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Targeting bacterial infections by using immunomodulatory host defence peptides and proteins

Kalle, Martina

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Targeting bacterial infections by using immunomodulatory
host defence peptides and proteins

Martina Kalle

LUND UNIVERSITY
Faculty of Medicine

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Faculty opponent:

Dr. Cornelis Van´t Veer

Center for Experimental and Molecular Medicine
Academic Medical Center
University of Amsterdam
Amsterdam, Netherlands

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Abstract:

Bacterial infections and sepsis are amongst the leading causes of death worldwide. Sepsis is caused by an uncontrolled systemic host response towards an invading pathogen. Due to the engagement of various systems including cell-mediated responses, the coagulation and complement cascades, treatment of sepsis remains challenging. This is reflected in mortality rates of about 50 % for patients with septic shock, despite improved health care and antibiotic usage.

Endogenous host defence peptides (HDPs) are essential components of the innate defence against invading pathogens. They exert multiple biological functions, and apart from their antimicrobial effects they may modulate inflammation, coagulation, and chemotaxis. Due to increasing bacterial resistance and due to the complex nature of severe bacterial infections, HDPs are today considered valuable candidates in the development of novel treatments for infections.

In papers I-III the ability of HDPs, derived either from the C-terminus of human thrombin (HVF18, GKY25) or human tissue factor pathway inhibitor 2 (EDC34), to modulate innate immune responses caused by bacteria or lipopolysaccharide (LPS), were investigated. Thrombin-derived peptides significantly blocked LPS-induced responses including tissue-factor driven coagulation *in vitro* and *in vivo* and thereby improved survival in experimental animal models of LPS-induced shock and *Pseudomonas aeruginosa* sepsis (Paper I). The data in Paper II, demonstrate that the inhibition of these pro-inflammatory responses by GKY25, is not solely dependent on extracellular LPS-scavenging, but involves interactions with macrophages and monocytes. The TFPI-2 peptide (EDC34) also significantly modulated the coagulation cascade *in vitro* and *in vivo* and efficiently killed bacteria by enhancing complement-mediated killing. The combination of these functions lead to improved survival in experimental models of *E. coli* or *Pseudomonas* sepsis (Paper III). In conclusion, the discovered immunomodulatory properties of these HDPs clearly indicate their potential for the development of new treatments for bacterial infections.

Finally, a novel role of the abundant plasma protein heparin cofactor II (HCII) in host defence was discovered. HCII belongs to the class of serine proteinase inhibitors (serpins) and specifically inhibits human thrombin. However, its precise physiological role remained enigmatic. Paper IV shows that cleavage of HCII by human neutrophil elastase induces a conformational change in the HCII molecule, thereby uncovering a previously unknown antibacterial function of HCII.

Key words: Host defence peptides, Sepsis, Heparin cofactor II, Immunomodulation

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Martina Kalle

LUND UNIVERSITY
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Martina Kalle
Department of Clinical Sciences
Division of Dermatology and Venereology
Faculty of Medicine
Lund University

Cover image:

The transmission-electron micrograph shows infiltrating immune cells and a LPS-induced fibrin clot in a lung section of a mouse treated with LPS.
The image was kindly provided by Matthias Mörgelin.

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Abstract

Bacterial infections and sepsis are amongst the leading causes of death worldwide. Sepsis is caused by an uncontrolled systemic host response towards an invading pathogen. Due to the engagement of various systems including cell-mediated responses, the coagulation and complement cascades, treatment of sepsis remains challenging. This is reflected in mortality rates of about 50 % for patients with septic shock, despite improved health care and antibiotic usage. Endogenous host defence peptides (HDPs) are essential components of the innate defence against invading pathogens. They exert multiple biological functions, and apart from their antimicrobial effects they may modulate inflammation, coagulation, and chemotaxis. Due to increasing bacterial resistance and due to the complex nature of severe bacterial infections, HDPs are today considered valuable candidates in the development of novel treatments for infections.

In papers I-III the ability of HDPs, derived either from the C-terminus of human thrombin (HVF18, GKY25) or human tissue factor pathway inhibitor 2 (EDC34), to modulate innate immune responses caused by bacteria or lipopolysaccharide (LPS), were investigated. Thrombin-derived peptides significantly blocked LPS-induced responses including tissue-factor driven coagulation *in vitro* and *in vivo* and thereby improved survival in experimental animal models of LPS-induced shock and *Pseudomonas aeruginosa* sepsis (Paper I). The data in Paper II demonstrate that the inhibition of these pro-inflammatory responses by GKY25, is not solely dependent on extracellular LPS-scavenging, but involves interactions with macrophages and monocytes. The TFPI-2 peptide (EDC34) also significantly modulated the coagulation cascade *in vitro* and *in vivo* and efficiently killed bacteria by enhancing complement-mediated killing. The combination of these functions lead to improved survival in experimental models of *E. coli* or *Pseudomonas* sepsis. In conclusion, the discovered immunomodulatory properties of these HDPs clearly indicate their potential for the development of new treatments for bacterial infections.

Finally, a novel host defence role of the abundant plasma protein heparin cofactor II (HCII) was discovered. HCII belongs to the class of serine proteinase inhibitors (serpins) and specifically inhibits human thrombin. However, its precise physiological role remained enigmatic. Paper IV shows that cleavage of HCII by human neutrophil elastase induces a conformational change in the HCII molecule, thereby uncovering a previously unknown antibacterial function of HCII.

Zusammenfassung

Bakterielle Infektionen gehören zu den häufigsten Todesursachen weltweit. Sepsis ist definiert durch das Vorhandesein einer Infektion, sowie der Erfüllung von 2 der 4 Kriterien eines systemischen Entzündungssyndroms. Die durch die Bakterien ausgelöste Immunantwort umfasst die Aktivierung der zellulären Immmunität, der Gerinnerungs- und Komplementsysteme, sowie die Produktion körpereigener antimikrobieller Peptide. Bei Sepsis mit zusätzlichem Organausfall und kontinuierlich niedrigem Blutdruck spricht man von septischem Schock. Im Fall eines septischen Schocks liegt die Sterblichkeitsrate bei mehr als 50 %, trotz verbesserter medizinischer Versorung und Benutzung von Antibiotika. Ursächlich dafür sind zum einen die Komplexität der Krankheit, sowie das verhäufte Auftreten von antibiotikaresistenten Bakterien.

Antimikrobielle Peptide (APs) sind ein essentieller Bestandteil des angeborenen Immunsystems. Sie haben die Fähigkeit Bakterien direkt zu töten, und Reaktionen des Immunsystems zu beeinflussen. Diese Eigenschaften machen APs zu einem wichtigen und interessanten Ausgangspunkt für die Entwicklung neuer Medikamente zur Behandlung von schweren backteriellen Infektionen. Antimikrobielle Peptide werden von Immun-zellen produziert oder durch das enzymatische Zerschneiden von Proteinen freigesetzt. Die in dieser Arbeit untersuchten APs enstehen durch das Zerschneiden des Gerinnungsproteins *Thrombin* bzw. eines weiteren Proteins namens *Tissue Factor Pathway Inhibior 2* (TFPI-2).

Der Hauptschwerpunkt der Arbeiten I-III lag auf der Charakterisierung dieser neu entdeckten Peptide im Bezug auf ihre Fähigkeit, die durch Bakterien induzierte Immunantwort zu modulieren. Die Daten der Arbeit I zeigen, dass Thrombinpeptide die Zytokin-freisetzung aktivierter Immunzellen reduzieren. Darüber hinaus können diese Peptide die Aktivierung der Gerinnungskaskade minimieren und Bakterien direkt abtöten.

Durch das Zusammenspiel der oben beschriebenen Eigenschaften konnten Thrombinpeptide die Sterblichkeitstsrate in bakteriellen Infektionen im Tiermodell drastisch senken.

Arbeit II beschäftigte sich mit der Frage, wie genau Thrombinpeptide die Immunantwort von Monozyten und Macrophagen beeinflussen. Die Ergebnisse zeigen, dass diese Peptide direkt an Bakterien und deren Bestandteile, aber auch an die Immunzellen binden. Damit können sie die Erkennung der Bakterien durch die Immunzelle unterbinden und somit auch die Immunzellaktivierung.

Das in Arbeit III charakterisierte TFPI-2 Peptid EDC34 inhibierte die Aktivierung des Gerinnungssytems. Darüber hinaus stimulierte es die Abtötung der Bakterien durch das Komplementsystem. Auch EDC34 konnte die Sterblichkeitstsrate in Tiermodellen mit *Escherichia coli* bzw. *Pseudomonas aeruginosa* induzierter Sepsis signifikant reduzieren.
Zusammenfassend konnten wir zeigen, dass beide Peptidgruppen signifikante antibakterielle und antikoagulante Funktionen aufweisen und sie sich deshalb für die Ent-wicklung neuer, Peptid-basierter Therapien eignen könnten.

Die Experimente der Arbeit IV konzentrierten sich auf die Erforschung neuer Eigenschaften des Proteins *Heparin Cofactor II* (HCII). HCII reguliert die Aktivierung von *Thrombin* und trägt damit zur Modulierung der Gerinnungskaskade bei. Frühere Studien haben gezeigt, dass die Konzentration von HCII im Blut, während bakterieller Infektionen abnimmt, allerdings war eine Funktion von HCII in Infektionen bisher noch nicht bekannt. Die Daten in dieser Studie demonstrieren eine bislang nicht bekannte antibakterielle Aktivität des Proteins, die erst nach Abschneiden, bestimmter Proteinteile aktiviert wird. Mit Hilfe von Experimenten im Tiermodell und der Analyse von Patientenproben konnten wir bestätigen, dass HCII tatsächlich an der Bekämpfung bakterieller Infektionen beteiligt ist.

List of papers and manuscripts

This thesis is based on the following articles, which will be referred to in the text by their Roman numerals (I-IV):

Paper I

Martina Kalle, Praveen Papareddy, Gopinath Kasetty, Matthias Mörgelin, Mariena J.A. van der Plas, Victoria Rydengård, Martin Malmsten, Barbara Albiger, Artur Schmidtchen.

Host defense peptides of thrombin modulate inflammation and coagulation in endotoxin-mediated shock and Pseudomonas aeruginosa sepsis. *PLOS ONE* 2012, 7(12):e51313

Paper II

Martina Kalle, Mariena J.A. van der Plas, Ann-Charlotte Strömdahl, Martin Malmsten, Matthias Mörgelin, Artur Schmidtchen.

The thrombin-derived peptide GKY25 modulates endotoxin-induced responses through direct interactions with macrophage/monocyte cell membranes. *Manuscript*

Paper III

Praveen Papareddy, **Martina Kalle**, Ole E. Sørensen, Martin Malmsten, Matthias Mörgelin, Artur Schmidtchen.

The TFPI-2 derived peptide EDC34 improves outcome of Gram-negative sepsis. *Manuscript* (submitted)

Paper IV

Martina Kalle[#], Praveen Papareddy[#], Gopinath Kasetty, Douglas M. Tollefsen, Martin Malmsten, Matthias Mörgelin, Artur Schmidtchen.

Proteolytic activation transforms heparin cofactor II into a host defense molecule. *Manuscript* (submitted) ([#]equal contribution)

List of publications not included in the thesis

Praveen Papreddy, **Martina Kalle**, Ole E. Sørensen, Katarina Lundquist, Matthias Mörgelin, Martin Malmsten, Artur Schmidtchen.
Tissue factor pathway inhibitor 2 is found in skin and its C-terminal region encodes for antibacterial activity.*PLOS ONE* 2012, 7(12):e52772

Gopinath Kasetty, Praveen Papareddy, **Martina Kalle**, Victoria Rydengård, Björn Walse, Bo Svensson, Matthias Mörgelin, Martin Malmsten, Artur Schmidtchen.
The C-terminal sequence of several human serine proteases encodes host defense functions. *Journal of Innate Immunity* 2011, 3, 471-482

Gopinath Kasetty, Praveen Papareddy, **Martina Kalle**, Victoria Rydengård, Matthias Mörgelin, Barbara Albiger, Martin Malmsten, Artur Schmidtchen.
Structure-activity studies and therapeutic potential of host defense peptides of human thrombin *Antimicrobial Agents and Chemotherapy* 2011, 55, 2880-2890

Praveen Papareddy, **Martina Kalle**, Gopinath Kasetty, Matthias Mörgelin, Victoria Rydengård, Barbara Albiger, Katarina Lundqvist, Martin Malmsten, Artur Schmidtchen.
C-terminal peptides of tissue Factor pathway inhibitor are novel host defense molecules. *Journal of Biological Chemistry* 2010; 285, 28387-2839

Abbreviations

AB	Antibody
AMP	Antimicrobial peptide
APC	Activated Protein C
AprA	Pseudomonas alkaline protease
aPTT	Activated partial thromboplastin time
ATIII	Antithrombin III
ATP	Adenosine-5'-triphosphate
BK	Bradykinin
C-terminal	Carboxy terminal (COOH-terminal)
C(x)	Complement factor (x)
CD	Cluster of differentiation
CRP	C-reactive protein
DAMPs	Damage-associated molecular patterns
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
FACS	Fluorescence-activated cell sorting
FDA	US Food and Drug Administration
FVII-FXIII	Coagulation factors VII-XIII
GAGs	Glycosaminoglycans
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPI	Glycosylphosphatidylinositol
hBD	Human β-defensins
hCAP-18	Human cationic antimicrobial protein 18
HCII	Heparin cofactor II
HD	Human α-defensin
HDP	Host defence peptide
HK	High molecular weight kininogen
HLE	Human leukocyte elastase
HMGB-1	High-mobility group box 1 protein
HNP	Human neutrophil peptides

HRG	Histidin-rich glycoprotein
ICU	Intesive care units
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IRAK	IL-1 receptor-associated kinase 4
IRF	Interferon regulatory factor
LBP	LPS-binding protein
LF	Lactoferrin
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MAC	Membrane attack complex
MAL	MyD88-adaptor-like
MAP kinases	Mitogen-activated protein kinase
MASPs	MBL-associated serine proteinases
MBL	Mannose-binding lectin
MCP-1	Monocyte chemoattractant protein-1
MD-2	Myeloid differentiation factor-2
MHC	Major histocompatibility complex
MKK6	Mitogen-activated protein kinase 6
MSR	Macrophage scavenger receptor
MyD-88	Myeloid differentiation factor protein 88
N-terminal	Amino-terminus (NH_2-terminus)
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NK cells	Natural killer cells
NLRs	NOD nucleotide-binding domain-like receptors
NO	Nitric oxide
NOD	Nucleotide binding domain
P. aeruginosa	*Pseudomonas aeruginosa*
PAI-1	Plasminogen activated inhibitor-1
PAMPs	Pathogen-associated molecular patterns
PAR-receptors	Proteinase-activated receptors
PCI	Protein C Inhibitor

PGN	Peptidoglycan
PK	Plasma kallikrein
PRR	Pattern recognition receptor
PT	Prothrombin time
RIG-I	Retinoic acid inducible gene I
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SERPIN	Serine proteinase inhibitor
SIRS	Systemic inflammatory response syndrome
TAFI	Thrombin-activatable fibrinolysis inhibitor
TAK1	TGF-β-activated kinase 1
TAMRA	Tetramethylrhodamine
TATc	Thrombin-antithrombin complex
TCP	Thrombin C-terminal peptide
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TIR	Toll-interleukin-1 receptor
TIRAP	TIR domain-containing adaptor protein
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor-α
tPA	Tissue plasminogen activator
TRAF6	Tumour necrosis factor receptor-associated factor 6
TRAM	TRIF related adapter molecule
TRIF	TIR domain-containing adaptor inducing interferon-β
uPA	Urokinase-like plasminogen activator
WBC	White blood cells

1   Introduction

Throughout our lifetime our body continuously interacts with different kinds of microbes, including commensal bacteria colonising our skin and mucosal surfaces, but also pathogens. In most cases invading pathogens will be effectively eradicated by our immune system, thereby preventing an infection. However, infectious diseases remain a major health care problem and cause of death worldwide [1]. Sepsis is the 11th leading cause of death in the United States [2] with estimated 751.000 cases of severe sepsis annually [3]. In an European study 24,7 % of the patients admitted to intensive care units (ICU) had sepsis [4]. Within the same study it was stated that 27 % of patients with sepsis and more than 50 % of patients with septic shock are dying in the ICU [4]. Reported mortality rates for severe sepsis and septic shock range from 22 % to above 50 % depending on disease severity, age, underlying diseases, access to care, genetic factors or cause of infection [4, 5, 6]. Interestingly, it has been shown that an infection caused by the Gram-negative bacterium *Pseudomonas aeruginosa* is related to an increased risk of mortality in patients with sepsis [4].

