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Novel actions of thrombin-derived host defense peptides

Novel actions of thrombin-derived host defense peptides

by Finja Catharine Hansen

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DOCTORAL DISSERTATION

By due permission of the Faculty of Medicine, Lund University, Sweden, this doctoral thesis will be defended on June 8th 2018 at 13:00 in Belfragesalen, Biomedical Center, Lund, Sweden

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Abstract
Thrombin-derived C-terminal peptides (TCPs) constitute a novel class of immunomodulatory host defense peptides, generated during infection and inflammation. Previous investigations have shown that TCPs of 2-3 kDa inhibit pro-inflammatory immune responses *in vitro* and *in vivo*, but their mode of action remained unclear. Paper I shows that the prototypic TCP GKY25 binds to LPS, thereby preventing TLR4 dimerization and subsequent activation of downstream signaling pathways in monocytes and macrophages. Furthermore, we found that GKY25 blocks TLR4- and TLR2-induced NF-κB/AP-1 activation in response to several other microbial-derived agonists as well. Whereas the first publication explains the effect of GKY25 on cell activation by purified bacterial components, the effect of GKY25 on whole bacteria and their interaction with immune cells remained unclear. In paper II we show the presence of TCPs in chronic and acute wound fluids. that GKY25 binds to Gram-negative bacteria in the extra- and intracellular environment and reduces pro-inflammatory immune response of monocytes/macrophages while preserving their important phagocytic function. During the investigations of the modes of action of TCPs, we observed that GKY25 is internalized in monocytes and macrophages. However, the exact uptake mechanism remained unknown. Therefore, in paper III we show that TCPs of 2-3 kDa are differently internalized by clathrin-dependent and -independent endocytosis pathways in monocytes and macrophages, depending on the type of cells, the length of the peptide, and the presence of LPS or bacteria. Internalized GKY25 was transported to lysosomes, where it remained detectable for up to 10 h. Finally, paper IV describes a TCP of about 11 kDa, which forms amorphous aggregates in the presence of LPS and Gram-negative bacteria, leading to scavenging, phagocytosis and killing. Taken together, herein we show that the multifunctional TCPs play a physiological role during infection and inflammation, and have therapeutic potential by modulating multiple interactions involving bacteria, endotoxins and inflammatory cell responses.

Key words
Host defense peptides, Thrombin, Monocytes/Macrophages, Gram-negative bacteria, LPS, TLR, Phagocytosis, Inflammation, Endocytosis

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Cover illustration front: Phagocytosis of *Escherichia coli* BioParticles (green) with bound thrombin-derived C-terminal peptides (red) by RAW264.7 cells (nucleus staining in blue).

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To my Dad and
my deceased Mom ♡

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This thesis is based on the following publications, referred to by their Roman numerals:

I **The thrombin-derived host defense peptide GKY25 inhibits endotoxin-induced responses through interactions with lipopolysaccharide and macrophages/monocytes**

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III **Differential internalization of thrombin-derived host defense peptides into monocytes and macrophages**

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Manuscript

IV **Aggregation of thrombin-derived C-terminal fragments as a previously undisclosed host defense mechanism**

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I **The thrombin-derived host defense peptide GKY25 inhibits endotoxin-induced responses through interactions with lipopolysaccharide and macrophages/monocytes**

M.K.-B., M.vd.P., A.S. and F.H. conceived the project and designed the experiments. F.H. performed RT-qPCR and phosphorylation assay. M.K.-B. and F.H. performed confocal microscopy analysis. M.vd.P., M.K.-B., and F.H. performed flow cytometer analysis; M.vd.P. and F.H. performed *in vivo* studies. M.vd.P. carried out the cytokine assays, M.K.-B. and M.Mö. performed electron microscopy analysis, AC.S. performed NF-κB assays. M.K.-B., A.S., and F.H. wrote the manuscript. All of the authors discussed the results and commented on the final manuscript.

II **Thrombin-derived host defense peptides modulate monocyte/macrophage inflammatory responses to Gram-negative bacteria**

M.vd.P., A.S., and F.H. conceived the project and designed the experiments. F.H. performed immunoblotting, confocal microscopy analysis, phagocytosis assays, cytokine analysis. AC.S. and F.H. carried out NF-κB assays. M.Mö. performed the electron microscopy analysis. M.vd.P. and F.H. wrote the manuscript. All of the authors discussed the results and commented on the final manuscript.

III **Differential internalization of thrombin-derived host defense peptides into monocytes and macrophages**

M.vd.P. and F.H. conceived the project and designed the experiments. F.H. performed immunoblotting, cytotoxicity assays, and flow cytometry analysis. A.N. and F.H. carried out confocal microscopy analysis. M.vd.P. and F.H. wrote the manuscript. All of the authors discussed the results and commented on the manuscript.

IV **Aggregation of thrombin-derived C-terminal fragments as a previously undisclosed host defense mechanism**

J.P. and A.S. conceived the project and designed the experiments. J.P. performed experiments for peptide production, sample preparation, anti-microbial assays, and fluorescence microscopy. J.P. and F.H. performed the confocal microscopy and immunoblotting. J.P. and M.vd.P. carried out the hemolysis assay. J.P. and M.Mö. performed the TEM. P.B. and R.H. contributed the *in silico* modeling data, and M.Ma. performed the ellipsometry experiments. J.P. and A.S. wrote the manuscript. All of the authors discussed the results and commented on the final manuscript.

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Summary

Nowadays, anti-infective treatments do not address the excessive activation of inflammatory pathways during infection and sepsis, and bacterial resistance problems are increasing. Hence, there is a substantial unmet need for novel therapies that go beyond mere classical antibacterial effects, and which also modulate pro- and anti-inflammatory pathways during infections. Host defense peptides might be possible candidates for the development of novel treatment strategies, as they facilitate clearance of pathogens and modulate various immune responses. The host defense peptides from the C-terminus of human thrombin (TCPs) constitute a novel class of immunomodulatory peptides, generated in humans during infection and inflammation. Previous investigations have shown that TCPs inhibit pro-inflammatory immune responses *in vitro* and *in vivo*, but their mode of action remained unknown.

In paper I, we show that the TCP GKY25 binds to LPS, thereby preventing TLR4 dimerization at the surface of monocytes and macrophages, and the subsequent activation of the NF-κB/AP-1 mediated signal transduction pathways and the release of pro-inflammatory cytokines. Furthermore, we found that GKY25 blocks TLR4- and TLR2- induced NF-κB/AP-1 activation in response to several other microbial-derived agonists, including lipoteichoic acid, peptidoglycan, and zymosan. Whereas the first publication explains the effect of GKY25 on cell activation by purified bacterial components, the peptide's effect on whole bacteria and their interaction with immune cells remained unclear.
In paper II we show the presence of TCPs in chronic and acute wound fluids. Moreover, we found that GKY25 binds to Gram-negative bacteria in the extra- and intracellular environment and reduces pro-inflammatory immune response of monocytes/macrophages while preserving their important phagocytic function. In conclusion, the first two publications demonstrate a multifunctional C-terminal thrombin-derived host defense peptide (GKY25), which reduces pro-inflammatory responses during LPS stimulation and Gram-negative bacterial infection by interacting with endotoxins, bacteria, and inflammatory cells. During the investigations of the modes of action of TCPs, it was observed that the GKY25 is internalized in monocytes and macrophages. However, the exact uptake mechanism remained unknown.
In an ongoing manuscript (paper III) the results reveal that TCPs of 2-3 kDa are differently internalized by clathrin-dependent and -independent endocytosis pathways in monocytes and macrophages, depending on the type of cells and the length of the peptide, as well as the presence of LPS and bacteria. Internalized GKY25 was transported to late endosomes and lysosomes, where it remained

detectable for up to 10 h.
Finally, paper IV describes a C-terminal thrombin fragment of about 11 kDa, which forms amorphous aggregates in the presence of LPS and Gram-negative bacteria. These larger TCPs facilitate bacterial scavenging, killing and increase phagocytic clearance of *E. coli* BioParticles in RAW264.7 cells by binding to LPS and Gram-negative bacteria. Furthermore, electron and confocal microscopy analysis showed that these TCPs are present in wound fluids and form aggregates in the presence of *E. coli* and LPS.
Taken together, herein we show that the multifunctional TCPs plays a physiological role during infection and inflammation, and have therapeutic potentials by modulating multiple interactions involving bacteria, endotoxins, and inflammatory cell responses.

Zusammenfassung

Antimikrobielle Peptide spielen eine wichtige Rolle im angeborenen Immunsystem. Sie besitzen die Fähigkeit eindringende Pathogene direkt zu töten und verschiedene Immunantworten zu modulieren. Darüber hinaus steigt die Anzahl der Antibiotikaresistenzen, außerdem behandeln antibakterielle Therapien nicht die übermäßige Immunantwort während einer Infektion und Sepsis. Aus diesem Grund besteht ein dringender Bedarf für die Entwicklung neuer Behandlungsmethoden, die über die klassischen antibakteriellen Wirkungen hinausgehen und auch die pro- und anti-entzündlichen Immunreaktionen während einer Entzündung beeinflussen. Antimikrobielle Peptide vom C-Terminus des Gerinnungsproteins Thrombin (TCPs) bilden eine neue und bisher nicht bekannte Klasse von immunmodulatorischen Peptiden, die beim Menschen während einer Infektion und Entzündung freigesetzt werden und Behandlungspotential gegen Infektion und septischen Schock besitzen.
In der ersten Veröffentlichung zeigen wir, dass das prototypische antimikrobielle Peptid vom C-Terminus des Thrombins (GKY25) an Lipopolysaccharide von Gram-negativen Bakterien bindet. Diese Interaktion verhindert eine Dimerisierung des *Toll-like* Rezeptors 4 (TLR) an der Oberfläche von Monozyten und Makrophagen und hemmt anschließend die Aktivierung der NF-κB/AP-1 Signalkaskade und die damit verbundende Freisetzung von pro-inflammatorischen Zytokinen. Darüber hinaus konnte die Aktivierung der TLR4- und TLR2-induzierten NF-κB/AP-1 Signalkaskade durch andere mikrobielle Agonisten von GKY25 blockiert werden. Die Ergebnisse in der zweiten Veröffentlichung zeigen die Anwesenheit von TCPs in chronischen und akuten Wundflüssigkeiten. Desweiteren zeigen wir, dass GKY25 an Gram-negative Bakterien in der extrazellulären und intrazellulären Umgebung binden und die pro-entzündliche Immunantwort in Monozyten und Macrophagen reduzieren, aber gleichzeitig ihre phagozytische Leistung beibehalten. Zusammenfassend zeigen die ersten beiden Veröffentlichungen ein multifunktionelles antimikrobielles Peptid vom C-Terminus des Thrombins, dass die pro-entzündiche Immunantwort während einer Lipopolysaccharid und Gram-negativen bakteriellen Infektion reduziert und dabei mit Endotoxinen, Bakterien und Immunzellen interagiert.
Im dritten Projekt verdeutlichen wir, dass TCPs über die Clathrin-abhängige und die Clathrin-unabhängige Endozytose von Monozyten und Makrophagen aufgenommen werden. Zusätzlich ist der Endozytoseweg abhängig von der Art der Zellen und der Länge des Peptides sowie der Anwesenheit von Lipopolysacchariden oder Bakterien. Das internalisierte antimikrobielle Peptid GKY25 wird nach der Aufnahme zu späten Endosomen und Lysosomen transportiert und ist bis zu 10 Stunden im Zellinneren nachweisbar, ohne einen Abbau in kleinere

Fragmente aufzuweisen.
Schließlich wird im vierten Projekt ein C-terminales Thrombinfragment von etwa 11 kDa beschrieben, das in Anwesenheit von Lipopolysacchariden und Gram-negativen Bakterien amorphe Aggregate bildet. Durch die Interaktion des größeren TCPs mit Lipopolysacchriden und Gram-negativen Bakterien wird das Einfangen und die Tötung von Bakterien erleichtert. Des Weiteren werden vermehrt *Escherichia coli* BioParticles von RAW264.7 Zellen phagozytiert. Elektronen- und Konfokal-mikroskopische Analysen zeigen, dass TCPs in Wundflüssigkeiten vorhanden sind und Aggregate in Gegenwart von *Escherichia coli* und Lipopolysacchariden bilden. Zusammenfassend wird im letzten Projekt demonstriert, dass Thrombin in der Anwesenheit von Lipopolysacchariden und Bakterien amyloide Strukturen bildet.

