Cell-specific pattern recognition receptor signaling in antibacterial defense
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Chapter 1

General introduction and outline of this thesis

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**General introduction**

**Infection and sepsis**

**Sepsis** is the syndrome of infection complicated by acute organ dysfunction. It is a leading cause of morbidity and mortality worldwide, both in developing countries and the developed world (1, 2). The most common cause of sepsis is pneumonia, followed by abdominal and genitourinary infections (1). Despite all efforts in the past decades to improve the outcome of sepsis, the mortality rate remains as high as 20-40% (3-5). Moreover, antimicrobial resistance rates of common pathogens are increasing, including *Streptococcus (S.) pneumoniae*. Especially alarming is the emergence of extended-spectrum β-lactamases producing strains of *Escherichia (E.) coli* and *Klebsiella (K.) pneumoniae*, as well as multi-drug resistant and extreme drug resistant strains of other gram-negative bacteria including *Pseudomonas (P.) aeruginosa* (5-10). These resistant strains are often confined to health-care institutions, but some become increasingly prevalent in the community. Infections with these resistant pathogens are associated with increased morbidity, mortality and economic costs. These major health concerns call for the development of new therapies against infection and sepsis.

**Host defense against infection**

In pneumonia, there are several lines of host defense against the infective pathogen. The respiratory tract is a large surface within the body that mediates gas exchange with the environment but therefore also is a large area of potential contact with pathogens. The first lines of defense consist of the pseudostratified mucosal barrier of the tracheobronchial tree where ciliated and secretory cells (goblet and Clara cells) work together to protect the airways. Mucus produced by secretory cells contains antimicrobial peptides, enzymes and surfactant proteins and entraps pollutants and pathogens, after which it is transported by the cilia in ascending direction. The lower airway surface is covered by type I alveolar cells that have gas exchange as primary function and type II alveolar cells that maintain the alveolar space by the secretion of several surfactant proteins that also serve an antimicrobial function by opsonizing pathogens (11). When these lines of defense fail, the innate and adaptive immune systems are the next defense mechanisms to induce an antibacterial response to eliminate pathogens (12, 13). Alveolar macrophages and dendritic cells reside in the lungs and therefore function in addition to airway epithelial cells as sentinel cells of the innate immune system (12, 13). When pathogens are detected, sentinel cells attract larger numbers of phagocytes such as neutrophils from the bloodstream to the site of infection in interplay with respiratory epithelial cells, via the secretion of various chemokines and cytokines, thereby also enhancing their effector functions of phagocytosis and killing (12-15). Moreover, alveolar macrophages themselves can also engulf pathogens and apoptotic neutrophils and in this way eliminate pathogens and contribute to the resolution of pneumonia (16). The secretion of interferon (IFN)-γ by both cells of the innate and adaptive system is known to
powerfully enhance these macrophage effector functions (17).

The abdominal cavity is normally sterile and as such, has fewer defense mechanisms than the respiratory tract. In the case of infectious peritonitis, reticuloendothelial cells, mesothelial cells, and peritoneal macrophages detect pathogens and contribute to host defense.

**Role of pattern recognition receptors**

**Innate immune cells** detect pathogens by recognition of conserved microbial molecules (**pathogen associated molecular patterns or PAMPs**) with sensors called **pattern recognition receptors (PRRs)** (13, 18, 19). **Toll-like receptors (TLRs)** prominently feature herein, detecting a variety of conserved microbial patterns as well as “danger signals” released from host cells as a consequence of injurious inflammation. As such, TLRs play an important role in the initiation and amplification of the host response (14, 18, 19). To date, 10 human TLRs have been identified, located on the cell surface (TLR1,2,4-6,10) or in membranes of endosomes and lysosomes (TLR3, TLR7-9) (14, 18, 19). Different microbial and endogenous ligands have been identified for most TLRs and for each one pathogen there are a variety of molecular patterns detectable by different TLRs (14).
Once TLRs are activated, they propagate their signal via intracellular adapters, activating nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and Mitogen-activated protein kinases (MAP kinases). The universal adapter for all TLRs - except TLR3 - is myeloid differentiation primary response gene (MyD)88 (18, 19). In addition, MyD88 mediates IL-1β and IL-18 receptor signaling (20). TIR-domain-containing adapter-inducing interferon-β (TRIF) is the sole adapter for TLR3 and contributes to TLR4 signaling (18, 19). Unrestrained activation of TLRs may result in excessive inflammation and collateral tissue damage. Several negative regulators of TLR signaling are known to balance the inflammatory response. Single immunoglobulin IL-1 receptor-related molecule (SIGIRR) has been shown to inhibit TLR-dependent and IL-1R like receptor induced NFκB activation (21).