Sepsis is a complex and dynamic disease characterised by an uncontrolled systemic immune response of the host towards invading pathogens or their toxins [7, 8]. This immune response comprises the recognition of the pathogen by immune cells, which release inflammatory mediators, host defence peptides and proteins, as well as the systemic activation of the complement and coagulation cascades [7]. Even though health care procedures have improved, the incidence of severe sepsis is rising and mortality remains high [3, 6]. Attempts to improve the outcome by using alternative treatments, which target specific pro-inflammatory pathways or molecules were not successful in clinical trials [9, 10]. Therefore it is of importance to investigate novel treatment possibilities, and host defence peptides and proteins might be excellent candidates.

The main aim of this work was to investigate the functions of novel endogenous host defence peptides, derived from human thrombin and tissue factor

pathway inhibitor-2 in the context of innate immune response modulation and treatment of bacterial infections, caused by Gram-negative bacteria and their main endotoxin lipopolysaccharide. Furthermore the role of heparin cofactor II, an abundant plasma serine proteinase inhibitor, was investigated.

1.1 The immune system - an overview

To effectively prevent invasion and colonisation of pathogenic microbes, the human body harbours an arsenal of defence strategies including external barriers as parts of the immune system. In an initial step, the invading pathogen has to bypass physical barriers such as the skin and the mucosal surfaces, but has also to overcome chemical barriers formed by antimicrobial peptides present in the skin and other mucosal linings [11]. Another barrier is provided by the ionicity and pH of body fluids, such as sweat and saliva creating a hostile environment. Moreover commensal microorganisms compete for nutrients and space with the invading pathogen [11]. However, pathogenic microbes developed mechanism to overcome these barriers and are able to enter the host. Upon invasion bacteria are sensed by the immune system which initiates a complex system of immune responses.

The immune system is divided into two interlinked parts, the innate and adaptive or acquired immunity. While the innate immune defence is based on germ line encoded receptors recognising distinct patterns, the adaptive system generates receptors somatically by clonal selection [12]. The innate defence system consists of different blood cells including professional phagocytes, endothelial and epithelial cells, antimicrobial peptides as well as the complement and coagulation systems, and is immediately activated to attack and eradicate invading microbes. This activation is also necessary in order to trigger the slower, but more specific adaptive system that provides a long-term protection by creating a memory for specific pathogens [13].

The main components of the adaptive system are T- and B-cells. T-cell activation is dependent on antigens, presented by antigen-presenting cells (e.g. dendritic cells) with the help of the major histocompatibility complex (MHC) and co-stimulatory signals mediated by CD80 or CD86 at the surface of the antigen-presenting cell. The upregulation of co-stimulatory molecules is controlled by the innate system. Recognition of a microbe or microbial toxin induces the expression of these co-stimulatory molecules. Activated T-cells then either directly attack damaged or infected host cells or secrete signalling

molecules that lead to the activation of other effector cells. B-cells are important for the humoral defence, a non-cell based response mediated by immunoglobulins, proteins or peptides found in body fluids. B-cells produce and release specific antibodies which bind to microbes and their products facilitating phagocytosis and elimination of the harmful microbe. Innate immune responses are not only induced by pathogens, but also by damaged or modified cells releasing damage-associated molecular patterns (DAMPs) [14]. These danger signals are also recognised by pattern recognition receptors on innate immune cells leading to activation of these and also to activation of professional antigen-presenting cells [15].

All processes involved in clearing bacterial infections, are tightly regulated in order to prevent systemic inflammation and activation of the plasma cascades, responses which significantly contribute to the fatal outcome in severe infections such as sepsis [16].

1.2 Inflammation as response to infection

Inflammation can be understood as a complex host response initiated in order to eliminate invading pathogens, but also to ensure healing and repair of injured tissue [17, 18]. The inflammatory response caused by microbes can be summarised in the following steps: recognition of infection, recruitment and delivery of blood components such as complement and coagulation factors as well as immune cells including monocytes and neutrophils to the site of infection, elimination of the microbe and finally restoration of homoeostasis [19]. An acute inflammatory response is initiated either by direct recognition of bacteria and bacterial patterns (exogenous inducers), or indirectly through sensing signals released through cell death or tissue damage caused by bacteria (endogenous inducer) [18]. The recognition of invading microbes by pattern recognition receptors, triggers signalling cascades leading to the release of various inflammatory mediators including cytokines, chemokines, reactive oxygen species, but also proteases or antimicrobial peptides which are important for killing and clearance of pathogens by phagocytosis.

2 Recognition of bacteria

The recognition of extreme heterogeneous microbes is based on highly conserved structures present within the microorganisms, but not the host [12, 20]. These pathogen-associated molecular patterns (PAMPs) are usually invariant, essential structures allowing recognition of an entire class of microbes. For example the Lipid-A portion of lipopolysaccharide (LPS), a main component of the cell wall of all Gram-negative bacteria, presents the invariant pattern recognised independently of the distinct subspecies. Other examples are lipoteichoic acid (LTA), peptidoglycan (PGN) or bacterial proteins like flagellin, but also bacterial DNA or double stranded RNA are recognised [21, 22, 23].

2.1 Pattern-recognition receptors

The sensing perception of pathogen-associated molecular patterns is mediated by specific pattern recognition receptors (PRRs) expressed by cells of the immune system (e.g. macrophages, monocytes, neutrophils). In general PRRs are found at cell surfaces, in intracellular compartments, but also secreted into the blood stream or other bodily fluids. Several different structural types of PRRs have been identified. This includes the Toll-like receptors (TLRs), the RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs) as well as NOD (nucleotide-binding domain)-like receptors (NLRs) and macrophage scavenger receptors (MSR) [17, 24, 25]. Their principal functions are opsonisation and phagocytosis, activation of inflammation facilitating production of various antimicrobials such as antimicrobial peptides or reactive oxygen species, but also activation of the coagulation and the complement system [26].

Based on their function, PRRs can be divided into 3 classes [12]:

- secreted receptors

- endocytic receptors

- signalling receptors

An example for secreted receptors is the mannose-binding lectin (MBL), which upon binding to bacterial carbohydrates, activates the lectin-pathway of the complement cascade (see also section 3.2 The complement system) [27] resulting in cleavage of complement component C3 and the formation of the antimicrobial anaphylatoxin C3a [28]. Moreover, the contact system (see section 3.1 The coagulation system) as part of innate immunity [29, 30] is an another example for secreted pattern-recognition receptors. Recognition of bacteria or bacterial components (e.g. LPS) by direct binding to coagulation factors [31, 32] initiates a cascade resulting in the formation of antimicrobial fragments [29]. The already mentioned macrophage scavenger receptors (MSRs) are an example of endocytic receptors localised at cell surfaces. MSRs bind e.g. lipopolysaccharide thereby facilitating the uptake and clearance of bacteria. This process is interlinked with the TLRs which belong to the signalling receptors [25, 33].

2.2 Toll-like receptors

The Toll-like receptors are probably the most studied pattern recognition receptors. They are named in analogy to the Toll-receptors discovered in the fruit fly *Drosophila melanogaster* [34]. They directly link microbial recognition to the initiation of inflammatory responses e.g. via NF-κB, production of antimicrobial peptides and the control of the adaptive immune system [26]. In humans 10 TLRs (TLR1-10) have been identified and 12 in mice (TLR1-9 and 11-13) [23]. Each human TLR recognises different molecular patterns as summarised in Figure 1 [35]. TLRs are type 1 transmembrane glycoproteins consisting of multiple external horse-shoe shaped leucine rich repeats (LRRs) responsible for pattern recognition and a single intracellular signalling domain homolog to the interleukin-1 receptor (IL-1R) called Toll/IL-1 receptor (TIR) domain [23, 36, 37, 38]. Most of the TLRs form dimer structures upon binding of the respective PAMP. Some TLRs such as TLR4 form homodimers while others such as TLR2 form heterodimers [36].

Furthermore TLRs are expressed at different cell locations (Figure 1), dependently on whether they detecting cell wall components or proteins, or sensing nucleic acids. The latter group has an endosomal localisation [37].
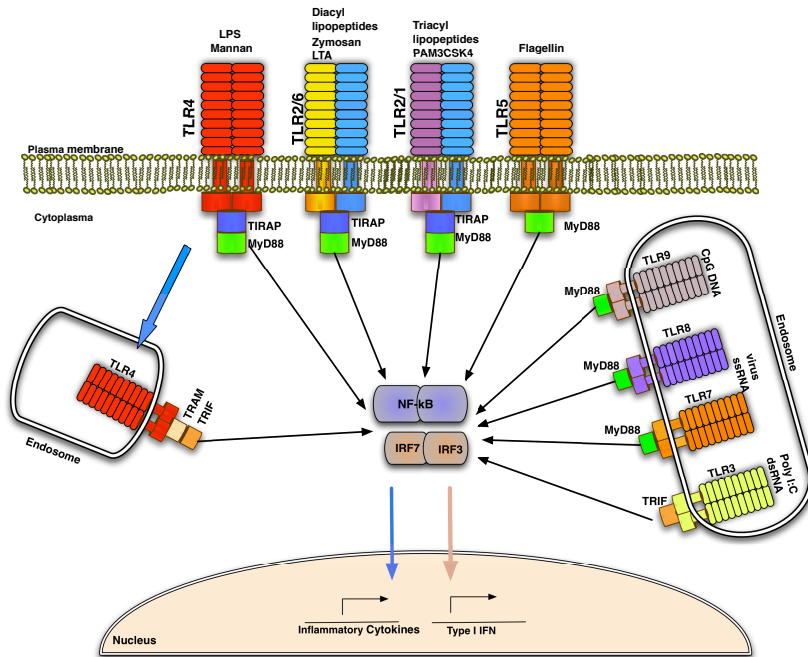
Figure 1: Human Toll-like receptors. Schematic representation of human Toll-like receptors showing adapters, cellular location, and examples of signalling pathways and ligands.

2.3 Toll-like receptor signalling

Upon interaction with bacterial patterns an immune response is initiated by dimerisation of the TLRs. This leads to the recruitment of the following TIR domain containing-cytoplasmic proteins: MyD-88 (myeloid differentiation factor protein 88), TIRAP (TIR domain-containing adaptor protein)

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or MAL (MyD88-adaptor-like), TRIF (TIR domain-containing adaptor inducing interferon-β) and TRAM (TRIF-related adaptor molecule) [20]. The specificity for individual TLRs is realised through receptor-dependent recruitment of various TIR domain-containing adaptors (see Figure 1) [39]. All TLRs, except TLR3, signal via the the MyD88-dependent pathway leading to the production of pro-inflammatory cytokines. TLR1/2, TLR2/6 and TLR4 signalling further involves a combination of the MyD88 and TIRAP/MAL adapters. Moreover, TLR4 and TLR3 trigger responses using a MyD88-independent pathway which includes recruitment of the TRIF (TLR3) or TRIF and TRAM adaptors (TLR4). This pathway is mainly involved in the production of type-1 interferons [23, 39].

Upon assembly of the TIR domain-containing adaptor molecules, MyD88 recruits IL-1 receptor-associated kinase 4 (IRAK4) which binds to the N-terminal death domain of the protein. Phosphorylation of IRAK4 initiates the phosphorylation of IRAK1 which binds to TRAF6 (tumour necrosis factor receptor-associated factor 6) initiating further phosphorylation and ubiquitination of several cytosolic signal proteins including TAK1 (TGF-β-activated kinase 1) and MKK6 (mitogen-activated protein kinase 6). This then activates the transcription factors NF-κB, AP-1 and MAP kinases which then finally induce the expression of pro-inflammatory cytokines such as TNF-α or IL-6. TRAF6 is also important for signalling via the MyD88-independent pathway which leads to activation of IRF3 or IRF7 inducing the expression of interferon -α or β [20, 26, 39].

2.3.1 Recognition of LPS by TLR4

TLR4 recognises multiple pathogen-associated molecular patterns (PAMPs) as well as damage-associated molecular pattern (DAMPs)(e.g. mannan from yeast, virus envelope proteins, HMGB-1)[35], but it is mostly known and studied as the main recognition receptor for lipopolysaccharides (LPS) [40]. In order to sense LPS, three additional proteins are required; the LPS-binding protein (LPB) [41], CD14 and the myeloid differentiation factor-2 (MD-2)[42]. At first LPS is bound by LPB in plasma or at the cell membrane, which subsequently catalyses the transfer of LPS to CD14 [43]. CD14, a glycosylphos-

phatidylinositol (GPI)-linked protein, present as soluble or membrane bound form, further transfers LPS to the TLR4/MD-2 receptor complex. MD-2 is essential for the recognition and cell signalling induced by LPS [42]. Upon binding of LPS to MD-2, which is associated with TLR4, the formation of a homodimer initiates the signalling cascade by transducing the signal to the intracellular TIR domain leading to recruitment of adapter molecules, the activation of NF-κB and MAP kinases [44], and finally to the production of cytokines, chemokines, interferons, co-stimulatory molecules or reactive oxygen species as part of the inflammatory response.

3   Bacterial killing and clearance

Upon recognition of invading pathogens, eradication and clearance become the main focus of the immune defence system. In this stage the coagulation and complement cascades, as well as antimicrobial peptides, proteins and professional phagocytes work in synergy with each other.

