Reviewer’s report

Title: The importance of the PD-1/PD-L1 pathway at the maternal-fetal interface

Version: 0 Date: 11 Jan 2019

Reviewer: Denise Cornelius

Reviewer’s report:

Immunotolerance during pregnancy is critical for protection of the fetus from attack by the maternal immune system. The mechanisms regulating maternal immunotolerance during pregnancy are not clearly defined. The authors set out to investigate a role for the PD-1/PD-L1 signaling pathway in regulating maternal immunotolerance during pregnancy.

Major Concerns:

1. The abstract states that 20 healthy pregnant women were involved in the project. However, the methods and results only indicate that a maximum of 13 healthy pregnant women were recruited. Please clarify.

2. 7 samples of DIC and 10 of PMBCs from pregnant women. Were any samples lost of were cell irretrievable from tissue? Were the PBMCs and DICs isolated from different patients? Were DICs and PBMCs from pregnant women matched?

3. Line 60: The authors state that the innate rather than the adaptive immune system is dominant at the maternal fetal interface. However, the study seems to focus more on CD8+ T cells (adaptive immunity). Perhaps should revise sentence to "at the maternal-fetal interface suggests an important role of both the innate and adaptive adaptive immune systems".

4. The introduction is too long. Lines 85-107 should be condensed into 1 paragraph.

5. Authors need to introduce/discuss NKG2D, its function and how it relates to PD-1/PD-L1 expression and function. The section in the results on NKG2D is the first you hear of it and readers may not be aware of this receptor and its function.

6. Are data normally distributed or did authors test for normality? If so, please state this in the statistics. With such small Ns this is a concern.

7. Was power analysis performed for this study?

8. Was the T test paired or unpaired?
9. According to sample flow cytometry gating strategy, Tregs were identified from the same sample of cells stained for NK cells. In the methods, both CD56 and Foxp3 are on APC, how could the authors distinguish these cells types with two markers in the same panel on the same Fluor? Wouldn't the Tregs appear as CD56+ and be identified as NKT cells?

10. Line 299: How do CD8+ T cells give a Th1 immune effect? Do the authors mean type 1 instead of Th1? Similar question for Line 329.

11. Lines 351-354: If soluble ligands for NKG2D bind to the receptor how does this reduce cytotoxicity? This seems contradictory to the authors previous description of NKG2D as an activating receptor. Does binding to NKG2D trigger cytotoxic function or inhibit it?

Minor Concerns:

1. English type editing required

2. Line 110-111: What did the study say about how PD-L1 influences Treg cells? Did the influence promote survival or demise of the fetus?

3. Ethical information should be included in participants section of Methods

4. Intracellular staining method should be before flow cytometry method

5. Authors should identify NK cells as CD56+NK, CD56Dim NK and CD56brightNK rather than just NK, NKDim and NKbright

6. What type of controls were used for the flow cytometry? FMOs, isotype controls?

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? 
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown? 
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

**Quality of written English**
Please indicate the quality of language in the manuscript:

- Needs some language corrections before being published

**Declaration of competing interests**
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal