

## 2 Peer-review report

### RESPONSE TO REVIEWER 1

*The manuscript is good, but LC-MS/MS data must be provided as the authors used. Also, the manuscript need English language correction throughout.*

**AUTHOR RESPONSE & ACTION TAKEN:** As suggested, the LC-MS/MS data have now been added to the supplementary materials and explained in line 212. In addition, we have now had a professional medical editor (Brian Quinn, Japan Medical Communication) whose native language is English proofread the revised manuscript.

### RESPONSE TO REVIEWER 2

*1) English should be thoroughly reviewed, some paragraphs are in a good form, others contain many mistakes, please check especially the materials and methods section.*

**AUTHOR RESPONSE & ACTION TAKEN:** Thank you very much for your professional and detailed comments, which have helped markedly improve the quality of the manuscript. As suggested, we have now had a professional medical editor (Brian Quinn, Japan Medical Communication) whose native language is English proofread the revised manuscript.

*2) The authors use many acronyms, and the most are well known to the scientific community, nevertheless they should mention the entire meaning of every acronym the first time they mention it in the text, or add a paragraph explaining all the acronyms used and their meaning, depending on the policy of the journal.*

**AUTHOR RESPONSE & ACTION TAKEN:** The acronyms used in this article have now been spelled out in full the first time they are mentioned in the text.

*3) The abstract seems very long, it is not possible to shorten it to a more usual length for a research article?*

**AUTHOR RESPONSE & ACTION TAKEN:** The abstract has now been rendered more concise. The main parts that were revised are the BACKGROUND and RESULTS.

*4) In contrast, the Introduction could be longer, the state of the art is not completely described, for example no mention to the relevance during intestinal infection of the blood cytokines investigated by the authors, and what is already known on the rise of these specific cytokines during *S. flexneri* intestinal infection.*

**AUTHOR RESPONSE & ACTION TAKEN:** The Introduction has been expanded (line 93-108). We have now summarized previously published papers concerning elevations in cytokine levels during *S. flexneri* intestinal infection in different models and patients. We also pointed out that this article explores trends in not only the cytokines IL-1, IL-6, IL-10 and TNF $\alpha$  but also IL-17 $\alpha$ , which plays important roles in host immunity against intracellular pathogens.

5) In the Results section there are several points to be clarified by the authors: -In the first paragraph, “Orally administered *S. flexneri* can provoke pathogen colonization and intestinal inflammation in rats”, line 294 referring to (Figure 1a), please check if the sentence describes this panel because panel a) refers to feces bacterial count and not inflammation, furthermore for what concerns the histological panels from b to g (Fig. 1) they should be described better in the text, with a short description referring to each panel.

**AUTHOR RESPONSE & ACTION TAKEN:** Thank you for your suggestion. We have now briefly described each panel in the text (line 268-274). We have also removed Figure 1a from line 273.

-In the second paragraph, “Changes of the WBC count and CRP level during the course of infection” the authors should carefully check CRP concentration measured because numbers are, for example,  $4.87 \pm 1.59 \times 10^6$  ng/mL and that means a blood concentration of 5 mg/mL, I frankly think that this is not possible, CRP is usually in the order of mg/L, maybe there is an error in the unit of measure?

**AUTHOR RESPONSE & ACTION TAKEN:** After reading your comments, we reviewed references concerning the rat CRP level. DeBeer et al (1982) reported that the serum concentration of CRP in adult rats maintained in a pathogen-free environment was 0.41 mg/ml (410 mg/L). Mumomura et al. (1990) reported that in normal rats, the mean concentration of CRP was  $3.6 \pm 0.8$   $\mu$ g/ml at birth, reaching 0.1 mg/ml (100 mg/L) at day 30 of life and further increasing gradually to the adult level of 0.4-0.8 mg/ml (400-800 mg/L). In Padilla et al.’s study (2003), it was pointed out that, in contrast to humans, rats have much higher plasma CRP concentrations under basal conditions (about 300-500 mg/L).

In our study, the CRP level of uninfected rats was between 120 and 543 mg/L, as shown in the table below. After *S. flexneri* infection, the CRP level increased sharply, peaking at 4869 mg/L. The number marked in red in the table is the CRP level after infection. Checking again, we confirmed that there was no mistake in the CRP values described in this study. The infection induced a 20- to 50-fold increase in the CRP level, and after the infection resolved, the CRP level returned to normal.

As you mentioned, CRP is generally expressed by mg/L. Therefore, in this paper, we

changed the unit of CRP to mg/L.

**Table. Changes in the CRP level during infection in this study**

CRP/days	PBS group(ng/mL)	PBS group(mg/L)	Infection group(ng/mL)	Infection group(mg/L)
Day 0	120655.0	120.655	259992.0	259.992
Day 1	125937.0	125.937	4.869179×10 <sup>6</sup>	4869.179
Day 5	182674.0	182.674	4.850417×10 <sup>6</sup>	4850.417
Day 9	154674.0	154.674	1.467589×10 <sup>6</sup>	1467.589
Day 18	191226.0	191.226	543496.0	543.496

## References

DeBeer FC, Baltz ML, Munn EA, et al. Isolation and characterization of C-reactive protein and serum amyloid P component in the rat. *Immunology*, 1982,45:55-70.

