Pneumonia: an investigation of host defence mechanisms
Rijneveld, A.W.

Citation for published version (APA):
Rijneveld, A. W. (2003). Pneumonia: an investigation of host defence mechanisms
Summary and general discussion
SUMMARY AND GENERAL DISCUSSION

Summary
Community acquired pneumonia caused by *Streptococcus (S.) pneumoniae* is a major cause of morbidity and mortality especially in the elderly, and is responsible for the death of 40,000 people a year in the United States. Emergence of penicillin-resistant *S. pneumoniae* has become a worldwide problem. Therefore, in order to develop new treatment options, it is essential to study the host response during pneumonia caused by *S. pneumoniae*.

*Chapter one* is a general introduction discussing the respiratory pathogens used in this thesis and gives an overview of the immune responses against pneumonia. *Chapter two* represents a literature review on the role of cytokines in innate immunity against respiratory pathogens. Two limitations for future cytokine modulating therapies are mentioned. First, the cytokine network is a cascade, which means that cytokines influence each other in production and activity. Targeting only one cytokine may be just a too simple approach. Second, this review shows different immune responses against different pathogens, which may result in difficulties to develop new strategies for pneumonia.

It is very important to realize that much of our knowledge of the role of the cytokine network in bacterial infection is deducted from experimental sepsis studies in which animals were injected with live bacteria or endotoxin, resulting in an acute and fulminant immune response. Pro-inflammatory cytokines play an essential role herein. However in clinical trials with anti-inflammatory agents in septic patients, such as anti-tumor necrosis factor-α (TNF) antibodies, soluble TNF receptors and recombinant interleukin-1 receptor antagonist (IL-1ra), have not demonstrated any beneficial effect. One possible explanation for this failure is that in clinical sepsis the systemic response develops gradually after a localized infection in the majority of cases. In *Chapter three* we investigated the role of IL-1, a potent proinflammatory cytokine, in the pathogenesis of pneumococcal pneumonia by making use of IL-1 receptor type I gene deficient (IL-1R<sup>−/−</sup>) mice. This study revealed that in IL-1R<sup>−/−</sup> mice infected with *S. pneumoniae*, a delayed early inflammatory response was found together with a transiently impaired antibacterial defense, but no influence on mortality. However, when IL-1R<sup>−/−</sup> mice were treated with a neutralizing anti-TNF antibody, a strongly diminished survival occurred, suggesting that the combined action of endogenous IL-1 and TNF is required for an effective pulmonary defense against *S. pneumoniae*. *Chapter four* addressed the role of interferon-γ (IFN) in the innate immune response to respiratory tract infection by *S. pneumoniae*. The main finding of this chapter was that IFNγ R<sup>−/−</sup> and IFNγ<sup>−/−</sup> mice were not more susceptible to, or even slightly protected against pneumococcal pneumonia.

*Chapter five* describes the effects of pneumolysin (PLY) in the mouse lung. PLY is a toxin produced by all clinical isolates of *S. pneumoniae* and a major contributor to the virulence of the bacterium. We found that PLY is able to induce an acute inflammation in the lung after intranasal instillation, characterized by granulocyte influx in bronchoalveolar lavage fluid (BALF) and lung injury which is mediated by the chemokine macrophage inflammatory protein (MIP)-2 and to a lesser extent by IL-6. PLY has cytolytic and complement-activating activities, each encoded by different regions in the PLY gene. By using PLY mutants, we
demonstrated that the cytolytic activity of PLY is most important for the induction of an inflammatory response.

The extrinsic, tissue factor (TF)-FVIIa dependent, pathway is responsible for coagulation activation in many infectious diseases. This has mainly been studied in sepsis. Coagulation has also been implicated in the pathogenesis of pulmonary diseases. We describe in Chapter nine that unilateral CAP patients demonstrated a procoagulant state with activation of the TF-FVIIa pathway at the site of the infection. TF expression and local coagulation activation could also be demonstrated in the lungs of mice with *S. pneumoniae* pneumonia. Inhibition of the TF-FVIIa pathway with rNAPc2 reduced the pulmonary procoagulant response, but did not influence anti-bacterial host defense.

