Immunotolerance during bacterial pneumonia and sepsis
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Citation for published version (APA):
Hoogerwerf, J. J. (2010). Immunotolerance during bacterial pneumonia and sepsis

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Chapter 9

Summary and General Discussion
Summary

Bacterial infections are a major cause of morbidity and mortality worldwide. The innate immune response is regarded the first line of defense against invading pathogens. As such, the host response is needed for an effective clearance of infections; however, it can also cause tissue damage when uncontrolled. During sepsis, the innate immune response is dampened, which makes the patient more vulnerable to nosocomial infections, in particular pneumonia. The increasing resistance to antibiotics in combination with the ongoing high mortality of pneumonia and sepsis urges us to develop new therapeutic strategies, for which more insight into the host response to bacterial agents is needed. This thesis aims to gain more insight into the host response during pneumonia and the pathogenesis of immunotolerance during sepsis. Chapter 1 is a general introduction. It describes the recognition of pathogens by the host and the ensuing inflammatory response in the lung and it discusses the different mechanisms known to be involved in immunotolerance during sepsis.

In the first part of this thesis we characterized the host response to either lung inflammation induced by bacterial products in the human lung or lung infection in mice induced by intact *Klebsiella pneumoniae*. In Chapter 2 we sought to compare the inflammatory response elicited by two different Toll-like receptor (TLR)-agonists: lipoteichoic acid (LTA, TLR2-agonist, component of gram-positive bacteria) and lipopolysaccharide (LPS, TLR4-agonist, component of gram-negative bacteria). Unlike LPS, the human response to LTA in vivo was never studied before. Knowledge of the effects of LTA in humans is important considering the prominent place of gram-positive pathogens in both community-acquired and nosocomial infections. Therefore, healthy subjects were given LTA or LPS and saline (control) using the well-established model of segmental instillation by bronchoscope. As determined in bronchoalveolar lavage fluid (BALF), marked differences were detected between LTA- and LPS-induced lung inflammation. Whereas both TLR agonists elicited neutrophil recruitment, only LPS instillation was associated with activation of neutrophils and consistent rises of chemo-/cytokine levels. Moreover, LPS but not LTA activated alveolar macrophages. Remarkably, only LTA induced complement factor C5a release. Thus, these data suggest that stimulation of TLR2 or TLR4 results in differential pulmonary inflammation in the human lung.

In a simultaneous study, we report on the effect of LTA versus LPS on hemostasis in the human lung (Chapter 3), since inflammation is frequently associated with changes in coagulation and fibrinolysis in the bronchoalveolar space. Unlike the inflammatory response, LTA induced similar procoagulant changes in the human bronchoalveolar space as LPS, characterized by activation of coagulation with concurrent inhibition of anticoagulant and fibrinolytic pathways. These results suggest that LTA is an
important bacterial component in inducing hemostatic changes in the lower airways during gram-positive pneumonia.

The same study of bronchial instillation was used to investigate the responsiveness of alveolar macrophages after LPS stimulation in vivo in the human lung (Chapter 4). Considering that the airways are continuously exposed to respiratory pathogens in combination with the fact that recurrent exposure to microbial products can lead to tolerance of immune cells, we hypothesized that LPS instillation would lead to decreased responsiveness of alveolar macrophages. On the contrary, however, in vivo LPS-exposed alveolar macrophages were primed as reflected by increased ex vivo LPS- and LTA-induced cytokine gene expression and production compared to in vivo saline-exposed alveolar macrophages. LPS instillation did not impact on TLR2 or TLR4 expression and did not influence on the expression of several extracellular and intracellular regulators of TLR signaling. However, LPS instillation resulted in sustained phosphorylation of p38 mitogen-activated protein kinase (MAPK) in alveolar macrophages. These results demonstrate that LPS instillation in the human lung primes alveolar macrophages for further stimulation with either LPS or LTA possibly by sustained p38MAPK activation.