Abbreviations

ADP	Adenosine diphosphate
AMP	Antimicrobial peptides
AP-1	Activator protein 1
AP2	Adaptor protein 2
ARF6	ADP-ribosylation factor 6
ATP	Adenosine triphosphate
BAR domain	Bin, amphiphysin and Rvs
CALM	Clathrin assembly lymphoid myeloid leukemia protein
C(x)	Complement factor (x)
CCR	CC chemokine receptor
CD	Cluster of differentiation
CIE	Clathrin-independent endocytosis
CHDPs	Cationic host defense peptides
CLRs	C-type lectin receptors
CME	Clathrin-mediated endocytosis
CpG	Cytidine-phosphate guanosine
CREB	Cyclic AMP-responsive element-binding protein
DAMPs	Damage associated molecular patterns
DNA	Desoxyribonucleic acid
dsRNA	Double-stranded RNA
EDH2	Eps-15 homology domain-containing protein 2
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPI	Glycosylphosphatidylinositol
GTP	Guanosine triphosphate
HDP	Host defense peptides
HMGB1	High mobility group box-1
HNPs	human neutrophil peptides
HSPs	Heat shock proteins
IFN	Interferon
Ig(x)	Immunoglobulin
IKK	IκB kinase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRAK	IL-1 receptor-associated kinases
IRF	Interferon-regulatory factor
JNK	c-Jun N-terminal kinase
LBP	LPS binding protein
LPS	Lipopolysaccharides
LRR	Leucine-rich repeat

LTA	Lipoteichoic acid
MAL	MyD88-adaptor-like
MAPKs	Mitogen-activated protein kinases
MBL	Mannose-binding lectin
MCETs	Mast cell-derived extracellular traps
M-CSF	Macrophage colony-stimulating factor
MD-2	Myeloid differentiation factor-2
MHCII	Major histocompatibility complex II
MKK	Mitogen-activated protein kinase kinase
MyD88	Myeloid differentiation factor protein 88
NETs	Neutrophil-derived extracellular traps
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B cells
NK cells	Natural killer cells
NLRs	NOD-like receptors
NO	Nitric oxide
NOD	Nucleotide binding protein
PAMPs	Pathogen-associated molecular patterns
PARs	Protease-activated receptors
PGN	Peptidoglycan
proHNPs	pro-protein HNPs
PRR	Pattern recognition receptor
RIG	Retinoic acid-inducible
RLRs	RIG-I-like receptors
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ssRNA	single-stranded RNA
TAB	TAK1-binding protein
TAFI	Thrombin activatable fibrinolysis inhibitor
TAK	Transforming growth factor-β-activated kinase
TCPs	Thrombin-derived peptides
TIR	Toll-interleukin-1 receptor
TIRAP	TIR domain-containing adaptor protein
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-α
TRAF	Tumor necrosis factor receptor-associated factor-1
TRAM	TRIF-related adaptor molecule
TRIF	TIR domain-containing adaptor inducing interferon-b

1 Introduction

The human skin is continuously interacting with different microorganisms, and it undergoes several injuries during a lifetime. Bacterial colonization and infection of a wound may lead to delayed healing and increases the risk for deeper infections, which in the worst case can lead to sepsis [1]. Wound healing is a complex and dynamic process characterized by sequential phases involving initial hemostasis, followed by inflammation, proliferation, and remodeling [2]. During wound healing, there is a constant interaction between residing microorganisms and our host immune system [3]. Phagocytic cells, like monocytes and macrophages, play an essential role in wound healing. These cells have various functions in host defense, including the release of pro-inflammatory cytokines and reactive oxygen species (ROS), to clear the infection at the site of injury [4, 5]. Additionally, macrophages remove apoptotic cells and promote cell proliferation of endothelial cells and fibroblasts through the release of growth factors and cytokines [6]. Besides the immune cells, the coagulation system also plays a fundamental role in host defense. The central role of coagulation system is to stop bleeding after wounding. However, the coagulation system also protects the host from invading pathogens, by trapping them, and regulates inflammation [7]. One central player of the coagulation system is the serine protease thrombin. Upon activation during tissue injury, it can form a clot through the cleavage of fibrinogen to fibrin [8]. Furthermore, thrombin is also a source of host defense peptides [9]. Host defense peptides may exert multifunctional properties including bacterial killing, modulation of pro- and anti-inflammatory immune responses, promotion of the differentiation of macrophages and modulation of wound repair [10, 11, 12, 13].

In general, the main function of these inflammatory responses is to restore homeostasis during infection and tissue injury. However, a dysfunctional immune system may lead to a high release of cytokines and destructive inflammatory mediators, which can cause tissue damage and in the worst case organ failure, and sepsis [11]. For this reason, the reduction of pro-inflammatory responses is an essential mechanism to reduce tissue damage during infection [14, 15].

2 The immune system

Throughout life, a human body is exposed to different kinds of microbial pathogens that may cause life-threatening infections. In order to survive, the human body has developed various defense mechanism, to protect itself against invading pathogens [16]. The first line of defense is a physical barrier consisting of the epidermis and the mucosal epithelium of the respiratory, gastrointestinal, and reproductive tracts. In addition, commensal microbes, present on the epidermis, in the gut and other mucosal membranes, compete with invading pathogens for nutrients and location. A further line of defense is built by the acidic environment of the skin surface, the production of antimicrobial peptides from keratinocytes and the release of body fluids like sweat, saliva, and tears containing amongst others, the antibacterial enzyme lysozymes and salt [16, 17]. However, in some cases pathogens manage to overcome this physical and chemical barrier to enter the host. These invading pathogens are recognized by the immune system, which activates various complex immune responses. The immune system is traditionally divided into two parts, the innate immunity and adaptive immunity, which interact with each other during immune responses.

2.1 Innate Immunity

The innate immune system is an ancient defense mechanism in comparison to the adaptive immune system. It is part of almost all multicellular organisms (metazoans), and most organisms on this planet survive on an innate immune system alone [18]. Vertebrates are the only ones that have developed an adaptive immunity [19]. The main function of the innate immune system is the detection of invading pathogens and the induction of mechanism to eliminate potential infectious threats [16]. Furthermore, the innate immune system is not only active against pathogens, but it also recognizes endogenous molecules released by stressed cells, undergoing necrosis, and damaged tissue. These molecules are known as damage-associated molecular patterns (DAMPs), such as the high mobility group box-1 (HMGB1), heat shock proteins (HSPs), desoxyribonucleic acid (DNA), and adenosine triphosphate (ATP). Both DAMPs and pathogens can be recognized by specific receptors, which is explained in more detail in chapter 3 'Recognition of pathogens' [20, 21]. The innate immune system includes different immune cells, like phagocytes, (neutrophils, macrophages, and dendritic cells) mast cells, eosinophils, natural killer (NK) cells, and natural killer T cells, as well as endothelial cells, epithelial cells, and fibroblasts [22]. Furthermore, the complement system and the coagulation system are part of in-

nate immunity and promote the clearance of invading pathogens [23]. Moreover, the innate immune system is able to activate the adaptive immune responses.

2.2 Adaptive Immunity

Adaptive immune responses are highly specific and facilitate long-term protection by developing a memory effect against specific pathogens. However, this immune response needs time to be efficiently active. Adaptive immunity involves B cells and T cells, which recognizes specific antigens, present on the surface of macrophages, dendritic cells, and mast cells, as well as antibodies and cytokines. Macrophages and dendritic cells internalize self and non-self proteins, which are degraded into peptides. The peptides are loaded onto the major histocompatibility complex class II (MHC class II) molecules, which are transported to the cell surface for antigen presentation [19]. In order to activate naïve T cells at the lymph node site, these antigen presenting cells also need to express CD80 and CD86 on the cell surface, to ensure that T cells are only activated in the presence of pathogens together with the costimulatory mediators CD80 and CD86 [24]. Activated T cells can either produce cytokines, to activate further effector cells, or actively attack infected host cells. B cells are essential for the humoral immune response by producing highly specific antibodies that bind to the surface of pathogens and facilitate their elimination by phagocytes. Besides antibody production, B cells generate immunological memory, present antigens, and regulate the production of different cytokines [25].

2.3 Inflammation

Inflammation is a complex and protective host response which facilitates clearing of infection and subsequent healing processes. The main inducers of inflammation are microbial infections and damaged tissue through injury. The classical signs of inflammation, caused by microbial infection, are redness, swelling, heat, pain, and loss of function [26].
In the initial steps of infection, microbes and microbial products are recognized through pattern recognition receptors (PRR) on immune cells. The recognition of microbes triggers inflammatory responses through the activation of various mediators, such as vasoactive amines released from mast cells or vasoactive peptides derived from the coagulation system, to facilitate vascular permeability and vasodilation. The activation of the complement system and their fragments (C3a, C4a, and C5a) initiate recruitment of monocyte and granulocytes and degranulation of mast cell. In addition, the release of cytokines from macro-

phages, mast cells, and other inflammatory cells, activate the endothelium and leukocytes. Chemokines recruit further inflammatory cells, such as neutrophils, monocytes, and dendritic cells, to the site of infection and tissue injury, to eliminate pathogens and to facilitate tissue repair [27, 28].

2.4 Immune cells - Monocytes and Macrophages

Monocytes and macrophages are mononuclear, heterogeneous cell populations, which are originally derived from common myeloid progenitors of the red bone marrow (mesoderm). The human blood consists approximately of 4-10% of mature monocytes within the leukocyte cell population. These cells can differ in size (5-20 μm), as well as nucleus morphology and degree of granularity [29]. Monocytes are mainly present in the peripheral blood, but the spleen and lung contain storage of monocytes, which can be released when they are needed [30]. The monocyte population in humans can be divided into three different classes based on the expression of CD14 and CD16. The inflammatory monocytes $CD14^{+}CD16^{+}$ and $CD14^{+}CD16^{-}$ express CC-chemokine receptor 2 (CCR2) and eliminate pathogens and regulate the pro-inflammatory response at the site of infection or inflammation [31]. In addition, the human $CD14^{low}CD16^{+}$ express CX3CR1 and role on the luminal endothelium, to patrol healthy tissue, and also contribute to wound healing and phagocytosis of damaged cells [32, 33]. In mice, monocytes are also divided into different subsets, based on the expression of the receptors Ly6C, CX3CR1, and CCR2. The $Ly6C^{high}$ inflammatory monocyte population expresses high levels of CCR2 and low levels of CX3R1, whereas the patroling monocytes express high levels of CX3CR1 and low levels of Ly6C and CCR2 [34].

Under steady state conditions, the inflammatory blood monocytes circulate between the bone marrow and bloodstream. However, during infection pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and type 1 interferon (IFN), enhance the recruitment of monocytes from the bone marrow to the site of infection [35]. In order to clear the infection, monocytes exert antimicrobial effects through the release of TNF-α and the inducible nitric oxide synthase (iNOS), which leads to the production of nitric oxides (NO) to promote bacterial killing in phagosomes [36]. Furthermore, monocytes may transport antigens of pathogens to the lymph node to present the antigen for the activation of specific T cells [37].

During inflammation, monocytes migrate from the bloodstream to the tissue to differentiate into 'inflammatory macrophages'. Under steady state conditions, however, the main macrophage population is not monocyte-derived, but consists of tissue-resident cells that have developed during embryogenesis from

embryonic precursors, which are distributed in the tissue before birth and can maintain their population by self-renewal [30, 37]. In general, a high diversity of different macrophage populations exist and depending on the different anatomical location in the tissue, macrophages fulfill their special functions [29]. Tissue-resident macrophages express various receptors to recognize pathogen-associated molecular patterns (PAMPs) and DAMPs, including Toll-like receptors (TLRs), NOD-like receptors (NLRs), retinoic acid-inducible (RIG)-I family receptors (RLRs) and lectin receptors. Depending on the microenvironment, tissue-resident macrophages express a unique phenotype of receptors for the activation of initial immune responses [38]. Moreover, the two growth factors macrophage colony-stimulating factor (M-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) are key players in the regulation of the amount and function of macrophages. The growth factor M-CSF regulates differentiation and proliferation of macrophages, as well as the survival and self-renewal of tissue resident-macrophages, whereas GM-CSF activates monocytes-derived macrophages during inflammation and tissue injury, to promote the pro-inflammatory immune response [39]. Inflammatory monocytes and macrophages play an essential role in innate immunity by eliminating apoptotic cells, cell debris and pathogens during inflammation, whereas the primary function of tissue-resident macrophages is maintaining tissue homeostasis and resolving inflammation [40].