3.1   The coagulation system

The coagulation system is an evolutionary highly conserved cascade of reactions based on the rapid and sequential activation of serine proteinases. Coagulation cascades similar to the human system have been identified in various species including Drosophila or the horseshoe crab [45]. The main function of the system is to rapidly form a stable fibrin clot which prevents blood loss, but also the entry and spreading of invading microbes by trapping them inside the clot. Moreover, the coagulation system is involved in the regulation of inflammation [46] and complement cascades via interactions with proteinase-activated receptors (PAR-receptors) [47] or proteins of the complement cascade [48]. Coagulation factors are a source of antimicrobial peptides with the potential to kill invading microbes directly, within a wound environment [29, 49, 50]. Thus, coagulation is an important part of innate immunity aiding in recognition and killing of invading microbes.

The coagulation process can be separated into primary haemostasis involving activation of platelets which form an initial cellular plug and secondary haemostasis, based on the activation of the proteolytic cascade [45]. The initiation of the proteolytic cascade is triggered at negatively charged surfaces such as bacterial membranes (contact activation) [31], but also upon exposure of tissue factor, expressed at the cell surface of endothelial cells or monocytes after stimulation by bacteria or bacterial products (TF-pathway) [51].

Both pathways lead to the activation of factor X (FX) to FXa which transforms prothrombin to thrombin. Thrombin is the central serine proteinase converting soluble fibrinogen to insoluble fibrin. Activated factor XIII (FXIII) is required to stabilise the clot by cross-linking the generated fibrin fibers [52]. This last part of the cascade is referred to as the common pathway. In order to avoid uncontrolled and systemic coagulation, a common complication of severe infections, the initiation of coagulation is tightly regulated by factors that include Kunitz-type protease inhibitors like TFPI-1 or serine proteinase inhibitors (serpins) such as antithrombin III or heparin cofactor II as well as protein C (see Figure 2 for overview) [53].

3.1.1 The contact system

The contact system or intrinsic pathway of coagulation is comprised of three serine proteinases: factor XII (FXII), factor XI (FXI), plasma kallikrein (PK) and the non-enzymatic cofactor, high-molecular weight kininogen (HK) [31]. Under normal conditions these factors are present in blood as zymogens or bound to endothelial cells, platelets or neutrophils [31]. The assembly and activation of coagulation factors takes place at various cell surfaces and on negatively charged non-physiological surfaces e.g. kaolin or glass, but more importantly at bacterial surfaces [31], bacterial membrane components such as LPS [32] as well as at extracellular DNA present in neutrophil extracellular traps (NETs) [54]. The activation of the cascade at physiological surfaces is still not fully understood, but the activation at non-physiolocial surfaces is well described. Initially, FXII is activated by an auto-catalytic reaction resulting in the formation of FXIIa. FXIIa further facilitates the conversion of PK and FXI into their proteolytically active forms.
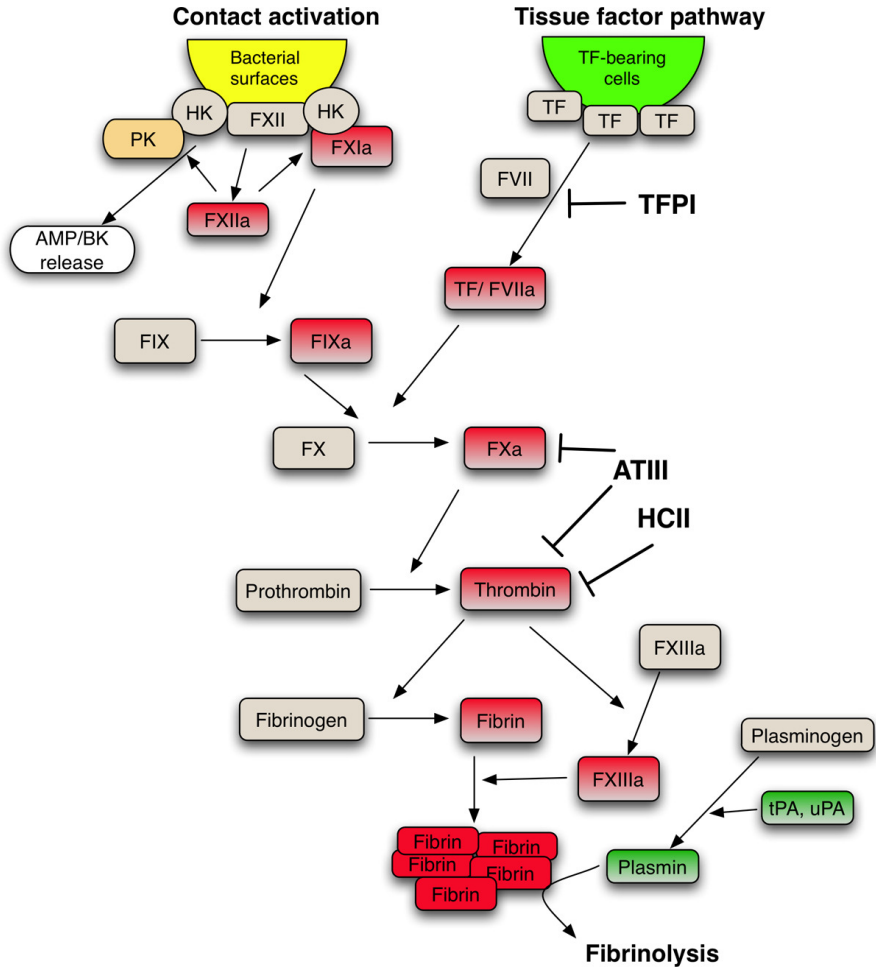
Figure 2: Schematic and simplified representation of the human coagulation system.

FXIa further activates the coagulation cascade leading to activation of FX and thrombin and ultimately clot formation, whereas activated plasma kallikrein cleaves HK. This cleavage leads to the release of bradykinin (BK), a potent pro-inflammatory molecule, mediating an increase of vascular permeability, the generation of nitric oxide (NO) and other inflammatory mediators [31, 55]. Therefore BK plays an important role in the pathogenesis of severe infections. Despite BK, antimicrobial peptides (AMPs) are generated by cleavage of domains D3 and D5 of HK [29, 56]. Interestingly, not only host cell produced enzymes such as neutrophil-derived elastase, but also bacterial-derived enzymes like *Pseudomonas aeruginosa* elastase can generate these antimicrobial fragments [56]. In summary, the contact system has several roles during bacterial infections comprising sensing and entrapment of bacteria, promoting inflammation and finally direct bacterial killing.

3.1.2 Tissue factor pathway

The extrinsic or tissue factor pathway of coagulation is the primary initiator of coagulation *in vivo*. Tissue factor (TF) is a membrane-bound glycoprotein constitutively expressed by extravascular cells e.g. fibroblasts or subendothelial cells which are not in contact with blood [57, 58]. Upon tissue injury TF is exposed to blood and interacts with factor VII (FVII) promoting the activation of FVII to FVIIa which then activates factor IX (FIX), FX and finally thrombin, leading to the formation of a fibrin clot [59, 60]. Moreover, blood circulating monocytes express tissue factor at their surface upon stimulation with bacterial components such as LPS, LTA, PGN or M1 as well as in response to cytokines, especially IL-6 [61, 62, 63, 51]. Furthermore, monocytes can release TF-coated microparticles [64]. TF is the central mediator of systemic inflammation-induced coagulation. Failures in regulating this system have severe consequences. One such coagulopathy, caused by bacterial infections, is disseminated intravascular coagulation (DIC). DIC is characterised by the systemic formation of intravascular thrombi, resulting in reduced platelet counts and reduced levels of coagulation factors. These parameters are directly connected to an increasing risk of organ failure and death [65, 66]. Therefore, the activity of the TF/FVIIa complex and FXa is

normally regulated by tissue factor pathway inhibitor-1 (TFPI-1) [67, 68, 69]. Interestingly, TFPI-1 can be cleaved by neutrophil elastase resulting in the release of antimicrobial peptides from its C-terminus [50].

Furthermore, there is a positive feedback loop connecting coagulation and inflammation. Inflammation is catalysed by the activation of proteinase-activated receptors (PARs). PARs belong to the class of G protein-coupled receptors and 4 types (PAR1-4) have been identified, at the surface or various cell types including endothelial cells, mononuclear cells, platelets or fibroblasts [70]. PAR 1, 2 and 4 can be activated by thrombin and PAR 2 via FXa or the TF/VIIa complex leading to increased inflammation due to augmented release of cytokines and growth factors [70, 47]. These mechanisms further emphasise the pivotal role of coagulation within the inflammatory network triggered by infiltrating bacteria.

3.1.3 Fibrinolysis

The main function of the fibrinolytic system is to balance the formation and degradation of fibrin. During bacterial infections and DIC an impaired fibrinolysis is observed [66]. The most important protease of the fibrinolytic system is plasmin. Plasmin is generated from its precursor plasminogen upon activation by tissue plasminogen activator (tPA) or the urokinase-like plasminogen activator (uPA). The main substrate for plasmin is fibrin. Fibrin regulates its own cleavage through binding to plasminogen or tPA and thereby enhancing plasmin formation. This mechanisms can be regulated by the thrombin-activatable fibrinolysis inhibitor (TAFI). Further plasminogen is inhibited by the plasminogen activated inhibitor-1 (PAI-1) and plasmin is blocked by $\alpha2$-plasmin inhibitor and α-2 macroglobulin [71].

3.2 The complement system

The complements system is a highly preserved, complex proteolytic cascade and plays a crucial role in innate defence against bacteria, but also in the adaptive immune system. The main complement functions are: recognition of pathogens, opsonization, killing and clearance of pathogens and cellular debris or apoptotic cells and activation and recruitment of leukocytes [72, 73]. Furthermore, complement factors also modulate the coagulation system and inflammatory responses, by interacting with coagulation proteins and cells [74]. Upon initiation of the system, a sequence of complement factors cleavage occurs. Cleavage of the complement components C3, 4 or 5 results in small (designated a) and bigger (designated b) fragments with different biological functions. The small fragments are released and can bind to their specific receptors at cell surfaces of multiple cells e.g. monocytes, macrophages, neutrophils, T-cells or dendritic cells, and thereby mediating inflammation and cell recruitment [74]. The larger moieties like C5b and C3b, directly bind to microbes promoting killing and phagocytosis through opsonization (Figure 3).
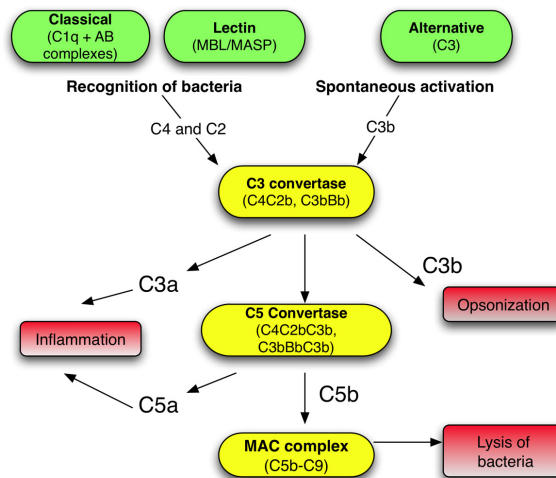

Figure 3: The human complement. Schematic summary of the human complement system illustrating, initiation, central cascades and functions.

3.2.1 Complement activation

There are three main pathways that lead to initiation of the complement cascade: the lectin, the classical and the alternative pathway.

Lectin pathway: The lectin pathway of complement serves as pattern recognition receptor. Mannose-binding lectins (MBL) or ficolins recognise specific oligosaccharide moieties (e.g. mannose or N-acetyl-glucosamine) at the bacterial membrane, based on their steric and spatial organisation. However MBLs do not recognise carbohydrates commonly found at mammalian cell surfaces such as galactose or sialic acid [75]. Correspondingly, it is not surprising that recognition of different Gram-negative bacteria by MBL depends on the structure of their LPS [75]. Subsequently, upon bacterial recognition MBL-associated serine proteinases (MASPs) are activated.
These MASPs cleave complement components C4 and C2 to form the C3 convertase complex consisting of C4bC2a. Alternatively, MASPs directly cleave C3 which can activate the alternative complement pathway [27].

Classical pathway: This pathway is primarily initiated by forming an antibody-mediated immune complex. The complement component C1q is the recognition subunit of this complex and binds to a variety of targets including IgM or IgG-bearing immune complexes. Upon recognition by C1q a sequence of cleavage of complement factors (e.g. C2 and C4) by the serine proteinases C1s leads to formation the C3 convertase, C5 convertase and the terminal MAC complex (for review see [76, 77]). Despite antibody-dependent activation, it was also shown that other molecules like for example the C-reactive protein (CRP) or LPS can activate the classical pathway in an antibody-independent process [78, 79, 80].

Alternative pathway: This pathway is spontaneously activated at the surface of invading pathogens or apoptotic cells. Cell surface bound C3b interacts with the plasma protein factor B resulting in cleavage and activation of factor B by the serine proteinase factor D. Whereas the resulting fragment Ba is

released into the plasma, Bb remains in complex with C3b and forms another C3 convertase (C3bBb) complex. This C3 convertase also cleaves C3 into C3a and C3b, forming the alternative C5 convertase (C3bBb3b), thereby amplifying complement cascades induced via the classical or the lectin pathway [74, 81].

In summary, all three pathways lead to the formation of the C3 convertase complex resulting in cleavage of the third complement component (C3) into C3a and C3b and further in the formation of a C5 convertase complex resulting in fragmentation of the fifth complement component (C5) into C5a and C5b. Additionally, to the already mentioned activation pathways, thrombin can directly cleave C3 or C5 and thereby also activate the system [48, 82]. Finally, in the lytic or termination pathway, the lipophilic membrane-attack complex (MAC) will be assembled at the pathogen surface. Pore formation by the MAC finally leads to cell death (summery figure 3). In addition, it was discovered that the C-terminal regions of the released anaphylatoxins C3a and C4a, but not C5a are also directly antimicrobial [28, 83]. The importance of an intact complement system is illustrated by various studies showing a direct link between complement deficiency and increased susceptibility for certain bacterial infections e.g. meningitis caused by *Streptococcus pneumoniae* or *Neisseria meningitidis* [84, 85] or post burn infections with *Pseudomonas aeruginosa* [86].

3.2.2 Complement evasion by bacteria

Although the complement system is highly efficient in killing and clearing invading microbes, bacteria have developed mechanisms to avoid or hijack the system. They use various strategies to counteract the complement system which can be summarised as follows: recruitment or mimicry of complement regulators, modulation/inhibition of complement components by direct interaction or enzymatic cleavage, prevention of lysis by the MAC through their specific cell wall structure (e.g. thick peptidoglycan layer of Gram-positive bacteria) or production of capsules which protect them from lysis (as reviewed by [87]). *Pseudomonas aeruginosa* strains are able to evade complement me-

diated killing by degradation of complement factors. C3 can be cleaved by Pseudomonas elastase [88, 89] or as recently discovered the Pseudomonas alkaline protease (AprA) cleaves complement component C2 thereby inhibiting activation of the classical and lectin pathway as well as the formation of the C3 convertase and killing by the MAC [90].