To further investigate the innate immune response in the lung against invading pathogens, we hypothesized that Interleukin-1-receptor-associated kinase (IRAK)-M – as a centrally positioned inhibitor of TLR signaling – plays a pivotal role in host defense against K. pneumoniae pneumonia (Chapter 5). IRAK-M was strongly upregulated in lungs of mice infected with K. pneumoniae. The absence of IRAK-M (studied by using IRAK-M−/− mice) resulted in lower bacterial counts, diminished dissemination to distant body sites, less peripheral tissue injury and better survival rates. These data indicate that IRAK-M impairs host defense during pneumonia caused by a common gram-negative respiratory pathogen.

In part II of this thesis different mechanisms that are thought to underlie immunotolerance in patients with sepsis are investigated, in particular the influence of apoptosis of immune cells and the role of TLR inhibitor ST2 during sepsis. Dysregulation of apoptosis in immune cells is an important feature of the host response to sepsis. Extensive apoptosis of lymphocytes was found in both experimentally-induced sepsis in animals and post-mortem in humans. A decrease in apoptosis of granulocytes during sepsis has been implicated in the pathogenesis of organ injury and failure through their uncontrolled release of oxygen radicals and proteolytic enzymes. Knowledge of factors that attribute to the altered apoptosis in immune cells during sepsis is limited. In Chapter 6 the expression profiles of genes encoding key regulators of apoptosis in highly purified monocytes, granulocytes and CD4+ T-lymphocytes are described using the multigene-system multiplex ligation-dependant probe amplification (MLPA) in a case-control study with 16 patients with sepsis and 24 healthy individuals. Relative to healthy controls, monocytes and granulocytes of patients with sepsis displayed anti-apoptotic profiles, while CD4+ T-
lymphocytes displayed a foremost pro-apoptotic mRNA profile, indicating that the alterations in apoptosis of circulating leukocytes occur in a cell-specific manner in patients with sepsis.

In **Chapter 7** we investigated the release of soluble ST2 (sST2), a decoy receptor that inhibits membrane-bound ST2-signaling, during sepsis. Serum sST2 levels were measured on multiple days from onset of sepsis in 95 patients. Sepsis patients had higher sST2 levels than healthy controls from onset of sepsis until 14 days later, which correlated with disease severity scores and cytokine levels. Moreover, patients who did not survive sepsis displayed elevated sST2 levels compared to survivors.

Animal models have revealed that sepsis leads to an attenuated antibacterial lung host defense and enhanced susceptibility to secondary pneumonia. Recently, absence of TLR-inhibitor IRAK-M was found to result in an improved survival and bacterial clearance from the lungs in septic mice exposed to *P. aeruginosa* via the airways. In **Chapter 8** we studied the role of TLR-inhibitor ST2 in lung host defense during sepsis by inducing abdominal sepsis in ST2<sup>-/-</sup> mice using cecal ligation and puncture (CLP), followed by a secondary intrapulmonary challenge with *P. aeruginosa*. Septic ST2<sup>-/-</sup> mice demonstrated improved bacterial clearance, increased local tissue damage in lungs, reduced dissemination and distant organ damage and higher survival rates when compared to normal wild-type mice. Sepsis led to decreased capacity to release pro-inflammatory cytokines by T-lymphocytes of septic WT mice. This phenomenon was reversed by the absence of ST2, suggesting that the presence of ST2 on T-lymphocytes is essential in the immunotolerance observed in WT animals. In contrast, sepsis did not impact the capacity to release TNF-α by alveolar macrophages in both presence and absence of ST2. ST2 deficiency did not impact on host defense during primary *Pseudomonas* pneumonia. These findings indicate that ST2 and possibly its presence on T-lymphocytes contribute to the immunocompromised state during sepsis and the ensuing disturbed homeostasis of lung host defense.
An adequate host response during severe infection relies on a balanced reaction of inflammatory pathways to an invading pathogen. Bacteria are recognized by the innate immune system by pattern recognition receptors among which TLRs prominently feature. TLRs recognize conserved motifs expressed by pathogens collectively named “pathogen associated molecular patterns” or PAMPs. The research in this thesis describes several aspects of the host response upon exposure to bacteria or PAMPs, both in the lungs (Chapters 2-5 and 8) and in the circulation (Chapters 6 and 7). Chapters 2 and 3 are the first to describe the effects of LTA, a PAMP expressed by gram-positive bacteria, in humans. The results obtained indicate that some responses to LTA are similar to those elicited by LPS, a PAMP expressed by gram-negative bacteria. In particular, the bronchoalveolar hemostatic changes produced by intrabronchial instillation of LTA resembled the alterations induced by LPS, characterized by concurrent activation of coagulation and inhibition of anticoagulant mechanisms (Chapter 3). On the other hand, the effect of LTA on local inflammation differed from that induced by LPS (Chapter 2), which sheds new light on the interaction between coagulation and inflammation in the lungs. First of all, these data point to differential induction of essential components of the host response after triggering of TLR2 (by LTA) or TLR4 (by LPS) in the human lung. Further, these findings suggest that inflammation and coagulation are initiated by distinct pathways in the pulmonary compartment. In this respect it should be noted that tissue factor is the main inducer of coagulation, both in the lung and in the circulation; although proinflammatory cytokines can induce tissue factor expression, apparently direct stimulation by LTA or LPS is sufficient to initiate a procoagulant response in the lungs. Our data do not exclude an interaction between inflammation and coagulation during a more severe and/or sustained host response. Indeed, rodent studies have suggested that during sepsis coagulation amplifies inflammation only during severe and late stage sepsis.