3 Recognition of pathogens

Immune cells such as macrophages and neutrophils play an essential role in protecting the host against infection, by the recognition and elimination of invading pathogens. Microbes are recognized by their very specific and conserved molecular patterns on the cell surface termed PAMPs. PAMPs are important for the survival of the microbe and are only produced by microbes and not by the host cells. Each class of microorganisms contains specific structures on the cell surface that can be recognized by host cells [41], for example the lipopolysaccharides (LPS) [42], lipoteichoic acid (LTA) [43], peptidoglycan (PGN) [44], bacterial proteins like flagellin [45], bacterial DNA or viral RNA [46, 47] (Figure 1).

3.1 Pattern-recognition receptors

During inflammation, PAMPs are recognized by specific PRRs. These receptors can be found as secreted receptors present in serum or as expressed receptors on the cell surface or in the cytoplasm of immune cells, such as monocytes, macrophages, and dendritic cells, but also of endothelial cells and fibroblasts [48]. Several different structural and functional proteins have been identified that are involved in pattern recognition, including TLRs, C-type lectin receptors (CLRs) and RLRs and NLRs [41, 49]. The TLRs belong to the membrane-bound receptors and sense invading pathogens on the cell surface or intracellular in endosomes or lysosomes (see section 3.2 'Toll-like receptors') [48]. RLRs, NLPRs, and CLRs are all located in the cytoplasm. RLRs recognize dsRNA from a virus, whereas NLRs and CLRs sense different PAMPs and DAMPs [26]. Mannose-binding lectins (MBL) are soluble receptors, activating the lectin-pathway of the complement cascade by their interaction with bacterial polysaccharides [50]. The main functions of PRRs are the induction of various immune responses, including the release of cytokines, chemokines, antimicrobial peptides, and enzymes, as well the uptake of pathogens [41].

3.2 Toll-like receptors

Originally, the Toll receptor was discovered being responsible for the dorsal-ventral patterning in embryos of the fruit fly *Drosophila melanogaster* [51]. Later on, it was shown that TLRs are involved in the inflammatory response by activating nuclear factor 'kappa-light-chain-enhancer' of activated B cells (NF-κB) and activator protein 1 (AP-1) pathways [52]. To date, there are ten different

TLRs found in humans (Figure 1) and 12 in mice and they are one of the most studied pattern recognition receptors. [53]. Toll-like receptors are type I integral membrane glycoproteins consisting of the extracellular leucine-rich repeat (LRR) protein and the Toll/IL-1R (TIR) domain [54]. The LRR consists of an evolutionary conserved motif 'LxxLxLxxN', which is structurally shaped like an arc or horseshoe and is sensing PAMPs [55]. The intracellular TIR domain recruits and interacts with various adaptor molecules to activate downstream signaling pathways [54]. However, each TLR is specialized in recognition of specific PAMPs [56, 57] (Figure 1). In detail, TLR4 recognizes LPS of Gram-negative bacteria and is located on the cell membrane and in endosomes [56, 57]. Furthermore, TLR5 senses flagellin and TLR2, which forms heterodimers with TLR1 or TLR6 or TLR10, recognizes peptidoglycan, triacylated lipopeptides or diacylated lipopeptides at the cell surface [57, 58, 59]. TLR3 recognizes retroviral double-stranded RNA (dsRNA), TLR7-TLR8 viral single-stranded RNA (ssRNA) and imidazoquinolines, whereas TLR9 senses bacterial cytidine-phosphateguanosine (CpG) and viral DNA motifs. These last four TLRs are localized in endosomes [53, 56].

3.3 Toll-like receptor signaling

The interaction of the TLRs with a specific microbial pattern, leads to the recruitment of TIR domain-containing adapters, like myeloid differentiation factor protein 88 (MyD88), TIR domain-containing adaptor inducing interferon-β (TRIF), TIR domain-containing adaptor protein (TIRAP)/MyD88-adaptor-like (MAL) and TRIF-related adaptor molecule (TRAM) [60]. TLR signaling pathways can be divided into MyD88-dependent and TRIF-dependent pathways. All TLR signaling, except TLR3, are conducted through MyD88-dependent pathways [56] (Figure 1). However, TLR4 is the only TLR that is interacting with all four TIR domain-containing adaptors by activating both the MyD88-dependent and the TRIF-dependent pathways [61]. Activation of the MyD88 dependent pathways induces the recruitment of IL-1 receptor-associated kinases IRAK4, IRAK1, and IRAK2 which interact with tumor necrosis factor receptor-associated factor-1 (TRAF6). TRAF6 activates TAK1-binding protein (TAB2 and TAB3) together with the associate transforming growth factor-β-activated kinase (TAK1). Those lead to the activation of different mitogen-activated protein kinase kinase (MKK) and the inhibitor of NF-κB (IKK), as well as the mitogen-activated protein kinases (MAPKs) p38 and c-Jun N-terminal kinase (JNK). Further, the MAPKs p38 and JNK, and IKKα/IKKβ induce the activation of the transcription factors NF-κB, cyclic AMP-responsive element-binding protein (CREB) and AP-1, which finally leads to the expression of

pro-inflammatory cytokines, such as IL-6, IL-12p40, and TNF-α [53, 61].
The recruitment of TRAM and TRIF initiates the activation of the TRIF dependent pathway also known as the MyD88-independent pathway, which is associated with TRAF6 and TRAF3. The signaling cascade via TRAF6 leads to the induction of MAPKs, NF-κB and pro-inflammatory cytokines as described for the MyD88-dependent pathways. In contrast, the activation of TRAF3 involves the regulation of interferon-regulatory factors (IRF3, IRF7) followed by the translocation of type I IFN genes and the expression of IFN-α and IFN-β, as well as the anti-inflammatory cytokine IL-10 [53, 56, 57].

3.4 LPS recognition by TLR4

TLR4 recognizes various DAMPs (HMGB1 and HSPs) and PAMPs, of which LPS is the most prominent interactor and inducer of intracellular pro-inflammatory signaling [62]. LPS are mainly released from the outer cell wall of Gram-negative bacteria and bind at first to soluble LPS-binding proteins (LBP) [63, 64]. LBP is a shuttle protein, which facilitates the transfer of LPS to the glycosylphosphatidylinositol (GPI)-linked protein CD14, which is found as soluble and membrane-bound protein, and promotes the transfers of LPS to TLR4/MD2-receptor complexes [65, 66]. The soluble protein MD-2 binds to LPS and is directly associated with TLR4 and induces the dimerization of the TLR4. Subsequently, the TIR domain recruits the intracellular adaptor molecules MyD88, TIRAP, TRIF, and TRAM, and activates either the MyD88-dependent or the MyD88-independent pathways [67]. These two pathways induce the activation of different MAP kinases, NF-κB/AP-1, IRF3 and IRF7, followed by the final production and release of pro-inflammatory cytokines, chemokines, and interferons [57].

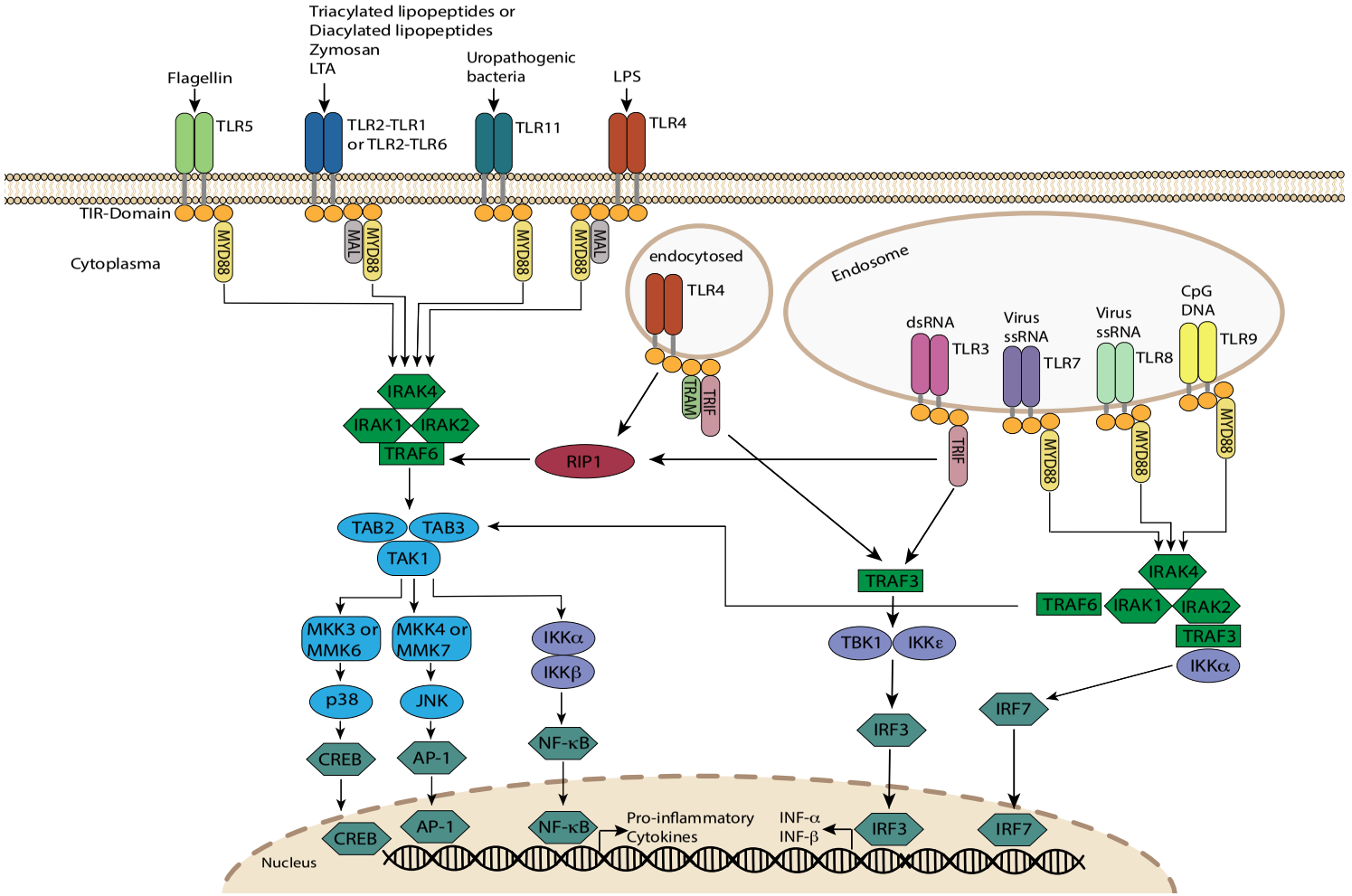

Figure 1: Toll like receptor pathways. Schematic overview of the different TLR signaling pathways (adapted from O'Neil et al., 2013, [53]).

4 Host defense peptides

Host defense peptides (HDPs) are short peptides (usually between 12 and 50 amino acids) which are positively charged and amphipathic molecules. Originally, HDPs were known as antimicrobial peptides (AMPs) due to the observation, that AMPs exhibit broad antimicrobial properties against bacteria, fungi, viruses and parasites *in vitro* [10, 68]. However, recent studies have shown that the antimicrobial properties of most HDPs were compromised under physiological conditions, such as the presence of NaCl or plasma, whereas their immunomodulatory actions remained preserved [69, 70]. For this reason, the terms HDPs or cationic host defense peptides (CHDPs) are more often used than AMP nowadays.
HDPs are produced in all multicellular organisms, including plants, insects, and vertebrates, in order to maintain homeostasis and to protect the host from microbial infection. Particularly, invertebrates such as insects, octopus, and starfish rely on host defense peptides to fight against invading pathogens [71].
HDPs can be broadly categorized into different groups according to their secondary structure: the α-helical and the β-sheet peptides, which are stabilized by disulfide bridges [72]. The most studied example of α-helical HDPs is the cathelicidin family, including the only human HDP LL37. In addition, the family of TCPs including GKY25, HVF18, and FYT21, belong to the α-helical HDP group [9, 73, 74]. The α-defensins and β-defensins are the best characterized HDP that belong to the class of β-sheets containing peptides.

