Pneumonia: an investigation of host defence mechanisms
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Introduction
Bacterial pneumonia is a serious clinical problem, in both developed and developing countries. Community acquired pneumonia can be caused by a variety of bacterial and viral pathogens. Bacteria are the most common cause and have traditionally been divided into two groups: typical and atypical. Typical include *Streptococcus (S.) pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and other Gram-negative and anaerobic bacteria. Atypical refers to pneumonia caused by *Legionella* species, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. The mortality rate is still 5% worldwide. While remarkable advances have been made in the treatment of pneumonia by the use of broad-spectrum antibiotic regimens, these approaches have also resulted in the emergence of multi-drug resistant bacteria. In 1967 the first penicillin resistant strains of *S. pneumoniae* were reported. Since then resistance has expanded around the world. It is important to obtain knowledge of the pathogenetic processes during pneumonia for the development of alternative treatment modalities. To understand better the role of the host immune response to pulmonary bacterial infections, several in vivo animal models have been developed using different bacterial pathogens. In this thesis two acute pneumonia models with *S. pneumoniae* and *Klebsiella (K.) pneumoniae* are used.

**Respiratory pathogens**

*S. pneumoniae* is a Gram-positive diplococcus that exists in encapsulated and unencapsulated forms. Only the encapsulated serotypes can cause disease. The capsule is composed of polysaccharides which are antigenic and there are more than 90 distinct serotypes. The virulence of the capsule is mainly attributable to its antiphagocytic properties. On the contrary, the cell wall is a potent inducer of inflammation. Another potent virulence factor of the pneumococcus is pneumolysin, a toxin which lyse cholesterol containing cell membranes and activates the complement cascade. Compromised patients are highly susceptible to infections with *S. pneumoniae*. Of the approximately 4 million cases of pneumonia each year in the United States, the pneumococcus is the most common agent leading to hospitalization in all age groups. In a 20-year US study, the overall lethality in pneumococcal pneumonia with bacteremia was 20%; very old patients (over 80 years) had the highest mortality rate (38%). There has been a resurgence of outbreaks of pneumococcal pneumonia, particularly in chronic care centers and involving antibiotic resistant strains. Pneumococcal pneumonia is the paradigm of classical lobar bacterial pneumonia. The patient becomes suddenly ill with fever, chills, cough and chest pain. There is rapid multiplication and invasion of the bacteria and therefore bacteremia is common. A remarkable feature is the lack of structural pulmonary damage.

*K. pneumoniae* is a Gram-negative rod and is encapsulated. *K. pneumoniae* is a rare cause of acute lobar pneumonia and usually affects severely immunocompromised hospitalized individuals, who suffer from underlying diseases such as diabetes or chronic pulmonary diseases.
Defense against pneumonia

The resolution of pulmonary bacterial infections needs an adequate inflammatory response, characterized by the infiltration and activation of inflammatory cells, complement activation and coagulation and fibrinolytic system activation in lung tissue. These reactions are mediated in a large part by specific mediators, primarily cytokines and chemokines, that orchestrate a well-coordinated defense protecting the host against potentially dangerous infections. Organisms need to become opsonized by complement products (innate defense) or by specific immunoglobulin binding (specific response). In this thesis we focus on the innate immunity. Several aspects of the innate immunity will be discussed here.

Mechanical barrier; every day the lung is exposed to more than 10,000 litres of air containing pathogens and also resident flora from the nasopharynx. The first defense encountered by micro-organisms are the mechanical barriers (i.e. anatomical barriers), which limit exposure of the lung to pathogenic organisms, whereas the mucociliary movement, cough and sneeze reflexes work to expel microbes that may bypass these initial defenses.

