Author's response to reviews

Title: Pulmonary immune responses to 2009 pandemic influenza A (H1N1) virus in mice

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Author's response to reviews: see over
Dear Miss Sheryl Ramos,

It is our pleasure to submit the revised manuscript (MS# 1368457039115825) entitled “Pulmonary immune responses to 2009 pandemic influenza A (H1N1) virus in mice” to the BMC infectious disease.

All the comments of the reviewers and editor have been fully considered. Our point-by-point responses to the comments are provided below.

We would be grateful if the manuscript could be considered for publication.

Sincerely yours
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Responses to Reviewer: Justin Julander

1. The article needs to be thoroughly edited by someone who is fluent in english, as there are grammatical errors throughout, which severely impedes the communication of results and their significance.

[Response]: Thank you for your suggestion. This article has been edited by a Professor who is fluent in English.

2. The statistical analyses described in materials and methods do not appear sufficient to test all the data properly. I doubt a one-way ANOVA was used to show significance in mortality curves. In addition, some of the longitudinal analysis would be ill-suited for analysis with this method. Better or more applicable statistical analyses should be used and described.

[Response]: Thank you for your question. We have clarified the statistic method used in this study in the material and method part (Page 11, Line 209 to 212). The detail information is: Statistical differences at each time point within one group were determined by one-way ANOVA test, while statistical differences of different group at the same time-point were determined by independent-sample T Test with SPSS19.0. Values of p < 0.05 were considered significant.
3. There are several publications that describe mouse models of influenza. Although there are some interesting data on cytokine increase and immune cell activation, it would be helpful if a functional analysis or demonstration of biological significance of such data was included in this manuscript.

[Response]: Thank you for your suggestion. We have added some published studies with related data in the discussion (Page 16 to 17, Line 301 to 302, 311 to 313, and 322 to 326). The detail information is: “whereas depletion of IL-6 did not directly protect against death from lethal H5N1 influenza virus infection”, “Furthermore, IL-17 deficiency or treatment with monoclonal antibodies against IL-17-ameliorated acute lung injury induced by the 2009 H1N1 virus in the mice.” And “Daniela Damjanovic and his colleagues suggested that TNF-α was dispensable for influenza clearance. Their study showed that TNF-α was up-regulated in the lung after influenza infection and TNF-α deficiency led not only to a greater extent of illness but also to heightened lung immunopathology and tissue remodeling.”

Level of interest: An article whose findings are important to those with closely related research interests.

Quality of written English: Not suitable for publication unless extensively edited.

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests.
Responses to Reviewer: Florian Krammer

Major points:

1. The authors need to calculate LD50 values for both viruses in order to compare the pathogenicity of the viruses.

[Response]: Thank you for your suggestion. We have given LD50 values for both viruses in the results (Page 11, Line 215 to 217). The detail information is: In this study, we used the same infectious doses of two strain influenza viruses, although the LD50 was different between PR8 viruses and BJ501 viruses (the LD50 of PR8 was 5.0×10^3 p.f.u / 25µL while BJ501 was 7.76×10^3 p.f.u / 25µL respectively).

2. The results paragraph "Molecular analysis of 2009 H1N1 viruses" should be deleted. The authors just made a list of sequence differences between strains - these differences are widely known in the field and are not really 'results'. I would recommend keeping the Table 1 since it gives a quick overview.

[Response]: Thank you for your question. We have deleted "Molecular analysis of 2009 H1N1 viruses" parts in results and kept Table1 as your suggestions in the text.

3. Discussion, line 338-340: The authors should either show experimentally that the
lysine in PB2 position 627 is causing the higher virulence in mice (by introducing the mutation into BJ501) or they should remove this statement.

[Response]: Thank you for your suggestion. We have removed this statement in discussion as your suggestions.

4. The pH1N1 isolate that the authors used was passage in eggs. It is widely known that egg passage changes residues near the receptor binding site in pH1N1 viruses. The authors should sequence the HA gene of the virus they used last passage) and compare it to the sequence of the original isolate.

[Response]: Thank you for your valuable suggestion. The pH1N1 used in this study was the third passage for PR8 and the second for BJ501 in eggs. We had extracted and sequenced HA gene of PR8, and found no mutations.

5. Figure 1B: It looks like the mice had to suffer severe weight loss of up to 40% of their initial body weight. IACUC protocols usually call for a humane endpoint at 25% weight loss and mice that cross this line have to be euthanized. Nothing about pre-defined humane endpoints is stated in the materials and methods section.

[Response]: Thank you for your suggestion. The animal experiments were approved by the Animal Subjects Research Review Board of the Beijing Institute
of Microbiology and Epidemiology and were conducted according to the institution’s guidelines for animal husbandry. It’s a pity there is no clear pre-defined human endpoints in the current ethics statement.

Minor points:

1. The quality of Figures 1, 2, 4 and 5 is pretty bad, the authors should consider to replace them by figures with a higher resolution.

[Response]: Thank you for your suggestion. We have redrawn figure 1, 2, 4 and 5 with higher resolution, and hope it would satisfied the requirement of your publication.

2. The manuscript needs major editing by a native English speaker (or a commercial editing agency).

[Response]: Thank you for your suggestion. We have modified as your suggestions.

3. Line 30: the pandemic is over, the virus strain is now seasonal, it lost its pandemic potential.

[Response]: Thank you for your suggestion. We have modified as your suggestion (Such as: Page 4, Line 54, 56 et al.)
4. Line 45 and throughout the text: BALB/c, not BALB/C

[Response]: Thank you for your correction. We have corrected the mistake in the revised manuscript (Page 2 to 3, 6, and 27, Line 33, 45, 49, and 113).

5. Line 71: The authors should explain what they mean with 'associated with its 2.6 glycan binding properties'.

[Response]: Thank you for your correction. We have deleted this unclear sentence.

6. Line 143: matrix, not metrix

[Response]: Thank you for your correction. We have corrected the mistake in the revised manuscript (Page 8, Line 143).

**Level of interest:** An article whose findings are important to those with closely related research interests.

**Quality of written English:** Needs some language corrections before being published.

**Statistical review:** No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.

Responses to Editor: Miss Sheryl Ramos

1. A new statistical analysis of the data needs to be including proper survival analysis and use of ANOVA when comparing two or more groups to a placebo.

[Response]: Thank you for your suggestions. We have clarified the statistic method used in this study in the material and method part (Page 11, Line 209 to 212). The detail information is: Statistical differences at each time point within one group were determined by one-way ANOVA test, while statistical differences of different group at the same time-point were determined by independent-sample T Test with SPSS19.0. Values of $p < 0.05$ were considered significant.

2. The manuscript needs to be reviewed by an English speaking individual.

[Response]: Thank you for your suggestion. This article has been edited by a Professor who is fluent in English.

3. In addition, please name the specific ethics committee that approved your study.
[Response]: Thank you for your suggestion. We have given the name of the ethics committee in the text as your suggestions (Page 7, Line 135 to 137). The detail information is: the animal experiments were approved by the Animal Subjects Research Review Board of the Beijing Institute of Microbiology and Epidemiology and were conducted according to the institution’s guidelines for animal husbandry. It’s a pity there is no clear pre-defined human endpoints in the current ethics statement.