Author's response to reviews

Title: Extracellular polysaccharides produced by Ganoderma formosanum stimulate macrophage activation via multiple pattern-recognition receptors

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Author's response to reviews: see over
Dear Dr. Rowles,

Please find enclosed a revised version of our manuscript (1637635574685304) entitled “Extracellular polysaccharides produced by *Ganoderma formosanum* stimulate macrophage activation via multiple pattern-recognition receptors” by Cheng-Li Wang, Chiu-Ying Lu, Chia-Chen Pi, Yu-Jing Zhuang, Ching-Liang Chu, Wen-Hsiung Liu, and Chun-Jen Chen. The referees’ comments were helpful. We have addressed all of the comments raised in review with additional data, changes to the text and clarifications. Our specific response is detailed below and required relatively limited changes that are highlighted on the manuscript itself. We believe the revised manuscript is improved and hope that you find it acceptable for publication.

Response to referee 1’s comments

1. “*The Dectin-1 and CR3 are well-characterized in fungal PAMP recognition. In Figure 2A of this study, the authors used cells from C3H/HeJ, in comparison with cells from C3h/HeN, to demonstrate the involvement of TLR4 in PS-F2-mediated stimulation. However, the induction of TNF in the BMDMs derived from both types of mice are significantly lower (in pg/ml levels) than the results shown in RAW264.7 cells (in ng/ml levels). The authors should discuss the potential causes for this difference.*”

As the reviewer pointed out, we have routinely observed that PS-F2 stimulated a significantly higher level of TNF-α production in RAW264.7 cells than in BMDMs (derived from C3H/HeJ or C57BL/6 strains). Besides the difference in cell origins (cell line vs. primary cell), we speculate that the relative expression levels of various PRRs may be different between these two types of macrophages, resulting in the difference in response to PS-F2 stimulation. We have discussed these potential causes in the revised manuscript (lines 177-182).

2. “*In addition, rather than TLR4, it has been suggested that TLR2 plays critical roles in mediating fungal stimulation. The role of TLR2 in PS-F2-mediated stimulation is not characterized in this study. It may be informative to compare the function of TL2 and TLR4 in PS-F2-stimualted RAW264.7 cells by using specific antagonistic antibodies for TL2 and TLR4.*”

To address whether TLR2 is involved in PS-F2 recognition, we have performed an additional experiment by stimulating wild-type and TLR2\textsuperscript{−/−} BMDMs with PS-F2, and found that TLR2 deficiency did not affect the response of macrophages to PS-F2 stimulation. We have described this new result in the revised manuscript (lines 163-165 and Additional file 2).

3. “*In the signaling study of this research, the author use piceatannol as the specific Syk inhibitor to demonstrate the involvement of Syk in PS-F2-mediated signaling and responses. However, it is always a concern about the specificity and off-target effect when using a chemical blocker on signaling. It will more convincing to*
include a negative control that shows piceatannol does not have any non-specific blocking effect on a Syk-independent signaling (such as poly I:C-TLR3 signal) in Figure 1E and Figure 4D.”

We have performed the experiment suggested by reviewer. We have also changed the order of data presentation (as suggested by referee 2, see below) and grouped the original Figure 1E and Figure 4D into a new Figure 5. The new Figure 5A shows that piceatannol specifically blocks PS-F2-stimulated signaling and has minimal effect on poly (I:C)-stimulated TNF production.

Response to referee 2’s comments

1. “This manuscript reports that Dectin-1, CR-3 and TLR4 play role in mediating cell activation by polysaccharides from Ganoderma formosanu., but only the Dectin-1 signaling is reviewed in the background section. Some background description of CR-3 and TLR4 would be useful to readers.”

In revision, we have provided more information regarding TLR4 and CR3 signaling in the background section (lines 62-78 and 94-97).

2. “p3-4, lines 68-70, “Several receptors recognize #glucans, including the C-type lectin receptor Dectin-1 [15], CR3 [16], scavenger receptors [17], lactosylceramide [70 18], and TLR 2 [19].” It would be better to include TLR4 in this sentence as that in the p6, line 133. Since the author’s study is mainly focusing on TLR4 not TLR2.”

In revision, we have included TLR4 and cited references in this sentence (line 86-87).

3. “p8, lines 168-169, the rationale of why the data can exclude the possibility of LPS contamination in PS-F2 is not clear”.

The rationale is that if the observed macrophage activation was mainly caused by LPS contaminated in the sample, we would expect to observe the “LPS tolerance” phenomenon in the experiment.

4. “Figure 1 is too small to be clearly seen.”

We expect that Figure 1 will appear as double column. To ensure legibility, we have increased the size of label text in the revised Figure 1.

5. “Figure 4D is not quite compatible with Figure 4A-C. This Figure 4D might be able to be grouped together with Figure 1E to a new figure 5.”

In revision, we have grouped the original Figure 1E and Figure 4D into a new Figure 5 and also reorganized the data description in text (lines 225-236).
6. “A schematic figure of receptors and signaling involved in PS-F2 activation would be useful for summarizing the results.”

In revision, we have provided a graphical summary of the findings (Figure 6) as suggest by reviewer.

Sincerely,

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