3.3 Host defence peptides and proteins

Host defence peptides

Host defence peptides (HDPs) are short (< 60 amino acids), mostly gene encoded, cationic, amphipathic molecules with heterogeneous structures and multiple modes of action in regard to bacterial killing, but also immunomodulation. These important components of innate immunity were discovered and caught interest due to their rapid and broad antimicrobial activity (antibiotic-like) and were therefore termed antimicrobial peptides (AMPs). But due to emerging evidence that these peptides exert various biological functions including chemotaxis, modulation of immune responses and coagulation apart from bacterial killing the term host defence peptides is often used today [91]. Antimicrobial peptides are produced by various organisms including bacteria, fungi, insects, plants, amphibia, fish or mammals, and form a first line of non-specific defence, against invading pathogens [92]. According to their amino acid composition, charge and structure, they can be classified into the following groups:

- cationic α-helical peptides (e.g. LL-37 [93], GKY25 [49])

- peptides with β-sheet structures stabilised by disulfide bridges (e.g. defensins [94])

- peptides with an over presentation of one or more amino acid (e.g. histatin [95])

- anionic peptides (e.g. dermicidin [96])

In humans, the most reviewed and prominent host defence peptides are defensins, cathelecidins and histatins. These peptides are expressed/stored as

precursors and will be processed and released upon various stimuli [92, 97]. Moreover, work by Schmidtchen and others demonstrated that the group of human endogenous peptides generated by cleavage from other proteins belonging for example to the complement or coagulation cascade are also important in innate defence against pathogens [28, 50, 49, 56, 98]. A main characteristic of these peptides is the heparin-binding region, which can be used to screen for these novel endogenous peptides [99]. In the following section only the two major classes of expressed human host defence peptides and peptides generated by cleavage will be discussed.

3.3.1 Defensins

Defensins are a highly abundant class of HDPs, expressed in cells and tissues closely linked to host defence against bacterial infections. High concentrations of defensins are present in granules of neutrophils and Paneth cells [94, 100]. Further, human defensins have been found in monocytes, macrophages, dendritic cells, keratinocytes and various epithelial cells [101, 102, 94, 103]. Mature defensins are short peptides (ranging from 18-45 amino acids), containing six cysteines which form three intramolecular disulfide bonds, have a cationic net-charge and lack glycosyl or acyl-side chain modifications. Defensins are synthesised as prepropeptides consisting of a signal peptide, an anionic pro-segment and a cationic C-terminus. Depending on their number of amino acids and structure, defensins are divided into three subclasses: α-defensins, β-defensins and θ-defensins. The latter class of cyclic defensins are produced in rhesus macaques (*Macaca mulatta*) and baboons (*Papio anubis*), but not in humans, due to a stop codon within the signal peptide [104]. The human α-defensins and β-defensins share an overall triple-stranded β-sheet conformation, but are different in their disulfide bridge pattern and spatial distance between their cysteine residues [94, 100].

α-*Defensins*: Six human α-defensins have been discovered. Four of these are produced in the azurophil granules of neutrophils, therefore also termed human neutrophil peptides (HNP 1-4) [105, 106]. More recently they were also discovered in monocytes and natural killer cells (NK cells) [100]. The

other two (HD5 and 6) were discovered in Paneth and multiple epithelial cells [101, 103]. α-Defensins consist of 29-34 amino acids with the disulfide pattern aligned between cysteines 1-6, 2-4, and cysteines 3-4 [107]. Functionally, they play a role in inflammation, wound repair and bacterial killing, probably within the phagolysosome in which they are highly concentrated [108, 109].

β-Defensins: Compared to α-defensins, these peptides consist of 36-42 amino acids and the cysteine motifs are stabilized by disulfide bridging between cysteines 1-5, 2-4 and cytsteines 3-6 [108]. Moreover, they possess a longer N-terminal region. Four β-defensins (hBD 1-4) have been discovered in human epithelial-tissues including skin and the respiratory tract as well as leukocytes, where they are either constitutively expressed (e.g. hBD1) or induced by various inflammatory stimuli like LPS, TNF-α or bacteria [100, 110]. β-Defensins possess antimicrobial properties in vitro, but whether this is true for the in vivo situations needs to be further investigated [110]. The immunomodulatory properties of β-defensins are more defined. It has been shown that β-defensins can interact with cell surface receptors on for example leukocytes or keratinocytes, thereby inducing pro-inflammatory mediators such as IL-6 or MCP-1 [110, 111]. However it was also shown that for example hBD3 can block LPS-induced cytokine responses in vitro and in vivo [112]. Other possible functions are in wound healing, fertility and cancer (for review see [110]).

3.3.2 Cathelicidins

Cathelicidins are the second important group of antimicrobial peptides produced by mammals. Similarly to defensins they are expressed as prepropeptides. All precursors consist of a highly conserved preproregion containing the signal peptide at the N-terminus, a conserved pro-region in the middle and a highly variable C-terminus which contains the antimicrobial sequence [113]. The name of this group of peptides was termed by Zanetti et al. and is based on the similarity of the pro-sequence with cathelin [113], a Cathepsin L inhibitor isolated from porcine neutrophils [114]. The only human member is the human cationic antimicrobial protein (hCAP-18, 18kDa) [115]. hCAP-18 is stored in the specific granules of neutrophiles [116] and expressed in subpopulations

of lymphocytes and monocytes [117], various epithelial cells [118, 119, 120], but also in seminal plasma [118], by keratinocytes during inflammation [121], mesenchymal stem cells [122] as well as cancer cells [123]. The mature antimicrobial peptide LL-37 is released from the C-terminus of hCAP-18 upon extracellular cleavage with neutrophil-derived proteinase 3 [124]. Another hCAP-18-derived antimicrobial peptide ALL-38 is released by cleavage with gastricsin, a prostatate-derived protease [125].

LL-37: The cationic, α-helical peptide LL-37 exerts a broad range of different functions, including antimicrobial activity against Gram-negative and Gram-positive bacteria, fungi, as well as viruses. LL-37 can bind LPS and inhibit LPS-induced responses of various cells including monocytes/macrophages and neutrophils *in vitro* and *in vivo*, function as a chemoattractant for e.g. monocytes and neutrophils, but is also involved in wound healing, angiogenesis and cancer (for review see [126, 127, 128]). Considering data showing, that the antibacterial effect of LL-37 can be blocked by high concentrations of NaCl, glycosaminoglycans (GAGs) (present in wound fluids) [129], DNA [130] or in presence of saliva [131], the *in vivo* relevance of the described broad antimicrobial effects exerted by LL-37 *in vitro* might be questionable. Finally, due to the broad spectrum of biological functions, especially in response to infection, LL-37 serves as template for the generation and development of novel synthetic peptide antibiotics for the treatment of severe infections [132, 133].

3.3.3 Proteolytically generated HDPs

During an infection, not only the described HDPs are released, but also other molecules including proteases like elastase (host or bacterial-derived) which can cleave for example complement and coagulation factors, in their surrounding and thereby releasing antimicrobial peptides, of which some will be briefly described in the following section. Interestingly, fragments containing heparin-binding sequences, were demonstrated to exert antimicrobial activities [99, 134].

Complement-derived HDPs: During activation of the complement the ana-phylatoxins C3a, C3a-derived peptides and C4a are generated. These peptides possess antimicrobial activity against various bacteria and fungi and have im-munomodulatory functions [28, 83, 135].

Lactoferrin-derived HDPs: Lactoferrin (LF) is an iron-binding glycoprotein, found in mammalian milk, saliva, bronchial mucus, seminal plasma and in the specific granules of neutrophils. Lactoferrin-derived HDPs called lactoferri-cins are released by pepsin, but also by bacterial and mammalian proteases [136, 137].

Coagulation-derived HDPs: The cleavage of domain D3 of high molecular weight kininogen (HK) by neutrophil elastase, releases fragments containing the antimicrobial sequence NAT26 [29]. Moreover cleavage of domain D5 of HK by neutrophil or *Pseudomonas aeruginosa* elastase releases antimicrobial peptides [28]. Cleavage of tissue factor pathway inhibitor-1 and 2, regulators of the TF pathway, also leads to the release of C-terminal peptides which execute various HDP functions [50, 138]. The properties of the TFPI-2 derived peptide EDC34, are described in **paper III** of the thesis. Moreover, cleavage of thrombin by neutrophil elastase, revealed the release of HDPs from the C-terminus (e.g. HVF18) *in vitro* and *in vivo* [49]. The functions of HVF18 and the prototypic GKY25, especially the anti-endotoxin as well as anticoagulant effects are discussed in **papers I and II** of the thesis. Finally, cleavage of fibrinogen by thrombin and plasmin results in fibrinogen-derived peptides that have host defence functions [139, 140, 141].

3.3.4 Mode of action of HDPs

HDPs execute multiple functions [93, 142]. There are several mechanisms de-scribed showing how these peptides kill bacteria. Less data are provided for the anti-inflammatory functions executed by various peptides. This section will briefly describe the antimicrobial mechanisms and anti-endotoxin proper-ties.

Models of antimicrobial activity: HDPs can kill bacteria either by binding to the membrane and thereby disturbing the structure, but also by targeting intracellular processes essential for bacterial survival [143]. How a peptide is acting depends on its amino acid sequence influencing hydrophobicity, charge and structure, membrane lipid composition and also the peptide concentration. The selectivity of peptides for prokaryotic membranes, compared to eukaryotic membranes is essentially based on differences in the lipid composition. Whereas bacterial membranes are composed of negatively charged phospolipids creating an overall negatively charged surface, are eukaryotic membranes composed of more neutral phospolipids and cholesterol. There are different models proposed how these cationic, amphipatic peptides interact with bacterial membranes leading to pore formation and lysis of the bacterium. But for all in common, is the initial step in which the peptides interact with the negatively charged lipids, adopting an orientation parallel to the membrane [143].

The aggregate model: This model suggests that the peptide interaction with the membrane, results in the formation of informal channels with different sizes and lifetime. In contrast to e.g. the toroidal model, peptides do not adopt a particular orientation, but also span the membrane by forming aggregates with micelles-like complexes of peptide and lipids. Further due to the informal nature of the channel, the peptide might be able to translocate through the bilayer as the aggregate collapses. This was shown for the horseshoe crab-derived peptide polyphemusin [144, 143].

The barrel-stave model: Upon aggregation at the membrane, multimeres of helical peptides form a stable transmembrane pore which leads to leakage of intracellular material and thereby to death of the cell. The peptides insert themselves into the membrane bilayer in a way that the hydrophobic part aligns with the lipid core and the hydrophilic part is directed inwards forming the inner pore region [145, 146]. A well described example is alamethicin a 20-residue peptide isolated from the fungus *Trichoderma viride* [147]. Also dermicidin, a human peptide found in sweat was shown to kill bacteria by forming barrel-stave like channels [148].

The toroidal model: The toroidal or worm whole model, is similarly to the barrel-stave model in respect of forming a transmembrane pore. A major difference is the structure of the pore formed. In this model the hydrophilic part of the peptide monomers associates with the polar head group of the lipids inducing the lipid monolayers to bend inwards, leading to a curvature strain in the membrane. Therefore the polar head groups of the lipids as well as the hydrophilic part of the peptides form a hydrophilic lining through the pore [146, 149]. Magainin 2, a peptide found in the african clawed frog as well as the human LL-37 have been shown to act via this model [149, 150].

The carpet model: The cationic peptides are spread out across the membrane, and their hydrophilic parts interact with the polar head groups of the lipids. This leads to changes in membrane fluidity, bilayer curvature and integrity. Upon reaching a certain critical concentration the membrane disrupts in a detergent-like fashion, which might lead to micelle formation and leakage [145, 146]. Interestingly, LL-37 was also shown to kill bacteria by this mechanism [151]. Another example is dermaseptin a frog-derived peptide [145].

Non-membrane mechanisms: There are peptides which are able to traverse into the cell and inhibit nucleic acid synthesis, protein synthesis, enzyme functions or cell wall synthesis, thereby killing the bacterium [146]. An example is the human histatin 5, a cationic histidine-rich peptide. Histatin 5 binds to a receptor at the cell surface and is actively taken up into the cell, where its targeting mitochondria, inducing efflux of ATP and potassium ions [97] (for review see [146]).

Immunomodulation: As already mentioned, most HDPs exert various biological roles within the immune system. These functions include recruitment of cells like monocytes, neutrophils and immature dendritic cells, inhibition of pro-inflammatory responses, stimulation of cell proliferation, angiogenesis, promotion of wound healing, but also modification of eukaryotic cell, gene and protein expression as well as killing of cancer cells. [95, 152, 93].

LPS-inhibition: LPS is a key initiator of septic shock by Gram-negative bacteria. Even though it is a well established property of various HDPs to inhibit LPS-induced cell responses it is not clarified how the inhibition works. The most obvious scenario is scavenging of extracellular LPS by the peptide through direct interactions. Various studies have proposed that peptide binding to LPS changes the aggregate structure of LPS [153, 154] which is necessary to induce cell responses [155]. Furthermore, peptide binding to LPS, might prevent LPS interactions with the LPS-binding protein (LBP), which shuttles LPS to its receptor [154, 156]. Further, the interaction between LPS and CD14 might be blocked leading also to the prevention of LPS signalling [154]. All these mechanisms aiming at extracellular quenching of LPS. Another possibility of preventing LPS-induced responses is by targeting molecules within the TLR4 pathway. The relevance of this was shown for LL-37 and also β-defensin 3. Both peptides inhibit LPS induced responses by targeting parts of the NF-κB pathway [157, 158]. LL-37 binds to GAPDH which inhibits MAP kinase signalling [159]. How and whether LPS-inhibition is performed by thrombin-derived and TFPI-2-derived peptides is content of **papers I-III** of the thesis.

3.3.5 Host defence proteins

In addition to HDPs, proteins also have antimicrobial as well as immunomodulatory properties, aiding in killing and clearance of invading pathogens. One example is the heparin-binding histidin-rich glycoprotein (HRG) (for review see [160]). HRG is antimicrobial, but has also antifungal properties *in vitro* and *in vivo* [161, 162, 163]. Furthermore HRG binds, amongst various other proteins, IgG and these complexes facilitating phagocytosis and clearance of apoptotic and necrotic cells [164]. Another multitasking protein is activated protein C (APC). APC is antithrombotic, but also anti-inflammatory and profibrinolytic [71]. It is generated by the thrombin-thrombomodulin complex from its precursor protein C, which is reduced in patients with sepsis [166]. Studies showed reduced sepsis mortality in animals and initially in humans by treatment with APC [165, 166]. Interestingly, the inhibitor of protein

C (PCI), which belongs to the class of serine proteins inhibitors (serpins), is antimicrobial [167]. In line with this, are the findings in **paper IV** showing a novel antimicrobial activity of the serpin heparin cofactor II, which otherwise blocks thrombin activation.