Furthermore, the thrombin-thrombomodulin (TM) complex plays a central role in the regulation of coagulation by converting PC to its activated form. This APC inhibits coagulation by inhibition of factor Va and VIIIa, but has also on its own anti-inflammatory effects. Recently it has been reported that treatment with APC reduced mortality in septic patients. Also in the pulmonary compartment APC exerts strong anti-inflammatory effects; intravenous infusion of APC protected rats from sepsis induced lung injury. Furthermore, intratracheal administration of APC attenuated bleomycin-induced lung inflammation in mice. By making use of TM mutated mice, with a decreased capacity to generate APC, we showed in Chapter eight that APC generation is not necessary for host defense in *S. pneumoniae*, *K. pneumoniae* and LPS induced lung injury.

During lung inflammation there is increased fibrin formation resulting from coagulation activation and concomitant inhibition of the fibrinolysis. Plasminogen activators (PA) convert plasminogen into plasmin, and are controlled by specific inhibitors (PA inhibitor type I (PAI-1) is considered most important). In Chapter seven we described that PA activity is decreased in BALF of the infected lung in unilateral CAP, associated with increased PAI-1 antigen levels at the same site. We further showed that mice lacking uPAR have a deficient migration of neutrophils and are therefore more sensitive to pneumococcal pneumonia (Chapter six). On the contrary, uPA deficient mice showed enhanced cell recruitment and less bacterial outgrowth in their lungs, resulting in the hypothesis that uPA negatively influences the function of uPAR as a chemotactic receptor. PAI-1 can affect cell migration by inhibiting uPA mediated pericellular plasmin generation, or by inhibiting uPAR mediated cell adhesion and migration through interaction with VN. However, PAI-1 deficiency did neither influence cell migration nor host defense during pneumococcal pneumonia in mice. We also investigated the inflammatory response in Plg−/− mice and these mice did not show a difference in host defense during system pneumococcal pneumonia. These studies propose that the influence of the uPA/uPAR/PAI-1 upon cell migration is not dependent on the fibrinolytic function of these components.

In Chapter ten we evaluated the effect of anti-TNF given as a therapeutic agent together with an antibiotic. In particular, we treated mice 25h after induction of pneumonia with ceftriaxone and added anti-TNF. The background of this approach is that anti-TNF has been
administered to sepsis patients, many of whom with pneumonia, in a number of clinical trials. We found that anti-TNF impaired the therapeutic effect of ceftriaxone, suggesting that endogenous TNF contributes to an adequate antibacterial defense in the host with progressing pneumonia receiving antibiotic therapy. In *Chapter eleven* endogenous IL-1 activity was partially blocked by treatment with recombinant IL-1ra, mimicking the use of this compound in the treatment of inflammatory diseases. IL-1ra influenced the host response in a similar way as described for IL-1RA-/- mice in *Chapter three*, although less profound. The investigations described in Chapters three, ten and eleven also provide evidence for the fact that endogenous TNF activity is more important for the antibacterial host response to pneumococcal pneumonia than endogenous IL-1 activity.

Platelet activating factor (PAF) is a lipid mediator of the inflammatory response. The activity of PAF is mainly determined by phosphorylcholine (PC) that binds specifically to the PAF receptor (PAFR). PC is also a part of the cell wall of *S. pneumoniae*. The pneumococcus is also able to bind to the PAFR expressed on respiratory cells, which facilitates entry of the bacterium into these cells. We demonstrate in *Chapter twelve* that the PAFR is used by *S. pneumoniae* to induce pneumonia, as reflected by decreased mortality, enhanced bacterial clearance and a diminished dissemination of the infection in PAFR-/- mice.