Cathelicidins: Cathelicidins consist of a signal peptide region at the N-terminus, a highly conserved cathelin-like pro-domain and a variable C-terminus, that encodes for HDPs, which can be released through proteolytic cleavage. hCAP18 is the only cathelicidin precursor in humans, which releases the HDP LL37 [75]. LL37 is mainly produced during infection and inflammation from immune cells, for example, neutrophils, monocytes, mast cells, and epithelial cells. Moreover, high concentrations of LL37 are released in the local environment from degranulating neutrophils during infection and inflammation and fulfill various immunomodulatory functions [10].

Defensins: Defensins are HDPs consisting of 30-45 amino acid, including six cysteines that form three disulfide bridges between each other, which are rich in arginine and aromatic amino acid residues [76]. The defensins are grouped in α-defensins and β-defensins, which are expressed in humans, and θ-defensins, that are only present in nonhuman primates like macaques and baboons [72, 77]. α-Defensins are also known as human neutrophil peptides (HNPs) and are secreted

as a pro-protein (proHNPs) from different cell types, like neutrophils, peripheral blood mononuclear cells, bone marrow, spleen and thymus [78]. The β-defensins are also expressed as pro-proteins, mainly in epithelial cells, and are converted to the active form by proteolytical cleavage. Additionally, immune cells like monocytes, macrophages, and dendritic cells release β-defensins [79]. The main structural difference between α-defensins and β-defensins is the organization of the disulfide bridges between the cysteines [80].

4.1 Modes of action of HDPs

HDPs are multifunctional molecules of the innate immune system that may facilitate bacterial killing and modulate various immune responses during infection and inflammation [81, 82, 83]. This section will briefly focus on the immunomodulatory functions of HDPs.

HDPs, like LL-37, can modulate the release of pro-inflammatory cytokines, like TNF-α, IL-6, and IL-12p70, during TLR stimulation with LPS, through the direct binding to LPS and thereby neutralizing LPS induced TLR4 dimerization or directly influencing the NF-κB pathway [11, 84, 85]. Moreover, under certain circumstances the HDP LL-37 can enhance the release of anti-inflammatory cytokine, like IL-10 and thereby balancing the pro- and anti-inflammatory immune responses during infection [86]. In order to clear an infection, HDPs such as HNP-1, IDR-1, and IDR-1002 are able to recruit immune cells directly, by operating as a chemoattractant, or indirectly by promoting the expression of specific chemokines in monocytes, macrophages, epithelial, cells or mast cells [87, 88, 89, 90]. Next, to the recruitment of neutrophils, monocytes, and macrophages, HDPs may also be able to enhance phagocytosis of bacteria in these phagocytic cells [91, 92]. Furthermore, HDPs may enhance bacterial killing intracellular by facilitating an increased production of ROS and NO [91].

Bacterial infection and other inflammatory stimuli trigger the formation of neutrophil-derived extracellular traps (NETs) or mast cell-derived extracellular traps (MCETs), which are a network containing antimicrobial molecules and DNA. In this network, HDPs may also enhance bacterial killing in the extracellular environment, as is shown for LL-37 and HNP [93].

HDPs form a link between the innate and adaptive immune system. They may influence adaptive immunity indirectly, by promoting the differentiation of human monocytes into dendritic cells and by generating an enhanced T helper cell response [94]. HDPs also affect B and T cells directly, by modulating the production of immunoglobulin (Ig)G and M in B cells, and the corresponding cytokine production in T cells [95, 96].

At the site of injury, various HDPs are expressed by keratinocytes, endothelial

cell, and immune cells, and promote wound healing. HDPs may induce cell migration of for example keratinocytes, cell proliferation, re-epithelialization, and may modulate angiogenesis, which supports closure and healing of the wound [97].

4.2 Thrombin and its derived C-terminal host defense peptides

The coagulation cascade plays a central role in host defense during tissue injury and infection. Through the activation of various proteins in the coagulation cascade, the serine protease prothrombin gets proteolytically cleaved into thrombin, which is mainly known for converting soluble fibrinogen into an insoluble fibrin clot [98, 99]. This clot formation stops bleeding, traps microbes to protect the host from invading pathogens and activates inflammatory immune responses [8]. Besides functioning as a pro-coagulant, thrombin can also act as anticoagulant via the activation of the thrombin activatable fibrinolysis inhibitor (TAFI) [100]. Thrombin also interacts with almost all cells. This interaction is mediated through protease-activated receptors (PARs), which are localized on endothelial cells, monocytes, fibroblasts, T-lymphocytes, smooth muscle cells, neurons and various other cell types, but not erythrocytes [101]. Cell activation through the PARs initiates various processes which promote wound healing after tissue injury [102]. Examples of this are that thrombin activates chemokine production via the PARs to recruit inflammatory cells to the site of injury and that it enhances angiogenesis locally, by activating cell migration, endothelial cell and smooth muscle cell proliferation and vascular tube formation [101, 103].

Previously our lab showed that proteolytic cleavage of thrombin by neutrophil elastase forms thrombin-derived C-terminal peptides (TCPs). In agreement, various low molecular weight TCPs in the range of 2-15 kDa have been identified in wound fluids, including HVF18 (HVFRLKKWIQKVIDQFGE) and the 11 kDa TCP [9]. Moreover, it was shown that the metalloproteinase elastase from *P. aeruginosa* cleaves the TCP FYT21 (FYTHVFRLKKWIQKVIDQFGE) [74] from thrombin, and the same peptide was also generated in the presence of the metalloproteinase aureolysin from *S. aureus* [104], together indicating that bacteria can mimic the formation of these smaller TCPs.
Initial studies showed that the prototypic peptide GKY25 (GKYGFYTHVFRLKKWIQKVIDQFGE) has antimicrobial activity *in vitro* against the Gram-negative bacteria *E. coli*, and *P. aeruginosa*, the Gram-positive bacteria *S. aureus*, and the fungus *Candida albicans*, through bacterial membrane lysis [9, 105]. Furthermore, it was shown that GKY25 exerted the strongest immun-

omodulatory effect during the stimulation of macrophages with LPS, LTA and zymosan as compared to other N- and C-terminally truncated peptides [105]. In a mouse model of *P. aeruginosa*-induced sepsis and LPS-induced shock, GKY25 significantly increased the survival of the mice by reducing pro-inflammatory immune responses while showing only a moderate reduction on the amount of bacteria in kidney, spleen, and liver [9, 14, 105]. In addition, GKY25 reduced the excessive activation of the clotting cascade during LPS-induced inflammation, by inhibiting contact activation and modulating LPS-induced tissue factor mediated coagulation. GKY25 also reduced pulmonary leakage and fibrin deposition in the lungs of mice during *P. aeruginosa*-induced sepsis [9, 14, 105]. Together, these observations highlight the important immunomodulatory and anti-coagulative function of GKY25 *in vivo* during LPS-induced shock and *P. aeruginosa* sepsis.
From a structural viewpoint, GKY25 is able to form an amphipathic helical conformation in the presence of LPS or negatively charged liposomes [9, 106]. Furthermore, GKY25 contains a helix-stabilizing N-cap motif and an interspersed hydrophobic residue, which enables this helix formation [107]. In comparison, the 12 amino acid internal fragment of GKY25 (VFRLKKWIQKVI) is also binding to LPS but does not have anti-endotoxic effects [105]. This together might indicate that the length of the peptide influences helix formation and that a combination of the binding to LPS/lipid A and the resulting helix conformation is mediating anti-endotoxic effects [106].

Since previous investigations have shown that TCPs reduce the pro-inflammatory response during bacterial infection *in vivo* and increase the survival of mice in an LPS-induced shock or *P. aruginosa* induced sepsis model, the overall aim of this work was to investigate the detailed modes of action of TCPs, underlying the reduced cytokine response in monocytes and macrophages during LPS induced inflammation and bacterial infection.

5 Endocytosis

Cells communicate with each other and their environment through the interaction of ligands with signaling receptors on the cell membrane. Endocytosis plays an essential role in the regulation of signal transduction, cell migration, uptake of nutrients, and uptake (phagocytosis) of pathogens and removal of apoptotic cells [108, 109]. Endocytosis is described as the internalization of extracellular molecules and transmembrane proteins into the interior of eukaryotic cells. The vesicle trafficking of endocytosis can be divided into two central pathways, the clathrin-dependent and the clathrin-independent endocytosis (CIE) [110, 111].

5.1 Clathrin-dependent endocytosis

The clathrin-mediated endocytosis (CME) pathway is the most studied and best-characterized uptake mechanism among the different endocytosis pathways. It controls the internalization of nutrients, pathogens and growth factors, regulates antigen presentation and the recycling of receptors [112, 113]. The CME is induced through the interaction of the cargo with transmembrane proteins on the outer leaflet of the cell membrane and the recruitment of specific adapter molecules [114]. The adapter molecules, for example, the adapter protein 2 (AP2), AP180, clathrin assembly lymphoid myeloid leukemia protein (CALM) and epsins, bind to the inner leaflet of the plasma membrane and the cargo [115, 116, 117, 118]. This promotes the recruitment of clathrin and other scaffolding proteins, to interact with the clathrin adapter molecules, and mediates the formation of clathrin-coated pits [119]. The constriction of the clathrin-coated vesicles is facilitated by polymerization of actin filaments at the neck of the vesicle. Finally, the GTPase dynamin mediates recruitment of the Bin, amphiphysin and Rvs (BAR domain) proteins for vesicle scission into the intracellular environment [120]. In the cytoplasma, chaperones, protein kinases, and lipid phosphatases uncoat the vesicle and fuse it together with early endosomes [111]. Early endosomes, also known as sorting endosomes, determine the route of the specific cargo [121]. Early endosomes can mature to late endosomes and fuse together with lysosomes, leading to degradation of the cargo (Figure 2). Instead of degradation, the internalized molecules can also be delivered to the trans-Golgi network or get recycled by endosomal carriers that transport the cargo back to the cell membrane [122].
CME controls different functions: it may influence the activation of the signaling pathways, regulation of cell growth, expression of proteins on the cell membrane, cellular homeostasis, cell differentiation, and signal transmission by

synaptic vesicle recycling [119]. One example during immune responses is that the TLR4-MD2 complex binds LPS and is internalized into endosomes by the CME pathway, leading to the activation of the NF-κB signaling pathways and the subsequent release of cytokines, such as TNF-α, IL-10 and IFN-β [123].

5.2 Clathrin-independent endocytosis

Several different endocytosis pathways have been identified, that are independent of clathrin and adapter molecules for cargo internalization. The clathrin-independent endocytosis pathways are until today not fully understood, but includes a broad range of different endocytotic pathways, which are difficult to categorize [124]. Moreover, the same endocytic cargo can be internalized by different endocytosis pathways in different cell types or, in the same cell type, the uptake mechanisms change under different conditions [125].
Among the CIE pathways, the caveolin-dependent endocytosis is the most investigated pathway. Caveolae are cave-like invaginations of the cell membrane, which are enriched in sphingolipids, cholesterol, and the cholesterol-binding proteins caveolin 1-3, which are connected with the four different cavin proteins [126, 127]. Caveolae are formed through oligomerization of the integral membrane protein caveolin and they are stabilized by the cytoplasmic cavin proteins. In the end, the Eps-15 homology domain-containing protein 2 (EDH2) and the GTPase dynamin regulate the caveolae budding from the cell membrane [128]. After reaching the cytoplasm, caveolae can fuse together with early endosomes, mature to late endosomes and fuse together with lysosomes, or they are recycled from early endosomes to the cell membrane [129, 130] (Figure 2). Besides endocytosis, caveolae are involved in various biological processes, including the regulation of lipids, signal transduction and transcytosis [131]. Recent investigations indicate that caveolae mainly function in mechanotransduction, especially in adipocytes, muscle and endothelial cells [132, 133]. However, until today no receptor, ligand or pathogen was identified that is specifically internalized in cells through caveolae [127].
Besides caveolin-dependent endocytosis, various clathrin- and caveolin- independent pathways exist for the internalization of different cargos. For example, the GTPase RhoA endocytosis route is a dynamin-dependent pathway that internalizes cytokine receptors with bound ligands via small non-coated invaginations [134]. Another uptake mechanism is the ADP-ribosylation factor 6 (ARF6) associated pathway, which is dependent on cholesterol but not on clathrin and dynamin [135]. The vessicle scission during the dynamin-independent endocytic processes are mainly driven by the polymerization of actin, and the cargos are sorted by early endosomes either for recycling or degradation [136].

