Author's response to reviews

Title: Changes of cytokines in a mouse model of post-infectious irritable bowel syndrome

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Author's response to reviews: see over
Dear Editor,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We greatly appreciate the editor and reviewers’ positive and constructive comments and suggestions on our manuscript entitled “Changes of the T cells-related cytokines on post-infectious irritable bowel syndrome mouse model” (MS: 1874287444123182).

We have studied the reviewers’ comments carefully and made revision in the places marked in red in the paper. We have tried our best to revise our manuscript according to the comments. Attached please find the revised version, which we would like to submit for your kind consideration. We have respond the comments of Reviewer in the “Cover letter”.

We would like to express our gratitude again to you and reviewers for the comments on our paper.

I am looking forward to hearing from you.

Yours sincerely,
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Responses to the comments of Reviewer

Reviewer: Patrick Hughes

Reviewer’s report:

Dear Prof. Patrick Hughes:

Thank you for your kind instructive comments and suggestions.

Comment 1: *Trich. spiralis* infection is recognised as causing a mainly TH2 type response from the immune system. The interesting findings here indicate this may switch to a TH1 type response after clearance. While the authors show that histology is cleared, these findings would be much stronger if TH1 and Th2 type cytokines were evaluated during infection to provide an indication of the immune phenotype of the infection.

Response: Thank you for your insightful comment. *Trich. spiralis* infection is indeed a mainly TH2 type response, which was confirmed in our pre-tests. Before a formal test, IL-10, IL-1β, and IFN-γ were measured by ELISA at three points in time (1 week, two weeks, and four weeks). We found IL-10 concentrations of infected mice were all lower than these of control mice, however, compared to control mice, the concentration of IFN-γ was lower at 1 week, and were increasing gradually from 2 weeks, the IL-1β concentration was higher in 2 weeks, and was decreased to normal level. Therefore, we think that the course of *Trich. spiralis* infection is a mainly TH2 type response in the acute infection phase, especially from 1 week to 2 weeks, and some TH1 cytokines were gradually increased after the acute infection phase, which caused imbalanced shift of cytokine expression between Th1 and Th2. This phenomenon has been found in PI-IBS patients and PI-IBS model mice which were infected by *Trich. Spiralis*1, 2.

Comment 2: Previous work investigating *Trich. spiralis* infection was not adequately discussed in the introduction or the discussion.

Response: We agree with the reviewer’s opinion. Accordingly, a few sentences which are focus on *Trich. spiralis* infection have been added in the introduction and discussion section of the revised
Comment 3: T cells were not specifically investigated as part of this study, and other immune cell types are capable of secreting cytokines. Therefore the title gives the wrong impression and needs to be changed.
Response: We feel really sorry to give you the wrong impression because that we did not provide a clear title in our initial manuscript. My co-authors and I decide to change “Changes of the T cells-related cytokines on post-infectious irritable bowel syndrome mouse model” into “Changes cytokines in a mouse model of post-infectious irritable bowel syndrome”.

Comment 4: Histology is presented as evidence of inflammation, but individual regions of the small intestine and colon were not scored so there is no indication of the severity of the inflammation caused.
Response: We are grateful for your kind suggestions to our paper. Why we did not scored the inflammatory severity of the small intestine and colon are mainly as follows: Firstly, PI-IBS mouse model induced by *Trich. spiralis* infection has been frequently used in the study of mechanisms of PI-IBS. Secondly, we consulted Prof. Xiaohua Hou (Division of Gastroenterology, Union Hospital of Tongji Medical College) and Dr. Baoquan Fu (Chinese Academy of Agricultural Sciences) about the establishment of the PI-IBS mouse model induced by *Trich. spiralis* infection. Furthermore, in this study, we just needed to prove a transient infection or acute inflammatory reaction in the mucosa of the intestinal tract so as to simulate the infection history of PI-IBS patients. Prof. Yu Li and Mr. Bing Liu (Department of Anatomic Pathology, Chongqing Medical University, China) have confirmed inflammatory reaction indeed existed at 1 week and 2 weeks post-infected, and we also obtained *Trich. spiralis* larvae from C57L/B6 mice infected. It needs a more in-depth research to found whether the levels of cytokines is related to the severity of the inflammation caused.

Comment 5: AWR scores were altered during low / mid pressures, but did not significantly differ from healthy mice at high pressures. This indicates low/medium threshold nerves but not high threshold nerves are altered, but this is not discussed adequately.
Response: Thank you for raising this important question. This point has been discussed and a paragraph has been added in the DISCUSSION section of the revised manuscript.

Comment 6: The standard of English needs improvement and proof reading.
Response: We are grateful for the positive comments regarding our paper. According to your kind suggestion, we have sought the assistance of a fluent English speaking colleague to polish our manuscript.

Comment 6: It is well written, but I am doubtful if the results would be of any relevance to patients with post-infectious IBS.
Response: a few sentences have been added in the DISCUSSION section of the revised manuscript.

References:
2. Chen J, Zhang Y, Deng Z. Imbalanced shift of cytokine expression between T helper 1 and T helper 2 (Th1/Th2) in intestinal mucosa of patients with post-infectious irritable bowel syndrome. BMC