Nunomura W. C-reactive protein in rat: In development, pregnancy and effect of sex hormones. *Comparative Biochemistry and Physiology Part A: Physiology*, 1990, 96(4):489-493.

Padilla ND, Bleeker WK, Lubbers Y, et al. Rat C-reactive protein activates the autologous complement system. *Immunology*, 2003, 109(4): 564–571.

*-In the third paragraph, “Induction of inflammatory cytokine production”, every time the authors describe a panel in Fig. 2 (referring to the quantification of a certain cytokine), they should mention in the text which panel they are referring to, to facilitate the reading and interpretation of the data by a reader.*

**AUTHOR RESPONSE & ACTION TAKEN:** In the third paragraph, “Induction of inflammatory cytokine production”, the related figure panel number was added to the text (line 290- line 302), referring to the quantification of certain cytokines. This has now been made clearer and may help readers interpret the data.

We also added relevant figure panel numbers to lines 323-329, lines 341-349.

*-In the “Correlation analysis” paragraph the word “predicative”, line 352, and also in the discussion, should be corrected with “predictive”.*

**AUTHOR RESPONSE & ACTION TAKEN:** The word “predicative” had been changed to “predictive” in the Results and Discussion.

6) *Figures and Legends*: in some legends, sometimes there is a description of the results. This is not the appropriate section to describe results, in the legend only the description of the content of the figures and panels and eventually the type of test and/or of instrument used should be present, together with the statistical analysis, and nothing more. -In Figure 1i where CRP is quantified, as already mentioned for the results, there is probably an error in the unit of measure, CRP can't be 4 million ng/ml in the blood. -In Figures 4 and 5, in the graphs referring to the quantification of 8-oxoG and of 8-oxodG, the Y-scale reports the acronym IOD, please specify in the legend what IOD means and how this quantification was obtained from the analysis of the images.

**AUTHOR RESPONSE & ACTION TAKEN:** The description of the results in the Figures and Legends has been deleted. In Figure 1i, where the CRP was quantified, the CRP results have been checked and explained in comment 5. In Figures 4 and 5, in the graphs referring to the quantification of 8-oxoG and 8-oxodG, the Y-scale reports the integrated optical density (IOD). The IOD was a representative parameter for assessing the immunostaining quantification. The explanation of IOD has now been added to the Figure Legend of Figures 4 and 5.

## **5 EDITORIAL OFFICE'S COMMENTS**

*Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:*

**(1) Science editor:** *1 Scientific quality: The manuscript describes a basic study of the increased systemic RNA oxidative damage and the diagnostic value of their metabolites during Shigella flexneri-induced intestinal infection. The topic is within the scope of the WJG. (1) Classification: Two Grades B; (2) Summary of the Peer-Review Report: The article is informative and useful for the setup of a new and reliable diagnostic tool. The experiments are well targeted and demonstrate the authors' hypotheses. The questions raised by the reviewers should be answered; (3) Format: There are 6 figures; (4) References: A total of 22 references are cited, including 12 references published in the last 3 years; (5) Self-cited references: There are 3 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations (i.e. those that are most closely related to the topic of the manuscript) and remove all other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated; and (6) References recommendations: The authors have the right to refuse to cite improper references recommended by the peer reviewer(s), especially references published by the peer reviewer(s) him/herself (themselves). If the authors find the peer reviewer(s)*

request for the authors to cite improper references published by him/herself (themselves), please send the peer reviewer's ID number to [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com). The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately. 2 Language evaluation: Classification: Two Grades B. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form. No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by National Natural Science Foundation of China, National Key R&D Program of China, the CAMS Innovation Fund for Medical Sciences. The topic has not previously been published in the WJG. 5 Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (3) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text. 6 Recommendation: Conditional acceptance.

**AUTHOR RESPONSE & ACTION TAKEN:** (1) The funding agency copy of the approval documents has been uploaded with the revised manuscript. (2) The original figure documents in PowerPoint were uploaded with the revised manuscript. (3) The "Article Highlights" were added to the end of the main text.

**(2) Editorial office director:**

**(3) Company editor-in-chief:** *I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before its final acceptance, please upload the primary version (PDF) of the Institutional Review Board's official approval in official language of the authors' country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the document, and authors from the United States and the United Kingdom should upload the English version of the document, etc. 作者提供的伦理文件不是一个正式的批准文件。*

**AUTHOR RESPONSE & ACTION TAKEN:** Our animal experiment was conducted in the experimental animal room of the Institute of Pharmaceutical Biotechnology. The last line of the file included the approval opinion of the ethics committee. We have now asked them to

stamp their official seal and upload the document with the official seal to the system.