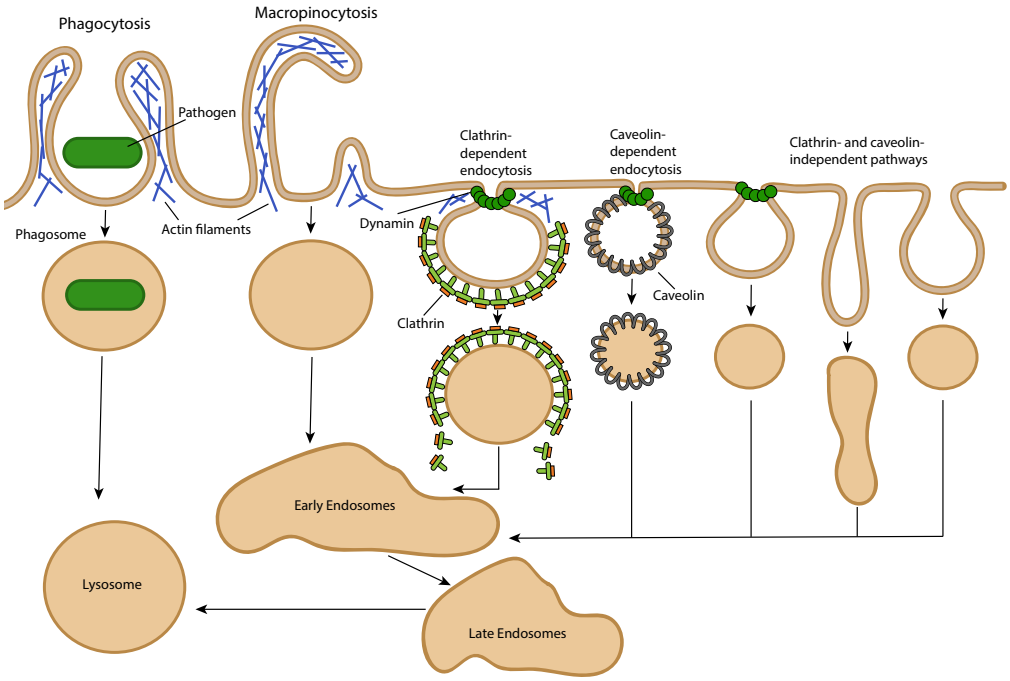

Figure 2: Endocytosis pathways. Schematic overview of the different endocytosis mechanisms, showing phagocytosis, macropinocytosis, clathrin-dependent endocytosis, caveolin-dependent endocytosis and clathrin- and caveolin-independent endocytosis pathways (adapted from Mayor and Pagano 2007, [125]).

5.3 Phagocytosis

Phagocytosis plays a key role in the innate and adaptive immune responses to pathogens, in which phagocytic cells engulf and kill pathogens. This process was described the first time by Elias Metchnikoff in 1891 [137].
At the site of infection, bacteria, fungi or parasites are recognized by PRRs present on the cell surface of amongst others neutrophils, macrophages, and dendritic cells. Furthermore, pathogens can be recognized by opsonins such as IgG or components of the complement cascade (C3b, C1q), which enhance the interaction with the receptors on phagocytes [138]. The binding of the pathogens with receptors induces an actin-dependent remodeling of the cell membrane and the cytoskeleton by forming a phagocytic vesicle, which leads to the engulfment of the pathogen. After a dynamin-dependent phagosome scission, the phagosome matures by fusion with endosomes and lysosome to a phagolysosome. In phagolysosomes, degradation of the pathogen through antimicrobial and degrading agents occurs, for example, antimicrobial peptides, different proteases, ROS, NO, and reactive nitrogen species (RNS) [139, 140] (Figure 2). The phagocytosis

of pathogens induces the release of pro-inflammatory cytokines, which recruits additional inflammatory cells to clear the infection. However, some pathogens have developed different strategies to evade their internalization and degradation in phagosomes. One strategy to avoid the recognition of pathogens by phagocytic cells is a polysaccharide-based capsule that prevents pathogens, like *Pseudomonas aeruginosa*, *Neisseria meningitidis* or *Strepococcus spp.*, from the binding of opsonins and internalization [141]. Another strategy that is used by *Staphylococcus aureus* is the expression of specific surface proteins that inhibit the binding to receptors [142].

6 Present Investigations

6.1 Paper I

The thrombin-derived host defense peptide GKY25 inhibits endotoxin-induced responses through interactions with lipopolysaccharide and macrophages/monocytes

Background:

Monocytes and macrophages play a fundamental role during infection, by eliminating invading microbes and releasing pro-inflammatory cytokines [143]. Monocytes, and macrophages recognize pathogens via pattern recognition receptors such as the toll-like receptors [48]. Binding of LPS to the TLR4/MD2 complex leads to dimerization of TLR4 followed by the activation of the NF-κB/AP-1 signaling pathway and the release of pro-inflammatory cytokines [144].
Previous work has disclosed a new function of thrombin-derived C-terminal peptides in host defense, which exert antimicrobial and immunosuppressive effects. The multifunctional HDP GKY25 (GKYGFYTHVFRLKKWIQKVIDQFGE), derived from the C terminus of human thrombin, exerts direct antimicrobial activities as well as antiendotoxic effects *in vitro* and *in vivo* [9, 14, 105]. It was shown that treatment with GKY25 in animal models of LPS-induced shock and *Pseudomonas aeruginosa* sepsis increased the survival of mice, by modulation of tissue factor-induced coagulation, but also by inhibition of pro-inflammatory responses in combination with antimicrobial effects [9, 14]. Furthermore, it was shown, that GKY25 is directly interacting with LPS, lipid A, and bacterial surface [106]. However, the detailed mode of actions remained unknown.

Aim of Paper I:

- To characterize the immunomodulatory effects of GKY25 during LPS-induced inflammation in monocytes and macrophages

Results and Conclusion:

Using monocytes and macrophages, the results show that interactions of GKY25 with LPS inhibits TLR4 dimerization, thereby preventing activation of NF-κB/AP-1 signaling pathways and the subsequent release of pro-inflammatory cytokines. Furthermore, the data demonstrate that GKY25 inhibits TLR4- and TLR2- induced NF-κB/AP-1 activation induced by other microbial stimuli such

as peptidoglycan, LTA, and zymosan as well. Flow cytometry analysis and confocal microscopy demonstrate a direct binding and internalization of GKY25 into monocytes and macrophages at 37°C *in vitro* and *in vivo*. However, the uptake was reduced at 4°C and during cytochalasin B treatment, suggesting that internalization of the peptide is dependent on membrane fluidity. Taken together, the data demonstrate a multifunctional thrombin-derived host defense peptide which reduces the pro-inflammatory cytokine response during LPS inflammation by interacting with endotoxins and inflammatory cells.

6.2 Paper II

Thrombin-derived host defense peptides modulate monocyte/macrophage inflammatory responses to Gram-negative bacteria

Background:

Monocytes and macrophages play an important role in infection and wounding. These phagocytic cells clear infections at the side of injury, by the engulfment of pathogens, the release of pro-inflammatory cytokines and reactive oxygen. Besides immune cells, host-defense peptides are also an essential part of innate immunity. Previous investigations have shown multifunctional thrombin derived host defense peptide GKY25 (GKYGFYTHVFRLKKWIQKVIDQFGE), that exert immunomodulatory and antimicrobial effects. In paper I, we clarify the mode of action GKY25 during LPS inflammation in monocytes and macrophages. We found that GKY25 binds to LPS, which prevents LPS-induced TLR4 dimerization on monocytes and macrophages, leading to inhibition of the downstream activation of transcription factors NF-κB and AP-1 and subsequent release of pro-inflammatory cytokines [145]. Furthermore, *in vivo* studies indicated that treatment with GKY25 increased the survival of mice after *Pseudomonas aeruginosa* infection, by inhibiting cytokine release and decreasing fibrin deposition and leakage in the lungs [9, 14]. However, as no antibacterial effect was observed under these physiological conditions, we hypothesized that GKY25 might influence phagocytosis and the activation of monocytes and macrophages during Gram-negative bacterial infection.

Aims of Paper II:

- To investigate the effect of GKY25 on bacterial uptake by and activation of monocytes, and macrophages during Gram-negative infection

Results and Conclusion:

The results revealed binding of C-terminal thrombin epitopes to Gram-positive and Gram-negative bacteria in the extracellular and intracellular environment of fibrin slough from a patient with a non-healing, infected wound. In agreement, live imaging showed binding of GKY25 to *E. coli* Bioparticles extracellularly, followed by phagocytosis by macrophages. However, GKY25 did not act as opsonin or negatively influence phagocytosis. Nevertheless, GKY25 did reduce NF-κB/AP-1 activation and the subsequent release of pro-inflammatory cytokines in response to heat-killed and live Gram-negative bacteria. Taken together, GKY25 binds to Gram-negative bacteria and reduces pro-inflammatory cytokine release by macrophages, while preserving their important phagocytic function.

6.3 Paper III

Differential internalization of thrombin-derived host defense peptides into monocytes and macrophages

Background:

Endocytosis is the internalization of extracellular molecules into the cytoplasm of eukaryotic cells. Endocytosis pathways are divided into two main groups: the clathrin-dependent and clathrin-independent endocytosis pathways [110, 111]. In paper I and II we have shown that the thrombin-derived host defense peptide GKY25 binds to and internalizes into monocytes and macrophages in a time- and temperature- dependent manner. In addition, we found that the endocytosis inhibitor cytochalasin B affects the internalization of GKY25 in macrophages [145]. However, the exact uptake mechanism of GKY25 remained unknown. In line with this, it was recently shown that the host defense peptide LL37, as well as other cell-penetrating peptides, are internalized by both clathrin-mediated and caveolin-mediated endocytosis pathways [146, 147, 148]. Based on these observations, we hypothesized that thrombin-derived host defense peptides may enter monocytes and macrophages via clathrin-dependent and -independent endocytosis pathways.

Aims of Paper III:

- To investigate which endocytosis pathway is responsible for the internalization of TCPs in monocytes and macrophages.
- To elucidate the intracellular localization of GKY25

Results and Conclusion:

Using specific endocytosis inhibitors in cell cultures, together with confocal microscopy and flow cytometry analysis, the data revealed that clathrin-dependent and clathrin-independent pathways are involved in the internalization of GKY25 in macrophages. Interestingly, the uptake of thrombin derived C-terminal peptides in monocytes was mainly dynamin-dependent. Comparisons of the internalization pathways of the prototypic thrombin derived C-terminal peptide GKY25, with the natural occurring thrombin derived C-terminal peptide HVF18, and FYT21 indicated that the endocytosis pathways of thrombin-derived C-terminal peptides are not only dependent on the type of cells but also on the length of the peptide. Moreover, the route of uptake was also affected by the presence of LPS or *E. coli* BioParticles. Internalized GKY25 was transported to late endosomes and finally to lysosomes, where it remained detectable for up to 10 h, without indicating degradation into smaller fragments. Taken together, the data show, that thrombin-derived host defense peptides are differently internalized by clathrin-dependent and clathrin-independent endocytosis pathways in monocytes and macrophages.

6.4 Paper IV

Aggregation of thrombin-derived C-terminal fragments as a previously undisclosed host defense mechanism

Background:

Paper I, II and III focus mainly on the function of the thrombin-derived C-terminal peptides of about 2 kDa. In these publications, it was shown, that the multifunctional thrombin-derived host defense peptide GKY25 reduces pro-inflammatory cytokine responses during LPS inflammation and Gram-negative bacterial infection in monocytes and macrophages. Furthermore, it was shown that thrombin-derived C-terminal peptides are differently internalized by clathrin-dependent and clathrin-independent endocytosis pathways in monocytes and macrophages. However, the cleavage of thrombin by the neutrophil elastase, besides the thrombin-derived C-terminal peptides of roughly 2 kDa, also a 11 kDa peptides. In this present investigation, we want to elucidate the function of this 11 kDa thrombin-derived C-terminal peptide in wound fluids.