Several chapters in this thesis focus on immunotolerance, a phenomenon characterized by a reduced responsiveness of leukocytes upon re-exposure to bacterial agonists. Immunotolerance, in the literature also referred to as “immunoparalysis” or “LPS tolerance”, has been extensively described for circulating leukocytes. In Chapter 5 we show for the first time that human alveolar macrophages, the predominant resident leukocyte present in the bronchoalveolar space, do not become tolerant for stimulation with either LPS or LTA after previous exposure to LPS. The absence of tolerance upon a primary stimulus in the lung contrasts with the previously described phenomenon of tolerance of blood leukocytes after exposure to bacteria or bacterial products. This organ-specific reaction could be beneficial for host defence against pneumonia; on the other hand, the increased responsiveness of alveolar macrophages may render the host more vulnerable to acute lung injury. Chapters 7 and 8 also investigate aspects of immunotolerance by
examining the extent of leukocyte apoptosis and the release of sST2 in patients with severe sepsis. Apoptosis is a physiological process by which cells are eliminated in a controlled manner ("programmed suicide") in order to limit damage of surrounding tissue. Deregulated apoptotic immune cell death has been implicated to play a major role in immune dysfunction in sepsis. Lymphocytes are considered the main cell type undergoing enhanced apoptosis in sepsis. The profound pro-apoptotic gene expression profile detected in purified CD4+ T cells described in Chapter 6 supports this notion. The pathogenetic significance of these findings has been illustrated in animal models of sepsis, in which prevention of apoptosis of lymphocytes improved survival. Chapters 7 and 8 focus on ST2, as a biomarker for severity of disease in patients with sepsis (sST2, Chapter 7) and as a functional mediator of immunotolerance in mice with polymicrobial sepsis (using ST2-/- mice, Chapter 8). Of note, although sST2 levels showed prognostic significance in clinical sepsis, our data did not directly link sST2 concentrations with the extent of immune suppression in these patients. As such, further research is warranted to clarify a possible connection between these two phenomena. Considering the work presented in Chapter 8 this potential future research should in particular focus on lymphocyte function. In addition, the fact that ST2-/- mice are relatively protected against secondary *Pseudomonas* pneumonia in the context of existing abdominal sepsis point to another future area of research, *i.e.* the design and development of strategies to inhibit ST2 signaling in the host that is prone to develop nosocomial pneumonia.

The host response to severe infection is highly complex and organ specific, involving both exaggerated inflammation and immune suppression. Several mediators and pathways have been demonstrated to play a role herein. This thesis only studied a small number of mechanisms implicated in this response. Clearly, many questions remain unanswered. Increasing our knowledge of the host response to sepsis is a challenging but potentially highly rewarding task for many researchers in the years to come.
References


