CFTR Function Impacts Proinflammatory Cytokine Expression of Bronchial Epithelial Cells During *Pseudomonas aeruginosa* and *Staphylococcus aureus* Infections

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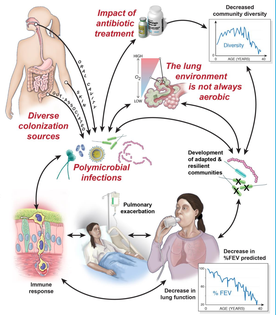
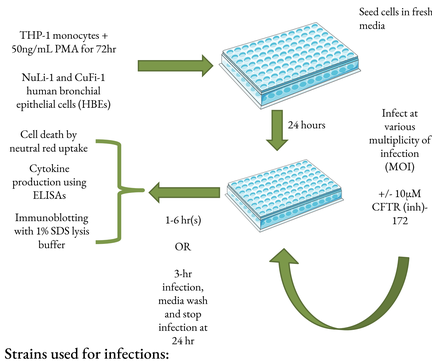
Introduction and Objectives

- CF is a recessive genetic disease that occurs in 1 out of 2,500 live births caused by a mutation in CFTR (1,2)
- Lung disease and subsequent respiratory failure are the leading causes of CF morbidity and mortality (2)
- Despite treatment advances in CF, tissue damage caused from hyper-inflammation and chronic respiratory infections can still contribute to patient morbidity
- It has been speculated that CFTR may play a role in cell death and immune response mechanisms (3,4)
- Objective: To explore how CFTR impairment may impact inflammatory mechanisms



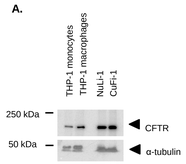
Filkins LM and, O'Toole GA, *PLoS Pathog*, 2015

Methods



- Strains used for infections:
  - PAO1, wild type PA14, and PA14 transposon insertion mutants for Type III secretion system (T3SS) regulator and flagellin
  - *S. aureus* 6538

Results



B.

Cells	Cq CFTR (mean ± SD)	Cq CFTR (no RT control) (mean ± SD)	Cq GAPDH (mean ± SD)	Cq GAPDH (no RT control) (mean ± SD)
NuLi-1	35.24 ± 0.04	36.3 ± 0.72	17.31 ± 0.13	25.80 ± 0.40
CuFi-1	33.98 ± 0.16	37.57 ± 1.43	17.7 ± 0.09	26.09 ± 0.04
THP-1 monocytes	34.31 ± 0.02	35.18 ± 0.48	18.10 ± 0.01	25.74 ± 0.59
THP-1 macrophages	33.96 ± 0.25	34.76 ± 0.59	18.66 ± 0.09	24.38 ± 0.08

Figure 1. CFTR expression can be detected via immunoblotting but not in active transcripts. (A) Immunoblotting of THP-1 monocytes, THP-1 macrophages, NuLi-1, and CuFi-1 for CFTR. **(B)** Cq values for THP-1 monocytes, THP-1 macrophages, NuLi-1, and CuFi- to detect CFTR transcripts.

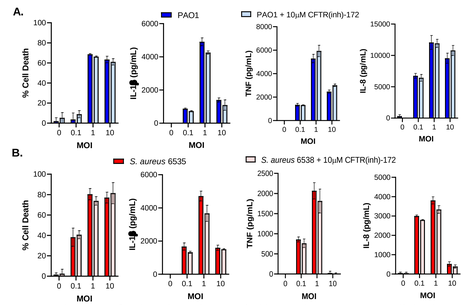


Figure 2. Inhibition of CFTR through CFTR(inh)-172 does not impact cell death or cytokine expression during THP-1 macrophages infections. *In vitro* infections of THP-1 macrophages were conducted with **(A)** PAO1 or **(B)** *S. aureus* 6538. Cell death was measured by neutral red assay and IL-1β, IL-8, and IL-6 production were measured by ELISA. Mean ± SD are shown.

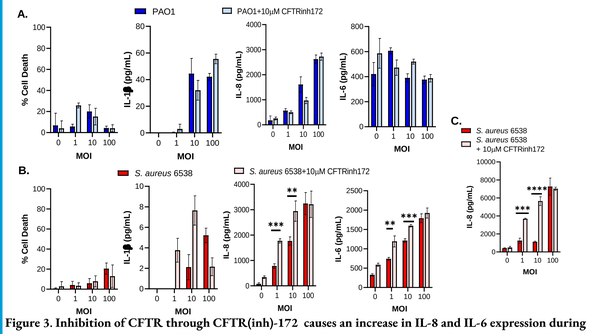


Figure 3. Inhibition of CFTR through CFTR(inh)-172 causes an increase in IL-8 and IL-6 expression during *S. aureus* infections through a CFTR-independent mechanism. NuLi-1 cells were infected at various MOIs with either **(A)** PAO1 or **(B)** *S. aureus* 6538 for 6 hours with or without 10μM CFTR(inh)-172. **(C)** CuFi-1 cells were infected with *S. aureus* 6538 at various MOIs for 6 hours with and without 10μM CFTR(inh)-172. Cell death and cytokine production were assessed through neutral red assay and ELISA, respectively. Mean ± SD are shown.

