Rat Model of Chronic Lung Infection using Pseudomonas Strain RP73

charles river

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1 Introduction

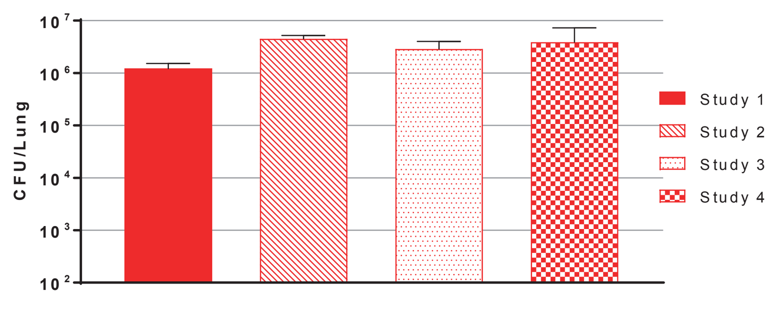
Pseudomonas aeruginosa is a major cause of morbidity and mortality in diseases such as ventilator associated pneumonia (VAP) and cystic fibrosis (CF). Currently it is difficult to eradicate pseudomonas from lungs, leading on to persistent infection that can ultimately cause respiratory failure. The data presented here demonstrates the development of a chronic model *P. aeruginosa* induced pneumonia using strain RP73 (kind gift from Jens Klockgether, Med Hochschule Hannover, Germany).

2 Methods

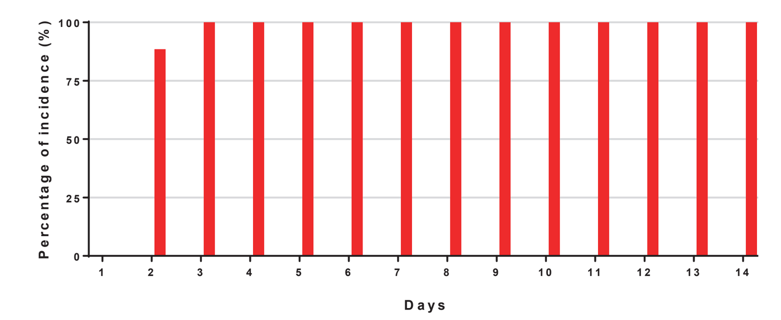
On Day 1, male Sprague Dawley rats (n=6) were administered Pseudomonas-embedded agar beads (4.6 x 10^7 CFU/mL) directly to the lungs via oropharyngeal aspiration. Control animals received sterile beads (n=3). Colony Forming Units (CFUs) were determined on Days 2, 7, 10 and 14 in lung tissue and in bronchoalveolar lavage fluid (BALF). Total and differential cell counts, as well as elastase activity, were determined in the BALF on Days 2, 7, 10 and 14. Body weights and respiratory clinical signs were monitored daily. Histopathological changes were assessed on Days 7 and 14. Data presented as Mean ± S.D. Statistical comparisons were performed against Vehicle Saline Control using ANOVA with Dunnett's post test analysis. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$

3 Results

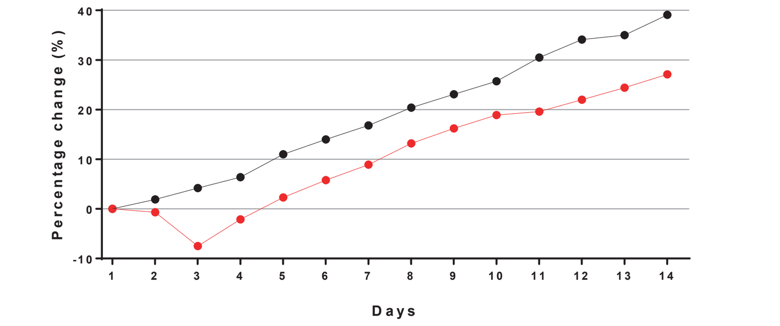
Reproducible infection 24 hours post instillation



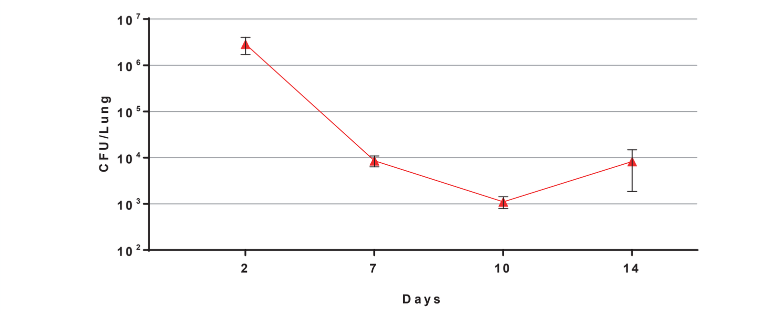
Incidence of Tachypnea (Increased respiratory rate) over 14 days