3.4 Phagocytosis

Phagocytosis is an essential mechanism that is part of the innate and also adaptive immunity. Phagocytosis is a complex receptor-mediated and actin-dependent process, leading to engulfment, uptake and destruction of microbes or particles [168]. Professional phagocytes like neutrophils, macrophages, and dendritic cells posses a set of specialised phagocytic receptors, such as the already described PRRs e.g. scavenging receptors. The destruction of internalised microbes or particles takes place in a membrane-bound vacuole called the phagosome. The phagosome matures by fusion with endosomes and lysosomes to the phagolysosome which is filled with antimicrobial and degrading agents [169]. The uptake of microbes can be enhanced by opsonisation with for example C3b,C1q or IgG. Phagocytosis of pathogens usually triggers the release of pro-inflammatory cytokines, whereas phagocytosis of apoptotic cells does not [168]. Even though phagocytosis is a highly effective mechanism to eradicate invading pathogens, some bacteria have developed mechanisms to prevent killing and or phagocytosis. These bacteria (e.g. *Pseudomonas aeruginosa, Streptococcus sp.*) either avoid phagocytosis by producing capsules thereby preventing uptake or they are internalised, but harbour strategies for intracellular survival as it has been shown for *Mycobacterium tuberculosis* and *Salmonella sp.* [169].

4 Severe bacterial infections

The innate immune defence comprises multiple mechanisms which in synergy recognise, destroy and finally clear the system from invading pathogens, as described above. Are these processes not properly controlled infections can rapidly progress into life threatening sepsis, severe sepsis and finally septic shock. Studies showing, that about 10 % of patients remitted to the emergency department will develop severe sepsis or septic shock within the first 24 hours from presentation [170, 171]. Therefore, early diagnostic and adequate treatment is important for survival of the patient [172, 173].

4.1 Definition of sepsis, severe sepsis and septic shock

Over the last 20 years international consortia agreed on definitions for the systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis and septic shock to provide consensus for clinical trials and guidelines for sepsis treatment [174, 173, 175] (Figure 4).

Systemic inflammatory response syndrome (SIRS) is the host response either to an infection, or a response triggered upon trauma, burns or pancreatitis. Patients are diagnosed with SIRS if they show at least two of the four minimum criteria: alterations in body temperature, changes in white blood cell counts, an increased heart rate or hyperventilation.

Sepsis is characterised by a systemic inflammatory response syndrome (SIRS) caused by an underlying infection.

Severe sepsis is defined as sepsis with disfunction of one or more organ.

Septic shock is defined by a state of acute circulatory failure characterised by a persistently low mean arterial blood pressure, despite adequate fluid resuscitation.
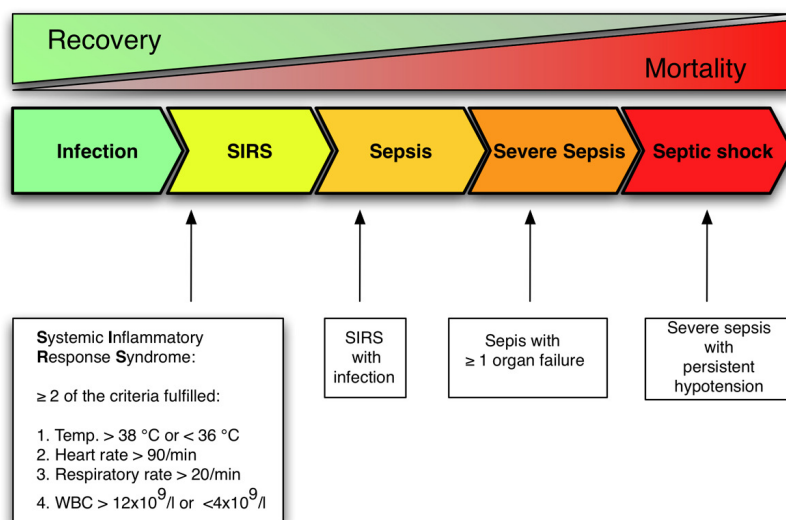
Figure 4: Illustration of the sepsis continuum.

4.2 Pathogenesis

Gram-positive and Gram-negative bacteria are the main inducers of sepsis, but also fungi, viruses and parasites can cause the disease. Amongst the most common bacteria to cause sepsis are the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumonia* and the Gram-negative *E. coli* bacteria [176].

The most common infections sites are the lungs, followed by the abdomen, the urinary tract, the skin and soft tissues [170, 177, 5]. During the initial state of sepsis, recognition of invading microbes and danger signals triggers systemic host responses including the release of cytokines (e.g. TNF-α), chemokines, acute phase proteins or other danger-associated molecules (e.g. HMGB-1). The excessive release of pro-inflammatory mediators, the so called cytokine storm, mediates the progression from an initially beneficial response towards a harmful one [7]. Invading bacteria and the presence of bacterial products in the blood then systemically activate the coagulation and complement cascades

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[178]. Tissue factor-induced coagulation is regarded as the primary initiator of coagulation during sepsis [179]. Further anti-coagulant mechanisms involving antithrombin III (ATII), tissue factor pathway inhibitor (TFPI) or protein C are impaired as well as fibrinolysis. This results in a systemic procoagulant state reflected by high plasma levels of prothrombin fragments (F1+2), prothrombin-antithrombin complexes (TATc) and decreased numbers of platelets. The consumption of coagulation factors results in prolonged prothrombin clotting times (PT) and a prolongation of the activated partial thromboplastin time [16].A consequence of uncontrolled inflammation-induced coagulation is disseminated intravascular coagulation (DIC) which can cause organ dysfunction [66]. Thus, uncontrolled coagulation significantly contributes to organ failure and death [180, 181]. An enhanced activation of endothelial cells results in increased vascular permeability, thereby augmenting neutrophil migration and vascular leakage. Recruited immune cells like neutrophils release proteinases or other enzymes as well as reactive oxygen species (ROS) which also contribute to tissue damage and organ failure. The cross-talk between cell-mediated inflammation, coagulation and complement creates a positive feedback loop further amplifying the pro-inflammatory response. Finally, the sustained pro-inflammatory condition, leads to a state of immunosuppression due to apoptosis of immune cells and the consumption of platelets, coagulation factors and other mediators. Patients in this stage have an increased susceptibility to secondary infections [7].

4.3 Treatment

Treatment of severe infections like sepsis to date, is based on mitigation of the occurring symptoms and the source of infection by using antibiotics, oxygen and fluid resuscitation [173]. Increasing insight into the pathophysiology of sepsis and the problematic of increasing antibiotic resistance, initiated the design and study of treatments aimed to block initiation of the pro-inflammatory responses. These concepts aim to prevent bacterial recognition by TLRs [182], but also inflammatory mediators such as TNF-α [10]. So far most of these strategies targeting one particular molecule failed in clinical

trials [183], probably explained by the complex nature of the disease. Even the FDA (Food and Drug Administration) approved anti-inflammatory, anti-coagulant and profibrinolytic activated protein C (APC), finally failed to show a significant reduction of mortality of sepsis patients at day 28 compared to regular sepsis treatments in clinical trials [9], but targeting several systems seems promising. Therefore, antimicrobial or host defence peptides gained interest. Most of them, directly target and kill a broad spectrum of bacteria, but posses also immunomodulatory functions. Recent experimental studies, including paper I and III of this thesis, provide promising data which suggest that treatment of sepsis by using endogenous or synthetic host defence peptides might be an option [91, 184, 185].

5 Present investigation

5.1 Paper I

Host defence peptides of thrombin modulate inflammation and co-agulation in endotoxin-mediated shock and *Pseudomonas aeruginosa* sepsis

Background: Systemic bacterial infections, causing sepsis and septic shock remain challenging and a leading cause of death world wide [1]. Although standard care procedures have improved, the mortality for sepsis and septic shock remains high, and the incidence of sepsis is increasing [186, 6]. The increase of antibiotic resistant bacteria and the current lack of other effective treatments, clearly illustrates the urgent need for the development of new treatment strategies. Host defence peptides are an important component of innate immunity facilitating eradication of invading microbes by being direct antimicrobial, but also immunomodulatory [92, 95, 93]. In a previous study by Papareddy et al. [49] it was shown that proteolysis of human thrombin by neutrophil elastase leads to the release of thrombin C-terminal peptides (TCPs) *in vitro* and *in vivo*. These peptides were antimicrobial against Gram-positive and Gram-negative bacteria as well as fungi. Furthermore, the prototypic thrombin C-terminal peptide (TCP), GKY25 was anti-inflammatory and reduced bacterial levels in initial *in vivo* experiments [49].

Aims of paper I :

- To investigate the role of peptide interactions with LPS as determined *in vitro* for the anti-inflammatory effects observed *in vivo*.

- To evaluate the importance of direct antimicrobial effects of TCPs for the outcome in a mouse model of *Pseudomonas aeruginosa* sepsis.

- To investigate other peptide mediated responses explaining the beneficial effects of TCPs *in vivo*.

Results and conclusions: The thrombin C-terminal peptides GKY25 and HVF18 abrogated LPS-induced pro-inflammatory responses *in vitro* and *in vivo*. Furthermore, experiments using a LPS-binding control peptide indicated that the anti-inflammatory effects of GKY25 are not solely due to direct LPS-peptide interactions. Moreover, we discovered that these thrombin-derived peptides significantly modulate LPS or bacteria-induced coagulation responses. Modulation of contact activation and especially tissue factor-mediated coagulation by GKY25 and HVF18 lead to normalisation of coagulation parameters *in vivo*. Finally, the results from a mouse model of *Pseudomonas aeruginosa* sepsis, suggest that the improvement of animal status (decreased fibrin deposition and leakage in the lungs) and survival is mainly dependent on the modulation of inflammation and coagulation by GKY25. Taken together, thrombin-derived peptides simultaneously modulate bacterial levels, pro-inflammatory responses, and coagulation and are therefore attractive therapeutic candidates for the treatment of invasive infections and sepsis.

5.2 Paper II

The thrombin-derived peptide GKY25 modulates endotoxin-induced responses through direct interactions with macrophage/monocyte cell membranes

Background: Monocytes and macrophages are important modulators of the inflammatory response induced by invading microbes [187]. They sense conserved pathogen-derived molecular patterns, with the help of pattern recognition receptors (PPRs). For example TLR4 recognises LPS, which is a main cell wall component of Gram-negative bacteria [17, 24]. It has been shown that cationic peptides like LL-37 can interfere in the recognition of LPS by blocking the binding of LPS to the LPS-binding protein (LBP) [156], but also by modulation of the TLR4 pathway [130, 158]. In paper I and other publications [49] we showed that GKY25 interacts with bacteria and LPS, but the detailed mode of action explaining the anti-inflammatory effects of GKY25 was not clarified.

Aims of paper II:

- To characterise and clarify how GKY25 modulates LPS-induced responses of monocytes and macrophages.

Results and conclusions: GKY25 significantly reduced NF-κB activation by various stimuli including zymosan, LTA and LPS. The peptide also prevented LPS-induced dimerization of the TLR4 receptor. Dimerization is important for LPS recognition and subsequent production of cytokines. Further, FACS studies using TAMRA-labelled GKY25 showed binding of GKY25 to monocytes and macrophages, independent of the presence of heparin or LPS. Less peptide-binding was observed at low temperatures, indicating an interaction of GKY25 with the cell membrane. In summary, the data in this study show that GKY25, apart from binding to lipopolysaccharide (LPS), directly interacts with monocytes and macrophages *in vitro, ex vivo* and *in vivo*. The data also suggest that the cell-binding property is important for the observed reduction in NF-κB activation and cytokine release.

5.3 Paper III

The TFPI-2 derived peptide EDC34 improves outcome of Gram-negative sepsis

Background: Tissue factor pathway inhibitor 2 (TFPI-2) belongs to the class of Kunitz-type serine proteinase inhibitors [188]. TFPI-2 is secreted by various cells including fibroblasts and endothelial cells [189, 190]. TFPI-2 can be cleaved by neutrophil elastase which leads to the release of C-terminal fragments [138]. C-terminal fragments were detected in wound material at the surface of bacteria indicating a role of TFPI-2 during wounding. EDC34, a prototypic peptide, representing the released C-terminal part was binding to bacteria as well as LPS. Furthermore EDC34 was antimicrobial against both Gram-negative and Gram-positive bacteria [138].

These properties make EDC34 an interesting novel candidate for the development of new strategies against bacterial infections.

Aims of paper III:

- To characterise the antimicrobial properties *in vitro* and *in vivo*.

- To determine whether EDC34 possess anti-coagulant activities.

- To investigate potential anti-inflammatory properties based on the demonstrated direct interactions with LPS.

Results and conclusions: EDC34 exerted direct bactericidal effects and boosted activation of the classical complement pathway including formation of antimicrobial C3a. Moreover EDC34 significantly reduced contact activation at negatively charged surfaces as well as at bacterial surfaces. This inhibition of contact activation also prevented cleavage of high molecular weight kininogen (HK) and the release of bradykinin, an important pro-inflammatory mediator. In mouse models of severe *E. coli* and *P. aeruginosa* infection, treatment with EDC34 reduced bacterial levels and modulated coagulation responses. In another study, treatment of severe *P. aeruginosa* infection with a combination of EDC34 and the antibiotic ceftazidime significantly improved survival. In conclusion, EDC34 boosts bacterial clearance and inhibits excessive coagulation, but does not have direct anti-inflammatory properties.

5.4 Paper IV

Proteolytic activation transforms heparin cofactor II into a host defence molecule

Background: Serine proteinase inhibitors (serpins) are the most abundant proteinase inhibitors in humans, regulating various proteolytic pathways including coagulation [191, 192, 193]. Heparin cofactor II (HCII) belongs to the class of serpins and specifically inhibits thrombin, probably within the extravascular space, and the inhibition is enhanced in the presence of dermatan

sulfate [68, 194]. Cleavage of HCII releases a chemotactic peptide from the N-terminal tail [195]. Interestingly, an inherited deficiency in antithrombin III, the other thrombin inhibitor is associated with thrombotic disorders, but humans or mice deficient in HCII do not present any evidence for thrombophilia under normal conditions [193]. Moreover, reduced plasma levels of HCII are primarily detected during infection. This lead to the hypothesis that HCII has a role in host defence against infections.

Aims of paper IV

- Comparison of intact and neutrophil elastase cleaved HCII in respect to interactions with bacteria.