Another family of PRRs are the nucleotide-binding and oligomerization-domain proteins, with NLR family, pyrin domain containing 3 (NLRP3) as the best characterized member. NLRP3, together with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), takes part in the formation of large multi-protein complexes called inflammasomes. These are crucial to the antimicrobial response because they activate caspase-1 dependent IL-1β and IL-18 maturation. Caspase-1 activation also leads to inflammatory cell death or pyroptosis (22, 23). Activation of caspase-1 by the NLRP3 inflammasome is a multi-signal process, requiring at least two signals (22, 23).

Although a brisk initiation and amplification of the host response is indisputably important, the inflammatory response may, also dependent on the virulence of the pathogen and host characteristics, induce local tissue injury and lead to a systemic inflammatory response that may contribute to organ injury (1). Little is known about the contributions of non-hematopoietic cells (i.e., lung epithelial cells, the vascular endothelium) to both the antibacterial response and potentially harmful side effects of the inflammatory response. Some evidence indicates that tissue and organ injury during sepsis may be negatively impacted by endothelial induced inflammation (24-27). With the aim of attenuating excessive inflammation during sepsis, several anti-TLR therapies have been developed in recent years (28, 29). Until now, results have only been very modest and not proven of additional value in the clinical setting.

**Infection models used in this thesis and the innate immune receptors involved**

Infections of the respiratory tract are in the top ten causes of death both nationally and globally; mortality affects mainly children and the elderly (30-32). In pneumonia, community-acquired infection is distinguished from pneumonia that is associated to hospital admission, mechanical ventilation or out-of hospital health care settings since they are very different with regard to microbial etiology and prognosis.

*Klebsiella (K.) pneumoniae* is a gram-negative pathogen of the Enterobacteriaceae family that frequently causes pneumonia and blood-stream infections, especially in hospitals and health-care related settings (33-35). In experimental *K. pneumoniae* respiratory infection particularly TLR4, that detects the gram-negative cell wall
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constituent lipopolysaccharide (LPS) as well as various endogenous danger signals, and TLR9, that detects bacterial DNA, were found to be protective (36-38). Universal TLR-adapter MyD88 is of crucial importance for host defense and survival in K. pneumonia respiratory infection (39). The adapter protein TRIF mediates TLR3 signaling in response to double-strand RNA and contributes to TLR4 dependent signaling. TRIF has been shown to be required for optimal host resistance in Klebsiella pneumonia (39).

Streptococcus (S.) pneumoniae is the most frequent cause of community-acquired pneumonia and responsible for a considerable part of the health burden that pneumosepsis places on society, especially among young children and the elderly (30, 34). More than 90 serotypes have been identified, several of which cause invasive and severe disease and mortality (40). In this thesis, two different pneumococcal strains are used: a serotype 3 strain (ATCC 6303) that causes lethal disease in mice after low-dose infection and a serotype 2 (D39) strain that is used at a high dose in experimental pneumonia thereby inducing about 25% mortality in immunocompetent mice (41-49). In humans, infections caused by serotype 3 pneumococci are common and associated with a complicated course and an increased risk of death (40, 50, 51), whereas infections with the serotype 2 are uncommon in the western world.

Several TLRs contribute to the host response during pneumococcal infection. TLR2 that detects lipoteichoic acid (LTA), a constituent of the pneumococcal cell wall (46, 52), has a modest role in the cytokine response during pneumococcal pneumonia after infection with a serotype 3 S. pneumoniae (47). TLR4 contributes to host defense during S. pneumoniae pneumonia by recognition of pneumolysin and serves a protective role during pneumococcal infection of the lower airways (37, 53). In the absence of pneumolysin, TLR2 limited bacterial growth during infection with the serotype 2 D39 pneumococcus (45). Finally, TLR9 deficient (Tlr9\(^{-}\)\(^{-}\)) mice showed enhanced bacterial growth and dissemination after induction of pneumococcal pneumonia (54). Myd88\(^{-}\)\(^{-}\) mice had a profoundly enhanced growth of pneumococci and a strongly reduced survival after intranasal infection with a serotype 4 S.pneumoniae strain (55). In recent years, the importance of the inflammasome components NLRP3, a member of the NOD like receptor family, and/or the adapter protein ASC for the host response during pneumococcal pneumonia was demonstrated in studies using S. pneumoniae strains with a relatively low virulence (42, 43, 56). This protective effect is hypothesized to be dependent on the activation of NLRP3 by pneumolysin, a crucial virulence factor expressed by S. pneumoniae (43, 57, 58).

