Acute *Pseudomonas aeruginosa* airway infection models in wild-type and *Phe508del* cystic fibrosis rats

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Introduction

- Cystic fibrosis (CF) lung disease is characterised by chronic and recurrent infections, inflammation, bronchiectasis and airway tissue damage.
- In humans, the CF lung is colonised early by a unique spectrum of microorganisms.
- One pathogen found in CF patients is *Pseudomonas aeruginosa*, which becomes more prevalent in the teenage years and plays a critical role in the development and progression of CF lung disease.
- CF mice and CF rats in clean specific pathogen-free animal facilities do not naturally acquire bacterial infections and therefore do not spontaneously develop CF lung disease.
- The *P. aeruginosa* bead model has been widely used in CF mice to study induced airway infections, however the lack of a representative model of human-like lung disease has been a significant hurdle to CF research.
- Using CRISPR/Cas9 gene editing technology, we have generated a CF rat model bearing the *Phe508del* CFTR mutation.

Aim

- To determine the airway inflammatory response induced by *P. aeruginosa* in *Phe508del* CF rats and whether there is a difference in response compared to wild-type (WT) rats.

Methods

- Anaesthetised WT and CF rats (8-12 weeks old) were inoculated with either 50 µl of sterile agar beads or ~1 x 10^6 CFU mucoid 20844 *P. aeruginosa* coated agar beads to the upper right main bronchus (RMB) of the lung via a miniature bronchoscope.

Post bead delivery:

- Animals were humanely killed at 1, 5 or 7 days post-infection.
- Whole lung bronchoalveolar lavage fluid (BALF) was collected and a cytospin was performed on each sample. Slides were stained with Giemsa to evaluate the percentage and type of immune cells produced in response to infection.
- The treated lobes were harvested, immersion fixed in 10% neutral buffered formalin, embedded and stained with hematoxylin and eosin (H&E) for histological assessment.

Results

Bronchoalveolar lavage fluid:

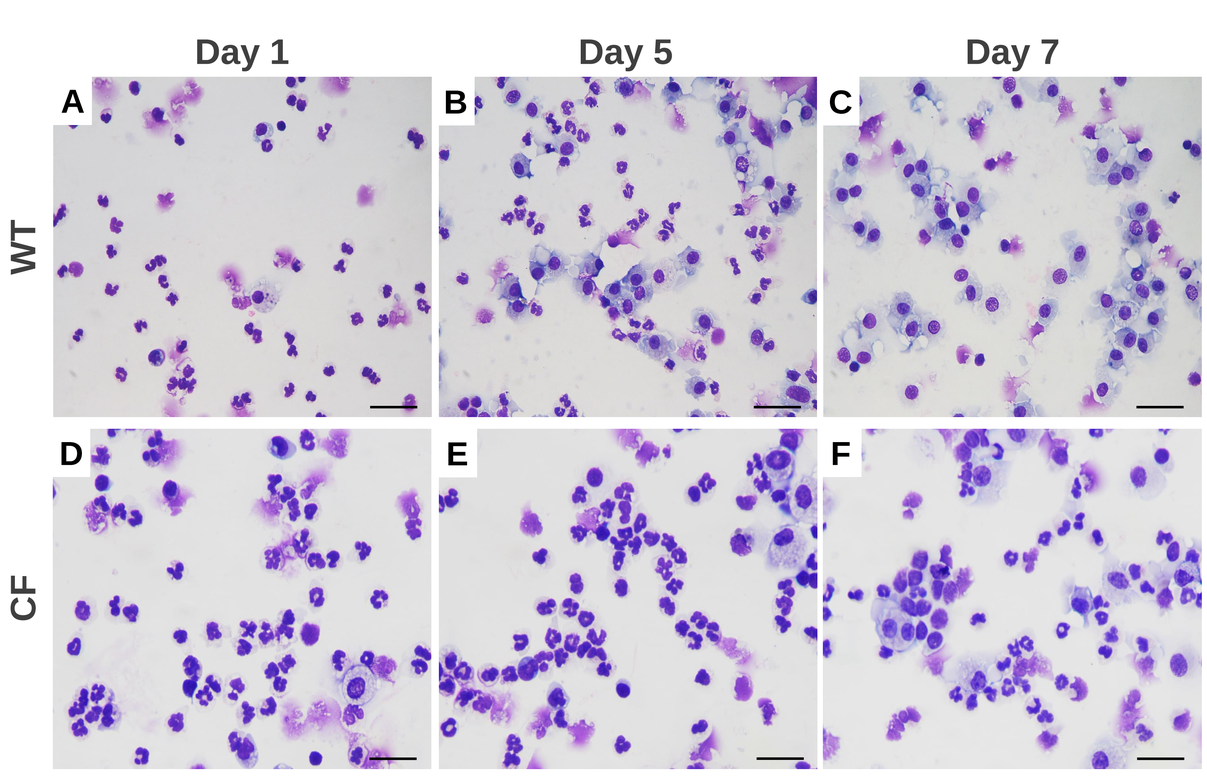

Figure 1: Example images of Giemsa stained immune cells in whole BALF following inoculation with *P. aeruginosa* coated agar beads 1, 5 and 7 days post delivery. CF rats produced a more neutrophilic-dominated response to infection compared to WT. **(A-C)** WT rats and **(D-F)** CF rats. Scale bar = 10 µm.

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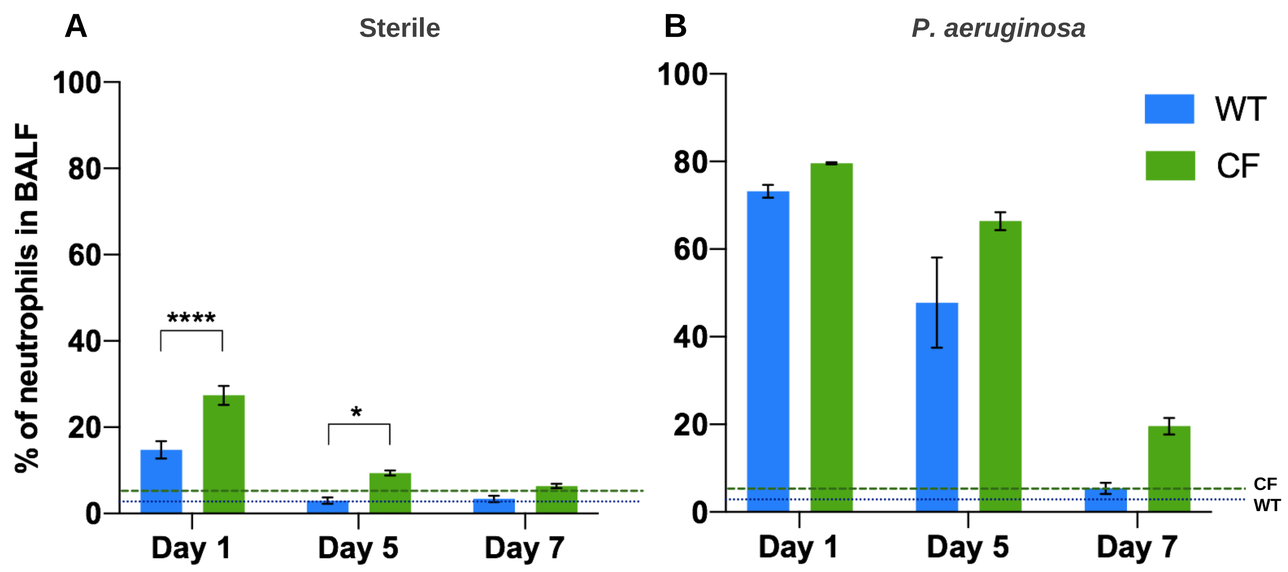

Figure 2: Percentage of neutrophils in whole lung BALF following inoculation with sterile agar beads or *P. aeruginosa* coated agar beads 1, 5 and 7 days post delivery. (A) *Sterile agar beads.* At 1 and 5 days post delivery, CF rats produced significantly higher percentages of neutrophils compared to WT. At day 7, the percentage of neutrophils for both WT and CF rats returned to the base line percentage. **(B)** *P. aeruginosa coated agar beads*. Both WT and CF rats mounted a notable immune response to *P. aeruginosa* coated agar beads at day 1 and 5. At day 7, the percentage of neutrophils for WT rats returned to baseline percentage, while CF rats did not. (Mean with SEM, ****p < 0.0001, *p < 0.05, one-way ANOVA with Tukey's multiple comparisons test, n = 5 per group). Dashed line indicates baseline percentage.

