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

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Version: 3 Date: 27 January 2015

Author's response to reviews: see over
Title: Changes of the T cells-related cytokines on post-infectious irritable bowel syndrome mouse model

Version: 2 Date: 27 December 2014

Reviewer: Patrick A Hughes

Reviewer's report:

General

The authors investigated whether acute inflammation induced by Trichinella has long term implications on immune and nerve function after the infection has cleared. The results show that there is an increase in Th1 type cytokines (but not IL-1β) and a decrease in IL-10 in regional segments of the small intestine but not the colon, and there is an increase response to balloon distension in post-infected mice but compared to healthy mice. This work is potentially very interesting, but suffers from several major issues:

Major Revisions:

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.

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*. The references are as follows:


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

We agree with the reviewer’s opinion. Accordingly, the following sentences have been added in the INTRODUCTION:

Many pathogens can produce PI-IBS. Bacteria and parasites are often used in PI-IBS animal models. But as for PI-IBS animal models caused by bacteria, the major characteristics of IBS such as visceral hypersensitivity, alterations in motility and secretion are weak sometimes even have not been found, so there is still it remains controversy whether the model infected by bacteria is a valid model of PI-IBS. *Nippostrongylus brasiliensis* and *cryptosporidium parvum* were used in rat models of PI-IBS. However, it was found that models infected by
**nippostrongylus brasiliensis** were lack of visceral sensitivity. The features of IBS, such as motility dysfunction and secretion alteration have not been evaluated in *cryptosporidium parvum* infection model[3]. Infection of *Trichinella spiralis* larvae induced changes in visceral sensitivity, alterations of intestinal smooth muscles, and secretion. These abnormalities persisted after recovered from infection, making this model feasible for the PI-IBS[4, 5].

Various animal models have been developed to get insight into the underlying mechanism of irritable bowel syndrome. A great number of studies demonstrated that some indicators such as visceral hypersensitivity and persistently dysfunction of intestinal muscle exited in mice infected with *Trichinella spiralis*[4, 16, 17]. Actually, the inflammation response to intestinal parasites has been regarded as a representative defense response against pathogens. For this reason experimental papasite *Trichinella* infection has been widely used to establish models for detecting the pathogenesis of intestinal dysfunction[18, 19].

And many sentences have been added in the DISCUSSION section of the revised manuscript:

*After* *T. spiralis* infection 8 weeks, GI system still have disturbed visceral hypersensitivity without any histological evidence of intestinal inflammation. It means *T. spiralis* infection models are acceptable model to represent post-infectious IBS[33].

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.

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 of cytokines in a mouse model of post-infectious irritable bowel syndrome”.

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.

We are grateful for your kind suggestions to our paper. In this study, A histopathological score was referenced by Dieleman (this sentence has been added in the Histological analysis section of the revised manuscript), and the results of this study were consistent with those of the reference edited by Dieleman. 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 wheather the levels of cytokines is related to the severity of the inflammation caused.

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.

Thank you for raising this important question. This point has been discussed and sentences have been added in the DUSCUSSION section of the revised manuscript:
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. This may be related to different different mechanical stimulation activated by different pressure expansion[34]. When distention volume was 0.25ml, the low pressure was too low to cause visceral sensation. When distention volumes was 0.65ml, the high pressure, as a kind of noxious stimulation to both group of mice, resulted in a strong gut feeling. When distention volumes was 0.35 or 0.5 ml, the AWR scores in model group were higher than in control group, and all the pain threshold in the model group were lower than the same time point in control group.

6: The standard of English needs improvement and proof reading.

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.

There are no minor essential revisions or discretionary revisions.

Level of interest: An article whose findings are important to those with closely related research interests.

Quality of written English: Not suitable for publication unless extensively edited.

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: None
Reviewer's report

Title: Changes of the T cells-related cytokines on post-infectious irritable bowel syndrome mouse model

Version: 2 Date: 24 August 2014

Reviewer: Cesare Cremon

Reviewer's report:

General

This is an interesting study assessing the role of immune mechanism in the pathophysiology of post-infectious irritable bowel syndrome (PI-IBS). In mice infected with Trichinella larvae, the authors assessed abdominal withdrawal reflex (AWR) scores, intestinal histology, and the expression (at day 56 post-infection) of pro- and anti-inflammatory cytokines. The levels of IFN-# and IL-17 were significantly increased in the duodenum and ileum, while those of IL-10 were decreased in jejunum, ileum, and colon of PI-IBS vs. control group. The study appears to be well designed and conducted, and the results are clearly reported. The discussion is consistent with the results.

However, English language is sometimes poor and needs consistent revision. All the sections should be thoroughly revised since some mistakes may have occurred.

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.

Level of interest: An article of importance in its field.

Quality of written English: Needs some language corrections before being published.

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests.
Reviewer's report

Title: Changes of the T cells-related cytokines on post-infectious irritable bowel syndrome mouse model

Version: 2 Date: 21 December 2014

Reviewer: Magdy El-Salhy

Reviewer's report:

General

The study represent an experimental animal model to study post-infectious changes in cytokines produced by T-cells. The study is interesting, well planed and conducted.

It is well written, but I am doubtful if the results would be of any relevance to patients with post-infectious IBS.

Thank you for raising this important question. Due to many limitations on clinic studies of PI-IBS patients, such as the collection of the whole intestinal tissue. PI-IBS animal model are often used to investigate the mechanism of IBS. Except same symptoms, many cytokines have a similar change between PI-IBS patients and PI-IBS animal model. For example, imbalanced shift of cytokine expression between Th1 and Th2 in PI-IBS model mice has been found in PI-IBS patients. Therefore, we will make further study about changes of cytokines in PI-IBS patients.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: None