Author’s response to reviews

Title: MORG1+/- mice are protected from histological renal damage and inflammation in a murine model of endotoxemia

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Answers to the reviewers and editorial points point by point in detail:

Answer to Reviewer 1: We thanks to the Review (Prof. Mark De Caestecker) for his supportive comments on the revised version of the manuscript.

Answer to Reviewer 2 (Prof. Charles Edelstein):

1. “It is noted that MORG1 +/- mice are histologically but not functionally protected. BUN and SCr were not decreased in MORG1+/- mice after LPS.

Title should be changed to say "histological renal damage and inflammation..."

• The data that are presented in the revised version of the manuscript in Fig. 2e show that plasma Creatinine (Cre) levels are significantly increased in both genotypes subjected to LPS treatment compared with the corresponding NaCL treated control mice. We would like to point here that the levels of plasma Cre what were measured in MORG1 +/- and MORG1 +/- mice treated with 5 mg/ml LPS were still in the normal levels of the plasma Cre for mice according to Dunn et al., Kidney International, Vol. 65 (2004), pp. 1959–1967, the authors found that a colorimetric measurement, as we have been using in our CHIP based analyses, demonstrate, citation: “mean plasma creatinine levels were significantly lower (P < 0.0001) by HPLC (0.128 ± 0.026 mg/dL) vs. Jaff´e (0.4 ± 0.12 mg/dL) for mice on a normal diet.” (Dunn et al., Kidney International, Vol. 65 (2004), pp. 1959–1967). Thus 5 mg/ml LPS i.p. application did not raise the plasma Cre levels to pathological values. Therefore, although in Fig. 2e are shown significantly
increased levels of plasma Cre in MORG1 +/+ and MORG1 +/- endotoxemic mice the presented data show only a statistical difference and cannot be used as a parameter for kidney damage at this time point and those LPS concentrations. Furthermore, it is also known that plasma creatinine levels increased later to pathological concentrations. In order to use an additional marker of renal injury we detected the Kidney injury molecule 1 [KIM 1] (Fig. 1c-e) which was also significantly more expressed in the renal sections of endotoxemic wild-type MORG1 +/- mice than in MORG1 +/- LPS treated animals demonstrating improvement in MORG1 +/- endotoxemic mice. We cannot explain the increased plasma BUN levels, but the plasma levels of BUN can be influenced as well from “changes caused by many other factors (volume status, catabolism, fever etc.) Nevertheless, This reviewer is correct that we did not really show functional improvement in MORG1 +/- mice treated with LPS. Therefore, we changed the title and abstract as suggested.

2. “The word "renoprotective" should be removed from abstract and conclusions and rest of manuscript, as there was only histological and not functional protection”

   • The word "renoprotective" was removed from the abstract and conclusions. The amendments can be found in: Abstract section, page 3, lane 55; and in Conclusions section, page 23, lanes 533-534.

   • Of note is that the word “renoprotective” is also present in Background section, page 4, lane 81 and Discussion section, page 19, lane 443, but is not replaced or removed from the text as it is not related to results or conclusions which are connected to the present manuscript.

3. “Add a few sentences to discussion why you think the mice were histologically but not functionally protected."

   • In order to address this issue we added in the discussion of the present revised form of the manuscript amendments, which could be found in Discussion section, page 20, lanes 459 to 469.

   • All changes are highlighted in yellow.

Answers to the editor:

• During the careful reading of the manuscript we found as well the “INFγ “ was not corrected in two other pages. The corrections made can be found in results section, page 16, lane 354 and in Abbreviations section, page 24, lane 545.

• We also find a typo “proceudre” and corrected it. Changes were made in the Methods section, page 6, lane 115.
We found a spelling mistake of the verb “analyzed” and corrected it. The changes are shown in Figure Legends section, page 34, lane 789 and page 35, lane 827.

All changes in the revised version of the manuscript are highlighted in yellow.

We hope that this second round of revision addresses all remaining issues and our manuscript will be now accepted for publication.

With best regards on behalf of all authors

Prof. Dr. Gunter Wolf, MD; MHBA