Percentage change in mean body weight over 14 days

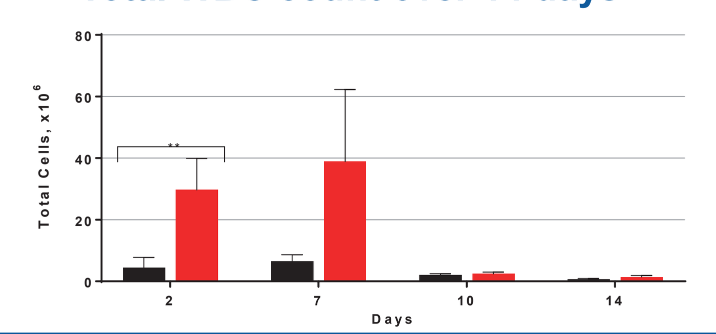


Persistent infection up to 14 days (Data from Lung homogenate)

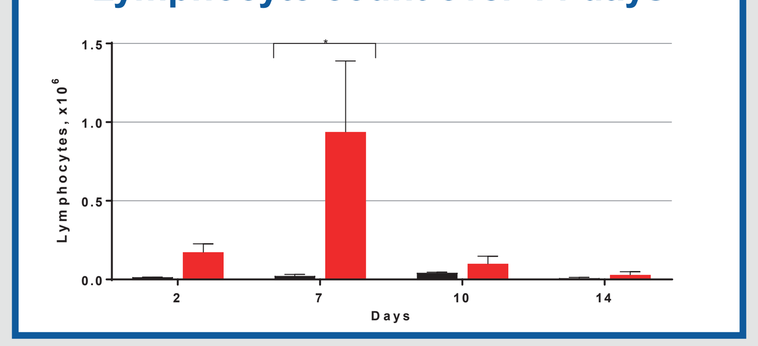


Infection persisted in the lungs of all infected animals up to 14 days after instillation. The infection model has be been shown to be reproducible. Infected animals demonstrated persistent tachypnea from Day 2 but bodyweights increased steadily over the 14 days after an initial drop. Quantitative microbiology was applied to BALF and lung homogenate illustrating the presence of a significant infection in lung tissue until Day 14 (>10^3 CFUs/lung). Animals dosed with sterile beads showed no signs of tachypnea and no CFU detected in the lungs and BALF. BALF CFU data not shown.

