Chapter 12

Summary & discussion
Summary

Infectious diseases are a major cause of morbidity and mortality worldwide. Pneumonia and tuberculosis are still accountable for a great number of deaths each year, despite antibiotic treatment. In addition, the emergence of resistance against antimicrobial agents has become a serious problem for the medical community. Therefore, in order to develop new therapeutic options, it is essential to obtain more knowledge of the mechanisms important for the pathogenesis of infectious diseases and the immune response against (myco)bacterial pathogens. In this thesis, several components considered important for the host immune response against (myco)bacterial infections, were studied.

Chapter 1 is a general introduction discussing the background of our studies and the experimental models used. The recognition of pathogens or pathogen-associated molecular patterns (PAMPs) is of pivotal importance for induction of an innate immune response during infection. CD14 and Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) that can bind PAMPs and subsequently induce intracellular signaling cascades resulting in activation of the immune system. The presentation of microbial PAMPs to CD14 is enhanced in the presence of lipopolysaccharide (LPS) binding protein (LBP). Mitogen Activated Protein Kinases (MAPKs) have been identified as an indispensable link in inflammation-induced signal transduction, transmitting signals from the cell membrane towards the nucleus of the cell resulting in cell activation and induction of immune responses. Three MAPK pathways have been involved in cellular responses during inflammation: p38 MAPK, p42-44 MAPK (ERK) and JNK. So far, little is known about the function of MAPKs in inflammation in vivo.

After recognition of pathogens and the activation of intracellular signaling pathways, immune cells release a range of inflammatory mediators including cytokines. Cytokines are important immunomodulators and act in a complex and dynamic network in which they influence each other's production and activity.

Several models of infection and inflammation were used in this thesis: low-grade endotoxemia, a model for acute systemic inflammation induced by intravenous LPS infusion in humans, and pulmonary tuberculosis or bacterial pneumonia, induced by intranasal inoculation of pathogens in mice.
Human endotoxemia is characterized by a typical cytokine and chemokine response, neutrophil activation, and activation of coagulation and fibrinolysis cascades. In chapter 2, this model was used to study the kinetics of the three major MAPK pathways. During a 24-hour time period after intravenous administration of LPS, peripheral blood leukocytes were obtained and assessed for their phosphorylation status and enzymatic activity of p38 MAPK, p42/44 MAPK and JNK. LPS induced a strong but transient phosphorylation and enzymatic activation of p38 MAPK and p42/44 MAPK, with a maximum activity 1 hour after LPS infusion, followed by dephosphorylation. However, no enhanced JNK phosphorylation or activity was seen in this model. This is in contrast with many in vitro studies showing an involvement of all three MAPK pathways in LPS-induced cell activation.

In chapter 3, the effect of an oral p38 MAPK inhibitor, BIRB 796 BS, on several inflammatory responses during human endotoxemia was evaluated. BIRB 796 BS, both low and high dose, inhibited p38 MAPK phosphorylation and enzymatic activity. Furthermore, BIRB 796 BS dose-dependently inhibited LPS-induced cytokine production (TNF, IL-6, IL-10, IL-1 receptor antagonist) and leukocyte responses including neutrophilia, release of elastase-α1-antitrypsin complexes and upregulation of CD11b with concurrent downregulation of L-selectin. Finally, LPS-induced C-reactive protein release was attenuated by BIRB 796 BS. These results identify p38 MAPK as a principal mediator of the inflammatory response to LPS in humans.

Chapter 4 describes the effect of p38 MAPK inhibition on the LPS-induced procoagulant response in humans. p38 MAPK inhibition, using a high dose of BIRB 796 BS, resulted in a strongly attenuated coagulation activation, as measured by the plasma concentrations of prothrombin fragment F1+2. In addition, activation of the fibrinolytic system (plasma tPA, PAPc, PAI-1) was dose-dependently diminished by BIRB 796 BS, as well as endothelial cell activation (plasma soluble E-selectin and von Willebrand factor). So, p38 MAPK plays an important role in the activation of coagulation, fibrinolysis and the vascular endothelium during human endotoxemia.

