact be the upstream event to an increased humoral anti-Leishmania response."
Thanking you again for your kind attention, I remain,

Yours sincerely,

Johan Van Weyenbergh