Platelets: versatile effector cells in pneumonia and sepsis

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General introduction

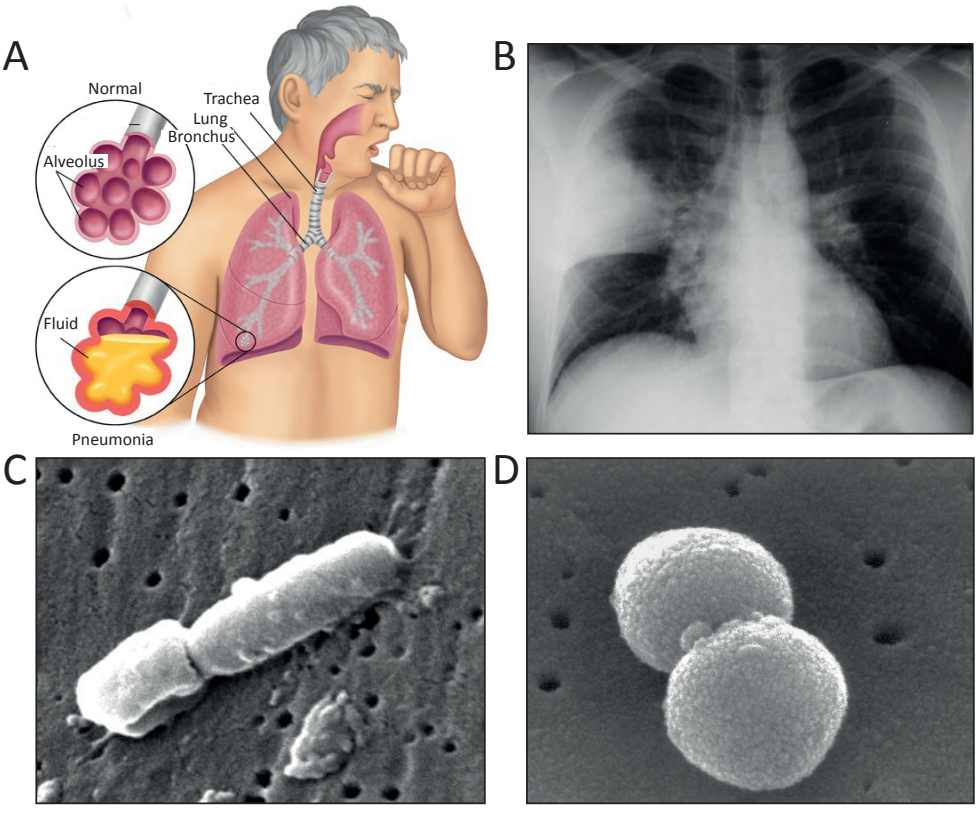
This thesis describes research on the role of platelets in pneumonia and sepsis. In this introductory chapter we first discuss the general background on pneumonia and sepsis, and the potential involvement of platelets in the host response to these infections. We end the introduction with an outline of the thesis.

Pneumonia

Lower respiratory tract infection is the most common infectious cause of death in the world and the third most common cause of death overall [1]. Pneumonia is caused by an invading pathogen elucidating an immune response - primarily in microscopic air sacs known as alveoli. This results in recruitment of immune cells and fluid deposition in alveoli, causing typical symptoms such as cough, increased sputum production, shortness of breath and chest pain. Pneumonia can occur at any age, but is most frequent in infants, the elderly and immune compromised patients. These groups of patients could present with atypical symptoms - confusion might be the only indicator of infection in elderly patients, causing diagnostic delay [2]. Treatment of pneumonia consists of the administration of antibiotics, starting as soon as the diagnosis is set [3].
Lower respiratory tract infection is usually caused by infection with bacteria or viruses and less commonly other microorganisms, certain drugs or autoimmune diseases. Pneumonia is distinguished into community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP). *Streptococcus (S.) pneumoniae* is the most frequent cause of CAP [4], while *Klebsiella (K.) pneumoniae* is a common causative agent in HAP [5]. These two bacteria are therefore used in the animal models presented in this thesis (Figure 1).

