Immunotolerance during bacterial pneumonia and sepsis
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Chapter 1

General Introduction
Introduction

Infectious diseases are a major cause of morbidity and mortality worldwide. Massive use of antibiotics promotes pathogen resistance and as a consequence, the incidence of drug-resistant bacteria is increasing (WHO; The world health report 2000, Health Systems: improving performance). Therefore, it is of the utmost importance to expand our comprehension of host responses against invading pathogens in order to develop new treatment strategies. This thesis focuses on the immune response against bacteria during (nosocomial) pneumonia and sepsis.

Bacterial pneumonia

Bacterial pneumonia is one of the most common infectious diseases and the most frequent source of sepsis. Depending on the circumstances in which the patient acquires pneumonia, community-acquired pneumonia can be distinguished from hospital-acquired (nosocomial) pneumonia occurring in patients with pre-existing conditions. The most frequent causative pathogen in community-acquired pneumonia is *Streptococcus pneumoniae*, whereas *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are prominent bacteria causing nosocomial pneumonia.

The host response against bacterial pneumonia

The airways are in direct contact with the outside environment and therefore continuously exposed to respiratory pathogens. The first line of defense in the upper respiratory tract is formed by physical mechanisms like coughing and sneezing. When respiratory pathogens overcome these structural defenses and enter the alveolar space, the innate immune response is primarily responsible for the elimination of these pathogens. Upon recognition of invading pathogens, innate immune cells like respiratory epithelial cells and resident alveolar macrophages will then orchestrate an innate immune response leading to the secretion of cytokines, chemokines and antimicrobial peptides (Figure 1.1). Moreover, alveolar macrophages are able to bind and phagocytose pathogens and subsequently kill them intracellularly. The secreted cytokines and chemokines mediate recruitment and activation of neutrophils from the circulation to the site of inflammation in the lung. Recruited neutrophils effectively phagocytose and eliminate pathogens (Figure 1.1). Besides the elimination of pathogens, alveolar macrophages are able to phagocytose apoptotic neutrophils and thereby contribute to the resolution of pneumonia. Furthermore, the innate immune response is thought to orchestrate the adaptive immune response that primarily consists of T- and B-cell responses that provide specific memory of infection.
Recognition of pathogens by Toll-like receptors

In the alveolar space, innate immune cells distinguish potential pathogens from self, using receptors that recognize highly conserved motifs (pathogen-associated molecular patterns; PAMPs) on pathogens that are not found in higher eukaryotes. The receptors recognizing these PAMPs have been termed “pattern recognition receptors” or PRRs. Among other receptor families, Toll-like receptors (TLRs) occupy a central position as PRRs in the initiation of cellular innate immune responses. TLRs are distinguished from other PRRs by their ability to recognize, but moreover, to discriminate between different classes of pathogens. Presently, thirteen TLRs are described, of which TLR2 and TLR4 are of great importance in bacterial pneumonia.
TLR4 recognizes lipopolysaccharide (LPS), part of the outer membrane of gram-negative bacteria\(^1\), whereas TLR2 recognizes lipoteichoic acid (LTA), a major constituent of gram-positive bacteria\(^12-17\) (Figure 1.2). Although many investigations have been published on the effects of LPS in humans\(^18,19\), the human response to LTA in vivo has never been studied. 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.

![Figure 1.2 Overview of PAMPs as part of the membrane of gram-positive and gram-negative bacteria (adapted from \(^20\))](image)

Coagulation and fibrinolysis

The acute inflammatory response is frequently accompanied by activation of coagulation and inhibition of fibrinolysis in the bronchoalveolar space during pneumonia\(^21-24\). These hemostatic effects can be considered host-protective in containing inflammation to the site of infection\(^25\). However, procoagulant activity can also be disadvantageous by modulating inflammatory activity, leading to excessive activation of inflammation in the alveolar compartment during pneumonia\(^26\). LPS have been demonstrated to reproduce the hemostatic alterations of pneumonia in the lungs of healthy humans when administered in the airways by bronchial instillation\(^27-29\). In contrast, knowledge of the hemostatic balance in inflammation caused by gram-positive pathogens is limited.

Sepsis

Sepsis is one of the leading causes of death in the Western world and its mortality rate remains unacceptably high between 20-40\(^%\)\(^30\). Sepsis is a heterogenous clinical
syndrome broadly defined as the systemic host response to an infection. Although any bacterial infection can progress and cause systemic inflammation, respiratory tract infections are the most common source for sepsis\textsuperscript{31,32}. Furthermore, patients with sepsis are prone to develop nosocomial infections, in particular pneumonia, which has a large impact on outcome.