Results and conclusions: Cleavage of heparin cofactor II with human leukocyte elastase (HLE) results in a major fragment with a size of about 50 kDa. Furthermore electron microscopy studies, in combination with LPS and bacterial binding assays, revealed that proteolytic cleavage of HCII induces a conformational change, thereby uncovering endotoxin-binding and antimicrobial properties in the molecule. These properties were mapped to the heparin binding helices A and D. Heparin cofactor II deficient mice showed increased susceptibility to invasive infection by *Pseudomonas aeruginosa* compared to wilde type animals. Wilde type animals challenged with bacteria or endotoxin had decreased levels of HCII. Cleaved forms of HCII were also detected in human wound samples in association with bacteria. In summary, the data in paper IV demonstrate a previously unknown role for HCII in host defence

5.5 Summary

In this work, interactions of human host defence peptides and proteins with the pro-inflammatory network, initiated during bacterial infections, have been investigated. The discovered properties are summarised in figure 5. The diagram illustrates that all three groups of host defence molecules directly target bacteria, as well as the coagulation system. Thrombin-derived peptides showed significant anti-inflammatory effects. The TFPI-2-derived peptide EDC34 enhanced complement-mediated bacterial killing, but did not have direct anti-inflammatory properties. In conclusion, host defence peptides of human thrombin and tissue factor pathway inhibitor 2, are promising candidates for the development of new therapies targeting severe bacterial infections.
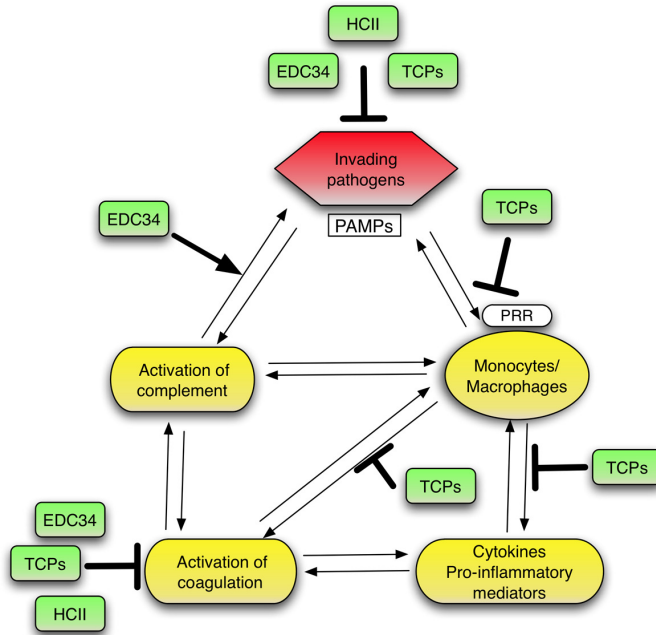

Figure 5: Summary of discovered interactions of the TFPI-2-derived peptide EDC34, thrombin C-terminal peptides (TCPs) and heparin cofactor II (HCII) with the inflammatory network in sepsis. (PRR - pattern recognition receptor)

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References

[1] C. Mathers, D. M. Fat, J. T. Boerma, and World Health Organization. *The global burden of disease : 2004 update.* Geneva, Switzerland : World Health Organization, 2008.

[2] S. L. Murphy, J. Xu, and K. D. Kochanek. Deaths: Preliminary Data for 2010. *National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System*, 2012.

[3] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Critical Care Medicine*, 2001.

[4] J.-L. Vincent, Y. Sakr, C. L. Sprung, V. M. Ranieri, K. Reinhart, H. Gerlach, R. Moreno, J. Carlet, J.-R. Le Gall, and D. Payen. Sepsis in European intensive care units: Results of the SOAP study*. *Critical Care Medicine*, 2006.

[5] M. M. Levy, A. Artigas, G. S. Phillips, A. Rhodes, R. Beale, T. Osborn, J.-L. Vincent, S. Townsend, S. Lemeshow, and R. P. Dellinger. Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study. *The Lancet infectious diseases*, 2012.

[6] V. Y. Dombrovskiy, A. A. Martin, J. Sunderram, and H. L. Paz. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: A trend analysis from 1993 to 2003*. *Critical Care Medicine*, 2007.

[7] D. Rittirsch, M. A. Flierl, and P. A. Ward. Harmful molecular mechanisms in sepsis. *Nature Reviews Immunology*, 2008.

[8] R. Namas, R. Zamora, R. Namas, G. An, J. Doyle, T. E. Dick, F. J. Jacono, I. P. Androulakis, G. F. Nieman, S. Chang, T. R. Billiar, J. A.

Kellum, D. C. Angus, and Y. Vodovotz. Sepsis: Something old, something new, and a systems view. *Journal of critical care*, 2012.

[9] A. J. Martí-Carvajal, I. Solà, C. Gluud, D. Lathyris, and A. F. Cardona. Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients. *Cochrane database of systematic reviews (Online)*, 2012.

[10] E. A. Panacek, J. C. Marshall, T. E. Albertson, D. H. Johnson, S. Johnson, R. D. MacArthur, M. Miller, W. T. Barchuk, S. Fischkoff, M. Kaul, L. Teoh, L. Van Meter, L. Daum, S. Lemeshow, G. Hicklin, C. Doig, and Monoclonal Anti-TNF: a Randomized Controlled Sepsis Study Investigators. Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab')2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Critical Care Medicine*, 2004.

[11] J. Harder, J.-M. Schröder, and R. Gläser. The skin surface as antimicrobial barrier: present concepts and future outlooks. *Experimental dermatology*, 2013.

[12] R. Medzhitov and C. Janeway. Innate immunity. *New England Journal of Medicine*, 2000.

[13] R. Medzhitov. Approaching the Asymptote: 20 Years Later. *Immunity*, 2009.

[14] P. Matzinger. The Danger Model: A Renewed Sense of Self. *Science*, 2002.

[15] R. Medzhitov. Decoding the Patterns of Self and Nonself by the Innate Immune System. *Science*, 2002.

[16] H. K. de Jong, T. van der Poll, and W. J. Wiersinga. The Systemic Pro-Inflammatory Response in Sepsis. *Journal of Innate Immunity*, 2010.

[17] O. Takeuchi and S. Akira. Pattern Recognition Receptors and Inflammation. *Cell*, 2010.

[18] R. Medzhitov. Origin and physiological roles of inflammation. *Nature*, 2008.

[19] G. M. Barton. A calculated response: control of inflammation by the innate immune system. *The Journal of clinical investigation*, 2008.

[20] B. A. Beutler. TLRs and innate immunity. *Blood*, 2009.

[21] M. S. Jin and J.-O. Lee. Structures of the Toll-like Receptor Family and Its Ligand Complexes. *Immunity*, 2008.

[22] E. M. Y. Moresco, D. LaVine, and B. Beutler. Toll-like receptors. *Current biology : CB*, 2011.

[23] B. Albiger, S. Dahlberg, B. Henriques-Normark, and S. Normark. Role of the innate immune system in host defence against bacterial infections: focus on the Toll-like receptors. *Journal of Internal Medicine*, 2007.

[24] H. Kumar, T. Kawai, and S. Akira. Pathogen Recognition by the Innate Immune System. *International Reviews of Immunology*, 2011.

[25] C. A. Janeway, Jr. and R. Medzhitov. Innate immune recognition. *Annual Review of Immunology*, 2002.

[26] R. Medzhitov. Toll-like receptors and innate immunity. *Nature Reviews Immunology*, 2001.

[27] T. Fujita. Evolution of the lectin–complement pathway and its role in innate immunity. *Nature Reviews Immunology*, 2002.

[28] E. A. Nordahl, V. Rydengård, P. Nyberg, D. P. Nitsche, M. Mörgelin, M. Malmsten, L. Björck, and A. Schmidtchen. Activation of the complement system generates antibacterial peptides. *Proceedings of the National Academy of Sciences of the United States of America*, 2004.

[29] I.-M. Frick, P. Akesson, H. Herwald, M. Mörgelin, M. Malmsten, D. K. Nägler, and L. Björck. The contact system–a novel branch of innate immunity generating antibacterial peptides. *The EMBO journal*, 2006.

[30] I.-M. Frick and H. Herwald. *Infectious Agents, the Contact System, and Innate Immunity*. KARGER, Basel, October 2008.

[31] I.-M. Frick, L. Björck, and H. Herwald. The dual role of the contact system in bacterial infectious disease. *Thrombosis and Haemostasis*, 2007.

[32] R. Perkins, M. D. Ngo, F. Mahdi, and Z. Shariat-Madar. Identification of lipopolysaccharide binding site on high molecular weight kininogen. *Biochemical and Biophysical Research Communications*, 2008.

[33] E. Amiel, A. Alonso, S. Uematsu, S. Akira, M. E. Poynter, and B. Berwin. Pivotal Advance: Toll-like receptor regulation of scavenger receptor-A-mediated phagocytosis. *Journal of Leukocyte Biology*, 2009.

[34] F. L. Rock, G. Hardiman, J. C. Timans, R. A. Kastelein, and J. F. Bazan. A family of human receptors structurally related to Drosophila Toll. *Proceedings of the National Academy of Sciences of the United States of America*, 1998.

[35] S. Uematsu and S. Akira. Toll-Like receptors (TLRs) and their ligands. *Handbook of experimental pharmacology*, 2008.

[36] B. Beutler. Microbe sensing, positive feedback loops, and the pathogenesis of inflammatory diseases. *Immunological reviews*, 2009.

[37] H. Kanzler, F. J. Barrat, E. M. Hessel, and R. L. Coffman. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. *Nature Medicine*, 2007.

[38] H. M. Kim, B. S. Park, J.-I. Kim, S. E. Kim, J. Lee, S. C. Oh, P. Enkhbayar, N. Matsushima, H. Lee, O. J. Yoo, and J.-O. Lee. Crystal Structure of the TLR4-MD-2 Complex with Bound Endotoxin Antagonist Eritoran. *Cell*, 2007.

[39] K. Takeda and S. Akira. TLR signaling pathways. *Seminars in Immunology*, 2004.

[40] A. Poltorak, X. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, and B. Beutler. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 1998.

[41] S. D. Wright, P. S. Tobias, R. J. Ulevitch, and R. A. Ramos. Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *The Journal of experimental medicine*, 1989.

[42] R. Shimazu, S. Akashi, H. OGATA, Y. Nagai, K. Fukudome, K. Miyake, and M. Kimoto. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *The Journal of experimental medicine*, 1999.

[43] E. Hailman, H. S. Lichenstein, M. M. Wurfel, D. S. Miller, D. A. Johnson, M. Kelley, L. A. Busse, M. M. Zukowski, and S. D. Wright. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *The Journal of experimental medicine*, 1994.

[44] R. Jerala. Structural biology of the LPS recognition. *International Journal of Medical Microbiology*, 2007.

[45] T. G. Loof, M. Morgelin, L. Johansson, S. Oehmcke, A. I. Olin, G. Dickneite, A. Norrby-Teglund, U. Theopold, and H. Herwald. Coagulation, an ancestral serine protease cascade, exerts a novel function in early immune defense. *Blood*, 2011.

[46] M. Levi and T. van der Poll. Two-way interactions between inflammation and coagulation. *Trends in cardiovascular medicine*, 2005.

[47] L. Ma and A. Dorling. The roles of thrombin and protease-activated receptors in inflammation. *Seminars in Immunopathology*, 2011.

[48] U. Amara, M. A. Flierl, D. Rittirsch, A. Klos, H. Chen, B. Acker, U. B. Bruckner, B. Nilsson, F. Gebhard, J. D. Lambris, and M. Huber-Lang.

Molecular Intercommunication between the Complement and Coagulation Systems. *The Journal of Immunology*, 2010.

[49] P. Papareddy, V. Rydengård, M. Pasupuleti, B. Walse, M. Mörgelin, A. Chalupka, M. Malmsten, and A. Schmidtchen. Proteolysis of Human Thrombin Generates Novel Host Defense Peptides. *PLoS Pathogens*, 2010.

[50] P. Papareddy, M. Kalle, G. Kasetty, M. Mörgelin, V. Rydengård, B. Albiger, K. Lundqvist, M. Malmsten, and A. Schmidtchen. C-terminal peptides of tissue factor pathway inhibitor are novel host defense molecules. *The Journal of biological chemistry*, 2010.

[51] M. Levi. The coagulant response in sepsis and inflammation. *Hämostaseologie*, 2010.

[52] T. G. Loof, O. Schmidt, H. Herwald, and U. Theopold. Coagulation Systems of Invertebrates and Vertebrates and Their Roles in Innate Immunity: The Same Side of Two Coins? *Journal of Innate Immunity*, 2011.

[53] H. H. Versteeg, J. W. M. Heemskerk, M. Levi, and P. H. Reitsma. New Fundamentals in Hemostasis. *Physiological Reviews*, 2013.

[54] S. Oehmcke, M. M ouml rgelin, and H. Herwald. Activation of the Human Contact System on Neutrophil Extracellular Traps. *Journal of Innate Immunity*, 2009.

[55] S. Oehmcke and H. Herwald. Contact system activation in severe infectious diseases. *Journal of molecular medicine (Berlin, Germany)*, 2010.

[56] E. A. Nordahl, V. Rydengård, M. Mörgelin, and A. Schmidtchen. Domain 5 of high molecular weight kininogen is antibacterial. *The Journal of biological chemistry*, 2005.

[57] R. A. Fleck, L. V. Rao, S. I. Rapaport, and N. Varki. Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. *Thrombosis Research*, 1990.

[58] S. H. H. F. Schoenmakers, P. H. Reitsma, and C. A. Spek. Blood coagulation factors as inflammatory mediators. *Blood cells, molecules & diseases*, 2005.

[59] B. Osterud and S. I. Rapaport. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proceedings of the National Academy of Sciences of the United States of America*, 1977.

[60] B. Dahlbäck. Blood coagulation. *Lancet*, 2000.

[61] K. Mészáros, S. Aberle, R. Dedrick, R. Machovich, A. Horwitz, C. Birr, G. Theofan, and J. B. Parent. Monocyte tissue factor induction by lipopolysaccharide (LPS): dependence on LPS-binding protein and CD14, and inhibition by a recombinant fragment of bactericidal/permeability-increasing protein. *Blood*, 1994.

[62] E. Mattsson, T. Hartung, S. Morath, and A. Egesten. Highly purified lipoteichoic acid from Staphylococcus aureus induces procoagulant activity and tissue factor expression in human monocytes but is a weak inducer in whole blood: comparison with peptidoglycan. *Infection and Immunity*, 2004.

[63] L. I. Påhlman, P. F. Marx, M. Mörgelin, S. Lukomski, J. C. M. Meijers, and H. Herwald. Thrombin-activatable fibrinolysis inhibitor binds to Streptococcus pyogenes by interacting with collagen-like proteins A and B. *The Journal of biological chemistry*, 2007.

[64] S. Oehmcke, M. Mörgelin, J. Malmström, A. Linder, M. Chew, H. Thorlacius, and H. Herwald. Stimulation of blood mononuclear cells with bacterial virulence factors leads to the release of pro-coagulant and pro-inflammatory microparticles. *Cellular microbiology*, 2012.

[65] M. Schouten, W. J. Wiersinga, M. Levi, and T. van der Poll. Inflammation, endothelium, and coagulation in sepsis. *Journal of Leukocyte Biology*, 2008.

[66] M. Levi and H. Ten Cate. Disseminated intravascular coagulation. *New England Journal of Medicine*, 1999.