Sepsis

When uncontrolled, pathogens can spread from the airways to distant body sites, causing sepsis. Pneumonia is the most common cause of sepsis [2,4]. Sepsis symptoms include those related to the initial infection, accompanied by high fever, elevated heart rate, hyperventilation, leukocytosis, low blood pressure and possibly altered mental status [6]. As with pneumonia, very young, elderly or immunocompromised patients can present with atypical symptoms [6].
The management of sepsis includes administration of antibiotics, and intravenous fluids to maintain blood pressure and close monitoring of the patient in order to provide vital organ support if required. Severe sepsis is typically lethal outside the setting of modern intensive care facilities. Even in Western countries, with advances in training, sophisticated monitoring and prompt therapy to treat the underlying infection and support failing organs, mortality is still 20 to 30% [7,8].
The pathophysiology of sepsis is to a large extent the result of dysregulation of the immune response to the invading pathogen by host-derived mediators of inflammation [8,9]. The proinflammatory response during sepsis leads to activation of the coagulation system with concurrent inhibition of anticoagulant mechanisms [10]. This can lead to the clinical syndrome



Source (from upper left to lower right): www. azitromicin-rx.net, www.thebody.com, www.de.wikipedia.org, www.ru.wikipedia.org

Figure 1: Pneumonia

Pneumonia is caused by pathogens invading the airways, after which their presence and host immune response cause fluid deposition in alveoli (A). Pneumonia infiltrates turn white in otherwise air-containing lungs on chest X-ray (B). The most common cause of HAP is rod-shaped *K. pneumoniae* (C), the most common cause of CAP is the diplococcus *S. pneumoniae* (D).

known as disseminated intravascular coagulation (DIC) characterized by concomitant microvascular thrombus formation and haemorrhage [10]. Other sepsis complications are the result of DIC in combination with hyperinflammation and shock and include multiple organ failure, acute lung injury and acute kidney injury. Immune suppression as a result of an exhausted immune system during sepsis is thought to render patients susceptible to opportunistic superinfections, further complicating disease management and outcome [9].

Platelets – the other white blood cells

Triggering of the inflammatory and coagulation cascade during infection, together with endothelial damage, leads to activation of platelets. This can be further stimulated by direct interactions with pathogens [11,12]. Platelets are small circulating anucleate cells that are of

crucial importance in haemostasis. Platelets derive from fragmentation of bone marrow precursor megakaryocytes. Circulating platelet numbers are between 150 - 400 x 10^9 per litre of blood and their average life span is 6 to 10 days [11]. In an intact circulatory system, platelets circulate at high shear rate and are maintained in an inactive state by prostacyclin, nitric oxide and ADPase secreted by endothelial cells. When platelets encounter damaged vessel endothelium, they accumulate at the site of injury, where they are activated by subendothelial collagen and von Willebrand Factor and will initiate blood clotting. Upon activation, platelets undergo shape change and release a number of soluble mediators from intracellular granules, leading to further platelet and immune cell recruitment. Surface molecules of activated platelets interact with fibrinogen, forming crosslinks, which is critical in a stable blood clot. Furthermore, the provision of a suitable phospholipid surface on the activated platelet membrane permits the assembly of complexes of activated coagulation factors, which catalyses the generation of thrombin several-fold and renders the coagulation system less susceptible to inhibitors [13].

Research over the last decades demonstrated an additional role for platelets - in infection and immunity. A first hint for platelets as immune cells came when platelets were found to be responsive to several bacteria and viruses in the early '70s [14,15]. Experimental evidence that platelets fulfill a central sentinel role in modulation of innate and adaptive immune responses has accumulated since then. For example, activated platelets mediate adhesion of neutrophils and monocytes to the endothelium and can up- or downregulate their pro-inflammatory functions [16]. In addition, platelets have a role in augmenting the adaptive immune response by recruitment and stimulation of dendritic cells to sites of tissue injury [17,18]; they enhance the generation of antigen-specific CD8 T-cells [19-22] and support B cell differentiation and isotype switching [20]. Platelets have the ability to secrete more than 300 different proteins following activation with thrombin [23], some of which are directly immunomodulatory or bactericidal [11]. Imperative in more innate immune mechanisms however, during the inflammatory and coagulation dysbalance that characterize sepsis, platelet activation actually contributes to sepsis pathogenesis. The role of platelets during sepsis pathogenesis is reviewed in detail in **chapter 2**.

Thesis outline

This thesis presents several studies aiming for better understanding of platelets in host defense against pneumonia versus their contribution to sepsis pathogenesis in progressed infection. The different research questions addressed are schematically depicted in Figure 2.

