Author's response to reviews

Title: Age-dependent differences in lung ventilation impact influenza-induced tachypnea in the cotton rat

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Author's response to reviews: see over
Response to reviewer’s comments:

We appreciate the constructive and insightful comments of both reviewers. The text of our manuscript has been amended as described below in our response to each comment:

Reviewer 1: Dr Fadi Xu

Major comments:

1. Significance: The reviewer agrees with our assessment that the results we present are interesting and clinically relevant.

2. Accuracy of title and abstract: The reviewer does not think our title is accurate; since our results show a correlation (and are not proof) of age-dependent changes in lung mechanics and differences in airway measurements during influenza-induced tachypnea, we have changed the title to accurately reflect our study: “Comparison of airway measurements during influenza-induced tachypnea in infant and adult cotton rats”.

   The reviewer states that it is difficult to understand how age-related differences in ventilation cause alterations in influenza-induced tachypnea. Our results show that dynamic elastance in infants is greater than adults. This provides a logical explanation for the reduced tidal volume in infected adults (reduced due to airway obstruction) but no reduction in tidal volume in infected infants (in spite of airway obstruction). This is the take-home message of our work and therefore we have clarified the text to make this point more clear. In addition, we have rephrased sentences so that the reader will know why non-anesthetized as well as ventilated cotton rats were used in this study.

3. The reviewer states that our hypothesis is not well addressed. We hypothesized that age-dependent differences in lung mechanics would result in differences in ventilatory characteristics following influenza infection of adult and infant animals. To address this hypothesis we compared many different respiratory parameters of infant and adult cotton rats before and after influenza infection. In our opinion, we have addressed the hypothesis but agree that there may be other age-dependent factors that contribute to differences in airway responses. Some of these alternate biological contributors are now referenced in the text.

4. Adequacy of experimental design and methods

Page 5 line 6: This sentence was poorly written and has been corrected. The animal body temperature was measured using a rectal probe; the temperature set on the whole body flow plethysymograph was 37.5 °C, this temperature is consistent with that measured in animals infected with influenza virus 2 days earlier.

Page 6: Statistical methods: We never compare more than 2 groups and do not consider the rate of change in values between these groups. The statistical differences in measurements shown in Table 1 and most figures compares measurements made for a single group (either infant or adult but not a comparison of the 2), with values generated at 2 time points (before infection and after infection). In
figure 4 we compare pathology scores of infants and adults at the same time point (and not the change over time). T tests are therefore appropriate for our analysis.

Page 7-parag2: we have clarified the text that refers to how means were calculated.

5. We did not include respiratory rate as this data is presented in Figure 2. However, since this has been requested by the reviewer, we have added it to Table 1. We have not included results from mock-infected animals as our published work clearly demonstrates that changes in respiratory rate require inoculation with live virus - even inoculation with inactivated preparation of virus does not increase respiratory rate (Eichelberger et al., 2004); we therefore include this reference in the text that discusses these results (page 7).

6. We agree with the reviewer’s comment that development of central respiratory drive may also contribute to the age dependent difference in airway measurements during infection. It would be beyond the scope of this publication to test this idea, but we have included this possibility in the discussion and provided appropriate references that point the reader to alternate mechanisms that may contribute to the differences in MV that we observe (page 10/11).

The explanation for difference in virus clearance, tachypnea and epithelial damage in animals inoculated with the same dose per 100g is now more clearly stated on page 11: simply put, the total virus inoculum when given in this fashion is approximately 4-times the amount of infectious virus in the adult animals. This results in greater titer of virus in the lungs, greater epithelial cell damage and correspondingly, prolonged tachypnea in adult animals.

The reviewer commented that the explanation regarding the infant and adult animals infected with equivalent virus dose resulting in difference in length of tachypnea and viral clearance is lacking. We have revised the sentences on page 12 that provide the explanation more clearly.

7. We have added in a discussion of age-dependent changes in ventilation following RSV infection (and references) as requested by the reviewer.

Minor comments

Table 1. We have simplified the footnotes.

Fig. 1. MV is not shown in this figure (uninfected) as the tidal volume and respiratory rate were controlled by the ventilator. MV for infant and adult cotton rats is provided before and after infection on Table 1.

Fig.2. does not include different ages of young animals; we performed these experiments with 14 day old animals since these are representative of ‘infants’ as these animals are too young to wean. We have clarified this in the text.

Figure 5. We now include the age (adult, 8 weeks old) of the animal section used in the control (uninfected) and the age of the animal used in panel B (infant) - 16 days (i.e. infected at 14 days old).
Reviewer 2: Dr Kevan Hartshorn

We appreciate this reviewer’s helpful comments and have followed his suggestions to:

1. Include some application points in the abstract

2. In the discussion of our paper, we propose that muscle fatigue is likely to result in respiratory failure in infants - we do not have data to show that there is muscle fatigue in flu-infected infant cotton rats – it is simply an idea that this could be the case. We do not want to mislead the reader to think that this is a fact and therefore have ‘softened’ this description.

3. We have not compared the ‘rate’ of change of parameters in infants and adults since it would be impossible to determine the basis for these differences (the differences could be due to ‘concentration’ of infection since the lung volume is different; differences in innate immune mechanisms; differences in lung mechanics; difference in sensory pathways). Our analyses are therefore restricted to demonstrating differences after infection in each group, and pointing out/discussing those differences that are significantly different in one age group but not another.

4. We have included a discussion of potential triggers of tachypnea (the reason for increased respiratory rate has not been demonstrated, so we provide hypothetical examples on page 4).

5. We would prefer not to add additional figures as all the relevant data is presented in Table 1: Fig 2 is essential as it is important to point out that respiratory rate, like adult animals, was increased in infant animals. We decided to show the data for experiments shown in Table 1 as this provides a snap-shot of the biggest differences observed.

Minor comments:

We have corrected grammatical and other errors in the text.