Aims of Paper IV:

- To characterize the biological role of the 11 kDa TCP in wound fluids

Results and Conclusion:

The cleavage of thrombin by neutrophil elastase generates 11 kDa thrombin-derived C-terminal peptides which form amorphous amyloid-like aggregates in the presence of LPS or Gram-negative bacteria. *In silico* molecular modeling using atomic resolution and coarse-grained simulations, confirm our experimental observations, altogether indicating increased aggregation through LPS-mediated intermolecular contacts between clusters of the recombinant 96-aa thrombin-derived C-terminal peptide (TCP) molecules. In addition to bacterial aggregate formation, TCPs facilitate bacterial killing and promote bacterial clearance by enhancing phagocytosis of bacterial aggregates in macrophages. TCPs were found in acute wound fluids, and incubation of acute wound fluids together with LPS or *E. coli* results in aggregate formation. Moreover, electron microscopy analysis showed colocalization of TCPs together with LPS in wound fluids, indicating the presence of TCP-LPS aggregates under physiological conditions. Taken together, these findings provide a novel link between the coagulation system, innate immunity, LPS scavenging, and protein aggregation/amyloid formation.

7 Conclusion

In this work, the modes of action of TCPs during LPS stimulation and bacterial infection of monocytes and macrophages have been investigated. In figure 3 the results of recent investigations are schematically illustrated, showing that the prototypic peptide GKY25 is binding to LPS and Gram-negative bacteria in the extracellular environment. It inhibits TLR4 dimerization, thereby preventing the activation of various MAPKs, the transcription factors AP-1 and NF-κB, and the subsequent production of pro-inflammatory cytokines including TNF-α, IL-6, IL-8 IL-12p40 and IFN-γ. Moreover, the investigations show that the HDP GKY25 is internalized through both clathrin-dependent and -independent pathways in monocytes and macrophages and was localized in endosomes and phagosomes, together with LPS and Gram-negative bacteria, as well in lysosomes. In addition, GKY25 was preserving the important phagocytic function of macrophages.

Besides the TCPs of roughly 2 kDa, larger fragments of 11 kDa were also found in wound fluids. These TCPs of 11 kDa form amorphous amyloid-like aggregates in the presence of LPS and Gram-negative bacteria. Furthermore, these TCPs facilitate bacterial killing and promote bacterial clearance through enhanced phagocytosis by macrophages.

Taken together, herein we show that the multifunctional, immunomodulatory TCPs plays a physiological role during infection and inflammation, have therapeutic potentials by modulating multiple interactions involving bacteria, endotoxins, and inflammatory cell responses.

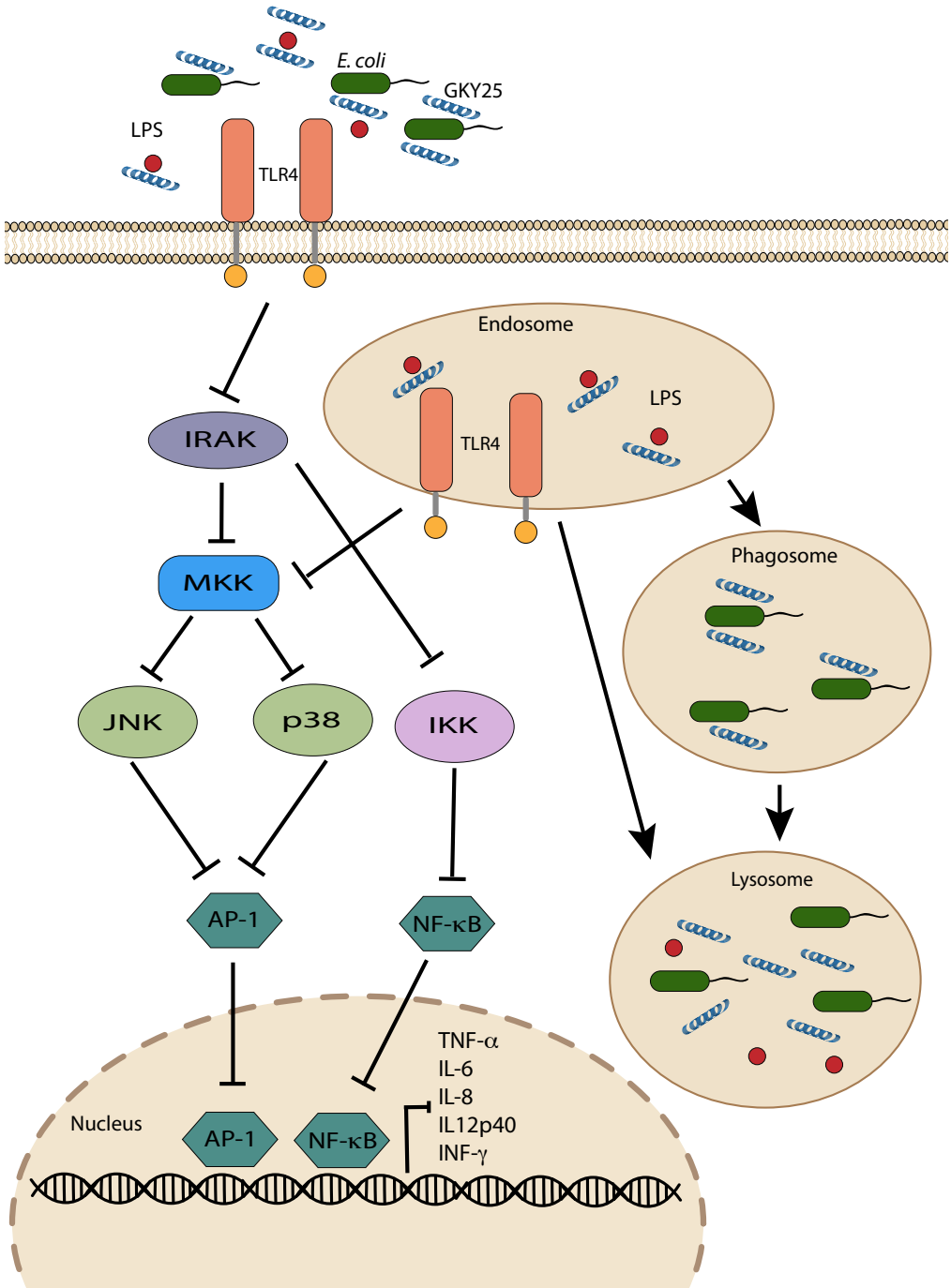

Figure 3: Mode of action of the prototypic thrombin derived host defense peptide GKY25. GKY25 is binding to LPS and Gram-negative bacteria, thereby inhibiting the TLR4 dimerization, which prevents the activation of the MAPKs, the transcription factors AP-1 and NF-κB, and the subsequent production of pro-inflammatory cytokines in monocytes and macrophages. Furthermore, GKY25 was localized in endosomes, phagosomes, and lysosomes together with LPS and Gram-negative bacteria.

8 References

[1] S. A. Eming, P. Martin, and M. Tomic-canic, "Wound repair and regeneration : Mechanisms, signaling, and translation," *Science Translational Medicine*, vol. 6, no. 265, pp. 1–16, 2014.

[2] G. C. Gurtner, S. Werner, Y. Barrandon, and M. T. Longaker, "Wound repair and regeneration," *Nature*, vol. 453, no. 7193, pp. 314–321, 2008.

[3] C. J. Holmes, J. K. Plichta, R. L. Gamelli, and K. A. Radek, "Dynamic role of host stress responses in modulating the cutaneous microbiome: Implications for wound healing and infection," *Advances in wound care*, vol. 4, no. 1, pp. 24–37, 2015.

[4] N. Bryan, H. Ahswin, N. Smart, Y. Bayon, S. Wohlert, and J. A. Hunt, "Reactive oxygen species (ROS)–a family of fate deciding molecules pivotal in constructive inflammation and wound healing," *European Cells and Materials*, vol. 24, pp. 249–265, 2012.

[5] T. J. Koh and L. A. DiPietro, "Inflammation and wound healing: the role of the macrophage.," *Expert reviews in molecular medicine*, vol. 13, pp. 1–12, 2011.

[6] P. J. Wermuth and S. A. Jimenez, "The significance of macrophage polarization subtypes for animal models of tissue fibrosis and human fibrotic diseases," *Clinical and Translational Medicine*, vol. 4, no. 2, pp. 1–19, 2015.

[7] M. Levi and T. van der Poll, "Two-way interactions between inflammation and coagulation," *British Journal of Haematology*, vol. 15, no. 7, pp. 254–259, 2005.

[8] U. Amara, D. Rittirsch, M. Flierl, U. Bruckner, A. Klos, F. Gebhard, J. D. Lambris, and M. Huber-Lang, "Interaction between the coagulation and complement system," *Advanced experimental medical biology*, vol. 632, pp. 68–76, 2008.

[9] 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*, vol. 6, no. 4, p. e1000857, 2010.

[10] S. C. Mansour, O. M. Pena, and R. E. Hancock, "Host defense peptides: front-line immunomodulators," *Trends in Immunology*, vol. 35, no. 9, pp. 443–450, 2014.

[11] 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. Hancock, "Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37," *The Journal of Immunology*, vol. 176, no. 4, pp. 2455–2464, 2006.

[12] O. M. Pena, N. Afacan, J. Pistolic, C. Chen, L. Madera, R. Falsafi, C. D. Fjell, and R. E. Hancock, "Synthetic cationic peptide IDR-1018 modulates human macrophage differentiation," *PLoS One*, vol. 8, no. 1, 2013.

[13] J. D. Heilborn, M. F. Nilsson, G. Kratz, G. Weber, O. Sorensen, N. Borregaard, and M. Stahle-Backdahl, "The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium," *Journal of Investigative Dermatology*, vol. 120, no. 3, pp. 379–389, 2003.

[14] M. Kalle, P. Papareddy, G. Kasetty, M. Morgelin, M. J. van der Plas, V. Rydengard, 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*, vol. 7, no. 12, p. e51313, 2012.

[15] M. Kalle, P. Papareddy, G. Kasetty, M. J. van der Plas, M. Morgelin, M. Malmsten, and A. Schmidtchen, "A peptide of heparin cofactor II inhibits endotoxin-mediated shock and invasive Pseudomonas aeruginosa infection," *PLoS One*, vol. 9, no. 7, p. e102577, 2014.

[16] R. Medzhitov and C. Janeway, "Innate immune recognition: mechanisms and pathways," *Immunological Reviews*, vol. 173, pp. 89–97, 2002.

[17] J. Harder, J. M. Schröder, and R. Gläser, "The skin surface as antimicrobial barrier: Present concepts and future outlooks," *Experimental Dermatology*, vol. 22, no. 1, pp. 1–5, 2013.

[18] M. D. Cooper and B. R. Herrin, "How did our complex immune system evolve?," *Nature Reviews Immunology*, vol. 10, no. 1, pp. 2–3, 2010.

[19] P. W. Dempsey, S. A. Vaidya, and G. Cheng, "The Art of War: Innate and adaptive immune responses," *Cellular and Molecular Life Sciences*, vol. 60, no. 12, pp. 2604–2621, 2003.

[20] P. Matzinger, "The danger model: A renewed sense of self," *Science*, vol. 296, no. April, pp. 301–305, 2002.

[21] S. Gallucci and P. Matzinger, "Danger signals : SOS to the immune system," *Current Opinion in Immunology*, vol. 13, pp. 114–119, 2001.

[22] S. E. Turvey and D. H. Broide, "Innate Immunity," *Journal of Allergy and Clinical Immunology*, vol. 125, pp. 1–4, 2006.

[23] K. Buchmann, "Evolution of innate immunity: Clues from invertebrates via fish to mammals," *Frontiers in Immunology*, vol. 5, pp. 1–8, 2014.

[24] R. Medzhitov and C. Janeway, "Advances in Immunology," *The New Englang Journal of Medicine*, vol. 343, no. 5, pp. 338–344, 2000.

[25] H. Eibel, H. Kraus, H. Sic, A.-K. Kienzler, and M. Rizzi, "B cell biology: An overview," *Current Allergy and Asthma Reports*, vol. 14, no. 5, p. 434, 2014.