Innate immunity; when microorganisms have reached the alveoli, the resident alveolar macrophages (AM) phagocytose the microorganisms before an active infection can be established. Only after the failure of these innate immune defenses to clear the bacterial challenge, an inflammatory response is mounted and pneumonia develops. AM orchestrate this inflammatory response by the secretion of cytokines, chemokines and bioactive lipids (including PAF). As a result inflammatory cells (i.e. granulocytes and monocytes) are attracted from the vascular space to the pulmonary interstitium and alveoli.

Cell influx; neutrophils are short-lived cells, comprising 40-70% of the total white blood cell count in peripheral blood. They are as actively phagocytic as AM and are heavily armed for killing ingested organisms by release of reactive oxygen products and proteolytic enzymes. Thus, these cells protect against infections, by moving towards invading bacteria, ingesting and destroying them. Adhesive interactions between neutrophils and endothelial cells are required for successful neutrophil emigration to the inflammatory site (Figure 2). First, they must adhere to vascular endothelial cells (rolling), which is mediated by selectins. This is followed by an interaction of β2-integrins (CD11b/CD18) on the neutrophil surface with endothelial ligands, resulting in tight adhesion. Next, neutrophils move between or
through the endothelial cells (diapedesis) and enter the interstitium under the guidance of locally produced chemotactic stimuli. Finally, neutrophils traver the lung epithelial cells to reach the alveolar space.

Figure 2. Migration of leukocytes through endothelial cells via the multi-step process of rolling (via selectins), adhesion (via CD11b/CD18-ICAM-1) and diapedesis towards a chemotactic gradient.

Cytokines are small proteins of which the production and activity are highly regulated. They act by binding to specific receptors on the cell membrane. Several cytokines have highly overlapping activities and moreover, they act in a complex network in which production of one cytokine will influence production of others. The role of cytokines in the defense against pneumonia is discussed in more detail in Chapter 2.\textsuperscript{11, 12}

Chemokines are leukocyte activators consisting of over 30 chemotactic molecules. Most can be classified into two groups CXC and CC chemokines, distinguished by the presence or absence of a single amino acid between the first two of four conserved cysteines. As the first CXC member described, IL-8, most other CXC chemokines activate neutrophils, whereas CC chemokines act toward different leukocyte subsets (monocytes, lymphocytes, eosinophilic and basophilic granulocytes).\textsuperscript{13-15} The murine equivalents of IL-8 are KC and macrophage inflammatory protein–2 (MIP-2).

Complement cascade; Complement plays an important role in the innate immunity against bacteria. A major function of this system is to cause lysis of bacteria. Another function is mediation of opsonization in which microorganisms are prepared for phagocytosis. Furthermore, complement factors are involved in the regulation of inflammatory and immune responses.\textsuperscript{16-18}

Coagulation system; pneumonia is associated with activation of the coagulation system in the lung, as in bronchoalveolar lavage fluid from pneumonia patients enhanced procoagulant
activity and reduced fibrinolytic activity was found. The extrinsic tissue factor mediated pathway is essential for coagulation activation in inflammatory diseases. This pathway starts with binding of tissue factor to factor VIIa. This results in thrombin generation and eventually fibrin formation, which both may exert pro-inflammatory functions. In experimental sepsis, tissue factor mediated blockage of coagulation, not only abrogated the coagulant response, but also prevented death. More downstream intervention, by blocking the generation of thrombin with DEGR-factor Xa or heparin, inhibited coagulopathy related to sepsis, but did not protect against death. It has therefore been postulated that tissue factor might influence the inflammatory response by a mechanism other than activating blood coagulation.

![Diagram](image.png)

**Figure 3. Activation of Protein C (APC) after binding of thrombin by thrombomodulin (TM).**

EPCR, endothelial protein C receptor, F, factor

The coagulation process is modulated by a number of natural occurring inhibitors, which limit the extent of the reaction. The thrombin-thrombomodulin complex plays a central role herein by converting protein C (PC) to its activated form (APC), a process that is facilitated by the endothelial protein C receptor (EPCR; Figure 3). Together with protein S (PS), APC inactivates Factor Va and VIIIa. In addition to these anti-coagulant properties, APC also attenuates several inflammatory responses. The anti-inflammatory activities of APC are mainly due to inhibition of leukocyte activation, tumor necrosis factor-α (TNF) production, and E-selectin mediated cell adhesion.