Respiratory tract infection with Pseudomonas (P.) aeruginosa, a flagellated gram-negative opportunistic pathogen, often occurs in hospitalized and/or mechanically ventilated patients and frequently results in severe disease (59, 60). Moreover this pathogen tends to induce chronic lung inflammation after colonization of the airways of patients suffering from chronic lung diseases thereby causing further decline in pulmonary function (61, 62). In experimental models of Pseudomonas infection the importance of TLR dependent clearance of this pathogen was clearly
illustrated. MyD88 deficient (Myd88−/−) mice were hypersusceptible to *Pseudomonas* pneumonia (63-65). TLR2, TLR4 and TLR5 (that detects flagellin) had redundant functions, but control of the bacterium required detection of either LPS or flagellin in a way that also depended on the infectious inoculum (66, 67).

Abdominal infection, together with urinary tract infection is the second most common cause of sepsis and *Escherichia (E.) coli* is among the most frequently cultured gram-negative bacteria in sepsis and peritonitis patients (68-70). Typically, peritonitis results from perforation of a hollow abdominal organ, spilling gut content into the normally sterile cavity leading to polymicrobial infection (secondary peritonitis). In certain susceptible patients however primary peritonitis may result from bacterial translocation (70-73). Since bacteria can quickly spread via the bloodstream from the peritoneal cavity, this can lead to the rapid onset of systemic inflammation and sepsis, resulting in a very high mortality rate of up to 60% (71, 72). Complicating treatment, *E. coli* has increasing extended antimicrobial resistance rates, both in hospitals and in the community (9). The experimental model of *E. coli* peritonitis used in this thesis has a low infectious inoculum of the virulent O18:K1 strain in contrast to frequently used models with high infectious doses of less virulent bacterial strains. In the model here used, TLR4 is important for the initial host defense, while during later stage infection the role of TLR2 becomes significant (74).

**Aim and outline of this thesis**

The general aim of this thesis is to advance our understanding of TLR-dependent, especially MyD88-dependent signaling in experimental models of sepsis, with a focus on pneumonia, its most common cause. We also explored the role of the “inflammasome” during pneumococcal pneumonia. Secondly, since innate immune sensors are widely distributed among different cell types in the airways and body, we aimed to gain insight in the contribution of different cell types and body compartments to TLR- and MyD88-dependent signaling during infection and sepsis. In chapter 2 we dissected the role of TLR2 and 4 and in chapter 3 of MyD88 and TRIF dependent signaling in hematopoietic and non-hematopoietic cells during *K. pneumoniae* airway infection by the use of bone-marrow chimeras. In chapter 4 we further dissected the role of MyD88 dependent signaling during *Klebsiella* pneumosepsis in myeloid and endothelial cells by the use of tissue specific conditional knockouts for MyD88. In chapter 5 we studied the importance of TRIF in the secretion of interferon (IFN)-γ in response to *K. pneumoniae* and the potential of recombinant IFN-γ to restore the impaired antibacterial defense in TRIF-deficient mice.

In chapter 6 we studied the role of MyD88 dependent signaling in lung epithelial cells versus myeloid cells during respiratory tract infection with *P. aeruginosaa*, and in addition used bone marrow chimeric mice to establish the different contribution of TLR5 on hematopoietic versus non-hematopoietic cells to the innate host response to this flagellated bacterium.

In chapter 7 we investigated the differential role of NLRP3 and ASC during
pneumococcal pneumonia with the serotype 2 D 39 strain. In more depth we analyzed the early in vivo immune response by whole-genome transcriptional profiling and the differential contribution of ASC and NLRP3. In chapter 8 we demonstrate an opposite role of NLRP3 and ASC compared to the previous chapter during infection with a serotype 3 pneumococcal strain. In addition, we investigated the role of TLR-dependent signaling during infection with this strain by the use of MyD88 deficient mice. Furthermore, in chapter 9 we investigated the role of SIGIRR, a negative regulator of TLR and IL-1 receptor dependent signaling, during lethal pneumococcal pneumonia. In chapter 10 we studied the effects of anti-TLR4 therapy on anti-bacterial defense, inflammatory response and organ injury in a delayed treatment model of *E. coli* peritonitis.
References


Chapter 1


