Endogenous danger signals in infectious diseases

Achouiti, A.

Citation for published version (APA):
Chapter 1
General introduction and outline of the thesis
**GENERAL INTRODUCTION**

Alarmins or damage associated molecular patterns (DAMPs) are endogenous molecules released by the host upon tissue injury or infection. They are recognized by pattern recognition receptors (PRRs), many of which also have key roles in the sensing of microbial pathogens. Alarmins activate innate immune cells and thereby act as endogenous danger signals to promote and perpetuate inflammation [1,2]. During infection, alarmins can be found in the systemic circulation as well as at invaded local sites. Uncontrolled release of alarmins may lead to a dysregulated inflammatory response [3].

In this thesis, we describe two well-characterized alarmins, High-mobility group Box (HMGB)1 and Myeloid-related Protein (MRP)8/14, and their receptors in several infectious diseases. We will begin this introductory chapter with a detailed description of these alarmins and their receptors. We proceed with the general background of relevant infectious diseases. We end the introduction with an outline of the thesis.

<table>
<thead>
<tr>
<th>Alarmin</th>
<th>Origin</th>
<th>Receptors</th>
<th>Extracellular actions</th>
<th>Implicated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGB1</td>
<td>All cell types</td>
<td>TLR2, TLR4, RAGE</td>
<td>(Subject to redox state) Proinflammatory response, chemotaxis, induction of adaptive immune response</td>
<td>Sepsis, auto-immune disease, acute lung injury, brain ischemia, epilepsy, I/R injury of the heart</td>
</tr>
<tr>
<td>MRP8/14 and S100A12</td>
<td>Epithelial cells</td>
<td>TLR4, RAGE</td>
<td>Proinflammatory response, neutrophil adhesion, migration and release from bone marrow, direct antimicrobial activity</td>
<td>sepsis, auto-immune diseases, acute lung injury, atherosclerosis</td>
</tr>
<tr>
<td></td>
<td>Phagocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP70 and HSP60</td>
<td>All cell types</td>
<td>TLR2, TLR4, SRA1</td>
<td>Regulation of inflammatory response</td>
<td>Sepsis</td>
</tr>
<tr>
<td>β-Defensins</td>
<td>Keratinocytes</td>
<td>GPCRs, e.g. CCR6</td>
<td>Direct antimicrobial activity, enhance adaptive immunity</td>
<td>Acute lung injury, CF, IBD</td>
</tr>
<tr>
<td></td>
<td>Epithelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cathelicidin</td>
<td>Keratinocytes</td>
<td>FPRL1</td>
<td>Direct antimicrobial activity, enhance adaptive immunity</td>
<td>Acute lung injury, CF, IBD</td>
</tr>
<tr>
<td>hCAP18/ LL37</td>
<td>Epithelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phagocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: A selection of alarmins and their receptors, partially adapted from (3). CCR6: C-C chemokine receptor 6; CF: Cystic fibrosis; FPRL1: formyl peptide receptor-like 1; GPCR, G protein–coupled receptor; hCAP18: human cationic antimicrobial protein 18; HMGB1: High-mobility Group Box 1; IBD: inflammatory bowel disease; I/R: ischemia/reperfusion; MRP8/14: Myeloid-related protein 8/14; RAGE: Receptor for advanced glycate endproducts; SRA1: steroid receptor RNA activator 1; TLR2/4: Toll-like receptor 2/4.
1. ALARmins

The list of alarmins in the literature is rapidly growing (table 1). They can activate the immune system and many of these proteins have additional extracellular innate immune functions, including chemotactic and antibacterial effects [3,4]. Alarmins have gained increasing interest, as they could serve as useful biomarkers and as potential immunomodulating targets in sterile or infectious inflammatory disorders [3]. In the text below we describe the alarmins that are relevant for this thesis.

1.1 High-mobility Group Box 1

HMGB1 is a highly conserved non-histone nuclear protein that serves various intracellular and extracellular roles. It can be rapidly released into the circulation during cell injury or upon inflammatory stimuli [5]. HMGB1 induces proinflammatory cytokine release via Toll-like receptor (TLR)4 by an interaction that requires a specific molecular conformation, determined by three redox-sensitive cysteines (C23, C45, and C106) [6]. In addition, HMGB1 can mediate cellular effects through other PRRs, including the Receptor for Advanced Glycation Endproducts (RAGE), TLR2 and TLR9 after binding of partner molecules such as interleukin (IL)-1β, bacterial ligands, DNA and histones [5]. Depending on posttranslational modifications, HMGB1 can act as a chemotactic factor as well, after formation of a heterocomplex with the chemokine CXCL12 via the chemokine receptor CXCR4 [6].

