Author's response to reviews

Title: Effect of praziquantel treatment of Schistosoma mansoni during pregnancy on immune responses to schistosome antigens among the offspring: results of a randomised, placebo-controlled trial.

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Author's response to reviews: see over
Thank you for giving us the opportunity to publish our work in your journal. Here below is the response to reviewers.

We thank all the reviewers for their very useful comments, which have helped us to make considerable improvements to the paper. We have addressed them as follows and the respective changes in the manuscript are highlighted in yellow.

**RESPONSE TO EDITOR’S COMMENT**

**Comment:** I was in agreement with one reviewer that endotoxin contamination of the schistosome antigens used is a likely explanation for the induction of IL-10 by cells other than lymphocytes. Was this tested for? I think this point merits further discussion, as I do not believe SEA induces IL-10 in APCs.

**Response:** We acknowledge this oversight and information on the antigens used for whole blood stimulation has been included (paragraph 5) in the methods. Endotoxin levels in SWA and SEA were measured using a Limulus Amebocyte Lysate Kit, QCL-1000 (BioWhittaker Inc, Walkersville, MD, USA) and concentration in antigen was 0.086EU/mg of SWA and 0.175EU/mg of SEA. This information has been included at the beginning of the section on whole blood culture and cytokine assays (paragraph 5) of the methods. The antigens were used at final concentrations of 10mg/ml. Thus in the whole blood cultures the endotoxin levels were negligible (<0.1ng/ml) and not likely to explain the IL-10 or the other cytokine responses.

We have no information on the cellular source of IL-10 and paragraph four of the discussion has been adjusted to reflect this.

**Comment:** Also I would be interested to know the actual levels of the cytokines produced

**Response:** I have submitted a new document for publication as supplementary material (additional files 1 and 2), showing the data corresponding to the figures.

**RESPONSE TO REVEIWER 1**

**Comment:** Table 1 indicates that there was no statistically significant correlation between rank correlation coefficients in any of the comparisons. The authors nonetheless to advance an (acknowledged) speculative reasoning for why certain cord-blood responses were correlated with maternal infection intensity in the placebo group but not the praziquantel group. It
would be simpler to acknowledge that the data were unable to demonstrate any statistically significant difference between the two groups, given that the large number of tests conducted in the analysis was very likely to have produced some aberrant results.

Response: We acknowledge that table 1 shows multiple comparisons and that there was no statistically significant modification of associations between cytokine responses and infection intensity by maternal praziquantel treatment. The study was not powered to assess such interactions. However, the apparent association between maternal infection intensity and type 2 cytokine responses to SWA in the cord blood in the placebo group was biologically plausible, and the interaction terms were borderline; we therefore consider that it is worthwhile to discuss the possibility that this may be an effect of interest in as far as in-utero sensitization may be concerned. We have modified the discussion (paragraph 5) to emphasize the limitations of this analysis.

Comment: The discussion could be improved by considering the bigger picture (e.g. the immunological properties of cord blood) and questioning the validity of the hypothesis

Response: We have addressed this concern by including more discussion and additional references regarding the properties of cord blood and the fetal default bias to tolerance (discussion, paragraph 4). We have also added a clear statement to the effect that, overall, our results do not support the hypothesis that praziquantel treatment during pregnancy influences the sensitization of the fetus to schistosome antigens (last sentence paragraph 5 of the discussion)

RESPONSE TO REVIEWER 2

Discretionary Revisions

Comment 1: Methods, Whole blood culture and….., last line. It would be useful to some readers if the authors would provide at least the range of control cytokine levels that were subtracted from the antigen-stimulated levels. Were they highly variable? Were they substantial? If so, were there differences between groups?

Response: The levels of cytokine in control wells were generally low, and did not differ between the treatment groups. This information has been added; paragraph three of the results.
Comment 2: The actual levels of eggs/gram of stool would be of considerable interest to some readers, and while it is impossible to give them all, at least a median and a range might be useful.

Response: This information has been added; first paragraph of the results.

Comment 3: The Journal may not wish to do so, but for me the “Additional Files” contain valuable information that adds to an understanding of the manuscript. If possible I would recommend their inclusion in the manuscript proper.

