Reviewer’s report

Title: Comparison of the effect of LPS and PAM3 on ventilated lungs

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Reviewer: Graeme Zosky

Reviewer’s report:

The authors compared the progression of ventilator induced lung injury (VILI) in mice exposed to lipopolysaccharide (LPS), as a model for gram-negative bacterial pneumonia, or PAM3, as a model for gram-positive bacterial pneumonia. VILI was assessed by monitoring lung mechanics and inflammation (neutrophils in the lung and cytokine mRNA production). Most previous studies have used intratracheal or systemic instillation of LPS prior to the initiation of mechanical ventilation. This study is novel in that it directly compared responses to gram-negative and gram–positive bacterial products after ventilation had begun. These aspects make the manuscript of interest, however there are a number of issues that need to be addressed by the authors.

General comments

Introduction – the content of the introduction is appropriate and the aims of the study are clear

Methods – the methods lack detail about critical aspects of the study and need to be clarified

Results – I had trouble assessing the lung function figures due to the small symbols, font and legend. This needs to be addressed.

Discussion - It is unfortunate that you did not use some of the more sophisticated measurements available with the flexivent system that can separate the contribution of the conducting airways and smaller airways (where gas flow occurs by diffusion) to lung resistance. The interpretation of the results also needs to be toned down as you do not have any data comparing responses to LPS and PAM3 in unventilated mice. These insults alone (in the absence of mechanical ventilation) could explain the differences in inflammation you observed.

Major Compulsory Revisions

Methods, animal preparation. The level of anaesthesia cannot be assessed without giving the doses of ketamine and xylazine (e.g. mg/kg) and the timing/criteria for giving additional doses of anaesthetic throughout the ventilation period.

Methods, animal preparation, last sentence. What were the animals covered with and how did you determine the body temperature was being maintained?
Methods, protocols. I am surprised at the low respiratory rate (120 bpm) that you were able to achieve. Was this done in the absence of paralysis which is usually reported in studies using similar respiratory rates?

Methods, protocols. How long was the nebulisation period required to deliver the 50uL solution of LPS/PAM3? Which version of the Scireq nebuliser was used for this study?

Methods, protocols, last sentence. Was any form of instillation/inflation (intratracheal or vascular) of fixative used or were the lung samples simply placed in formalin?

Methods, lung function measurements, last sentence. What was the volume displacement of the “forced manoeuvre” used to measure resistance and compliance?

Methods, histological measurements. As per my previous comments – was any form of standardised inflation of the lung used for fixation?

Methods, QRTPCR. Some justification needs to be provided for the choice of these cytokines in the context of mechanical ventilation and known responses to LPS and PAM3.

Methods, statistics. The description of the statistical methods used needs to be clarified. For example, serial measurements of lung function should be compared using repeated measures ANOVA – was this the case? Also, which statistical package was used to conduct the analysis? Please justify the use of SEM – in most cases you are simply reporting the variability of measurements within an individual group of mice so you should report the standard deviation not the SEM.

Results, lung function parameters. What is your explanation for the significant increase in lung mechanics for the saline exposed group compared to the controls? Looking at the associated figure it seems to me that the increase is transient and returns to control levels. It was extremely difficult to understand which groups were represented on this graph due to the minute symbols and low quality legend.

Results, cytokine expression. The fold increases in cytokine expression compared to unventilated animals are unclear in the figure as the mRNA expression in the controls appeared to be zero – I suggest changing these graphs to a log scale. Due to the substantial differences in variability between groups (and the apparent zeroes in the w/o group) the statistical comparisons made between groups here needed to be done on transformed data or an ANOVA on ranks – was this the case?

Results, neutrophil inflammation. See previous comments re: inflation during fixation. In order to compare these groups based on “fields” the level of inflation needs to be standardised. Even when inflation during fixation is standardised at
the very least the cell counts should be standardised to the area of the lung tissue (mm2), not the field of view as you have done here.

Discussion, second paragraph, last sentence. You cannot comment on the “synergistic” effect without having measured the response to LPS alone in unventilated mice.

Discussion, third paragraph. See previous comment – these interpretations of the results need to be discussed in light of the fact that you do not have data comparing inflammatory responses to PAM3 and LPS in unventilated mice.

Discussion, third paragraph, last sentence. Please expand on your discussion of the limitations of your results i.e. you did not measure cytokine production just mRNA expression.

Minor Essential Revisions

Introduction, paragraph 2, second sentence. Please insert the reference indicating the incidence rates of VAP in the ICU “…from 8% to 28%”.

Introduction, paragraph 3, first sentence. Insert comma after LPS and insert reference showing that gram-positive bacteria are important in VAP

Introduction, paragraph 3, last sentence. Delete “which has not been investigated very well until yet”.

Results, oxygen saturation. Spelling of “oxygen”.

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

**Declaration of competing interests:**

I declare that I have no competing interests