Toll-like receptor and associated regulators in pneumonia and sepsis

Blok, D.C.

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Chapter 1

General introduction
Introduction

Since time immemorial mankind has combatted the micro-organisms inhabiting its environment. Despite Alexander Fleming’s discovery of penicillin in 1928 and the subsequent development of additional antibacterial drugs this worldwide struggle is still ongoing and demands lives on a daily basis. Under pressure of antibiotic treatment regimens during the past decennia bacteria have developed multi-drug resistant traits that further hamper infectious disease control [1-3]. Advancing our understanding of pathogen evoked host responses is paramount in the development of new treatment strategies and promoting global health. This thesis focuses on the role of Toll-like receptor regulators and their ligands in lung infections and sepsis caused by common human pathogens.

Pneumonia & sepsis

Due to its prevalence and potentially life-threatening manifestations, infections of the lower respiratory tract (pneumonias) are a particular challenge to global health [2, 4, 5]. Disease burden projections towards 2030 predict pneumonia to remain among the top four leading causes of death worldwide [6-8]. The most common causative agent of community-acquired pneumonia and sepsis is the gram-positive diplococcus Streptococcus (S.) pneumoniae [9, 10]. Pneumococcal pneumonia occurs as a primary bacterial infection of the lung but causes superinfection during seasonal influenza epidemics as well [11-14]. The gram-negative rod shaped bacillus Klebsiella (K.) pneumoniae is an important causative agent in nosocomial (health-care/hospital-acquired) pneumonia and sepsis [15, 16]. Sepsis is the result of uncontrolled infection and characterized by an unbalanced systemic host response leading to cellular dysfunction and subsequently organ failure [17-20]. Though many infections can lead to sepsis, the most frequent source is pneumonia [19].

A very old and specific type of lung infection is caused by the acid fast Mycobacterium (M.) tuberculosis. Approximately one third of the world’s population is likely infected with this micro-organism and with 1.3 million deaths in 2012 M. tuberculosis remains the most lethal human bacterial pathogen to date [21]. Fortunately, not all infections lead to tuberculosis disease and death. Alveolar macrophages are the residence of choice of M. tuberculosis [21]. The microbe persists inside these phagocytes by arresting normal phagosome maturation and preventing phagolysosomal fusion [22]. A dynamic equilibrium between host immunity and the mycobacterium allows a latent/dormant disease state where encapsulated lesions (granulomas) effectively isolate the infection within the host but where eradication is nonetheless rarely achieved [22]. In less than 10% of cases latent disease develops into active disease [21-23].
Pulmonary host defense

The lung contains one of the largest surfaces of the body that maintains contact with the outside world. Anatomical (airway angulation, epithelium, mucus, surfactant) and physiological barriers (mucociliary clearance, coughing, sneezing) are there to limit and remove inhaled substances and pathogens [24] (Figure 1). When, despite these barriers, micro-organisms do invade the airways, the host has both innate and adaptive immune responses at its disposal to eliminate the threat [25]. Resident immune cells (i.e. alveolar macrophages) and respiratory epithelial cells [26, 27] organize this response through secretion of cytokines and chemokines, the production of anti-microbial peptides, the recruitment and activation of additional immune cells (i.e. neutrophils, additional phagocytes and antigen presenting cells) and the phagocytosis of micro-organisms [25, 28, 29] (Figure 2). Eventually, through antigen presentation and interaction with specific cells and cytokines, an adaptive immune response takes form (primarily composed of T- and B- lymphocytes) to further combat infection and to facilitate immune memory after infection has been resolved [25, 30, 31].
Recruited neutrophils especially are particularly proficient when it comes to host defense against bacteria [28, 30, 35, 36]. Firstly, neutrophils phagocytose extracellular pathogens which subsequently are killed and degraded intracellularly during phagosome maturation with the help of produced reactive oxygen species and hydrolytic enzymes. Secondly, extracellular killing is facilitated via degranulation (and thus the release of soluble anti-microbials), and NETosis: the kamikaze formation of neutrophil extracellular traps (NETs) with inherent bactericidal properties [30, 35]. In addition, neutrophils produce and release inflammatory mediators of their own, including complement components, cytokines and chemokines [30, 35], which add to and shape both innate and adaptive immune responses [36].

**Figure 2:** Depicted is a normal (left side) and inflamed (right side) alveolus. In the acute phase, there is sloughing of epithelial cells. In the air space an alveolar macrophage is secreting cytokines (i.e. IL-1, -6, -8, -10 and TNF-α), which activate neutrophils and stimulate chemotaxis. Neutrophils are shown adhering to injured epithelium and migrating to the site of inflammation. They produce inflammatory mediators (i.e. leukotrienes, oxidants, proteases, platelet-activating factor (PAF)) which are released in the protein-rich edema fluid. Adapted from [37]
Resident **alveolar macrophages** are particularly eager phagocytes. They internalize not only micro-organisms and pathogenic components, but also clear apoptotic cells, debris and inert particulates (like dust) from the airways [25]. Immunologically, they are (kept) relatively quiet to limit unnecessary lung injury [29]. This restraint lasts up until warning signals like tissue damage [38, 39] or an overwhelming infection [29] changes their fate and they develop into a more inflammatory phenotype, resulting in the production of regulatory cytokines, assisting the attraction of neutrophils to the site of infection.

