Reviewer’s report

Title: Modulation of innate immune responses at birth by prenatal malaria exposure and association with malaria risk during the first year of life

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Reviewer: Prasanna Jagannathan

Reviewer's report:

In this paper by Natama et al, the authors describe an ambitious analysis of cord blood innate cell responsivity to TLR stimulation among a birth cohort of children whose mothers had evidence of malaria in pregnancy. The authors had two overarching questions: 1) What impact do different manifestations of malaria in pregnancy have on both spontaneous and TLR-stimulated cytokine production? 2) Is spontaneous or TLR-stimulated cytokine production at birth associated with protection from malaria in infancy? The authors found that spontaneous cytokine, chemokine, and growth factor production were all significantly lower in samples with evidence of PME than in those that were unexposed, but, following TLR7/8 stimulation, samples with evidence of resolved placental malaria (pigment only) were "hyperresponsive" in comparison to samples without evidence of prenatal exposure. Furthermore, certain responses (both spontaneous and following TLR stimulation) were associated with differential malaria risk in infancy. Strengths of the manuscript include large numbers of samples studied (n=313), use of placental histopathology and longitudinal data in pregnancy to detail prenatal malaria exposure, ability to evaluate longitudinal associations with clinical malaria in infancy, and appropriate statistical analyses (with correction for numerous comparisons) employed.

Although the authors have described several interesting associations, I have several suggestions that would greatly enhance interpretability of the manuscript:

1) Was this cohort made up from both arms from the parent COSMIC study? Given the differences in follow-up between the treatment arms, how often was parasitemia assessed by RDT during pregnancy? Were these similar/different in parasite prevalence/treatment between the various PME arms? This should be reported and added to Table 1.

2) Figure 1 would greatly benefit from additional details regarding subject selection. Furthermore, there should be some mention as to how representative this subset is to the overall cohort (i.e., were associations between PME and subsequent child malaria risk also found in the subset of children where TLR responses were not measured?).
3) One major concern is the choice of the control group (Prenatal malaria exposure unexposed, n=22, or only 7% of the overall cohort) and whether this group is truly "unexposed."

   a. How was this group selected? How representative is this unexposed group in comparison to the unexposed group with the parent COSMIC cohort, since only a subset of the overall cohort was studied?

   b. Although this control group is utilized to report associations between various PME groups, given its small size (and the small size observed in the some of the other groups) lack of significant associations between groups may simply be a reflection of inadequate power. This should be discussed.

   c. Did the authors perform statistical analyses to evaluate whether there were differences in cytokine production between PME groups that were exposed? This was not reported. Relatedly: in Figure S3, several of the features appear to have a trend towards decreasing production among unstimulated samples with increasing "severity" of PME. Did the authors assess for trend across these categories?

4) Relatedly: categories of acute, chronic, past placental malaria. Both acute and chronic PM have evidence of active placental infection - did authors evaluate whether combining these 2 groups increased statistical power to observe for differences in TLR responses?

5) Regarding the category of exposed/no PM. Did these women have clinical malaria, asymptomatic parasitemia, or both during pregnancy? Please provide additional details.

6) The authors make no mention in the results as to which cells may be producing these cytokines, nor to whether differential admixture of cell types in the PME categories may be explanatory for any differences observed. Did the authors measure cellular frequencies? Would also expand the discussion of this (lines 378-380, 404-408)

7) Re: Prospective protection: figure one shows that children born to mothers with placental malaria have a lower risk of malaria during the first 6 months. That is in contrast to what has been reported in several epidemiologic studies. Why do the authors postulate this reduced risk? Does their data help to explain this observation? Authors should address this in the discussion

   a. Relatedly - authors only discuss time to clinical malaria. Did they observe any different associations between analytes measured and repeated malaria in infancy (incidence), parasite prevalence in infancy, or severity of symptoms if infected? Did the authors observe
associations with risk of non-malarial febrile illness given evidence of hyper-responsivity to TLR7/8 stimulation?

8) Overall the discussion section was fairly difficult to follow. The first paragraph didn't clearly summarize the study's main findings and this could be useful to setup the rest of the discussion. Furthermore, although the question of how prenatal malaria exposure may shape innate immune responses is interesting, I find the more critical question is how innate immune responsivity at birth may influence prospective risk. I would elevate this in the discussion. That paragraph should also be reorganized to discuss cytokines/chemokines/growth factors associated with protection followed by those associated with risk, since that may make the section easier to follow.

9) The paper lacks a limitation section in the discussion. Some of the points addressed above could be included.

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If not, please specify what is required in your comments to the authors.

Yes

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