HMGB1 is involved in the pathogenesis of dysregulated inflammation in severe infectious diseases [3,7]. In the setting of experimental sepsis, HMGB1 is implicated as a mediator of lethality and associated with delayed and sustained release, providing a clinically relevant time frame for pharmacological interventions [7]. Anti-HMGB1 treatment improved outcome in models of sepsis, even when administered after the onset of infection [8,9]. Anti-HMGB1 antibodies also improved outcome in severe pneumonia models, including airway infection with Pseudomonas (P.) aeruginosa [10] and treatment with intratracheal endotoxin [11]. In chapter 3 we investigated the role of HMGB1 in Escherichia (E.) coli abdominal sepsis, whereas the role of HMGB1 during Staphylococcus (S.) aureus lung infection is characterized in chapter 5.

1.2 S100 proteins (or Myeloid-related proteins)

The S100 protein family consists of more than 20 members with a regulatory role in a variety of intra- and extracellular processes. Several of these proteins are linked to innate immune functions, including S100A8 (also called MRP8), S100A9 (or MRP14) and S100A12 (MRP6) [12]. MRP8 and MRP14 are the most abundant cytoplasmic proteins of neutrophils and monocytes [13]. They form MRP8-MRP14 heterodimers (MRP8/14 or calprotectin), which are the biologically relevant forms of these proteins [14,15].
MRP8/14 is actively released upon stress stimuli and induces a variety of innate immune reactions [3,16,17]. It enhances cytokine release via TLR4 in response to lipopolysaccharide (LPS), contributing to endotoxin shock-induced lethality [18] and it promotes leukocyte recruitment [19-21]. Next to its proinflammatory effects, MRP8/14 possesses direct antimicrobial properties directed against bacterial and fungal pathogens [22-25]. MRP8/14 mediates at least part of these effects by chelation of manganese and zinc, elements that are important for microbial growth and virulence [24,25]. Recent studies revealed that MRP8/14 is a major component of neutrophil extracellular traps (NETs) [23], DNA-networks released by neutrophils that trap microorganisms and facilitate interaction with antimicrobial proteins [26,27]. In this thesis, we have examined the role of MRP8/14 in Gram-negative and Gram-positive pneumonia in chapters 8 to 10. In chapter 11 we investigated the role of MRP8/14 in local skin diseases induced by *S. aureus*.

Another member of the S100 family is the S100A12-protein. S100A12 is constitutively expressed in human neutrophils [28]. It has been found at high concentrations in pulmonary tissue and bronchoalveolar lavage fluid during acute respiratory distress syndrome [29]. S100A12 mediates cellular effects via interaction with RAGE [30] and TLR4 [31]. Circulating S100A12 levels during human sepsis and endotoxemia are described in chapter 12.

### 2. ALARMIN-RECEPTORS

PRRs are membrane bound or cytosolic proteins that respond to pathogens or alarmins. Several alarmin-receptors and their ligands are listed in table 1. Sensing of alarmins mediates the inflammatory response. We here discuss the two multiligand alarmin receptors, RAGE and TLR4, both of which are relevant for this thesis.

#### 2.1 The Receptor for Advanced Glycation Endproducts (RAGE)

RAGE is a membrane bound receptor of the immunoglobulin superfamily [32,33], which is highly expressed in lung tissue and on various cell types [34]. It binds several alarmins, including HMGB1 and S100A12, which are released during invasive disease [35,36]. Recently, RAGE was identified as a receptor for LPS as well [37]. Engagement of RAGE activates and perpetuates inflammation via the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase pathways [35,36]. In addition, RAGE contributes to infiltration of neutrophils, as it upregulates adhesive molecules [36] and acts as an adhesive molecule itself by binding to β₂-integrins on neutrophils [35,38,39]. Inhibition of RAGE signaling has been found to reduce the inflammatory response in several (non-infectious) models [40-42] and improved outcome in a model of polymicrobial abdominal sepsis [43].
Soluble RAGE (sRAGE) [32,33], is the truncated form of full length RAGE, lacking the cytosolic and transmembrane domains and can be found in plasma and in the bronchoalveolar compartment [34]. It can compete with cell-surface RAGE for ligand binding and may contribute to neutralization of circulating RAGE ligands [44]. In addition to functioning as a decoy receptor, sRAGE has been implicated in neutrophil recruitment [45]. sRAGE levels may increase during severe infectious diseases and has been suggested as a useful biomarker in sepsis and acute lung injury [46,47]. We have reviewed the role of RAGE and sRAGE during infection in chapter 2. In chapter 3 we describe the role of RAGE-ligands and their potential to be used as a therapeutic target in abdominal E. coli sepsis. Chapter 4 describes the role of RAGE in Streptococcus (S.) pneumoniae bacteremia. The role of RAGE in Klebsiella and staphylococcal pneumonia is described in chapter 5 and 6. We investigated the role in local and systemic S. aureus skin disease in chapter 7.

2.2 Toll-like receptor (TLR)4

The family of TLRs consists of 13 different members, of which 10 have been identified in humans. TLRs detect different kinds of pathogen associated molecular patterns (PAMPs) and endogenous molecules which activate the innate immune system. Different signaling pathways are involved that all culminate in NF-κB activation. TLR4 detects LPS from Gram-negative bacteria as well as numerous endogenous molecules that induce and amplify inflammatory responses, including MRP8/14, S100A12 and HMGB1 [6,18,48]. The role of TLR4 and MRP8/14 in Gram-negative sepsis is partly discussed in chapter 8. In chapter 5 we describe the role of TLR4 during S. aureus pneumonia.

