Detailed response to the reviewers' comments

Dear Editors and Reviewers:

Thank you very much for your constructive comments and suggestions concerning our manuscript entitled "Protective Effect of Toll-like receptor 3 agonist Poly I:C on Mucosal Injury and Epithelial Barrier Disruption in mouse models of DSS-induced Acute Colitis" (Manuscript No. 28907), and those comments are valuable and very helpful for revising and improving our paper.

We have studied comments carefully and have made correction as marked in the revised manuscript, which we sincerely hope will meet with your approval. The main corrections in the paper and the detailed response to the comments are as following:

Reviewer 1:

Comments to the Author

It is an interesting manuscript. A number of typographic errors through the manuscript should be corrected

Response: Thanks for your time and positive comments on our manuscript. We have carefully proofread our manuscript and have made the corrections, sincerely hoping it will meet with your requirement.

Reviewer 2:

Comments to the Author

Zhao et al here report that administration of the Toll-like receptor agonist Poly I:C

protects against inflammation in the mouse DSS colitis model, and that this seems to act through better preserved tight junctions. The effect of Poly I:C on DSS colitis has been described previously, in depth by Vijay-Kumar and coworkers (Inflammatory Bowel Diseases 2007), and the experimental setup in the present paper is virtually identical to this. The mechanistic aspects with respect to TJ protection have probably not been examined in this model before, however similar effects of TLR3 stimulation have been observed in other model systems (i.e. Moyano-Porcile Pharmacol Res 2015). In general, the present work is well done and the paper describes results of interest for the IBD research community working with animal models of gut inflammation. My specific comments are these:

(1) The main concern is that novelty is disputable, most of the observations here can be derived from previous papers. However, as mentioned above, the DSS model is widely used and the results can nevertheless be of interest.

Response: Thanks for your comments. The effect of Poly I:C on DSS colitis and on gut permeability did have been described previously in other model systems. However, activation of TLR-3 by Poly I:C seems to cause different effects on various epithelial barriers. We have added some information in the discussion section of our manuscript (Page 14 Line 20-27). Besides, as you mentioned, the potential mechanism of Poly I:C on mucosal injury and epithelial barrier disruption in DSS-induced acute colitis mouse model have not been well characterized. Our study may help to better understand protective mechanisms of Poly I:C in animal models of DSS-induced gut

inflammation.

(2) The Methods section (under Electron microscopy) seems to describe tissue prepared for both TEM and SEM; I am unable to find results from TEM in the paper.

Response: Thanks for your suggestions and sorry for our mistake. We have deleted the description about TEM from our manuscript (Page 9 Line 6-10), sincerely hoping it will meet with your requirement.

(3) Figure 1 panels A and B have time points also at 2 days, while the legend describes assessment 3-8 days. Please clarify.

Response: Thanks for your kind remind. The Rachmilewitz disease activity index (DAI) and body weight (BW) were assessed during 1 to 8 days observation. Since the data of DAI and BW were almost the same among the groups on observation day 2, and the gut inflammation and weight loss was noted in the model group during 3 to 8 days. Therefore, we only mentioned that the DSS-induced damage to the colon tissue was noted during observation days 3 to 8. The corresponding revision has been made as shown in the revised manuscript (Page 10 Line 13).

(4) Were the Zo-1 bands in Figure 4D really measured with densitometry? These look technically dubious, to say the least. I:C is not written correctly in this panel (capitalize C).

Response: Thanks for your comments. The zo-1 bands in Figure 4D were measured

with densitometry. It did look technically dubious. However, in view of the technological difficulty in extraction of membrane proteins from intestinal mucosa and the zo-1 as a large molecular weight protein, it is really difficulty for us to obtain the better results of zo-1 expressions, although the procedures have been repeated for several times. We sincerely hope it will still meet with your approval. Besides, the I:C has been correctly written in the panel, thanks for your kind remind.

(5) The Discussion section is somewhat unorganized; I would prefer that the authors move general information to the Introduction section and use established knowledge only in the context of discussing their own results.

Response: Thanks for your constructive comments and suggestions. We have made some revisions of the discussion section in our manuscript, sincerely hoping it will meet with your requirement.

(6) Please let the manuscript undergo a thorough language revision.

Response: Thanks. The manuscript has been proofread thoroughly, and we sincerely hope it will meet with your approval.

Besides, we have added Lili He MD, Qilu Hu MD, and Ziqian Dun MD, who have worked on experiment performance and manuscript revision, in the author list of our manuscript. We sincerely hope it will still meet with your approval.

Thank you and best regards.

Looking forward to hearing from you soon.

With kind regards,

Yours sincerely,

Hongwei Zhao,

Department of Gastroenterology, Hebei General Hospital, Shijiazhuang 050051, China

E-mail: hongweizhao788@163.com