The effect of p38 MAPK inhibition on neutrophil migration and activation was studied in chapter 5. Endotoxemia-induced downmodulation of neutrophil CXC receptor 1 and 2 expression was inhibited by a high dose of BIRB 796 BS, as determined by FACS analysis. The release of the chemokines IL-8 and GRO-α during human endotoxemia was dose-dependently inhibited by the kinase inhibitor. These results indicate a principal role for p38 MAPK in regulating essential factors for neutrophil activation and chemotaxis in vivo.
Chapter 6 and 7 describe the role of LBP and TLR4 respectively in a model of murine tuberculosis. In vitro studies have shown that LBP facilitates the binding of lipoarabinomannan (LAM), a major cell wall component of mycobacteria, to the pattern recognition receptor CD14 on immune cells. However, as shown in chapter 6, LBP does not influence the immune response against in vivo infection with M. tuberculosis, as reflected by similar survival curves and mycobacterial outgrowth in lungs and liver in LBP-deficient and normal wild type mice. Therefore, LBP does not contribute to an effective host response in M. tuberculosis infection.

In vitro studies have implicated TLR2 and TLR4 in the innate recognition of M. tuberculosis. In chapter 7, we investigated the role of TLR4 in the host defense against pulmonary tuberculosis in vivo. TLR4 mutant mice were more susceptible to pulmonary tuberculosis, as indicated by a reduced survival and an enhanced mycobacterial outgrowth. Furthermore, TLR4 mutant mice displayed an impaired Th1 helper immune response. These data imply that TLR4 does contribute to the immune response against M. tuberculosis infection in vivo.

Streptococcus pneumoniae is the most frequent cause of community acquired pneumonia. In nosocomial pneumonia, Klebsiella pneumoniae is a commonly isolated pathogen. We employed Streptococcus pneumoniae and Klebsiella pneumoniae to induce Gram-positive and Gram-negative pneumonia respectively in mice. Using these pneumonia models, the role of LBP (chapter 8) and TLR4 (chapter 9) in the host immune response was evaluated. Many in vitro studies have shown that LBP greatly enhances the presentation of LPS, a cell wall component of Gram-negative bacteria, to host inflammatory cells. In addition, evidence exists that LBP facilitates the transfer of Gram-positive cell wall components to inflammatory cells. In line with in vitro experiments, in pneumonia induced by K. pneumoniae, LBP deficient mice demonstrated a diminished survival together with an enhanced bacterial outgrowth in their lungs. In contrast, LBP did not alter the host immune response in pneumococcal pneumonia. Thus, LBP is important for a protective immune response in Klebsiella pneumonia, but does not contribute to an effective host response in pneumococcal pneumonia (chapter 8).

TLR4 has been widely accepted as a PPR for LPS. TLR4 has also been implicated in the recognition and signal transduction of cell wall components of Gram-positive bacteria. Chapter 9 shows that TLR4 is a factor of importance in the induction of an effective immune response in K. pneumoniae pneumonia, as shown by a shortened survival in combination with increased bacterial numbers in the lungs of TLR4 mutant mice. In
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Discussion

*S. pneumoniae* pneumonia, the protective effect of TLR4 was limited to respiratory tract infections induced by low bacterial doses. Our results suggest that other PRRs or receptor clusters also contribute to host defense mechanisms during bacterial pneumonia.

Antibacterial host defense in the pulmonary compartment is regulated by a complex interaction between immunocompetent cells and a network of cytokines and chemokines. In *chapter 10*, the role of the proinflammatory cytokines IL-18 and IL-12 was studied during pneumonia induced by *S. pneumoniae* in mice. IL-18 synergistically enhances IL-12-induced IFN-γ production. Endogenous IL-18 promoted bacterial clearance from the lungs and delayed the progression to systemic infection. In contrast, endogenous IL-12 did not affect the immune response during pneumococcal pneumonia. Our data indicate that IL-18 exerts its protective effect via IL-12- and IFN-γ-independent mechanisms.

