Author's response to reviews

**Title:** Enhanced upper genital tract pathologies by blocking Tim-3 and PD-L1 signaling pathways in mice intravagninally infected with Chlamydia muridarum

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**Version:** 2  **Date:** 14 November 2011

**Author's response to reviews:** see over
November 13, 2011

Dr. David Ojcius
Editor
Journal Editorial Office
BioMed Central

Re: Revision of manuscript#5087930485700208, entitled “Enhanced upper genital tract pathologies by blocking Tim-3 and PD-L1 signaling pathways in mice intravaginally infected with Chlamydia muridarum” by Bo et al.

Dear Dr. Ojcius,

Thank you and the reviewers for reviewing the above manuscript and providing constructive comments. We have carefully revised the manuscript and addressed the concerns raised by the reviewers. An item-by-item response to the reviewers’ comments is provided.

Comments from reviewer#1 (Dr. Li Shen):
Needs some language corrections before being published

We have carefully revised the manuscripts including language editing. The revised manuscript is now more concise (reduced from 26 to 23 pages).

Comments from reviewer#2 (Dr. Qing He):

1. Materials and method need to be organized. Author should reference their previous publications to replace the detailed description of experiments.

We agree. We have reorganized and significantly shortened the materials and methods section.

2. Figures are not easy to read and need to simplified, especially figure 1.

We disagree with these comments. The figures are in standard formats previously utilized by us and other chlamydiologists to report findings from Chlamydia-infected mice. Figure 1 panel A describes the mouse antibody treatment and infection time line, panel B lists the live organism shedding time course while panel C indicates the number of mice remaining positive for live organisms shedding. Although the information revealed is comprehensive, it is straightforward and easy to follow when I consulted with various nonchlamydial microbiologist colleagues in my home department.

3. Reduce number of pages.
We agree. The revised manuscript is now more concise (total pages reduced from 26 to 23).

4. Can authors give an explanation why double blocking of these two pathways did not alter C. muridarum infection time course

We propose that the primary reason is that the normal mouse immune responses to intravaginal infection with C. muridarum organism infection are already very robust and efficient in clearing the infection. Removal of some negative regulation loops may not be enough to see enhanced the clearance of infection. In addition, the Tim3 and PD-1-mediated negative regulation may mainly target CD8+ T cells while CD8+ T cells are known to play a minimal role in protective immunity against chlamydial infection. More importantly, CD8+ T cells have been proposed to significantly contribute to inflammatory pathology in the upper genital tract. These analyses may explain why blocking of Tim3 and PD1 only enhanced upper genital tract pathology without affecting lower tract infection course.

Comments from reviewer#3 (Dr. Huizhou Fan):
1. The rationale of selecting BALB/c mice instead of other mouse strains should be given.

Many different strains of mice are susceptible to chlamydial infection. The selection of Balb/c strain in the current study was due to the use of the same strain of mice in our chlamydial vaccine studies sponsored by Merck. Since we hope to apply knowledge on the immunological properties of chlamydial infection we have learnt from the current study to improve vaccine efficacy, using the same strain of mice in both studies will make the observations/data more comparable.

2. Although antibody treatment resulted in the worsening of hydrosalpinx, the effect is somewhat mild, and caution should be taken to not overstate the effect.

Yes, we agree that caution should be taken when interpreting our data. We have modified the description of the treatment effects.

3. Medroxyprogesterone treatment prior to chlamydial infection is to achieve a synchronized estrous cycle in mice, not the “menstrual cycle” as stated by the authors in p5. The latter term is for primates only.

Changed made as suggested.

4. p11, “during intravaginal infection with Chlamydia muridarum” should be deleted or be changed to “after…”.

The phrase is deleted.
5. p14, the effect of Tim-3 and PD-L1 antibody treatment should be referred as restoration of in vitro cytotoxicity towards HCV-bearing hepatocytes instead of inhibition of viral replication.

Changes made as suggested.

6. A few typographical errors should be corrected.

Corrections made.

7. Needs some language corrections before being published

We have carefully revised the manuscripts including language editing. The revised manuscript is now more concise.

We hope that our revision to the manuscript and responses to the reviewers’ concerns are adequate. However, if there are still additional concerns, please feel free to let us know.

Sincerely,

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