**TLR signaling**

The key to initiation of any immune response is recognition of a health risk. Toll-like receptors (TLRs) are part of the innate system and belong to the so called group of pattern recognition receptors (PRRs). TLRs are capable of recognizing both pathogen- and danger associated molecular patterns (PAMPs and DAMPs) [39-43]. As such they are at the first line of defense when it comes to preventing and combating infectious disease. Upon recognition of a PAMP (or DAMP) activation of TLRs ensues, entailing TLR dimerization and subsequently recruitment of adaptor and accessory proteins which initiate an intracellular signaling cascade resulting in nuclear factor (NF)-κB, mitogen-activated protein (MAP) kinase activation and/or interferon regulatory factor (IRF) activation, thus propagating a pro-inflammatory response [40, 42].

Myeloid differentiation primary response gene (MyD)88 is the universal adaptor for all TLRs (except TLR3) and the interleukin (IL)-1 receptor family (IL-1, -18 and -33 receptors) [41, 42, 44]. TLR3 is TIR domain-containing adapter-inducing IFN-β (TRIF) dependent; TLR4 signaling can proceed via either MyD88 or TRIF. In the absence of both MyD88 and TRIF no TLR (IL-1 or IL-18) signaling takes place [41, 42].

**Negative regulators**

TLR activation leads to a pro-inflammatory response involving the production of cytokines and other inflammatory mediators, and the recruitment of activated immune cells in principle culminating in a self-augmenting and expanding inflammatory reaction meant to clear pathogenic micro-organisms from the body. Unfortunately, a fulminant inflammatory response invariably leads to host cell damage, tissue destruction and possibly organ dysfunction. Consequently, rigorous regulation of inflammation is imperative. Several negative regulators of TLR signaling have been identified [45]. Single immunoglobulin IL-1R-related molecule (SIGIRR or TIR8) and membrane bound IL-1R-like 1 (ST2) are two of such negative regulators [46, 47] (Figure 3).
Figure 3: Negative regulation of Toll-like receptor signaling [43].
Both ST2 and SIGIRR are membrane bound regulators able to interfere with TLR adaptor- and associated molecule recruitment/ activation thus inhibiting downstream signaling. IRAK-M, SOCS-1 (suppressor of cytokine signaling-1) and MyD88s (myeloid differentiation primary-response-protein 88 short) are intracellular inhibitors. IκB, inhibitor of NF-κB; IKK, IκB kinase; IRAK, IL-1R associated kinase; TIRAP, TIR (Toll/IL-1R)-domain-containing adaptor protein; TRAF, tumor necrosis factor receptor associated factor.

ST2 & IL-33

The ST2 gene produces two different splice variants: the membrane bound ST2L and the soluble (s)ST2 which can be measured in the circulation. ST2L functions both as a negative regulator of TLR signaling and as part of the IL-33 receptor. sST2 has been suggested to act mainly as a decoy receptor for IL-33 [48], but has been shown to inhibit lipopolysaccharide (LPS) signalling and subsequent effector functions by binding to monocytes, macrophages and dendritic cells as well [49-52]. ST2 is found on many hematopoietic and immune cells. Nevertheless, a certain expression selectivity within the adaptive immune cell cluster is denoted since it is expressed specifically on type 2 not type 1 helper cells [48, 53]. In contrast, innate immune expression is more ubiquitous: ST2 is found on mast cells, granulocytes, monocytes, macrophages, natural killer cells, innate lymphoid cells, dendritic cells, even epithelia [48, 53-55].

IL-33 belongs to the IL-1 family of cytokines and is pleiotropic in its function [53]. IL-33 is expressed in many different cells’ nuclei where it exhibits transcriptional repressor capabilities by binding to NF-kB, thus negating any NF-kB effector function and dampening the immune response [53]. Once released from the cell (possibly actively
secreted but definitely due to necrosis [48, 56, 57] IL-33 triggers NF-κB and MAP kinases via ST2. By means of the induction of cytokines like IL-4, IL-5 and IL-13 from various cell types in various tissues the main function of extracellular IL-33 seems to be directing the immune system towards a predominantly Th2 polarized response [58, 59], although IL-33 induced/supported secretion of IL-1β, IL-6 and TNF has been reported as well [60-63]. Interestingly, SIGIRR may dampen IL-33 effects, providing an additional inhibitory circuit in innate immunity [64].

Outline of this thesis:
The general aim of this thesis is to improve our understanding of the (innate) immune system’s response to common human lung infections and sepsis; specifically the role of TLR signaling adaptors MyD88 and TRIF, and the role of negative regulators SIGIRR & ST2 and ST2’s additional functions are discussed.

In chapter 2 the role of SIGIRR during pneumococcal lung infection and sepsis is investigated by comparing the host response in SIGIRR deficient and normal wild-type mice after infection with S. pneumoniae via the airways or by intravenous injection. Chapter 3 discusses the role of ST2 in primary and secondary pneumococcal pneumonia; the latter using a murine model of secondary S.pneumoniae respiratory tract infection following non-lethal influenza A lung infection in wild-type and ST2 deficient mice. Chapter 4 describes studies seeking to determine the role of ST2 in sepsis caused by either S. pneumoniae or K. pneumoniae. Chapter 5 investigates the receptor function of ST2 by administering IL-33 to mice with K. pneumoniae pneumonia. Chapter 6 focusses on the TLR adaptor molecules MyD88 and TRIF and their possible differential roles in both resident and hematopoietic cells during murine K. pneumoniae infection. The last two chapters report human studies: chapter 7 reports on the expression of TLR inhibitors tuberculosis patients recruited in Chittagong, Bangladesh; furthermore, plasma sST2 is measured in these patients to evaluate ST2’s biomarker potential in tuberculosis. sST2 measurements are also reported in chapter 8, in a cohort of Dutch community-acquired pneumonia patients caused by either influenza A (H1N1), Coxiella burnetii (Q-fever) or S. pneumoniae patients.
Reference list

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