The haemostatic versus the immuno-modulatory role of platelets during *Klebsiella* and *S. pneumoniae* induced pneumosepsis is investigated in **chapters 3 and 4** by (dose dependent) depletion of the whole cell type. **Chapters 5 to 8** provide further insight into our findings from these experiments. In **chapter 5**, the contribution of platelet and endothelial cell P-selectin in *Klebsiella* pneumosepsis is further clarified. The role of toll like receptor (TLR)-

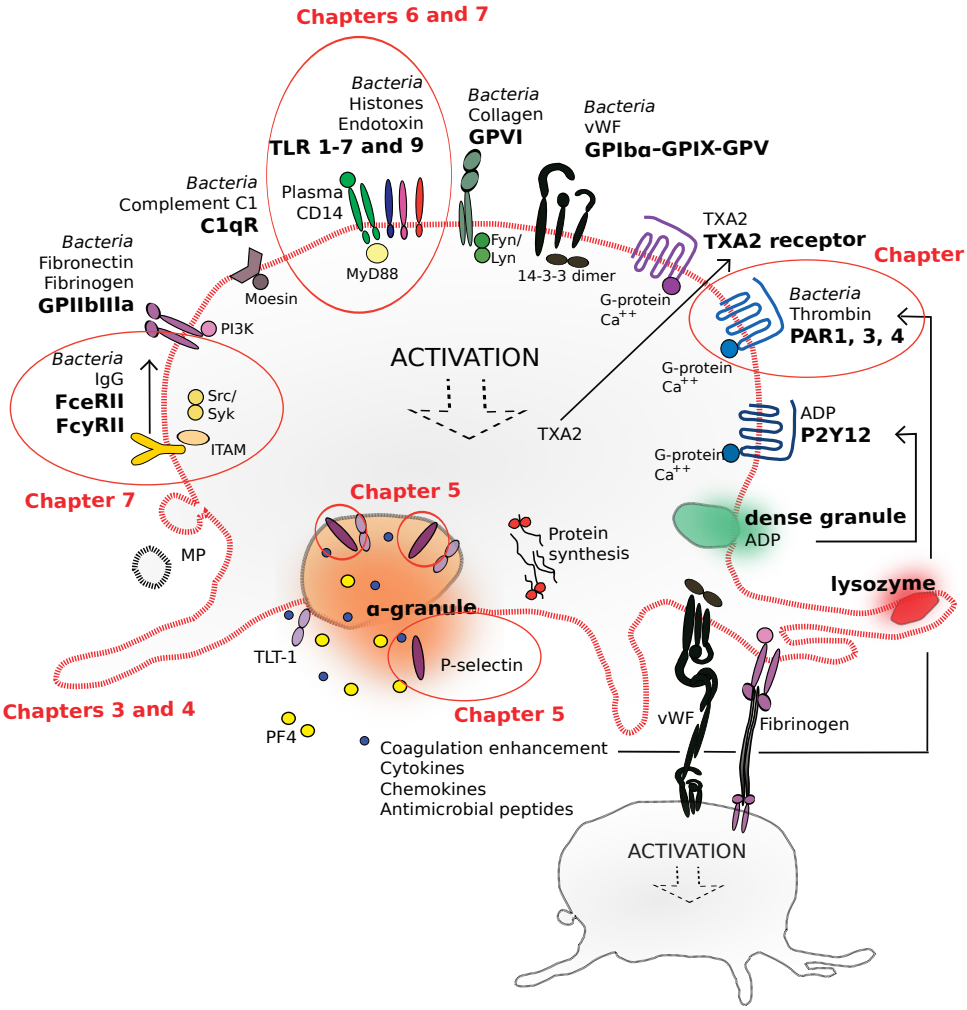


Figure 2: Thesis outline

Research strategies presented in this thesis are marked in red. We have investigated the platelet cell as a whole, platelet signalling via FcyRII, PAR4 and TLRs, and P-selectin, all in the context of pneumonia induced sepsis caused by *K. pneumoniae* or *S. pneumoniae*.

dependent platelet activation during *Klebsiella* pneumonia and sepsis is characterized in **chapter 6**; **chapter 7** explores the role of TLR and other signaling pathways in response to *S. pneumoniae in vitro* and *in vivo*. **Chapter 8** describes protease activated receptor (PAR)4 (the thrombin receptor on mouse platelets) in host immunity during *S. pneumoniae* pneumonia derived sepsis. Finally, **chapter 9** evaluates the effect of platelet-inhibitory therapy during sepsis in a patient sepsis cohort.

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