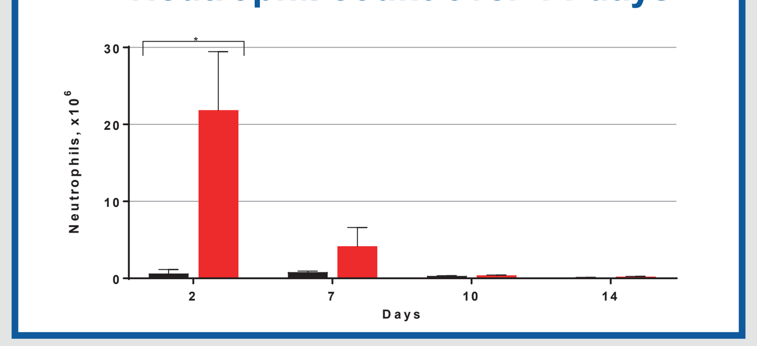
Total WBC count over 14 days



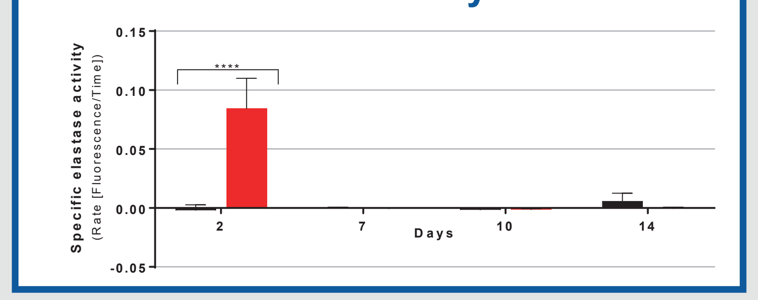
Lymphocyte count over 14 days



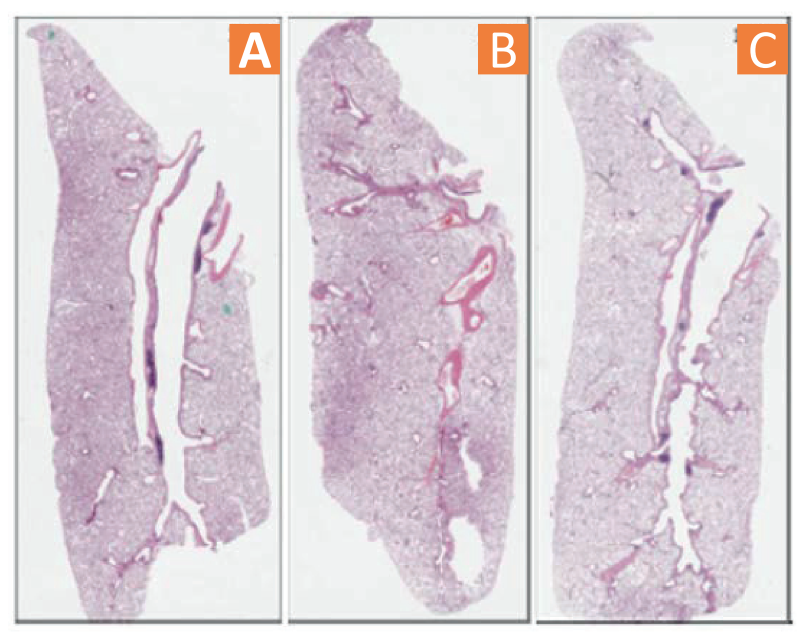
Neutrophil count over 14 days



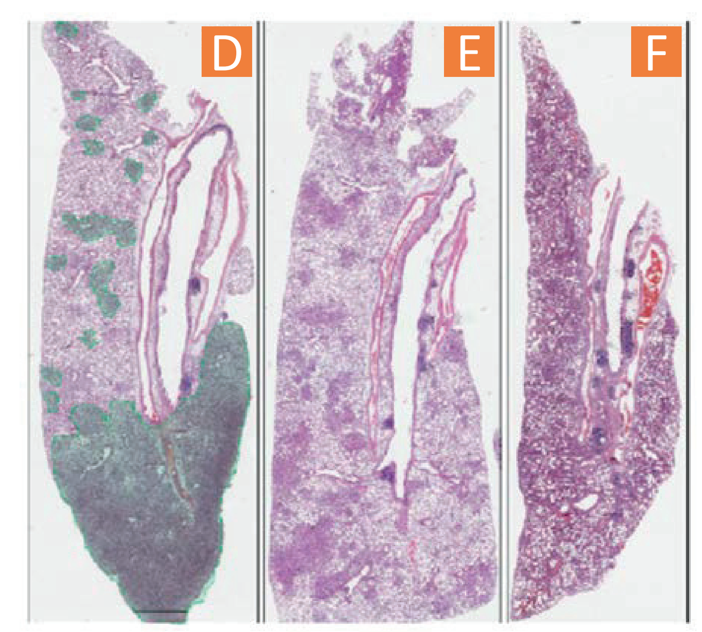
Neutrophil specific elastase activity over 14 days



Assessment of the total and differential WBC response in BALF showed a CFU-infection induced dose dependent increase in lymphocytes (>0.3x10^6 cells, peaking at D7) and neutrophils (>10^7 cells, peaking at D2). BALF elastase activity also peaked at Day 2 coinciding with the BALF neutrophil peak.



Day 7- Lungs of control animals administered sterile beads. Rare lesions in animal A highlighted in green. H&E stain



Day 7- Lungs of animals administered *P. aeruginosa* laden beads. Foci of inflammation are highlighted in green in animal D. H&E stain.

Microscopically, subacute inflammatory changes at Day 7 were locally extensive, of moderate to marked severity and contained a high proportion of neutrophils. By Day 14, chronic inflammatory foci were rare, of minimal severity and more organised with an increase in lymphocytes and a reduction in neutrophils. Day 7 inflammatory foci were positive for alcian blue staining which is indicative of biofilm formation. Administration of sterile beads was associated with minimal inflammation at Day 7 which had resolved by Day 14.

Sterile beads   Pseudomonas beads

4 Conclusion

The data presented characterises the cellular and tissue changes in a subacute to chronic model of pseudomonas pneumonia.
The model can be used for investigating both the pathobiology of chronic pneumonia as well as novel approaches for drug development, diagnosis and therapy.