Platelet-activating factor (PAF), a lipid with proinflammatory properties, exerts its biological effects by interacting with the PAF receptor (PAFR). The PAFR specifically binds phosphorylcholine, which is the biologically active component of PAF but also a component of the cell wall of non-typeable *Haemophilus influenzae* (NTHi). In vitro, the PAFR mediated the invasion of respiratory epithelial cells by NTHi. However, in *chapter 11*, we show that absence of the PAFR does not alter the innate immune response in pneumonia induced by NTHi, as indicated by similar bacterial counts and several other host inflammatory responses in PAFR−/− and wild-type mice. Our findings suggest that in NTHi pneumonia, the role of the PAFR is limited and that other receptors are responsible for the regulation of the innate immune response.

Discussion

The data in this thesis have been obtained by the use of human and animal models of inflammation and infection. The human endotoxemia model is a well-established and reproducible model of acute systemic inflammation induced by intravenous administration of low dose LPS. It enables us to study the effect of an intervention in a controlled setting. The endotoxemia model has been used as a simplified version of bacteremia and sepsis. However, the model has several limitations. First, infection with live bacteria has different dynamics in the host compared to LPS. Bacteria multiply and die within the host, shedding bacterial...
components. In contrast, LPS is a purified, nonvital agent that is cleared from the body in a relatively short period of time. Second, systemic infection or sepsis usually starts with an originally localized infection, while intravenous administration of LPS results in an acute and fulminant response. Third, in the human endotoxemia model, the intervention takes place in a controlled setting, i.e., before or a known period of time after LPS infusion, which is not the case in the clinical setting. These differences are illustrated by several studies showing an important role for proinflammatory cytokines for the development of experimental sepsis, while treatment with anti-inflammatory agents in clinical trials did not demonstrate any positive effect.

Experimental mouse models have been used for a long time. The underlying rationale is based on the high degree of evolutionary homology between species, making cautious extrapolation of data derived from mice to humans an acceptable scientific method. Using mouse models, we are able to study in vivo infections. Furthermore, manipulation of the mouse genome resulting in the overexpression or deletion of a potential mediator of the host response, enables us to isolate and study the role of this particular component in the complex network of the immune system. However, one must keep in mind that the deleted or dysfunctional gene may not only result in the absence of the gene related product but may also cause compensatory changes.

In this thesis, we demonstrated the involvement of p38 MAPK in inflammation during human endotoxemia. Furthermore, inhibition of this signaling pathway resulted in attenuation of virtually all host inflammatory responses. This makes p38 MAPK inhibition a promising therapeutic modality for many disease states caused by activation of the immune system. Crohn’s disease, caused by chronic inflammation of the gastrointestinal tract, and rheumatoid arthritis are examples of diseases for which p38 MAPK inhibition can be a novel treatment option. However, drugs suppressing the immune system, especially when used for longer periods of time, create an enhanced risk of infectious diseases. This phenomenon has been well described in patients using corticosteroids, but also in patients treated with anti-TNF-antibodies. To date, several p38 MAPK inhibitors have entered clinical trials. These trials will have to prove the clinical relevance of this treatment in combination with an acceptable risk of unwanted side-effects.
The clinical implications of the mouse studies described in this thesis may be less evident. Extrapolation of the described results to the clinical setting should be done with the greatest caution. However, these studies involving the pathophysiology of pulmonary infections in mice increased our knowledge of the host immune response. A thorough understanding of the host inflammatory response during infection is vital for the development of new immunomodulatory therapies for infectious diseases. Therefore, the continuation of research in this field is highly required.