**Immunotolerance in sepsis**

Until recently, the high mortality rate of sepsis was thought to be the result of an uncontrolled hyperinflammatory response of the host to an infection. However, failure of clinical trials with anti-inflammatory strategies in sepsis patients and the development of animal models more closely imitating clinical sepsis have led to the reconsideration of the pathogenesis of sepsis. Sepsis is currently considered a misbalance between hyperinflammatory responses and immunotolerance (Figure 1.3).

![Figure 1.3 Misbalance of hyperinflammation and immunotolerance in the host response during sepsis (adapted from \textsuperscript{20}).](image)

Hyperinflammation is designed to eliminate invading pathogens, but is at the same time responsible for tissue damage. In contrast, immunotolerance is believed to dampen excessive inflammation and subsequent tissue damage, but may contribute...
to the susceptibility of septic patients to nosocomial infections\textsuperscript{20,33-35}. Clear evidence of immunotolerance in sepsis comes from studies showing hyporesponsiveness of immunocompetent cells upon recurrent exposures to microbial agents or products (often referred to as tolerance to LPS)\textsuperscript{36-38}. Various mechanisms are thought to contribute to immunotolerance, among which anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth-factor (TGF)-\(\beta\). Likewise, deregulated apoptosis of lymphocytes, dendritic cells, monocytes/macrophages and granulocytes, has been implicated to play a role in immunotolerance\textsuperscript{20,33,34,39,40} (Figure 1.3). Alongside upregulation of anti-inflammatory mediators and deregulated apoptosis of immune cells, inhibitors of TLRs such as MyD88 short, A20, interleukin-1 receptor-associated kinase (IRAK)-M and ST2 are thought to play a role in the immunotolerance in septic patients\textsuperscript{41-43} (Figure 1.3 and 1.4).

**ST2**

The receptor ST2 emerges as a transmembrane variant (ST2L) and a soluble secreted variant (sST2). Originally described as a Th2 marker\textsuperscript{44}, several other cell-types also express ST2 including mast cells\textsuperscript{45}, eosinophils\textsuperscript{46} and macrophages\textsuperscript{47}. ST2L is linked to important Th2 effector functions\textsuperscript{48-51}, but concomitantly, ST2L has been shown to play an important negative regulatory function in TLR signaling\textsuperscript{43} (Figure 1.4). Therefore, ST2 is thought to play a role in the immunosuppression in septic patients. Soluble ST2 probably acts as a decoy receptor by binding IL-33 (ligand of ST2L), thereby inhibiting signaling by ST2L\textsuperscript{52,53}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Overview of extra- and intracellular Toll-like receptor regulators.}
\end{figure}
INTRODUCTION

IRAK-M

IRAK-M is an intracellular proximal inhibitor of TLR signaling expressed by epithelial cells and macrophages in the lung. IRAK-M inhibits the IRAK-1/IRAK-4 complex and thereby mitigates intracellular responses elicited by all MyD88 dependent receptors. Considering its central position in the regulation of TLR signaling and its expression in the two most prominent resident cells in the bronchoalveolar space, IRAK-M likely plays an important role in the host response to bacterial infection. Importantly, in septic mice, enhanced IRAK-M expression in pulmonary macrophages resulted in a strongly impaired host defense response during secondary (i.e. following sepsis) *Pseudomonas* pneumonia, suggesting that IRAK-M contributes to immunotolerance.

OUTLINE OF THIS THESIS

The general aim of this thesis is to enhance our knowledge of the host response to bacterial pneumonia and sepsis and to increase our insight into the underlying mechanisms of immunotolerance as a feature of patients with sepsis. In the first part we used a model of 1) lung inflammation: bronchial instillation of LTA or LPS in the human lung in healthy volunteers, in order to mimic the pulmonary response during gram-positive or gram-negative pneumonia respectively; and 2) lung infection: *K. pneumoniae* pneumonia in mice. **Chapter 2** describes the inflammatory host response to LTA versus known LPS-induced responses in the human bronchoalveolar space. In **Chapter 3** the effects of LTA on hemostasis in the human lung was described and compared with the known hemostatic effects to LPS. In **Chapter 4** we investigated the effect of *in vivo* LPS bronchial instillation on the responsiveness of alveolar macrophages to further stimulation with bacterial products. **Chapter 5** reports on the role of TLR-inhibitor IRAK-M in the host response during gram-negative pneumonia. In the next part the influence of apoptosis and TLR-inhibitor ST2 was investigated in sepsis. **Chapter 6** describes the gene expression profiles of apoptosis regulators in purified leukocyte subsets in human sepsis. The extent of soluble ST2 (a decoy receptor for TLR inhibitor ST2) release during human sepsis was investigated in **Chapter 7**. Last, the role of ST2 in modulating host defense in the lung during sepsis was investigated using a murine model of cecal ligation and puncture (CLP)-induced sepsis followed by secondary challenge with intranasal *P. aeruginosa* (**Chapter 8**).


