The tissue factor pathway in pneumonia
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Citation for published version (APA):
van den Boogaard, F. E. (2015). The tissue factor pathway in pneumonia

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CHAPTER 1

General Introduction
Infectious diseases are a major cause of morbidity and mortality worldwide\(^1\). As the respiratory tract is in continuous contact with the external environment, it is particularly prone to invading pathogens. Consequently, lower respiratory infections contribute strongly to infectious disease-related illness and mortality, with over 4 million deaths annually\(^2\).

*Streptococcus (S.) pneumoniae* is a Gram-positive diplococcus that resides asymptomatically in the nasopharynx of healthy carriers, but may become pathogenic in susceptible individuals. Community-acquired pneumonia (CAP) is a common illness worldwide with *S. pneumoniae* as the most frequently isolated causative pathogen, accounting for up to 60% of bacterial cases\(^3,4\). Mortality rates of CAP range from 2 up to 30% as it progresses into sepsis\(^5\). During sepsis, an uncontrolled host response to an infection results in tissue injury and organ failure. As such, sepsis is an important risk factor for acute lung injury, commonly referred to as the acute respiratory distress syndrome (ARDS), with mortality rates ranging between 26 and 40%\(^6-8\). Gram-negative pathogens, such as *Pseudomonas (P.) aeruginosa*, are the leading cause of hospital-acquired pneumonia\(^9,10\).

Morbidity and mortality of bacterial pneumonia have not improved over the past decades, despite the availability of an extensive arsenal of antibiotics, and with an emerging increase in antibiotic resistance\(^3\), expanding our understanding of the host response to respiratory infection is mandatory. This thesis is focussed on the role of Tissue Factor (TF) pathway induced coagulation and its interaction with inflammation during CAP caused by *S. pneumoniae*, mediated in part by Protease-Activated Receptor (PAR)-2.

**COAGULATION IN INFECTION**

The host inflammatory response to infection comprises activation of coagulation, reduced anticoagulant capacity and inhibition of fibrinolysis. The net procoagulant state with subsequent fibrin deposition is considered an essential element of host defence in its attempt to contain invading pathogens and the inflammatory response at the site of infection\(^11,12\). Coagulation is tightly regulated by mechanisms that warrant the blood to flow freely through the vasculature, and counterbalance the coagulation response upon infection in order to restore homeostasis. However, in severe infection, exaggerated inflammation and coagulation, with failing regulatory mechanisms may cause an ongoing and uncontrolled host response. Hemostatic misbalance in the lung leads to intrapulmonary fibrin deposition and lung injury, which undermines tissue integrity and poses a serious challenge to lung function\(^13\). In the most fulminant form, this hypercoagulable state results in microvascular thrombosis throughout the body, known as disseminated intravascular coagulation (DIC), which leads to paradoxical bleeding, compromised blood flow and multiple organ failure\(^14\). In the last decades intensive
research efforts have resulted in better understanding of the intricate triad of infection, inflammation and coagulation.

**Tissue Factor Pathway**

**Tissue Factor**

TF is the key initiator of infection- and inflammation-induced activation of the coagulation cascade. TF binds and activates factor (F) VII (a), and the newly formed TF-FVIIa complex activates factor IX (FIX) and factor X (FX), which both initiate positive feedback loops. Limited quantities of generated FXa catalyse the conversion of trace quantities of prothrombin to thrombin. These minute concentrations of thrombin enable feedback activation of cofactors FVIII and FV, which increases the efficiency of thrombin generation tremendously (Figure 1). Under physiological circumstances TF does not become exposed to circulating blood. TF resides at extravascular sites, yet along all blood-tissue barriers, where it can rapidly initiate coagulation upon disruption of the vascular endothelium. However, TF expression can be induced on the surface of endothelial cells,

Figure 1. Overview of the tissue factor-initiated coagulation cascade and PAR2 activation in the airway during lung infection. Abbreviations: *S. pneumoniae*, Streptococcus pneumoniae; PAR2, protease-activated receptor-2; TF, tissue factor; TFPI, tissue factor pathway inhibitor; K1, Kunitz-1 (Figure designed and drawn by I.E.M. Kos).
circulating monocytes and macrophages in response to bacteria or inflammatory stimuli, such as chemokines or cytokines\textsuperscript{15}. TF is also abundantly expressed in the lung where it plays a pivotal role in activation of coagulation upon lung injury, as illustrated by enhanced levels of TF in lavage fluid from the affected lung of healthy volunteers challenged with lipoteichoic acid, a major cell wall component of Gram-positive bacteria\textsuperscript{17}, and of patients with pneumonia\textsuperscript{18-22}.

\textit{Tissue Factor Pathway Inhibitor}

TF pathway inhibitor (TFPI) is the only known endogenous regulator of the TF-dependent pathway of coagulation. It consists of 3 Kunitz domains, that mimic the substrate of the target protease, and a carboxy (C)-terminal tail. TFPI targets the initiating procoagulant stimulus by forming a quaternary complex with TF-FVIIa-FXa, which prevents additional generation of FXa and the subsequent burst of thrombin generation (Figure 1)\textsuperscript{16}. TFPI is mainly produced by vascular endothelial cells and is expressed in the lung, where it is present along alveolar septae and epithelium, which allows direct release into the alveolar space upon lung injury. Besides its anticoagulant function, TFPI has recently been shown to have antibacterial properties, exerted by its carboxy-terminal peptides\textsuperscript{23}. Although during infection TFPI expression is upregulated, the TFPI molecule becomes inactivated and insufficient to counterbalance the procoagulant state\textsuperscript{24-26}. These observations prompted studies investigating the effect of treatment with recombinant human (rh)-TFPI. In experimental sepsis, primates treated with rh-TFPI were protected from lethality\textsuperscript{27, 28}. Despite these promising results, the OPTIMIST and CAPTIVATE trials failed to show a treatment benefit of rh-TFPI in septic patients\textsuperscript{29} or patients suffering from CAP\textsuperscript{30} respectively.

