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

Version: 0 Date: 17 May 2018

Reviewer: Christopher King

Reviewer's report:

This study aims to examine the impact of malaria in pregnancy on the innate immune response in whole cord blood in responses to different TLR agonists. Key findings show that certain TLR agonists are more likely to induced cytokine/chemokine responses than others, that newborns of mothers with chronic placental are more likely to have TLR-induced cytokine responses, and spontaneous cytokine production is reduced in cord blood of newborns exposed to malaria during pregnancy compared to those not exposed. In addition there is some association between innate immune response to certain cytokines/chemokine with either increased or decreased risk of malaria infection in the newborns over a year of followup. Similar studies have been performed in the past, but there are unique characteristics of this study, including better definition of the types of material exposure based on recent or past placental malaria, as determined by placental histology.

There are several concerns with this study;

General comments:

1. There could be better definition of malaria exposure during pregnancy of the mothers of the different groups. Samples for study were selected as part of larger study examining different treatment regimens during pregnancy. For example how often were mothers examined for presence of malaria? How often were they infected? When were mothers enrolled and how often did they receive malaria chemoprophylaxis during pregnancy? These are important variables that could affect exposure maternal and thus fetal exposure. Having this information is an asset the study.

2. Cytokine responses were performed with whole blood. However there is no reported measurement of WBC populations and lymphocyte subsets in cord blood at delivery. There is evidence that prenatal malaria exposure can alter myeloid subsets abundance could account for the differences observed to TLR agonist. If this information is not available, it should be stated as such and potential limitations in the interpretation of their data.
3. A challenge in evaluating malaria risk in the first year of life is exposure. Obviously mothers with malaria exposure during pregnancy are more likely to live in an environment with higher malaria exposures, which will impact their infants. This has always been a challenge in interpreting the impact of prenatal malaria exposure on subsequent malaria during infancy. For example their observation that offspring of PM+ mothers was decreased in the first 6 months would be a result of passive transfer of protective maternal antibodies and then increased risk from 6 to 12 months from increase malaria exposure. These factors can easily obscure any prenatal immune response. How exposure controlled for at all?

4. We usually do not think of innate immune response having memory. So how does malaria exposure earlier in pregnancy, influence innate immune responses observed at delivery? Impact more rapid maturation of innate immune response? There is some evidence for memory in NK cells, but there is also controversy whether NK cells express much TLR 7/8. There should be some discussion about the mechanisms of how prenatal malaria exposure in utero affects innate immune responses.

Specific comments:

1. Overall the paper was difficult to follow, lots of data. Admittedly parsing out all the cytokine/chemokine data is challenging. The abstract need significant revision. Key points to do not come out well.

2. The discussion is too long and not well focused on mechanisms as discussed above and limitations of the study.

3. Figure 2 showing principal components are not well described and does not help much in interpreting the data. If the PC analysis was able to group cytokine/chemokine responses in an unbiased fashion and the PC could be used a predicting variables rather an individual cytokine/chemokines, then this would be useful in the analysis. Otherwise Supp Fig 5 would be most helpful in understanding the immune responses.

4. Unclear how much Figs 4 and 5 contribute to results in an impactful way.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No
**Does the work include the necessary controls?**
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Not applicable

**Are the conclusions drawn adequately supported by the data shown?**
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No

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