Response: We would be grateful to see the additional material included and have revised the presentation of the mentioned “additional files” accordingly into table 2 and figure 4

Minor Essential Revisions

Comment 1: Results – cord blood cytokine responses. The responsiveness of the cells of 29% to 33% of the cord bloods to make IFNg and IL-2 and 58% to make IL-10 seems significant, but the authors rather dismiss this antigen-stimulated responsiveness. This seems odd to me. The IL-10 responsiveness is dismissed as being due to non-lymphoid cells, but as I understand the Methods section this is the level of IL-10 produced by stimulation with either SWA or SEA – with the levels produced without SWA or SEA subtracted out, i.e., representing the antigen-specific responsiveness. This confuses me, and I think the authors should reconsider their explanation and think in terms of what these presumably antigen-specific responses mean. A more trivial explanation, which they might be able to address, is that there are traces of LPS in the antigen preparations – then the non-lymphoid source of IL-10 would make more sense.

Response: We were concerned not to make too much of the cord and infant responses for most cytokines because (with the exception of cord IL-10) they were so low – the majority below 10 pg/ml – and the precision of the assay is not such as to allow great weight to be put on these findings. However, we appreciate the point that the IL-10 must be antigen specific, in some way, given that background was subtracted. The real issue is that we still do not know the cellular source – whether “innate” cell, T-cell or B-cell. We have now devoted a full paragraph (paragraph 4) to the discussion of the cord blood IL-10 response findings, and are grateful for this thought-provoking comment.

The question of LPS has been addressed above, in response to the editors comments and information has been included in methods (paragraph 5)

Comment 2: Abstract, Results; Results, 2nd paragraph; Results, last paragraph; Discussion, 5th paragraph.
(a) The authors state, in several places, that there is no evidence that any of the 1 year olds are infected with *S. mansoni*. I would submit that their own data argue against that conclusion. What would be correct would be to say that all the stool examinations by Kato-Katz (and they do not say in the manuscript how many were done) were negative for *S. mansoni* eggs. It is well known and has been documented many times that egg-positivity can often be a matter of how many stools and slides are examined.

Response: We have added information to the methods (first paragraph) on the number of slides and stool samples examined and have revised the statements about infection status of the infants to reflect egg detection, rather than definitive infection status (abstract and last line of paragraph 2 of the results).

(b) The IgG1 and IgE antibody responses to SEA, which the authors find “surprising” (last paragraph/Results), are not at all surprising to me. In fact, based on other literature from Ugandan lakeside villages, they are rather expected in a proportion (the authors only state “a number” but Figure 3D makes the IgG1 look like at least several) of 1 year olds in a such a setting. Early infections in this setting are much more common than previously thought, and a paper from Kenya indicates that anti-schistosome (in this case anti-worm) antibodies are detected before eggs are numerous enough to detect in the stool. If I might conjecture further, if a 6 or 8 month-old infant is exposed to *S. mansoni* cercariae their worm burdens by 1 year of age may still be quite low, although their one or two worm pairs might have been producing eggs for 4 or 5 months, which would fit nicely with the authors observations that anti-SEA levels are greater than anti-SWA levels. I strongly suggest that the authors reconsider their conclusions that none of their 1 year-olds are infected, and if possible it would be of interest to re-examine those who are anti-SEA+ (for IgG1 and IgE) in terms of their mother’s status (intensity), etc. It is quite likely that there is nothing more there than they already report, but this subgroup that I believe did get infected during their first year of life (based on their responsiveness) might be worth a second look.

Response: We greatly appreciate this interesting comment. We have added additional information on the numbers of infants with antibodies to SEA in the last paragraph of results and have modified the last paragraph of the discussion to address these issues.

Comment 3: The authors refer to Tables 4 and 5, but I can only find Table 1 and Additional File 1. This needs to be sorted out.

Response: Thank you for noticing this mistake. The concern has been rectified. The said information is now presented in additional file 2 and table 3 respectively.
RESPONSE TO REVIEWER 3

Comment 1: Background section, first paragraph—in two locations, the authors refer to “anti-idiotypic” antibodies. It should be simply “idiotypic” antibodies.

Response: This recommendation has been accepted and the change effected.

Comment 2: Presumably, the authors are using the standard cut-off of 100 epg to distinguish low intensity infections from moderate and high intensity infections but this is not specified anywhere in the manuscript.

Response: Yes; this information has now been provided in the first paragraph of the methods.

Comment 3: The authors should specify units in Figures 1-3 and additional table 1.

Response: We are grateful to the reviewer for pointing out the omission. The units pg/ml for cytokines and µg/ml have been included on the scale labels as well as in the respective legends.

Comment 4: “Table 5” that presents correlations between antibody levels and maternal infection intensity levels. This table was not included in the materials sent to reviewers. Perhaps it was part of an earlier version of the manuscript that got removed. However, it would be informative to include in the final publication either as a regular table in the paper or as an additional file.

Response: addressed as in comment 3 of reviewer 2.