Peer Review File

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Reviewer A:

The article of Qi-Fang Liu (LPS induces pyroptosis through regulation of autophagy in cardiomyocytes) is a pleasant surprise. The article is written by Chinese with such good English is welcome surprise. The author has described all sections (Abstract to Discussion) of manuscript very clearly, focused and to the point, except reference should be modified according to CDT guide to author (after names of three authors et.al instead of six authors). The manuscript should be accepted for publication.

Response: Thank you very much for careful reviewing of our manuscript, and also thank you for your recognition of our article. The references were revised in accordance with the CDT author's guidelines.

Reviewer B:

The present study conducted by He and co-workers exploring LPS induces pyroptosis through regulation of autophagy in cardiomyocytes. The authors explore the potential role and relationship of autophagy and pyroptosis in Lipopolysaccharide (LPS)-induced inflammatory response of cardiomyocytes. Furthermore, this study provides significant insights and novels findings in uncovering the pathophysiological factors and delivering a promising therapeutic avenue in treating multiple diseases, including cardiovascular diseases. However, the topic of conducting the study is novel deals. The manuscript is well written. Unfortunately, the present version of the manuscript is missing significant aspects and explanations. Thus, the authors may address the following comments and suggestions for further clarification of their investigations to meet the standard publication requirements for this prestigious journal:

Significant comments and suggestions:

1. The introduction description is cryptic in more detail on the fundamental mechanism of the non-canonical pyroptosis-signaling pathways. I suggest the authors to include this critical explanation to improve the manuscript draft more evidently and transparently.

Response: Thank you very much for careful reviewing of our manuscript. We have modified the manuscript as advised (see Page 5, line 64-68).

2. The authors may provide a statement in the abstraction segment that inhibiting LPS-induced pyroptosis could be a promising therapeutic target in treating cardiovascular diseases.

Response: Thank you very much for careful reviewing of our manuscript. We have add the statement in abstract (see Page 4, line 47-48).

3. I am confused why the authors considered only determining the canonical inflammasome signaling pathways-related protein expression in experimenting. Non-canonical inflammasome-signaling pathways and newly discovered caspase-3/8 dependent signaling pathways may also induce pyroptosis-mediated cell death. Unfortunately, the authors did not focus on these in their experimenting. Please clarify.

Response: Thank you very much for careful reviewing of our manuscript. Our preliminary experiments showed that appropriate concentration of LPS affected autophagy and NLRP3 pyroptosis process. At the same time, other studies have shown that LPS-induced autophagy can activate the NLRP3 pyroptosis pathway through certain systems. Therefore, the focus of this study is to explore the inflammasome-mediated pyroptosis pathway.

4. LPS agent only activates caspase-4/5/11 signaling axis, leading to the initiation of cardiomyocyte cell death. Is there

any evidence LPS may also induce the NLRP3/caspase-1 signaling axis, leading to the initiation of pyroptosis-mediated cell death?

Response: Thank you very much for careful reviewing of our manuscript. According to literature review, LPS may induce the NLRP3/caspase-1 signaling axis, leading to the initiation of pyroptosis-mediated cell death. As shown in the literature[1, 2].

[1] Tang YS, Zhao YH, Zhong Y, et al. Neferine inhibits LPS-ATP-induced endothelial cell pyroptosis via regulation of ROS/NLRP3/Caspase-1 signaling pathway. Inflamm Res 2019;68:727-738.

[2] Qiu Z, He Y, Ming H, et al. Lipopolysaccharide (LPS) aggravates high glucose-and hypoxia/reoxygenation-induced injury through activating ROS-dependent NLRP3 inflammasome-mediated pyroptosis in H9C2 cardiomyocytes. J Diabetes Res 2019;2019:1-12.

5. Pyroptosis is a form of GSDMD-mediated cell death, which further induces a massive pro-inflammatory response. Unfortunately, the authors did not determine the inflammatory markers, including IL-1β and IL-18 in cell culture medium. Please explain the critical reason.

Response: Thank you very much for careful reviewing of our manuscript. Inflammasomes are responsible for the maturation of pro-inflammatory cytokines such as IL-1β and IL-18. Under LPS stimulation, NLRP3 triggers the cleavage of pro-caspase-1 into its active and mature form of caspase-1 by forming protein complexes. GSDMD, IL-1β, and IL-18 are then cleaved to an activated form that promotes inflammation. This is the recognized pyroptosis pathway at present. Our current research is not focused on this, but on the link between autophagy and pyroptosis induced by LPS. Therefore, we did not measure the expression of IL-1β and IL-18.

6. Pyroptosis is named as a caspase-1 dependent pro-inflammatory cell death. I do not know why the authors did not evaluate caspase-1 protein expression levels in their experiment. How the authors can conclude the LPS promotes pyroptotic cardiomyocytes and support their outcomes. The authors may re-determine this protein expression levels by performing the western blotting or immunohistochemistry staining analysis to transparent their study to the scientific research community.

Response: Thank you very much for careful reviewing of our manuscript. According to your suggestion, we used western blot to detect Cleaved caspase-1 protein expression level, and the relevant experimental results have been added to the revised manuscript. We have add the data as advised (see Page 26, line 470-481).

7. Why did the authors not evaluate the GSDMD-NT protein expression levels? How did the authors claim the LPS agent promotes plasma membrane pore formation, leading to pyroptosis? Please clarify the critical concerns.

Response: Thank you very much for careful reviewing of our manuscript. I am very sorry, in this study, we did not detect the expression level of GSDMD-NT. It has been proven that LPS can induce cell pyroptosis. As for how LPS causes cell pyroptosis through the formation of plasma membrane pores, this is not the main content of this study.

8. How autophagy activates pyroptosis-signaling pathways its remained elusive in the discussion section. I suggest the authors to explain it more clearly and evidently.

Response: Thank you very much for careful reviewing of our manuscript. LPS induces autophagy and pyroptosis of cardiomyocytes and autophagy inhibits LPS induced pyroptosis. LPS regulates autophagy via PARP-1 in cardiomyocytes, and PARP-1 play roles by binding to LC3B. We have modified the text as advised (see Page 16-17, line 295-297).

9. The authors may draw a figure representing how LPS stimulates pyroptosis through the regulation of autophagy in

cardiomyocytes.

Response: Thank you very much for careful reviewing of our manuscript. We have added a graphical abstract to show how LPS stimulates pyroptosis through the regulation of autophagy in cardiomyocytes.