[67] T. J. Girard, L. A. Warren, W. F. Novotny, K. M. Likert, S. G. Brown, J. P. Miletich, and G. J. Broze. Functional significance of the Kunitz-type inhibitory domains of lipoprotein-associated coagulation inhibitor. *Nature*, 1989.

[68] C. van 't Veer and K. G. Mann. Regulation of tissue factor initiated thrombin generation by the stoichiometric inhibitors tissue factor pathway inhibitor, antithrombin-III, and heparin cofactor-II. *The Journal of biological chemistry*, 1997.

[69] A. C. Cunningham, K. A. Hasty, J. J. Enghild, and A. E. Mast. Structural and functional characterization of tissue factor pathway inhibitor following degradation by matrix metalloproteinase-8. *The Biochemical journal*, 2002.

[70] S. R. Coughlin. Thrombin signalling and protease-activated receptors. *Nature*, 2000.

[71] G. Cesarman-Maus and K. A. Hajjar. Molecular mechanisms of fibrinolysis. *British Journal of Haematology*, 2005.

[72] M. J. Walport. Complement. First of two parts. *New England Journal of Medicine*, 2001.

[73] M. J. Walport. Complement. Second of two parts. *New England Journal of Medicine*, 2001.

[74] P. F. Zipfel and C. Skerka. Complement regulators and inhibitory proteins. *Nature Reviews Immunology*, 2009.

[75] D. L. Jack, N. J. Klein, and M. W. Turner. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophago-cytosis. *Immunological reviews*, 2001.

[76] M. Kojouharova, K. Reid, and M. Gadjeva. New insights into the molecular mechanisms of classical complement activation. *Molecular Immunology*, 2010.

[77] R. Wallis, D. A. Mitchell, R. Schmid, W. J. Schwaeble, and A. H. Keeble. Paths reunited Initiation of the classical and lectin pathways of complement activation. *Immunobiology*, 2010.

[78] M. H. Kaplan and J. E. Volanakis. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. *Journal of immunology (Baltimore, Md. : 1950)*, 1974.

[79] D. C. Morrison and L. F. Kline. Activation of the classical and properdin pathways of complement by bacterial lipopolysaccharides (LPS). *Journal of immunology (Baltimore, Md. : 1950)*, 1977.

[80] S. W. Vukajlovich, J. Hoffman, and D. C. Morrison. Activation of human serum complement by bacterial lipopolysaccharides: structural requirements for antibody independent activation of the classical and alternative pathways. *Molecular Immunology*, 1987.

[81] R. C. Duncan, L. C. Wijeyewickrema, and R. N. Pike. The initiating proteases of the complement system: controlling the cleavage. *Biochimie*, 2008.

[82] M. J. Krisinger, V. Goebeler, Z. Lu, S. C. Meixner, T. Myles, E. L. G. Pryzdial, and E. M. Conway. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood*, 2012.

[83] M. Pasupuleti, B. Walse, E. A. Nordahl, M. Mörgelin, M. Malmsten, and A. Schmidtchen. Preservation of antimicrobial properties of complement peptide C3a, from invertebrates to humans. *The Journal of biological chemistry*, 2007.

[84] L. Skattum, M. van Deuren, T. van der Poll, and L. Truedsson. Complement deficiency states and associated infections. *Molecular Immunology*, 2011.

[85] B. Woehrl, M. C. Brouwer, C. Murr, S. G. B. Heckenberg, F. Baas, H. W. Pfister, A. H. Zwinderman, B. P. Morgan, S. R. Barnum, A. van der Ende, U. Koedel, and D. van de Beek. Complement component 5 contributes to poor disease outcome in humans and mice with pneumococcal meningitis. *The Journal of clinical investigation*, 2011.

[86] M. Møller-Kristensen, W. K. E. Ip, L. Shi, L. D. Gowda, M. R. Hamblin, S. Thiel, J. C. Jensenius, R. A. B. Ezekowitz, and K. Takahashi. Deficiency of mannose-binding lectin greatly increases susceptibility to postburn infection with Pseudomonas aeruginosa. *Journal of immunology (Baltimore, Md. : 1950)*, 2006.

[87] J. D. Lambris, D. Ricklin, and B. V. Geisbrecht. Complement evasion by human pathogens. *Nature Publishing Group*, 2008.

[88] A. Schmidtchen, E. Holst, H. Tapper, and L. Björck. Elastase-producing Pseudomonas aeruginosa degrade plasma proteins and extracellular products of human skin and fibroblasts, and inhibit fibroblast growth. *Microbial Pathogenesis*, 2003.

[89] Y. Q. Hong and B. Ghebrehiwet. Effect of Pseudomonas aeruginosa elastase and alkaline protease on serum complement and isolated components C1q and C3. *Clinical immunology and immunopathology*, 1992.

[90] A. J. Laarman, B. W. Bardoel, M. Ruyken, J. Fernie, F. J. Milder, J. A. G. van Strijp, and S. H. M. Rooijakkers. Pseudomonas aeruginosa Alkaline Protease Blocks Complement Activation via the Classical and Lectin Pathways. *The Journal of Immunology*, 2011.

[91] R. E. W. Hancock and H.-G. Sahl. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology*, 2006.

[92] M. Zasloff. Antimicrobial peptides of multicellular organisms. *Nature*, 2002.

[93] K.-Y. Choi, L. N. Y. Chow, and N. Mookherjee. Cationic Host Defence Peptides: Multifaceted Role in Immune Modulation and Inflammation. *Journal of Innate Immunity*, 2012.

[94] T. Ganz. Defensins: antimicrobial peptides of innate immunity. *Nature Reviews Immunology*, 2003.

[95] Y. Lai and R. L. Gallo. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends in immunology*, 2009.

[96] B. Schittek. The Multiple Facets of Dermcidin in Cell Survival and Host Defense. *Journal of Innate Immunity*, 2012.

[97] K. Smet and R. Contreras. Human Antimicrobial Peptides: Defensins, Cathelicidins and Histatins. *Biotechnology Letters*, 2005.

[98] M. Kalle, P. Papareddy, G. Kasetty, M. Mörgelin, M. J. A. van der Plas, V. Rydengård, M. Malmsten, B. Albiger, and A. Schmidtchen. Host defense peptides of thrombin modulate inflammation and coagulation in endotoxin-mediated shock and Pseudomonas aeruginosa sepsis. *PLoS ONE*, 2012.

[99] E. Andersson, V. Rydengård, A. Sonesson, M. Mörgelin, L. Björck, and A. Schmidtchen. Antimicrobial activities of heparin-binding peptides. *European Journal of Biochemistry*, 2004.

[100] M. E. Selsted and A. J. Ouellette. Mammalian defensins in the antimicrobial immune response. *Nature Immunology*, 2005.

[101] D. E. Jones and C. L. Bevins. Paneth cells of the human small intestine express an antimicrobial peptide gene. *The Journal of biological chemistry*, 1992.

[102] L. A. Duits, B. Ravensbergen, M. Rademaker, P. S. HIEMSTRA, and P. H. Nibbering. Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. *Immunology*, 2002.

[103] J. D. Spencer, D. S. Hains, E. Porter, C. L. Bevins, J. DiRosario, B. Becknell, H. Wang, and A. L. Schwaderer. Human Alpha Defensin 5 Expression in the Human Kidney and Urinary Tract. *PLoS ONE*, 2012.

[104] A. M. Cole, T. Hong, L. M. Boo, T. Nguyen, C. Zhao, G. Bristol, J. A. Zack, A. J. Waring, O. O. Yang, and R. I. Lehrer. Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. *Proceedings of the National Academy of Sciences of the United States of America*, 2002.

[105] T. Ganz, M. E. Selsted, D. Szklarek, S. S. Harwig, K. Daher, D. F. Bainton, and R. I. Lehrer. Defensins. Natural peptide antibiotics of human neutrophils. *The Journal of clinical investigation*, 1985.

[106] C. G. Wilde, J. E. Griffith, M. N. Marra, J. L. Snable, and R. W. Scott. Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family. *The Journal of biological chemistry*, 1989.

[107] M. E. Selsted, S. S. Harwig, T. Ganz, J. W. Schilling, and R. I. Lehrer. Primary structures of three human neutrophil defensins. *The Journal of clinical investigation*, 1985.

[108] F. Bauer, K. Schweimer, E. Klüver, J. R. Conejo-Garcia, W. G. Forssmann, P. Rösch, K. Adermann, and H. Sticht. Structure determination of human and murine beta-defensins reveals structural conservation in the absence of significant sequence similarity. *Protein science : a publication of the Protein Society*, 2001.

[109] S. van Wetering, P. J. Sterk, K. F. Rabe, and P. S. Hiemstra. Defensins: key players or bystanders in infection, injury, and repair in the lung? *The Journal of allergy and clinical immunology*, 1999.

[110] F. Semple and J. R. Dorin. β-Defensins: Multifunctional Modulators of Infection, Inflammation and More? *Journal of Innate Immunity*, 2012.

[111] F. Niyonsaba, H. Ushio, N. Nakano, W. Ng, K. Sayama, K. Hashimoto, I. Nagaoka, K. Okumura, and H. Ogawa. Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *Journal of Investigative Dermatology*, 2007.

[112] F. Semple, S. Webb, H.-N. Li, H. B. Patel, M. Perretti, I. J. Jackson, M. Gray, D. J. Davidson, and J. R. Dorin. Human β-defensin 3 has immunosuppressive activity in vitro and in vivo. *European Journal of Immunology*, 2010.

[113] M. Zanetti, R. Gennaro, and D. Romeo. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS letters*, 1995.

[114] A. Ritonja, M. Kopitar, R. JERALA, and V. Turk. Primary structure of a new cysteine proteinase inhibitor from pig leucocytes. *FEBS letters*, 1989.

[115] J. W. Larrick, M. Hirata, R. F. Balint, J. Lee, J. Zhong, and S. C. Wright. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. *Infection and Immunity*, 1995.

[116] O. Sorensen, K. Arnljots, J. B. Cowland, D. F. Bainton, and N. Borregaard. The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. *Blood*, 1997.

[117] B. Agerberth, J. Charo, J. Werr, B. Olsson, F. Idali, L. Lindbom, R. Kiessling, H. Jörnvall, H. Wigzell, and G. H. Gudmundsson. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. *Blood*, 2000.

[118] J. Malm, O. Sorensen, T. Persson, M. Frohm-Nilsson, B. Johansson, A. Bjartell, H. Lilja, M. Stahle-Backdahl, N. Borregaard, and A. Egesten. The Human Cationic Antimicrobial Protein (hCAP-18) Is Expressed in the Epithelium of Human Epididymis, Is Present in Seminal Plasma at High Concentrations, and Is Attached to Spermatozoa. *Infection and Immunity*, 2000.

[119] R. Bals, X. Wang, M. Zasloff, and J. M. Wilson. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proceedings of the National Academy of Sciences of the United States of America*, 1998.

[120] K. Hase, L. Eckmann, J. D. Leopard, N. Varki, and M. F. Kagnoff. Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. *Infection and Immunity*, 2002.

[121] M. Frohm, B. Agerberth, G. Ahangari, M. Stahle-Backdahl, S. Lidén, H. Wigzell, and G. H. Gudmundsson. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *The Journal of biological chemistry*, 1997.

[122] A. Krasnodembskaya, Y. Song, X. Fang, N. Gupta, V. Serikov, J.-W. Lee, and M. A. Matthay. Antibacterial Effect of Human Mesenchymal Stem Cells Is Mediated in Part from Secretion of the Antimicrobial Peptide LL-37. *Stem Cells*, 2010.

[123] S. B. Coffelt, R. S. Waterman, L. Florez, K. Höner zu Bentrup, K. J. Zwezdaryk, S. L. Tomchuck, H. L. LaMarca, E. S. Danka, C. A. Morris, and A. B. Scandurro. Ovarian cancers overexpress the antimicrobial protein hCAP-18 and its derivative LL-37 increases ovarian cancer cell proliferation and invasion. *International journal of cancer. Journal international du cancer*, 2008.

[124] O. E. Sorensen, P. Follin, A. H. Johnsen, J. Calafat, G. S. Tjabringa, P. S. Hiemstra, and N. Borregaard. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood*, 2001.

[125] O. E. Sörensen, L. Gram, A. H. Johnsen, E. Andersson, S. Bangsbøll, G. S. Tjabringa, P. S. HIEMSTRA, J. Malm, A. Egesten, and N. Borregaard. Processing of seminal plasma hCAP-18 to ALL-38 by gastricsin: a novel mechanism of generating antimicrobial peptides in vagina. *The Journal of biological chemistry*, 2003.

[126] D. Vandamme, B. Landuyt, W. Luyten, and L. Schoofs. A comprehensive summary of LL-37, the factoctum human cathelicidin peptide. *Cellular Immunology*, 2012.

[127] R. Bucki, K. Leszczyńska, A. Namiot, and W. Sokołowski. Cathelicidin LL-37: A Multitask Antimicrobial Peptide. *Archivum Immunologiae et Therapiae Experimentalis*, 2010.

[128] A. Nijnik and R. E. Hancock. The roles of cathelicidin LL-37 in immune defences and novel clinical applications. *Current Opinion in Hematology*, 2009.

[129] W. Baranska-Rybak, A. Sonesson, R. Nowicki, and A. Schmidtchen. Glycosaminoglycans inhibit the antibacterial activity of LL-37 in biological fluids. *The Journal of antimicrobial chemotherapy*, 2006.

[130] A. Scott, S. Weldon, P. J. Buchanan, B. Schock, R. K. Ernst, D. F. McAuley, M. M. Tunney, C. R. Irwin, J. S. Elborn, and C. C. Taggart. Evaluation of the Ability of LL-37 to Neutralise LPS In Vitro and Ex Vivo. *PLoS ONE*, 2011.

[131] R. Bucki, D. B. Namiot, Z. Namiot, P. B. Savage, and P. A. Janmey. Salivary mucins inhibit antibacterial activity of the cathelicidin-derived LL-37 peptide but not the cationic steroid CSA-13. *Journal of Antimicrobial Chemotherapy*, 2008.

[132] T. Sigurdardottir, P. Andersson, M. Davoudi, M. Malmsten, A. Schmidtchen, and M. Bodelsson. In Silico Identification and Biological Evaluation of Antimicrobial Peptides Based on Human Cathelicidin LL-37. *Antimicrobial Agents and Chemotherapy*, 2006.

[133] M. G. Scott, E. Dullaghan, N. Mookherjee, N. Glavas, M. Waldbrook, A. Thompson, A. Wang, K. Lee, S. Doria, P. Hamill, J. J. Yu, Y. Li, O. Donini, M. M. Guarna, B. B. Finlay, J. R. North, and R. E. W. Hancock. An anti-infective peptide that selectively modulates the innate immune response. *Nature Biotechnology*, 2007.

[134] G. Kasetty, P. Papareddy, M. Kalle, V. Rydengard, M. Morgelin, B. Albiger, M. Malmsten, and A. Schmidtchen. Structure-Activity Studies and Therapeutic Potential of Host Defense Peptides of Human Thrombin. *Antimicrobial Agents and Chemotherapy*, 2011.