**COAGULATION-INDUCED INFLAMMATION: PROTEASE ACTIVATED RECEPTORS**

Inflammation and coagulation are two important host defence mechanisms that interact to mount an adequate response against infectious agents. Inflammation activates coagulation via the TF pathway; conversely, the TF pathway can contribute to inflammation. PARs are recognised to play a central role in the functional link between coagulation and inflammation\textsuperscript{31}. These seven transmembrane G-protein coupled receptors bear their own ligand, which is unmasked by proteolytic cleavage of their extracellular amino-terminal domain\textsuperscript{32}. To date four PARs have been identified, each of which can be activated by a variety of proteases. From this family of receptors PAR2 is unique in its resistance to thrombin cleavage, but has emerged as a key mediator for the cellular effects of the coagulation proteases in the TF pathway. PAR2 is a substrate for TF-FVIIa
and FXa; other endogenous serine proteases that can cleave and activate PAR2 include trypsin, tryptase and granzyme A, as well as a number of bacteria-derived enzymes (Figure 1). PAR2 is abundantly expressed in the lung by epithelial cells, endothelial cells, airway and vascular smooth muscle cells, fibroblasts, and by non-resident cells such as macrophages and neutrophils, and therefore considerable interest has emerged in the role of PAR2 in airway inflammation. However, both host protective and detrimental effects of PAR2 activation in the lung have been demonstrated depending on the type of disease, and at present our understanding of the role of pulmonary PAR2 during pneumonia is still in its infancy.

**Tryptase**

Tryptase is a trypsin-like protease by which only PAR2 of the PAR family can be activated. Inhibition of trypsin reduced inflammation in infectious colitis, however the role of trypsin-like proteases in bacterial lung disease remains to be elucidated. Tryptase is a prominent mast cell product, stored in secretory granules along with other preformed, fully active proteases that can be released upon activation by invading pathogens or inflammatory mediators. In addition, mast cells recognise pathogens and can enhance host resistance during bacterial infections, mediated by enhancement of the recruitment or function of inflammatory cells, cytokine production, complement activation and phagocytosis. Mast cells are particularly prominent at the host-environment barrier and have become increasingly appreciated as important modulators in inflammatory lung diseases.

**Granzyme A**

Granzymes are a family of serine proteases stored in secretory granules of cytotoxic lymphocytes. Granzyme A (GzmA) can be classified as a trypsin based on the preferential amino acid at which it cleaves, and as such is a potential activator of PAR2. GzmA is constitutively expressed in Natural Killer cells and lymphocytes; however, the cytotoxic potential of GzmA is subject of debate. Instead, mounting evidence suggests a pro-inflammatory role for GzmA. Extracellular GzmA was shown to induce secretion and activation of cytokines and elevated levels of plasma GzmA were found in patients with various infectious diseases. Of interest for lung disease, GzmA expression was recently observed in lung epithelial cells, pneumocytes and alveolar macrophages and increased levels of GzmA were demonstrated in bronchoalveolar lavage fluid from patients with inflammatory lung disease.
PLATELETS

Platelets are mainly known as the chief cellular effectors of hemostasis. They immediately form a physical plug at the site of injury and propagate further coagulation by providing a suitable surface for the activation of clotting factors. However, it has become clear that platelets exert activities that extend beyond their traditional hemostatic properties and they are increasingly appreciated as key components of the inflammatory response. Next to their immunomodulatory effects mediated through coagulation, platelets are able to act on the host inflammatory response via several ways: they can release pre-formed proinflammatory peptides from their granules and interact with other inflammatory cells. In addition, platelets produce antimicrobial mediators, and can bind to and internalize microorganisms. In critically ill patients thrombocytopenia is common, and is associated with a worse outcome. Moreover, lower platelet counts were an independent risk factor of mortality in patients admitted to the ICU for severe CAP.
AIM AND OUTLINE OF THIS THESIS

The overall aim of this thesis is to expand our knowledge on the interaction between coagulation and inflammation in lung injury, with a special focus on the TF pathway and signalling via PAR2 during pneumonia caused by *Streptococcus pneumoniae*.

After the general introduction, **Part I** describes the role of coagulation in pneumococcal pneumonia, focusing on the TF pathway. In **chapter 2 and 3** the role of endogenous TF and TFPI respectively during murine pneumococcal pneumonia is addressed, which is followed by **chapter 4** studying the treatment effect of rh-TFPI either or not as an add-on to antibiotic treatment in this model. Next, **chapter 5 and 6** report on the feasibility and treatment effect of intra-alveolar administration of rh-TFPI by nebulization in rat models of pneumococcal pneumonia and Gram-negative lung injury respectively.

Since PAR2 has been described to mediate the interaction between TF-induced coagulation and inflammation, in **Part II** we aim to gain more insight in the role of PAR2 and various (cellular sources) of its archetypal activating proteases during pneumococcal pneumonia: in **chapter 7** we report on the effects of deficiency of PAR2, and in **chapter 8** we investigate the role of mast cells, which are able to release pre-stored tryptase, the main endogenous activating protease of PAR2. In **chapter 9** we report on the role of the protease granzyme A, another potential endogenous activator of PAR2, in pneumonia.

Finally, in **Part III** we extend our research on the interaction between coagulation and inflammation to the role of platelets herein, by investigating the effect of thrombocytopenia in murine pneumococcal pneumonia in **chapter 10**.

**Chapter 11** provides a general summary and discussion of the preceding chapters.
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