Kinome activity profiling and kinase modulation of pulmonary inflammation

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Chapter 8

Summary and general discussion
Summary

Despite the high standards of modern healthcare, infectious diseases are a major cause of death. Due to its constant contact with the environment, the lung is frequently affected by infections. In Europe over 200,000 deaths were attributed to respiratory infections in 2004, ranking as seventh leading cause of death [1].

Common causes of pneumonia include *S. pneumoniae*, *K. pneumoniae* and *S. aureus*. Increasing resistance of pathogens to antibiotics is a great concern and demands additional understanding of inflammatory processes in infection and inflammation [2]. While the inflammatory response is pivotal in host-defense, hyper-inflammation can be detrimental to the host [3]. Kinases (or the kinome if reflecting all kinases and their activities of one organism) regulate many signal transduction pathways [4]. In this thesis we set out to investigate and modulate (parts of) the kinome during pulmonary infection. In the general introduction, Chapter 1, the kinome, kinome profiling, kinase modulation, and inflammatory lung models are discussed in light of the studies within this thesis.

Kinase mediated phosphorylation events enable progression and regulation of signal transduction cascades [4]. In our study described in Chapter 2, we investigated the kinome profile in the lungs of mice with experimentally induced *S. pneumoniae* pneumonia. We observed changes in chemotoxic stress and Th1 pathway responses. Moreover, we found WNT signaling to be overall reduced. Also, cell cycle activity diminished during the course of severe *S. pneumoniae* pneumonia. We continued with profiling the lung kinome of *K. pneumoniae* pneumonia (Chapter 3). During this gram-negative pulmonary infection, we observed induction of classic innate immunity pathways, such as p38 and p42/44 MAPK’s and TGF-β signaling. We also found a reflection of the host response through SRC kinases and GSK-3β signaling. Apart from inflammatory signaling processes controlled by AKT and protein kinase A were induced by pneumonia.

From the obtained kinome profiles of pneumonia we selected potential targets for modulation: CDKs, p38 MAPK and AMPK. As written in Chapters 4-7, small molecule modulators of these kinase targets were applied in the setting of pulmonary inflammation and infection.

Based on the observation of alterations in CDK activity in gram-positive pneumonia and recent findings in the literature [5], we applied the CDK inhibitor r-roscovitine in sterile and infectious lung inflammation (Chapter 4). R-roscovitine treatment reduced inflammatory mediator levels in macrophage and lung epithelial cell lines. In vivo, we observed strongly r-roscovitine reduced cytokine and chemokine levels in response lipoteichoic acid (LTA) induced acute lung inflammation. Moreover, the intervention induced apoptosis in polymorphonuclear cells (PMNs). Postponed administration of this compound after induction of gram-positive pneumonia resulted in reduced PMN counts in lungs. This was accompanied by a primary increase and secondary decrease in lung bacterial loads.
In Chapter 5, we investigated the use of r-roscovitine in mechanical ventilator-induced lung inflammation. Mechanical ventilation is vital for the support of critically ill patients, but can induce or aggravate lung inflammation and damage [6,7]. During lung-injurious mechanical ventilation r-roscovitine treatment lowered the number of PMNs and lung damage marker levels RAGE and total IgM in bronchoalveolar lavage fluid. However, r-roscovitine did not impact cytokine or chemokine levels in the bronchoalveolar space.

Our data lead us to speculate that CDK inhibition is a potent method of reducing sterile inflammation, while its application in infectious settings should be approached with caution.

As reported in Chapter 6, we assessed the effects of intrapulmonary p38 MAPK inhibition by BIRB 796 on lipopolysaccharide (LPS) and LTA induced sterile lung inflammation. Oral administration of BIRB 796 reduced the inflammatory response in human endotoxemia [8]. BIRB 796 circumvented p38 MAPK phosphorylation and inhibited cytokine and chemokine production in lung epithelium and macrophage cell lines in response to LPS and LTA stimulation. Although local administration of BIRB 796, in vivo, prevented p38 MAPK phosphorylation and reduced inflammatory mediator levels in LPS-induced lung inflammation, PMN recruitment remained unaltered and coagulation activation was even enhanced.

Unlike the preceding chapters, in Chapter 7 we describe the use of a small molecule kinase activator for AMPK: AICAR. AMPK is a highly conserved kinase that classically is known for its key role in energy homeostasis [9,10]. AICAR, in vitro, reduced cytokine production in the alveolar macrophage cell line MH-S. In LTA induced lung inflammation treatment with AICAR diminished early PMN influx into the pulmonary compartment. Moreover, protein leakage and cytokine/chemokine levels in the bronchoalveolar fluid were reduced due to AICAR treatment after 6 hours of LTA lung inflammation.
General discussion

With the research presented in this thesis, we sought to elucidate the state of the kinome in experimental pneumonia of common pathogens in order to identify potential targets to modulate pulmonary inflammatory processes.

A clear distinction between modulation of sterile inflammation or infection is that in sterile inflammation inhibition of inflammatory processes is deemed desirable; while in the infectious setting, inflammation is crucial to fend of pathogens. Nevertheless, in pneumonia, the host response must be well balanced to prevent tissue damage and ensure a proper return to homeostasis.

In both *S. pneumoniae* and *K. pneumoniae* induced pneumonia, there was a remarkable impact on kinase activities compared to non-infected mice. Although major differences were apparent in both models, kinases associated with AKT signaling were observed for both pathogens. Strikingly, in *K. pneumoniae* pneumonia there was a clear fingerprint of classic inflammatory kinases, while in *S. pneumoniae* infection this was less evident. *S. pneumoniae* infection demonstrated a clear change in activity of cell cycle kinases. In *K. pneumoniae* the SRC-cassette was represented by various kinases. Given the gross differences between gram-positive and gram-negative bacteria and the dissimilarities in the dynamics of *S. pneumoniae* and *K. pneumoniae* pneumonia it is not surprisingly that such differences emerged. The non-hypothesis driven approach of these experiments facilitated the establishing of hypotheses for the rest of our studies.

A common reservation on the use of pharmacological interventions, as small molecule kinase modulators, is based on selectivity and resulting side effects of these molecules. E.g. an inhibitor may not be selective to one target, but may interact with other, often highly homologous, targets. However, given the tendency of biological systems to have redundancy, a single intervention with multiple targets can, in itself, be a strength. Nevertheless, it remains imperative to be aware of all possible targets of a small molecule kinase modulator to prevent undesirable outcomes.

Apart from the obvious differences between men and mice, there is a high homology and many processes are evolutionary conserved. We utilized murine models of lung inflammation and infections and *in vitro* cell lines from murine backgrounds. Despite that both BIRB 796 and r-roscovitine were designed with humans and human model systems in mind, these inhibitors functioned in isolated human cells (and clinical trials [11]) as well as in the murine based experiments. This does, however, not enable direct extrapolation of the results of the murine experiments found in this thesis to humans, due to essential interspecies differences.

Concluding, we profiled and successfully modulated the kinome in during pulmonary inflammation and infection by using small molecule kinase modulators. Further research is warranted before interventions as addressed in this thesis can potentially be applied in treatments strategies for lung inflammation.
References