Reviewer's report
Title: TLR4 Modulates Inflammatory Gene Targets in the Retina During Bacillus cereus Endophthalmitis

Version: 1 Date: 27 Feb 2018

Reviewer: Moran Homola

Reviewer's report:
The authors have convincingly addressed the issue raised regarding the relevance of the data for current therapeutics. However, minor but critical issues arise concerning the discussion. Although rewriting and improving some sections in the discussion, the discussion, in its current form, still requires modifications before publication. Some paragraphs are written in an unclear way, which makes it very hard for the reader to understand the hypotheses and arguments made by the authors. Furthermore, some of the statements made by the authors to support their hypotheses are based on inaccurate information and the relevance of the citation made in the discussion is not always clear. This is elaborated in the following points:

* Page 11:

Lines 4-5: "CXCL10 is secreted by monocytes, endothelium, and fibroblasts after IFN-γ stimulation in response to viral infection, and "after" LPS stimulation in response to Gram-negative infection." - Please add the word "after"
Lines 7-8: "Since LPS activates TLR4, the upregulation of CXCL10 could be the result of the activation of the TLR4 pathway by a novel ligand related to B. cereus infection [26]". - Please rephrase this sentence to better explain your meaning. How does the fact that LPS activates TLR4 support the notion that CXCL10 upregulation is attributed to the activation of the TLR4 pathway by a novel ligand?
Furthermore, please explain how reference #26 relates to your statement. It does not seem connected at all.
Lines 9-10: please explain why the fact that CXCL10 was not upregulated in a TLR2-dependent inflammation supports the hypothesis of the existence of a novel ligand for TLR4.

Line 17: This statement is not correct!
In the publication of Parkunan et al. (2015) no difference was detected in the expression of IL-6 between C57Bl mice and TLR4-/- mice at 4 hours post-infection.

Lines 17, 19: Please insert a citation
Line 20: Parkunan et al. did not show increased levels of IL-1β 4 hours post-infection

Lines 20-23: In order to make the point that the authors aim for, it would be advisable to mention again that the data from Parkunan et al. was obtained from whole globes.

* Page 12,

Lines 2-3: "While IL-6 has also been shown to be anti-inflammatory based on its effects on TNFα and IL-1β expression [60]" - Please specify which effects IL-6 has on TNFα and IL-1β.

Lines 6-7: "while not precluding its role as a signal to upregulate TNF-α and IL1- β expression at a later time point as a means to limit inflammation" - Should be "downregulate" in order to make sense! Or upregulate their antagonists, as mentioned in the study that was cited.

* Page 15,

Lines 15-17: The unlikeliness of the hypothesis refers to the statement "Excessive production of 11-cis-retinal is toxic for photoreceptor cells". While the toxicity of retinoid by-products is indisputable, toxicity via excessive amounts of 11-cis-retinal is not well established. The authors are requested to provide a citation to support this statement.

The citations used by Chucair-Elliott et al. to support a similar statement refer to studies focusing on the protective effects of downregulation of RPE65 and thereby of 11-cis-retinal in a light-induced retinal damage. In this model, mice (usually mice that carry a light sensitive variation of the enzyme RPE65) are exposed to high light intensities to induce retinal degeneration. Different genetic variations of the enzyme RPE65 provide lower/higher levels of 11-cis retinal leading to decrease/increase in rhodopsin regeneration. Therefore, different genetic variations of RPE65 provide resistance or susceptibility to the light-induced retinal degeneration. Making a
correlation between processes occurring in such a model with other conditions is somewhat problematic.

* In multiple positions in the text there is reference to infected mice in comparison to control uninfected mice (e.g. page 19, line 14; table 1; P.7, L.21; P.8, L.1, etc.). According to material and methods there was no use of uninfected mice in this study, but rather the use of one uninfected eye in each mouse as control.

* The authors use the timing of 4 hours post infection (hence prior to PMN/neutrophils infiltration) as an argument to support the suggested roles of the different mediators in an inconsistent way. In some places they claim that expression early during infection (viz. prior to infiltration) might indicate a different role than involvement in neutrophil infiltration (p. 14, l. 1-2), while in other places they hypothesize the involvement of other mediators in neutrophil infiltration and ignore the same early timing (p.13, L.5-8).

* In multiple locations in the text IL-1β is written as IL1-β. Please keep consistency throughout the text.

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

Yes

**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 am able to assess the statistics
Quality of written English
Please indicate the quality of language in the manuscript:
Acceptable

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