**General discussion**

The vast majority of the data presented in this thesis is obtained from a murine model of pneumonia caused by *S. pneumoniae*. In some studies *K. pneumoniae* was used as a pathogen, whereas in two chapters, the measurement of parameters in BALF of CAP patients seek to establish a link to the human situation.

The use of mouse models has several limitations. A model is an object of imitation, seeking to resemble something else, in which an induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans. It is important that the pathology and outcome of an induced disease in the tested species resembles the pathology in the target species. Because of the high degree of evolutionary conservation of many genes and pathways, confidence has been placed on animal models, principally the mouse. This animal has the advantage of being relatively inexpensive and having a rapid breeding time. Furthermore, the genome of the mouse can be easily manipulated and a wide variety of immunological reagents and assays are available to measure interrelated mediators. In our experimental studies we used genetically modified mice making them deficient for one potential mediator of the inflammatory response. This provides us with the opportunity of dissecting the complex network of cytokines and other mediators in the pathogenesis of the disease. The disadvantage of the use of gene deficient mice is the fact that they may differ from Wt mice not only with respect to the product of the deleted gene, but the hereditary deficiency of the protein may also result in compensatory changes.
The value of animal models is worthwhile when we are able to reconcile biological phenomena between species. I.e. we examine a specific mediator in one species and extrapolate this knowledge to another. The assumption that homo sapiens is identical to other animals has led to mistakes in the history of medicine. Galen (130-200 AD), a Greek physician and philosopher who has been described as the founder of experimental studies, based his ideas on the study of apes and pigs. Galen directly transferred his discoveries to humans, consequently introducing many major errors. The problem was that he drew wrong conclusions because of an uncritical interspecies extrapolation. It is still obvious that extrapolation of experimental findings to the human situation is not straightforward, as reflected by recent disappointments in clinical trials. This is not necessarily a problem with the use of mice itself, but may also result from an ignorance of the disease process. For example, experimental data showed that pro-inflammatory cytokines are important for the development of multiple organ failure and death during sepsis. However in clinical trials with anti-inflammatory agents in patients with sepsis, anti-TNF antibodies, soluble TNF receptors and II-1ra, have not demonstrated any positive effect. One likely explanation for the contradiction between the results found in experimental studies and clinical sepsis trials is that sepsis models (using intravenous administration of live bacteria or endotoxin) inadequately imitate the clinical situation. Namely, intravenous injection of LPS or live bacteria results in an acute and fulminant response, while clinical sepsis originates from an at least initially localized infection. Therefore, it is important to gain insight in the pathogenesis of localized infections as described in this thesis. It is important to bear in mind that no single animal model can ever duplicate the original condition (i.e. the model is never the same as the prototype), and models never provide final answers but offer an approximation.

What are the clinical implications of our findings? Exploring the pathogenesis of bacterial pneumonia in a murine model is important to elucidate the inflammatory response to this infection, which may give direction to the development of new treatment modalities. It is reasonable to evaluate new therapeutical options in animal models before evaluating them in clinical trials. We tried to mimic a therapeutic setting in Chapter ten and found that anti-TNF impaired antibacterial defense in the presence of antibiotic therapy. In addition, in Chapter nine, the effect of a TF inhibitor, developed for the use in patients with sepsis among other indications, was assessed. Other investigations were primarily done in genetically modified animals, and although such studies provide a valuable insight into the pathogenesis of the disease, direct extrapolation to possible implications for the treatment of patients with pneumonia should be done with extreme caution.

The studies presented in this thesis increased our knowledge on the role of a number of specific mediators and receptors in the pathogenesis of pneumococcal pneumonia. Some results provided insight into the effects of pharmaceutical interventions in the cytokine network and the coagulation system on the outcome of this important infectious disease. It is
clear that many more studies are necessary to extend our knowledge on the host response to bacterial pneumonia and possible manipulation hereof.