[26] O. Takeuchi and S. Akira, "Pattern recognition receptors and inflammation," *Cell*, vol. 140, no. 6, pp. 805–820, 2010.

[27] G. M. Barton, "A calculated response: control of inflammation by the innate immune system," *Journal of Clinical Investigation*, vol. 118, no. 2, pp. 413–420, 2008.

[28] R. Medzhitov, "Origin and physiological roles of inflammation," *Nature*, vol. 454, no. 7203, pp. 428–435, 2008.

[29] S. Gordon and P. R. Taylor, "Monocyte and macrophage heterogeneity," *Nature Reviews Immunology*, vol. 5, no. 12, pp. 953–964, 2005.

[30] F. Ginhoux and S. Jung, "Monocytes and macrophages: Developmental pathways and tissue homeostasis," *Nature Reviews Immunology*, vol. 14, no. 6, pp. 392–404, 2014.

[31] H. Xiong and E. G. Pamer, "Monocytes and infection: Modulator, messenger and effector," *Immunobiology*, vol. 220, no. 2, pp. 210–214, 2015.

[32] C. Auffray, D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau, and F. Geissmann, "Monitoring of blood vessels and tissues by a population of monocytes with patrolling behaviour," *Science*, vol. 317, pp. 666–670, 2007.

[33] G. Thomas, R. Tacke, C. C. Hedrick, and R. N. Hanna, "Nonclassical patrolling monocyte function in the vasculature," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 35, no. 6, pp. 1306–1316, 2015.

[34] F. Geissmann, S. Jung, and D. R. Littman, "Blood monocytes consist of two principal subsets with distinct migratory properties," *Immunity*, vol. 19, no. 1, pp. 71–82, 2003.

[35] T. Jia, I. Leiner, G. Dorothee, K. Brandl, and E. G. Pamer, "MyD88 and type I interferon receptor-mediated chemokine induction and monocyte recruitment during Listeria monocytogenes infection," *The Journal of Immunology*, vol. 183, no. 2, pp. 1271–1278, 2009.

[36] N. V. Serbina, T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel, and E. G. Pamer, "TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection," *Immunity*, vol. 19, pp. 59–70, 2003.

[37] C. V. Jakubzick, G. J. Randolph, and P. M. Henson, "Monocyte differentiation and antigen-presenting functions," *Nature Reviews Immunology*, vol. 17, no. 6, pp. 349–362, 2017.

[38] L. C. Davies, S. J. Jenkins, J. E. Allen, and P. R. Taylor, "Tissue-resident macrophages," *Nature Immunology*, vol. 14, no. 10, pp. 986–995, 2013.

[39] I. Ushach and A. Zlotnik, "Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage," *Journal of Leukocyte Biology*, vol. 100, no. 3, pp. 481–489, 2016.

[40] T. Lucas, A. Waisman, R. Ranjan, J. Roes, T. Krieg, W. Muller, A. Roers, and S. A. Eming, "Differential Roles of Macrophages in Diverse Phases of Skin Repair," *The Journal of Immunology*, vol. 184, no. 7, pp. 3964–3977, 2010.

[41] R. Medzhitov and C. Janeway, "Innate immune recognition: mechanisms and pathways," *Immunological Reviews*, vol. 173, pp. 89–97, 2000.

[42] J. Da Silva Correia, K. Soldau, U. Christen, P. S. Tobias, and R. J. Ulevitch, "Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. Transfer from CD14 to TLR4 and MD-2," *Journal of Biological Chemistry*, vol. 276, no. 24, pp. 21129–21135, 2001.

[43] A. Yoshimura, E. Lien, R. R. Ingalls, R. Dziarski, and D. Golenbock, "Recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2," *The Journal of Immunology*, vol. 163, pp. 1–5, 1999.

[44] R. Schwandner, R. Dziarski, H. Wesche, and E. Al., "Peptidoglycan- and lipoteichoic acid-induced cell-activation is mediated by Toll-like receptor 2," *Journal of Biological Chemistry*, vol. 274, no. 28, pp. 17406–17409, 1999.

[45] F. Hayashi, K. D. Smith, A. Ozinsky, T. R. Hawn, E. C. Yi, D. R. Goodlett, J. K. Eng, S. Akira, D. M. Underhill, and A. Aderem, "The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5.," *Nature*, vol. 410, no. 6832, pp. 1099–1103, 2001.

[46] H. Hemmi, O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, and S. Akira, "A Toll-like receptor recognizes bacterial DNA.," *Nature*, vol. 408, no. 6813, pp. 740–745, 2000.

[47] I. Botos, L. Liu, Y. Wang, D. M. Segal, and D. R. Davies, "The Toll-like receptor 3: dsRNA signaling complex," *Biochimica et Biophysica Acta*, vol. 1789, no. 9-10, pp. 667–674, 2009.

[48] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[49] S. W. Brubaker, K. S. Bonham, I. Zanoni, and J. C. Kagan, "Innate immune pattern recognition: A cell biological perspective," *Annual Review of Immunology*, vol. 33, no. 1, pp. 257–290, 2015.

[50] W. K. Ip, K. Takahashi, R. A. Ezekowitz, and L. M. Stuart, "Mannose-binding lectin and innate immunity," *Immunological Reviews*, vol. 230, no. 1, pp. 9–21, 2009.

[51] C. Hashimoto, K. L. Hudson, and K. V. Anderson, "The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein," *Cell*, vol. 52, pp. 269–279, 1988.

[52] R. Medzhitov, P. Preston-hurlburt, and C. A. J. Jr, "letters to nature A human homologue of the Drosophila Toll protein signals activation of adaptive immunity," *Nature*, vol. 388, pp. 6–9, 1997.

[53] L. A. J. O'Neill, D. Golenbock, and A. G. Bowie, "The history of Toll-like receptors - redefining innate immunity," *Nature Reviews Immunology*, vol. 13, no. 6, pp. 453–460, 2013.

[54] H. Kumar, T. Kawai, and S. Akira, "Toll-like receptors and innate immunity," *Biochemical and Biophysical Research Communications*, vol. 388, no. 4, pp. 621–625, 2009.

[55] A. Ng and R. J. Xavier, "Leucine-rich repeat (LRR) proteins," *Autophagy*, vol. 7, no. 9, pp. 1082–1084, 2011.

[56] M. Brown, J., Wang, H., Hajishengallis, G. N., and Martin, "TLR-signaling networks : An integration of adaptor molecules, kinases, and cross-talk," *Critical Review in Oral Biology and Medicine*, vol. 90, no. 4, pp. 417–427, 2011.

[57] T. Kawasaki and T. Kawai, "Toll-like receptor signaling pathways," vol. 5, no. 461, pp. 1–8, 2014.

[58] F. Hayashi, K. D. Smith, A. Ozinsky, T. R. Hawn, E. C. Yi, D. R. Goodlett, J. K. Eng, S. Akira, D. M. Underhill, and A. Aderem, "The innate immune response to bacterial fagellin is mediated by Toll-like receptor 5," *letters of nature*, vol. 410, pp. 1–5, 2001.

[59] L. Oliveira-Nascimento, P. Massari, and L. M. Wetzler, "The role ofTLR2 in infection and immunity," *Frontiers in Immunology*, vol. 3, no. 79, pp. 1–17, 2012.

[60] K. Takeda and S. Akira, "TLR signaling pathways," *Seminars in Immunology*, vol. 16, no. 1, pp. 3–9, 2004.

[61] T. Kawai and S. Akira, "The role of pattern-recognition receptors in innate immunity : update on Toll-like receptors," *Nature Immunology*, vol. 11, no. 5, pp. 373–384, 2010.

[62] J. da Silva Correia, K. Soldau, U. Christen, P. S. Tobias, and R. J. Ulevitch, "Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex.," *Journal of Biological Chemistry*, vol. 276, no. 24, pp. 21129–21135, 2001.

[63] C. R. H. Raetz and C. Whitfield, "Lipopolysaccharide endotoxins," *Annual Review of Biochemistry*, vol. 71, no. 1, pp. 635–700, 2002.

[64] 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*, vol. 170, no. 4, pp. 1231–41, 1989.

[65] S. D. Wright, R. A. Ramos, P. S. Tobias, R. J. Ulevitch, and M. J. C., "CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein," *Science*, vol. 249, no. 4975, pp. 1431–1433, 1990.

[66] 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*, vol. 189, no. 11, pp. 1777–1782, 1999.

[67] Y. C. Lu, W. C. Yeh, and P. S. Ohashi, "LPS/TLR4 signal transduction pathway," *Cytokine*, vol. 42, no. 2, pp. 145–151, 2008.

[68] A. L. Hilchie, K. Wuerth, and R. E. Hancock, "Immune modulation by multifaceted cationic host defense (antimicrobial) peptides," *Nature Chemical Biology*, vol. 9, no. 12, pp. 761–768, 2013.

[69] D. M. E. Bowdish, D. J. Davidson, Y. E. Lau, K. Lee, M. G. Scott, and R. E. W. Hancock, "Impact of LL-37 on anti-infective immunity," *Journal of Leukocyte Biology*, vol. 77, no. 4, pp. 451–459, 2005.

[70] R. E. W. Hancock and H. G. Sahl, "Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies," *Nature Biotechnology*, vol. 24, no. 12, pp. 1551–1557, 2006.

[71] M. Zasloff, "Antimicrobial peptides of multicellular organisms," *Macmillan Magazines*, vol. 415, pp. 389–395, 2002.

[72] M. Hemshekhar, V. Anaparti, and N. Mookherjee, "Functions of cationic host defense peptides in immunity," *Pharmaceuticals*, vol. 9, no. 40, pp. 1–10, 2016.

[73] J. Johansson, G. H. Gudmundsson, M. E. Rottenberg, K. D. Berndt, B. Agerberth, and E. Rottenberg, "Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37," *The Journal of Biological Chemistry*, vol. 273, no. 6, pp. 3718–3724, 1998.

[74] M. J. A. van der Plas, R. K. V. Bhongir, S. Kjellström, H. Siller, G. Kasetty, M. Mörgelin, and A. Schmidtchen, "Pseudomonas aeruginosa elastase cleaves a C-terminal peptide from human thrombin that inhibits host inflammatory responses," *Nature Communications*, vol. 7, no. 11567, pp. 1–13, 2016.

[75] K. Yamasaki, J. Schauber, A. Coda, H. Lin, R. A. Dorschner, N. M. Schechter, C. Bonnart, P. Descargues, A. Hovnanian, and R. L. Gallo, "Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin," *The FASEB Journal*, vol. 20, no. 12, pp. 2068–2080, 2006.

[76] D. M. E. Bowdish, D. J. Davidson, and R. E. W. Hancock, "Immunomodulatory properties of defensins and cathelicidins," *Springer*, vol. 306, pp. 27–66, 2006.

[77] R. I. Lehrer, A. M. Cole, and M. E. Selsted, "θ-Defensins: Cyclic peptides with endless potential," *Journal of Biological Chemistry*, vol. 287, no. 32, pp. 27014–27019, 2012.

[78] C. Zhao, I. Wang, and R. I. Lehrer, "Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells," *Federation of European Biochemical Societies*, vol. 396, pp. 319–322, 1996.

[79] M. Suarez-Carmona, P. Hubert, P. Delvenne, and M. Herfs, "Defensins: "Simple" antimicrobial peptides or broad-spectrum molecules?," *Cytokine and Growth Factor Reviews*, vol. 26, pp. 361–370, 2015.

[80] E. H. Mattar, H. A. Almehdar, H. A. Yacoub, V. N. Uversky, and E. M. Redwan, "Antimicrobial potentials and structural disorder of human and animal defensins," *Cytokine and Growth Factor Reviews*, vol. 28, pp. 95–111, 2016.

[81] M. Zasloff, "Antibiotic peptides as mediators of innate immunity," *Current Opinion in Immunology*, vol. 4, pp. 3–7, 1992.

[82] M. Pasupuleti, A. Schmidtchen, and M. Malmsten, "Antimicrobial peptides: Key components of the innate immune system," *Critical Reviews in Biotechnology*, vol. 32, no. 2, pp. 143–171, 2012.