3. INFECTIOUS DISEASES STUDIED IN THIS THESIS

We investigated the roles of alarmins in several disease models. In the text below we discuss the infectious diseases relevant for this thesis.

3.1 Sepsis

Sepsis is a heterogeneous clinical syndrome caused by a deregulated host response to an infection. It is a condition that can lead to multiorgan failure and/or refractory hypotension [49,50]. Sepsis is a cause of considerable morbidity and mortality despite the availability of effective antimicrobial therapy and supportive care [51,52]. Recent insights have made clear that the sepsis syndrome is complex, variable and characterized by both proinflammatory and anti-inflammatory responses, dependent on host and/or pathogen associated factors [50,53]. The (initial) intense inflammatory response during sepsis may lead to collateral tissue damage and necrotic cell death, which results in the
release of alarmins that perpetuate inflammatory processes (Figure 1) [49,50,53]. Other hallmark features of sepsis include activation of the coagulation system (in severe cases resulting in disseminated intravascular coagulation) and vascular dysfunction [49,50,53]. The respiratory tract and the abdomen are the most frequent sources for sepsis. The most common Gram-positive isolates in sepsis are *S. aureus* and *S. pneumoniae*, while *E. coli*, *Klebsiella* species, and *P. aeruginosa* are among the most common Gram-negative isolates [50].

### 3.2 Bacterial pneumonia

Pneumonia is the most common infectious cause of death in the world and the third most common cause of death overall. Bacterial pneumonia is the most frequent source of sepsis [50]. When bacteria invade the lower respiratory tract, various inflammatory mechanisms ensue which initiate the recruitment of neutrophils into the bronchoalveolar space. Transmigrated neutrophils are then activated to phagocytose pathogens and to produce proteases and reactive oxygen species that act on infected cells and induce necrosis [54]. Invasive infection and intense host defense mechanisms contribute to
extensive lung injury and release of alarmins. Uncontrolled release of alarmins may lead to an overwhelming inflammatory response in the lungs [3].

*S. pneumoniae* is the single most frequent pathogen causing community-acquired pneumonia, responsible for up to 60% of cases [55]. *K. pneumoniae* is a common causative agent in hospital-acquired pneumonia [56]. *S. aureus* infection of the respiratory tract is classically confined to health-care settings [57]. In recent years however, the prevalence of staphylococcal pneumonia in the general population has increased, in particular due to the emergence of highly virulent community associated methicillin resistant *S. aureus* [58].

### 3.3 Peritonitis

Peritonitis is caused by the presence of pathogenic bacteria in the peritoneal cavity. It is a potential life-threatening event, especially in elderly and in those with significant underlying disease. Bacteria can rapidly spread via the circulation and cause systemic inflammation and sepsis [59]. Intraperitoneal infections are mostly caused by members of the gastrointestinal flora. *E. coli* is the most frequently isolated organism in peritonitis [60].

### 3.4 Skin Infection

Skin and soft tissue infections are a frequent source for sepsis [50]. The cutaneous immune response against bacteria involves both the innate and adaptive immune system. The innate cutaneous immune response triggers the production of cytokines and chemokines. In addition, neutrophils are recruited from the circulation to form a neutrophil abscess at the infected site [61-63]. Neutrophil abscesses contain high quantities of antimicrobial proteins, which may be essential for bacterial control [61-63]. *S. aureus* is responsible for the vast majority of skin infections [64-67].

### 4. AIM AND OUTLINE OF THIS THESIS

The overall aim of this thesis is to expand our knowledge of the specific role of alarmins and their receptors in the innate immune response during severe infections. Part 1 discusses the role of RAGE and RAGE ligands in a number infectious disease models. Chapter 2 presents an overview of what was known thus far on RAGE during infection. Chapter 3 reports on the effects of sRAGE and anti-HMGB1 treatment during *E. coli* abdominal sepsis. In chapter 4 we describe the role of RAGE during bacteremia caused by pneumococci. We focused on the role of RAGE (and TLR4) in pneumonia caused by *S. aureus* and *K. pneumoniae* in chapters 5 and 6. The role of RAGE in local and systemic disease during subcutaneous *S. aureus* infection is studied in chapter 7.
In part 2 we discuss the role of S100 proteins, MRP8/14 (or S100A8/A9) and S100A12 during infection. In chapter 8-10 we investigated the role of MRP8/14 during pneumonia and pneumonia derived sepsis caused by *K. pneumoniae*, pneumococci and staphylococci. In chapter 11 we investigated the role of MRP8/14 in staphylococcal skin disease. Chapter 12 characterizes systemic S100A12 and sRAGE concentrations during human sepsis and experimental endotoxemia.
REFERENCES

General introduction and outline of the thesis