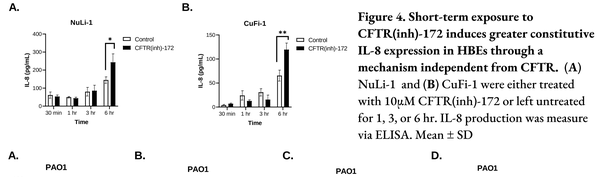


Figure 4. Short-term exposure to CFTR(inh)-172 induces greater constitutive IL-8 expression in HBEs through a mechanism independent from CFTR. **(A)** NuLi-1 and **(B)** CuFi-1 were either treated with 10μM CFTR(inh)-172 or left untreated for 1, 3, or 6 hr. IL-8 production was measure via ELISA. Mean ± SD

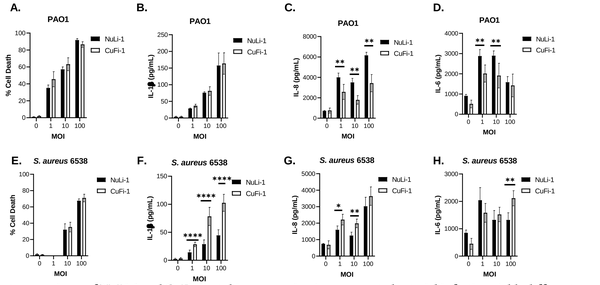


Figure 5. Direct comparisons of NuLi-1 and CuFi-1 cytokine expression in response to bacterial infection yields different trends. NuLi-1 cells and CuFi-1 were infected with PAO1 at various MOIs for 6 hr. **(A)** Cell death was measured by neutral red assay, and **(B-D)** IL-1β, IL-8, and IL-6 production were measured by ELISA. HBEs were also infected with *S. aureus* 6538 at various MOIs for 6 hr and **(E)** cell death and **(F-H)** cytokine production were measured as previously described. Mean of 3 biological replicates ± SEM are shown.

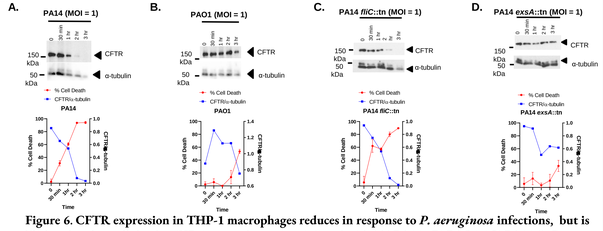


Figure 6. CFTR expression in THP-1 macrophages reduces in response to *P. aeruginosa* infections, but is likely related to cell death. THP-1 macrophages were infected with **(A)** PA14, **(B)** PAO1, **(C)** PA14 *fliC*::tn, or **(D)** PA14 *exsA*::tn at an MOI of 1. Cell death via neutral red assay and CFTR expression via immunoblotting were measured over time for up to 3 hr. Mean ± SD for cell death and mean for relative CFTR expression as assessed by densitometry are shown.

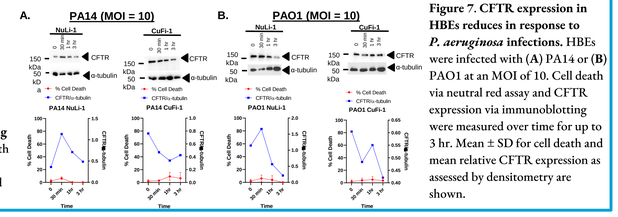


Figure 7. CFTR expression in HBEs reduces in response to *P. aeruginosa* infections. HBEs were infected with **(A)** PA14 or **(B)** PAO1 at an MOI of 10. Cell death via neutral red assay and CFTR expression via immunoblotting were measured over time for up to 3 hr. Mean ± SD for cell death and mean relative CFTR expression as assessed by densitometry are shown.

Results

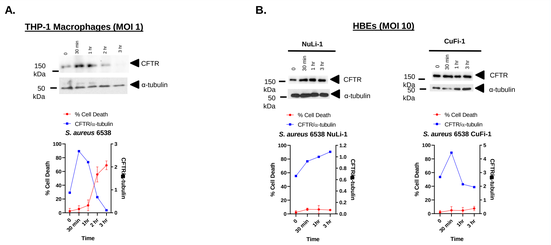


Figure 8. CFTR expression in THP-1 macrophages during *S. aureus* infections reduces with cell death. CFTR expression in HBEs during *S. aureus* infections changes, but in an inconsistent manner. **(A)** THP-1 macrophages were infected with *S. aureus* 6538 at an MOI of 1. CFTR expression via immunoblotting and cell death via neutral red assay were measured over time for up to 3 hr. **(B)** NuLi-1 and CuFi-1 cells were infected with *S. aureus* 6538 at an MOI of 10. CFTR expression and cell death were measured as previously described. Mean ± SD for cell death and mean relative CFTR expression as assessed by densitometry are shown.

Conclusions

- CFTR(inh)-172 treatment yields minor differences in HBE IL-8 expression but independently of endogenous functional CFTR
- NuLi-1 and CuFi-1 cells demonstrate some differences in cytokine expression with and without infection with PAO1 and *S. aureus* 6538
- Future directions include further characterizing CFTR expression levels in available cell lines and working towards developing CFTR KO cell lines for direct comparisons using CRISPR
- Understanding how CFTR impacts immune responses can shed light on adjuvant immunotherapy for CF

Acknowledgements

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References

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