Lung pathology:

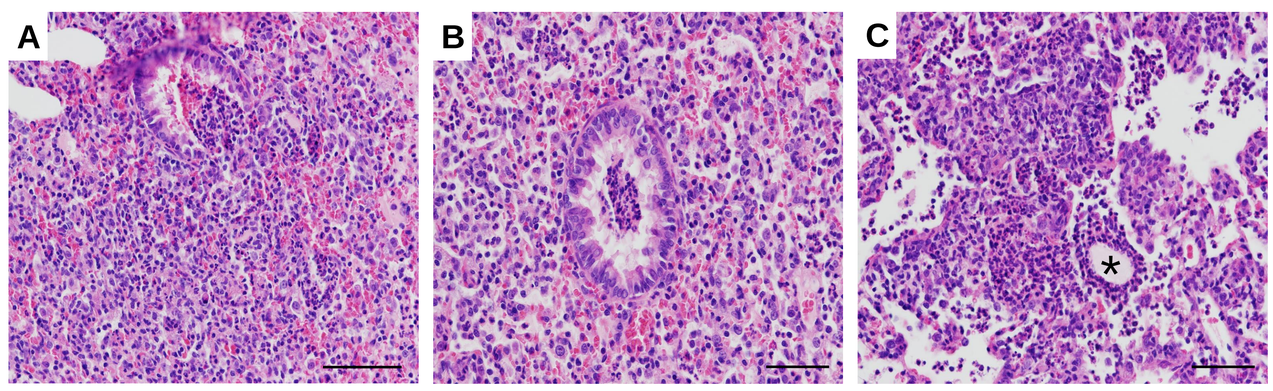

Figure 3: Representative H&E stained sections showing bronchopneumonia at day 1 in the *P. aeruginosa* treated lung region in CF rats. Histological analysis indicates an acute phase of infection with mild perivascular lymphohistiocytic cuffing and numerous neutrophils in bronchiolar lumina and alveolar spaces. **(A,B)** Bronchiolar lumen and alveolar spaces containing abundant neutrophils. **(C)** Exogenous bodies (agar bead = *) surrounded by intense neutrophilic aggregates. Scale bar = 100 µm.

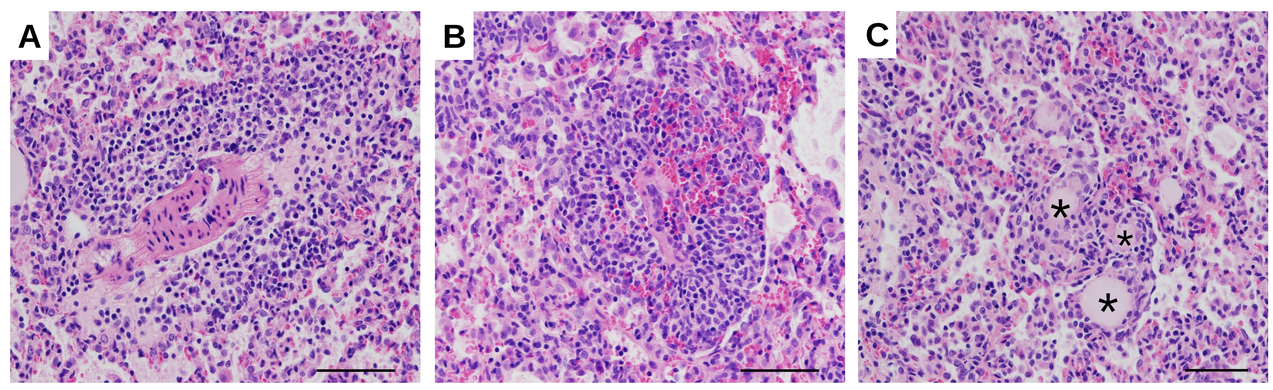

Figure 4: Representative H&E stained sections showing bronchopneumonia at day 5 in the *P. aeruginosa* treated lung region in CF rats. Histological analysis indicates a more severe infection compared to day 1 with marked perivascular lymphohistiocytic cuffing and bronchial-associated lymphoid hyperplasia. **(A)** Perivascular mononuclear and polynuclear cell cuffing. **(B)** Perivascular cuffing and chronic inflammatory cell infiltrate of pulmonary parenchyma. **(C)** Exogenous bodies (agar bead = *) surrounded by numerous macrophages and less neutrophils in alveolar interstitium spaces. Scale bar = 100 µm.

Conclusions

- We successfully established a lobe-specific acute infection in WT and CF rats.
- The local establishment of infection caused a notable immune response in both WT and CF rats. Day 7 results suggest a potential difference in clearance to infection in *Phe508del* CF rats compared to WT.
- Lung pathology demonstrates the development of bronchopneumonia in both WT and CF rats caused by *P. aeruginosa* infection.
- Future studies will determine whether we can achieve an animal model that exhibits progressive airway disease after exposure to *P. aeruginosa* to provide a representative CF lung disease animal model for respiratory research.