Author's response to reviews

Title: Polysaccharides from Agaricus bisporus and Agaricus brasiliensis show similarities in their structures and their immunomodulatory effects on human monocytic THP-1 cells.

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Answers to Reviewers questions:

Reviewer #1: No answers required.

Reviewer #2:
The figures 1 and 2 have been revised, and the labels were added. All the main signals of the spectra were checked carefully and assigned in the figures as ppm. The explanation of the main signals is described in the text.
ABSE is the remainder of Agaricus bisporus polysaccharide after α-amylase treatment (E is for enzyme). It is added to the legends.
Fig 3 legend has been changed as proposed by reviewer.
The last column of Table 1: the reviewer probably means Table 3 and there we adapted the last column because it was confusing.

Reviewer #3:
Minor changes:
Page 6 line 161: “thrice” changed for “three times” (although “thrice” is correct English)
Page 11 line 287: changed as requested.

Major issues:
Ad “No validation of q-PCR data by ELISA”: Two of us (W.C. and HJW) showed convincingly that the q-PCR data of cytokine induction in activated THP-1 cells quantitatively reflect transcription preceding the expression as measured by ELISA (Chanput W. et al. 2010. Food and Function 1 (3), pp. 254-261). Reviewer must be aware of the time schedule of transcription versus expression, and as
the kinetics of these two processes are not identical there will always remain room for doubt.

Ad “Figure 4 etc”: The expression of Cox-2 was tested later as an extra. TNF-a and IL1b are already convincing parameters of proinflammatory effects.

Ad “Experiments carried out in Figure 4”: We agree with the reviewer and will certainly follow this up in the near future. However, the experiments were done as they were planned with A. blazei. We have not foreseen the outcome of the experiment.

Ad “necessity to validate q-PCR by methods not relying on amplification”: As above.

Ad “need to explain possible mechanisms”: This is the ultimate esoterical question for any biologist: Explain the possible mechanisms behind your observations!

We have added 17 lines of discussion (lines 359-376) on why mushroom substances can enhance pro-inflammation on their own while in combination with LPS they show opposite effects. One of us (LvG) has shown that A. bisporus and A. blazei extracts are strongly ROS inducing in cell systems (Wei et al. 2008) and are therefore considered pro-inflammatory. LPS is a strong inducer of ROS, but this can be abolished by high glucose and /or by PPAR-g agonists., Whether glucans and other polysaccharides may be able to function as PPAR–g agonists or may compete with the binding site for LPS is unknown. Answering the up mentioned question remains speculative for the time being.