SUMMARY

Chronic lung inflammation caused by *Pseudomonas aeruginosa* is a major driver of pathogenesis in people with cystic fibrosis (pwCF; CF). Although *P. aeruginosa* downregulates many classical virulence factors during long-term colonization, inflammation persists, suggesting the involvement of additional, yet unidentified, bacterial mediators.

The main objective of this thesis was to elucidate the inflammatory roles of both known virulence factors and novel protein mediators expressed by *P. aeruginosa* during chronic CF lung infection. To accomplish this, physiologically relevant growth conditions and model systems that closely replicate the *in vivo* CF lung environment were employed to promote *P. aeruginosa* phenotypes and protein expression profiles characteristic of chronic infection.

Building on this, **Chapter III** examines the experimental conditions that most accurately reflect the infection dynamics and microenvironmental pressures experienced by *P. aeruginosa* in the CF lung. This chapter summarizes a range of *in vitro, ex vivo,* and *in vivo* animal model systems of CF lung infection that have been evaluated for their *in vivo*-like performance using (transcript)omics-based approaches. It also highlights the current lack of proteomic and metabolomic validation strategies and outlines future directions for improving *in vivo*-like model systems.

Chapter IV describes experimental work aimed at gaining novel insights into *P. aeruginosa*-driven lung inflammation under conditions that mimic the *in vivo* CF lung environment. Fourteen *P. aeruginosa* isolates obtained from two pwCF were cultured in synthetic CF sputum medium (SCFM) 2, and the capacity of the resulting cell-free supernatants to elicit host inflammation was assessed using an organotypic three-dimensional (3D) lung epithelial cell culture model.

Part 1 of Chapter IV investigates the contribution of *P. aeruginosa* proteases to CF lung inflammation. Isolates from the two patients exhibited substantially different levels of proteolytic activity, with isolates from one patient generally showing high proteolytic and elastolytic activity, while those from the other patient displayed minimal activity. Cell-free supernatants from isolates with proteolytic activity degraded cytokines and reduced cytokine release in the 3D model. Comparative proteomics analysis of cell-free supernatants from isolates with and without proteolytic activity identified Elastase B (LasB) as the most differentially expressed protease. Through the use of *P. aeruginosa lasB* knockout mutants, LasB was shown to degrade multiple key inflammatory mediators, confirming its immunomodulatory role under the tested

experimental conditions. Additional proteases were also identified that may act in concert with LasB to promote immune evasion.

Part 2 of Chapter IV focuses on uncovering previously unrecognized pro-inflammatory mediators that sustain chronic inflammation even when classical virulence factors are attenuated. Isolates varied significantly in their ability to induce interleukin (IL)-8 secretion, with a subset of isolates inducing a strong pro-inflammatory response despite low production of known virulence factors such as proteases, lipopolysaccharide (LPS), or rhamnolipids. The proteomic dataset generated in **Part 1** was leveraged to compare pro- and anti-inflammatory isolates, uncovering several candidate mediators not previously associated with inflammation. Among these, the transcription factor DksA emerged as a potential driver of persistent airway inflammation. Analysis of existing transcriptomic datasets of *P. aeruginosa* in CF sputum, confirmed consistent and robust *in vivo* expression of *dksA*, and its role in driving inflammation was further demonstrated by assessing host inflammatory responses elicited by a *P. aeruginosa dksA* knockout mutant in the 3D lung model.

Together, these findings advance our understanding of how *P. aeruginosa* modulates host immunity during chronic CF infection. This work highlights both proteolytic activity and previously unrecognized mediators as key contributors of the persistent inflammatory milieu characteristic of the CF lung, and provides a foundation for the development of new therapeutic strategies.