[135] A. Sonesson, L. Ringstad, E. A. Nordahl, M. Malmsten, M. Mörgelin, and A. Schmidtchen. Antifungal activity of C3a and C3a-derived peptides against Candida. *Biochimica et biophysica acta*, 2007.

[136] J. L. Gifford, H. N. Hunter, and H. J. Vogel. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. *Cellular and Molecular Life Sciences*, 2005.

[137] B. E. Britigan, M. B. Hayek, B. N. Doebbeling, and R. B. Fick. Transferrin and lactoferrin undergo proteolytic cleavage in the Pseudomonas aeruginosa-infected lungs of patients with cystic fibrosis. *Infection and Immunity*, 1993.

[138] P. Papareddy, M. Kalle, O. E. Sörensen, K. Lundqvist, M. Mörgelin, M. Malmsten, and A. Schmidtchen. Tissue Factor Pathway Inhibitor 2 Is Found in Skin and Its C-Terminal Region Encodes for Antibacterial Activity. *PLoS ONE*, 2012.

[139] L. I. Påhlman, M. Morgelin, G. Kasetty, A. I. Olin, A. Schmidtchen, and H. Herwald. Antimicrobial activity of fibrinogen and fibrinogen-

derived peptides – a novel link between coagulation and innate immunity. *Thrombosis and Haemostasis*, 2013.

[140] W. F. Skogen, R. M. Senior, G. L. Griffin, and G. D. Wilner. Fibrinogen-derived peptide B beta 1-42 is a multidomained neutrophil chemoattractant. *Blood*, 1988.

[141] R. M. Senior, W. F. Skogen, G. L. Griffin, and G. D. Wilner. Effects of fibrinogen derivatives upon the inflammatory response. Studies with human fibrinopeptide B. *The Journal of clinical investigation*, 1986.

[142] A. T. Y. Yeung, S. L. Gellatly, and R. E. W. Hancock. Multifunctional cationic host defence peptides and their clinical applications. *Cellular and Molecular Life Sciences*, 2011.

[143] H. Jenssen, P. Hamill, and R. E. W. Hancock. Peptide Antimicrobial Agents. *Clinical Microbiology Reviews*, 2006.

[144] M. Wu, E. Maier, R. Benz, and R. E. W. Hancock. Mechanism of Interaction of Different Classes of Cationic Antimicrobial Peptides with Planar Bilayers and with the Cytoplasmic Membrane of Escherichia coliâ€ . *Biochemistry*, 1999.

[145] Y. Shai and Z. Oren. From "carpet" mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides. *Peptides*, 2001.

[146] K. A. Brogden. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Publishing Group*, 2005.

[147] P. Pieta, J. Mirza, and J. Lipkowski. Direct visualization of the alamethicin pore formed in a planar phospholipid matrix. *Proceedings of the National Academy of Sciences*, 2012.

[148] C. Song, C. Weichbrodt, E. S. Salnikov, M. Dynowski, B. O. Forsberg, B. Bechinger, C. Steinem, B. L. de Groot, U. Zachariae, and K. Zeth. Crystal structure and functional mechanism of a human antimicrobial membrane channel. *Proceedings of the National Academy of Sciences*, 2013.

[149] B. Bechinger. Insights into the mechanisms of action of host defence peptides from biophysical and structural investigations. *Journal of Peptide Science*, 2011.

[150] K. A. Henzler Wildman, D.-K. Lee, and A. Ramamoorthy. Mechanism of Lipid Bilayer Disruption by the Human Antimicrobial Peptide, LL-37. *Biochemistry*, 2003.

[151] Z. Oren, J. C. Lerman, G. H. Gudmundsson, B. Agerberth, and Y. Shai. Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its non-cell-selective activity. *The Biochemical journal*, 1999.

[152] L. Steinstraesser, U. Kraneburg, F. Jacobsen, and S. Al-Benna. Host defense peptides and their antimicrobial-immunomodulatory duality. *Immunobiology*, 2011.

[153] Y. Kaconis, I. Kowalski, J. Howe, A. Brauser, W. Richter, I. Razquin-Olazarán, M. Iñigo-Pestaña, P. Garidel, M. Rössle, G. M. de Tejada, T. Gutsmann, and K. Brandenburg. Biophysical Mechanisms of Endotoxin Neutralization by Cationic Amphiphilic Peptides. *Biophysj*, 2011.

[154] Y. Rosenfeld, N. Papo, and Y. Shai. Endotoxin (lipopolysaccharide) neutralization by innate immunity host-defense peptides. Peptide properties and plausible modes of action. *The Journal of biological chemistry*, 2006.

[155] M. Mueller, B. Lindner, R. Dedrick, A. B. Schromm, and U. Seydel. Endotoxin: physical requirements for cell activation. *Journal of Endotoxin Research*, 2005.

[156] M. G. Scott, A. C. Vreugdenhil, W. A. Buurman, R. E. Hancock, and M. R. Gold. Cutting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein. *Journal of immunology (Baltimore, Md. : 1950)*, 2000.

[157] F. Semple, H. MacPherson, S. Webb, S. L. Cox, L. J. Mallin, C. Tyrrell, G. R. Grimes, C. A. Semple, M. A. Nix, G. L. Millhauser, and J. R. Dorin. Human β-defensin 3 affects the activity of pro-inflammatory pathways associated with MyD88 and TRIF. *European Journal of Immunology*, 2011.

[158] N. Mookherjee, K. L. Brown, D. M. E. Bowdish, S. Doria, R. Falsafi, K. Hokamp, F. M. Roche, R. Mu, G. H. Doho, J. Pistolic, J.-P. Powers, J. Bryan, F. S. L. Brinkman, and R. E. W. g. Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *Journal of immunology (Baltimore, Md. : 1950)*, 2006.

[159] N. Mookherjee, D. N. D. Lippert, P. Hamill, R. Falsafi, A. Nijnik, J. Kindrachuk, J. Pistolic, J. Gardy, P. Miri, M. Naseer, L. J. Foster, and R. E. W. Hancock. Intracellular Receptor for Human Host Defense Peptide LL-37 in Monocytes. *The Journal of Immunology*, 2009.

[160] I. K. H. Poon, K. K. Patel, D. S. Davis, C. R. Parish, and M. D. Hulett. Histidine-rich glycoprotein: the Swiss Army knife of mammalian plasma. *Blood*, 2011.

[161] V. Rydengård, A.-K. Olsson, M. Mörgelin, and A. Schmidtchen. Histidine-rich glycoprotein exerts antibacterial activity. *FEBS Journal*, 2007.

[162] V. Rydengård, O. Shannon, K. Lundqvist, L. Kacprzyk, A. Chalupka, A.-K. Olsson, M. Mörgelin, W. JAHNEN-DECHENT, M. Malmsten, and A. Schmidtchen. Histidine-Rich Glycoprotein Protects from Systemic Candida Infection. *PLoS Pathogens*, 2008.

[163] O. Shannon, V. Rydengard, A. Schmidtchen, M. Morgelin, P. Alm, O. E. Sorensen, and L. Bjorck. Histidine-rich glycoprotein promotes bacterial entrapment in clots and decreases mortality in a mouse model of sepsis. *Blood*, 2010.

[164] I. K. H. Poon, M. D. Hulett, and C. R. Parish. Histidine-rich glycoprotein is a novel plasma pattern recognition molecule that recruits IgG to facilitate necrotic cell clearance via Fc RI on phagocytes. *Blood*, 2010.

[165] G. R. Bernard, J. L. Vincent, P. F. Laterre, S. P. LaRosa, J. F. Dhainaut, A. Lopez-Rodriguez, J. S. Steingrub, G. E. Garber, J. D. Helterbrand, E. W. Ely, C. J. Fisher, and Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. *New England Journal of Medicine*, 2001.

[166] P. Della Valle, G. Pavani, and A. D'Angelo. The protein C pathway and sepsis. *Thrombosis Research*, 2012.

[167] E. Malmström, M. Mörgelin, M. Malmsten, L. Johansson, A. Norrby-Teglund, O. Shannon, A. Schmidtchen, J. C. M. Meijers, and H. Herwald. Protein C Inhibitor—A Novel Antimicrobial Agent. *PLoS Pathogens*, 2009.

[168] A. Aderem and D. M. Underhill. Mechanisms of phagocytosis in macrophages. *Annual Review of Immunology*, 1999.

[169] H. Sarantis and S. Grinstein. Subversion of Phagocytosis for Pathogen Survival. *Cell Host and Microbe*, 2012.

[170] S. W. Glickman, C. B. Cairns, R. M. Otero, C. W. Woods, E. L. Tsalik, R. J. Langley, J. C. van Velkinburgh, L. P. Park, L. T. Glickman, V. G. Fowler, Jr, S. F. Kingsmore, and E. P. Rivers. Disease Progression in Hemodynamically Stable Patients Presenting to the Emergency Department With Sepsis. *Academic Emergency Medicine*, 2010.

[171] M. S. Rangel-Frausto, D. Pittet, T. Hwang, R. F. Woolson, and R. P. Wenzel. The dynamics of disease progression in sepsis: Markov modeling describing the natural history and the likely impact of effective antisepsis agents. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 1998.

[172] A. Kumar, D. Roberts, K. E. Wood, B. Light, J. E. Parrillo, S. Sharma, R. Suppes, D. Feinstein, S. Zanotti, L. Taiberg, D. Gurka, A. Kumar, and M. Cheang. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock*. *Critical Care Medicine*, 2006.

[173] The Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup*, R. P. Dellinger, M. M. Levy, A. Rhodes, D. Annane, H. Gerlach, S. M. Opal, J. E. Sevransky, C. L. Sprung, I. S. Douglas, R. Jaeschke, T. M. Osborn, M. E. Nunnally, S. R. Townsend, K. Reinhart, R. M. Kleinpell, D. C. Angus, C. S. Deutschman, F. R. Machado, G. D. Rubenfeld, S. Webb, R. J. Beale, J.-L. Vincent, and R. Moreno. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock, 2012. *Intensive Care Medicine*, 2013.

[174] M. M. Levy, M. P. Fink, J. C. Marshall, E. Abraham, D. Angus, D. Cook, J. Cohen, S. M. Opal, J.-L. Vincent, and G. Ramsay. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Critical Care Medicine*, 2003.

[175] R. C. Bone, R. A. Balk, F. B. Cerra, R. P. Dellinger, A. M. Fein, W. A. Knaus, R. M. Schein, and W. J. Sibbald. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. In *Chest*, 1992.

[176] S. Hagel, M. W. Pletz, F. M. Brunkhorst, H. Seifert, and W. V. Kern. [Bacteremia and sepsis]. *Der Internist*, 2013.

[177] R. M. Daniels and T. Nutbeam. *ABC of sepsis*. Chichester, West Sussex ; Hoboken, NJ : BMJ/ Wiley-Blackwell, 2010.

[178] M. Levi and T. van der Poll. Inflammation and coagulation. *Critical Care Medicine*, 2010.

[179] A. A. Anas, W. J. Wiersinga, A. F. de Vos, and T. van der Poll. Recent insights into the pathogenesis of bacterial sepsis. *The Netherlands journal of medicine*, 2010.

[180] J.-F. Dhainaut, A. F. Shorr, W. L. Macias, M. J. Kollef, M. Levi, K. Reinhart, and D. R. Nelson. Dynamic evolution of coagulopathy in the first day of severe sepsis: Relationship with mortality and organ failure*. *Critical Care Medicine*, 2005.

[181] F. Fourrier. Severe sepsis, coagulation, and fibrinolysis. *Critical Care Medicine*, 2012.

[182] S. M. Opal, P.-F. Laterre, B. Francois, S. P. LaRosa, D. C. Angus, J.-P. Mira, X. Wittebole, T. Dugernier, D. Perrotin, M. Tidswell, L. Jauregui, K. Krell, J. Pachl, T. Takahashi, C. Peckelsen, E. Cordasco, C.-S. Chang, S. Oeyen, N. Aikawa, T. Maruyama, R. Schein, A. C. Kalil, M. Van Nuffelen, M. Lynn, D. P. Rossignol, J. Gogate, M. B. Roberts, J. L. Wheeler, J.-L. Vincent, and ACCESS Study Group. Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. *JAMA: The Journal of the American Medical Association*, 2013.

[183] N. C. Riedemann, R.-F. Guo, and P. A. Ward. Novel strategies for the treatment of sepsis. *Nature Medicine*, 2003.

[184] R. E. W. Hancock, A. Nijnik, and D. J. Philpott. Modulating immunity as a therapy for bacterial infections. *Nature Publishing Group*, 2012.

[185] A. Nijnik, L. Madera, S. Ma, M. Waldbrook, M. R. Elliott, D. M. Easton, M. L. Mayer, S. C. Mullaly, J. Kindrachuk, H. Jenssen, and R. E. W. Hancock. Synthetic Cationic Peptide IDR-1002 Provides Protection against Bacterial Infections through Chemokine Induction and Enhanced Leukocyte Recruitment. *The Journal of Immunology*, 2010.

[186] G. S. Martin, D. M. Mannino, S. Eaton, and M. Moss. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med*, 2003.

[187] N. V. Serbina, T. Jia, T. M. Hohl, and E. G. Pamer. Monocyte-Mediated Defense Against Microbial Pathogens. *Annual Review of Immunology*, 2008.

[188] H. S. Chand, D. C. Foster, and W. Kisiel. Structure, function and biology of tissue factor pathway inhibitor-2. *Thrombosis and Haemostasis*, 2005.

[189] M. Iino, D. C. Foster, and W. Kisiel. Quantification and characterization of human endothelial cell-derived tissue factor pathway inhibitor-2. *Arteriosclerosis, thrombosis, and vascular biology*, 1998.

[190] V. Neaud, J. G. Duplantier, C. Mazzocco, W. Kisiel, and J. Rosenbaum. Thrombin up-regulates tissue factor pathway inhibitor-2 synthesis through a cyclooxygenase-2-dependent, epidermal growth factor receptor-independent mechanism. *The Journal of biological chemistry*, 2004.

[191] J. A. Huntington. Shape-shifting serpins–advantages of a mobile mechanism. *Trends in biochemical sciences*, 2006.

[192] D. van Gent, P. Sharp, K. Morgan, and N. Kalsheker. Serpins: structure, function and molecular evolution. *The International Journal of Biochemistry & Cell Biology*, 2003.

[193] J. C. Rau, L. M. Beaulieu, J. A. Huntington, and F. C. Church. Serpins in thrombosis, hemostasis and fibrinolysis. *Journal of thrombosis and haemostasis : JTH*, 2007.

[194] D. M. Tollefsen. Vascular dermatan sulfate and heparin cofactor II. *Progress in molecular biology and translational science*, 2010.

[195] M. Hoffman, C. W. Pratt, R. L. Brown, and F. C. Church. Heparin cofactor II-proteinase reaction products exhibit neutrophil chemoattractant activity. *Blood*, 1989.