Fibrinolytic system; activation of the fibrinolytic system results in dissolution of fibrin, the end product of the coagulation cascade. The basic reaction is the conversion of plasminogen into plasmin by plasminogen activators. Plasmin then is able to degrade fibrin into fibrin degradation products. Like in the coagulation cascade, the fibrinolytic activity is regulated by inhibitors that inhibit both the activation of plasminogen and the proteolytic effects of
The main plasminogen activator inhibitor is plasminogen activator inhibitor type I (PAI-1). Apart from the above mentioned intravascular functions of the fibrinolytic system, it also serves a role in cellular movement through the extracellular matrix. This is especially shown for urokinase plasminogen activator receptor (uPAR), a glycophosphatidyl-inositol-linked receptor which binds urokinase plasminogen activator (uPA), resulting in the generation of cell surface-associated plasmin activity, which is critical for pericellular proteolysis of extracellular matrix proteins.\textsuperscript{35-37} uPAR also contributes to cell migration in a plasmin-independent way; by a functional linkage and physical association with integrins. Indeed, uPAR is able to form complexes with the β2-integrin CD11b/CD18, thereby modulating migration. Furthermore, by binding of uPA to uPAR, uPAR transforms into a cell-associated chemokine, resulting in chemotaxis. uPAR can also bind to vitronectin, an extracellular matrix protein, and interfere with the migration of cells.

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**Lung interstitium**

Opsonization by complement, IgG, surfactant

Bacteria

Activated AM

Phagocytosis

Alveolus

Positive chemotaxis on PMN's

**Figure 4. The resident and recruited defenses of the lung against bacteria.** Bacteria are opsonized by complement factors, IgG and surfactant and recognized by alveolar macrophages (AM), which phagocytose the bacteria and then either kill them or amplify the host defense by secreting several mediators, among which TNF-α, IL-1, IFN-γ, PAF. These mediators induce positive chemotaxis on polymorphonuclear cell (PMN) from pulmonary endothelium to the lung interstitium.

**Aim and outline of the thesis**

The overall objective of the research described in this thesis is to obtain more insight into the pathogenesis of pneumococcal pneumonia. The resident and recruited defenses of the lung against bacteria investigated in the present thesis are depicted in Figure 4. Several host defense mechanisms during bacterial pneumonia were studied, using clinical and experimen-
The first part of this thesis focuses on the roles of the pro-inflammatory cytokines tumor necrosis factor-α (TNF, Chapter 3), interleukin-1 (IL-1) (Chapters 3 and 11) and interferon-γ (Chapter 4) in acute bacterial pneumonia caused by *S. pneumoniae*. For this, we used a pneumonia model in a number of genetically modified mice. Chapter 10 evaluates treatment of ceftriaxone in combination with anti-TNF against pneumococcal pneumonia in mice. Furthermore, the role of pneumolysin (a toxin of *S. pneumoniae*) in the induction of pulmonary inflammation was determined and the roles of IL-6 and MIP-2 herein were evaluated (Chapter 5). Chapter 12 describes the role of platelet activating factor (PAF) receptor in pneumococcal pneumonia by the use of PAFR deficient mice. We assessed the possible attribution of tissue factor-factor VIIa complex in the host defense against pneumococcal pneumonia (Chapter 9) and the role of thrombomodulin (Chapter 8) in the response to intranasal instillation of *S. pneumoniae, K. pneumoniae* and lipopolysaccharide (LPS). Chapters 6 and 7 focus on the role of urokinase, uPAR, PAI-1 and plasminogen in cell migration during pneumococcal pneumonia.

References
INTRODUCTION