[83] T. Schuerholz, K. Brandenburg, and G. Marx, "Antimicrobial peptides and their potential application in inflammation and sepsis," *BioMed Central*, vol. 16, no. 207, pp. 1–8, 2012.

[84] Y. Rosenfeld, N. Papo, and Y. Shai, "Endotoxin (lipopolysaccharide) neutralization by innate immunity host-defense peptides: Peptide properties and plausible modes of action," *Journal of Biological Chemistry*, vol. 281, no. 3, pp. 1636–1643, 2006.

[85] M. G. Scott, A. C. E. Vreugdenhil, W. A. Buurman, R. E. W. Hancock, and M. R. Gold, "Cutting Edge: Cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein," *The Journal of Immunology*, vol. 164, no. 2, pp. 549–553, 2000.

[86] J. Yu, N. Mookherjee, K. Wee, D. M. E. Bowdish, J. Pistolic, Y. Li, L. Rehaume, and R. E. W. Hancock, "Host defense peptide LL-37, in synergy

with inflammatory mediator IL-1 , augments immune responses by multiple pathways," *The Journal of Immunology*, vol. 179, no. 11, pp. 7684–7691, 2007.

[87] G. S. Tjabringa, D. K. Ninaber, J. W. Drijfhout, K. F. Rabe, and P. S. Hiemstra, "Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors," *International Archives of Allergy and Immunology*, vol. 140, pp. 103–112, 2006.

[88] M. Dürr and A. Peschel, "Chemokines meet defensins: The merging concepts of chemoattractants and antimicrobial peptides in host defense," *Infection and Immunity*, vol. 70, no. 12, pp. 6515–6517, 2002.

[89] A. Nijnik, J. Pistolic, N. C. J. Filewod, and R. E. W. Hancock, "Signaling pathways mediating chemokine induction in keratinocytes by cathelicidin LL-37 and flagellin," *Journal of Innate Immunity*, vol. 4, pp. 377–386, 2012.

[90] M. M. Welling, P. S. Hiemstra, M. T. Van Den Barselaar, A. Paulusma-Annema, P. H. Nibbering, E. K. Pauwels, and W. Calame, "Antibacterial activity of human neutrophil defensins in experimental infections in mice is accompanied by increased leukocyte accumulation," *Journal of Clinical Investigation*, vol. 102, no. 8, pp. 1583–1590, 1998.

[91] S. M. Alalwani, J. Sierigk, C. Herr, O. Pinkenburg, R. Gallo, C. Vogelmeier, and R. Bals, "The antimicrobial peptide LL-37 modulates the inflammatory and host defense response of human neutrophils," *European Journal of Immunology*, vol. 40, no. 4, pp. 1118–1126, 2010.

[92] M. Wan, A. M. van der Does, X. Tang, L. Lindbom, B. Agerberth, and J. Z. Haeggstrom, "Antimicrobial peptide LL-37 promotes bacterial phagocytosis by human macrophages," *Journal of Leukocyte Biology*, vol. 95, no. 6, pp. 971–981, 2014.

[93] M. von Köckritz-Blickwede, O. Goldmann, P. Thulin, K. Heinemann, A. Norrby-teglund, M. Rohde, and E. Medina, "Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation," vol. 111, no. 6, pp. 3070–3081, 2008.

[94] D. J. Davidson, A. J. Currie, G. S. D. Reid, D. M. E. Bowdish, K. L. MacDonald, R. C. Ma, R. E. W. Hancock, and D. P. Speert, "The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T Cell Polarization," *The Journal of Immunology*, vol. 172, no. 2, pp. 1146–1156, 2004.

[95] R. P. Allaker, "Host defence peptides-a bridge between the innate and adaptive immune responses," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 102, no. 1, pp. 3–4, 2008.

[96] N. W. Kin, Y. Chen, E. K. Stefanov, R. L. Gallo, and J. F. Kearney, "Cathelin-related antimicrobial peptide differentially regulates T- and B-cell function," *European Journal of Immunology*, vol. 41, no. 10, pp. 3006–3016, 2011.

[97] M. L. Mangoni, A. M. Mcdermott, and M. Zasloff, "Antimicrobial peptides and wound healing: Biological and therapeutic considerations," *Experimental Dermatology*, vol. 25, no. 3, pp. 167–173, 2016.

[98] S. Krishnaswamy, "The transition of prothrombin to thrombin," *Journal of Thrombosis and Haemostasis*, vol. 11, no. 1, pp. 265–276, 2013.

[99] P. Thiagarajan and A. Narayanan, "Thrombin," *eLS Biochemistry*, pp. 1–7, 2014.

[100] S. Narayanan, "Multifunctional roles of thrombin," *Annals of clinical and laboratory science*, vol. 29, no. 4, pp. 275–280, 1999.

[101] O. Dery, C. U. Corvera, M. Steinhoff, and N. W. Bunnett, "Proteinase-activated receptors: novel mechanisms of signaling by serine protease," *The American Physiological Society*, pp. 1429–1452, 1998.

[102] L. G. Licari and J. P. Kovacic, "Thrombin physiology and pathophysiology," *Journal of Veterinary Emergency and Critical Care*, vol. 19, no. 1, pp. 11–22, 2009.

[103] R. Bar-Shavit, A. Kahn, J. Fenton, and G. Wilner, "Receptor-mediated chemotactic response of a macrophage cell line (j774) to thrombin.," *Lab Investigations*, vol. 49, no. 6, pp. 702–707, 1983.

[104] R. Saravanan, S. S. Adav, Y. K. Choong, M. J. Van Der Plas, J. Petrlova, S. Kjellström, S. K. Sze, and A. Schmidtchen, "Proteolytic signatures define unique thrombin-derived peptides present in human wound fluid in vivo," *Scientific Reports*, vol. 7, no. 1, pp. 1–11, 2017.

[105] G. Kasetty, P. Papareddy, M. Kalle, V. Rydengård, M. Mörgelin, B. Albiger, M. Malmsten, and A. Schmidtchen, "Structure-activity studies and therapeutic potential of host defense peptides of human thrombin," *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 6, pp. 2880–2890, 2011.

[106] S. Singh, M. Kalle, P. Papareddy, A. Schmidtchen, and M. Malmsten, "Lipopolysaccharide interactions of C-terminal peptides from human thrombin," *BioMacromolecules*, vol. 14, pp. 1482–1492, 2013.

[107] P. Luo and R. L. Baldwin, "Origin of the different strengths of the (i,i+4) and (i,i+3) leucine pair interactions in helices," *Biophysical Chemistry*, vol. 96, no. 2-3, pp. 103–108, 2002.

[108] A. Sorkin and M. Von Zastrow, "Endocytosis and signalling: Intertwining molecular networks," *Nature Reviews Molecular Cell Biology*, vol. 10, no. 9, pp. 609–622, 2009.

[109] C. Rosales and E. Uribe-Querol, "Phagocytosis: A fundamental process in immunity," *BioMed Research International*, vol. 2017, 2017.

[110] C. Le Roy and J. L. Wrana, "Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 2, pp. 112–126, 2005.

[111] G. J. Doherty and H. T. McMahon, "Mechanisms of endocytosis," *Annual Review of Biochemistry*, vol. 78, no. 1, pp. 857–902, 2009.

[112] K. Takei and V. Haucke, "Clathrin-mediated endocytosis: Membrane factors pull the trigger," *Trends in Cell Biology*, vol. 11, no. 9, pp. 385–391, 2001.

[113] A. Sorkin, "Cargo recognition during clathrin-mediated endocytosis: A team effort," *Current Opinion in Cell Biology*, vol. 16, no. 4, pp. 392–399, 2004.

[114] C. R. Hopkins, K. Miller, and J. M. Beardmore, "Receptor-mediated endocytosis of transferrin and epidermal growth factor receptors: a comparison of constitutive and ligand-induced uptake.," *Journal of cell science*, vol. 3, pp. 173–86, 1985.

[115] S. E. Rundlett, A. A. Carmen, N. Suka, B. M. Turner, and M. R. Grunstein, "Epsin is an EH-domain-binding protein implicated in clathrin-mediated endocytosis," *Nature*, vol. 394, no. 1997, pp. 831–835, 1998.

[116] A. Motley, N. A. Bright, M. N. J. Seaman, and M. S. Robinson, "Clathrin-mediated endocytosis in AP-2-depleted cells," *Journal of Cell Biology*, vol. 162, no. 5, pp. 909–918, 2003.

[117] J. Samaj, B. Baluska, FrantisekVoigt, M. Schlicht, D. Volkmann, and D. Menzel, "Endocytosis, actin cytoskeleton, and signaling," *Plant physiology*, vol. 135, pp. 1150–1161, 2004.

[118] S. E. Miller, S. Mathiasen, N. A. Bright, F. Pierre, B. T. Kelly, N. Kladt, A. Schauss, C. J. Merrifield, D. Stamou, S. Höning, and D. J. Owen, "CALM regulates clathrin-coated vesicle size and maturation by directly sensing and driving membrane curvature," *Developmental Cell*, vol. 33, no. 2, pp. 163–175, 2015.

[119] H. T. McMahon and E. Boucrot, "Molecular mechanism and physiological functions of clathrin-mediated endocytosis," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 8, pp. 517–533, 2011.

[120] M. Kaksonen and A. Roux, "Mechanisms of clathrin-mediated endocytosis," *Nature Reviews Molecular Cell Biology*, vol. 19, no. 5, pp. 313–326, 2018.

[121] D. J. Yamashiro and F. R. Maxfield, "Acidification of morphologically distinct endosomes in mutant and wild- type Chinese hamster ovary cells," *The Journal of cell biology*, vol. 105, no. 6, pp. 2723–2733, 1987.

[122] B. D. Grant and J. G. Donaldson, "Pathways and mechanisms of endocytic recycling," *Nature Reviews Molecular Cell Biology*, vol. 10, no. 9, pp. 597–608, 2009.

[123] G. M. Barton and J. C. Kagan, "A cell biological view of toll-like receptor function: Regulation through compartmentalization," *Nature Reviews Immunology*, vol. 9, no. 8, pp. 535–541, 2009.

[124] K. Sandvig, M. L. Torgersen, H. A. Raa, and B. Van Deurs, "Clathrin-independent endocytosis: From nonexisting to an extreme degree of complexity," *Histochemistry and Cell Biology*, vol. 129, no. 3, pp. 267–276, 2008.

[125] S. Mayor and R. E. Pagano, "Pathways of clathrin-independent endocytosis," *Nature Reviews Molecular Cell Biology*, vol. 8, pp. 603–612, 2007.

[126] J. P. Cheng and B. J. Nichols, "Caveolae: One function or many?," *Trends in Cell Biology*, vol. 26, no. 3, pp. 177–189, 2016.

[127] A. P. Ferreira and E. Boucrot, "Mechanisms of carrier formation during clathrin-independent endocytosis," *Trends in Cell Biology*, vol. 28, no. 3, pp. 188–200, 2017.

[128] R. G. Parton and M. A. Del Pozo, "Caveolae as plasma membrane sensors, protectors and organizers," *Nature Reviews Molecular Cell Biology*, vol. 14, no. 2, pp. 98–112, 2013.

[129] A. Hayer, M. Stoeber, D. Ritz, S. Engel, H. H. Meyer, and A. Helenius, "Caveolin-1 is ubiquitinated and targeted to intralumenal vesicles in endolysosomes for degradation," *Journal of Cell Biology*, vol. 191, no. 3, pp. 615–629, 2010.

[130] L. Pelkmans and M. Zerial, "Kinase-regulated quantal assemblies and kiss-and-run recycling of caveolae," *Nature*, vol. 436, no. 7047, pp. 128–133, 2005.

[131] B. Han, C. A. Copeland, A. Tiwari, and A. K. Kenworthy, "Assembly and turnover of caveolae: What do we really know?," *Frontiers in Cell and Developmental Biology*, vol. 4, no. 68, pp. 1–7, 2016.

[132] A. F. Dulhunty and C. Franzini-Armstrongt, "The relative contributions of the folds and caveolae to the surface membrane of frog skeletal muscle fibers at different sarcomere lengths," *Journal of Physiology*, vol. 250, pp. 513–539, 1975.

[133] B. Sinha, D. Köster, R. Ruez, P. Gonnord, M. Bastiani, D. Abankwa, R. V. Stan, G. Butler-Browne, B. Vedie, L. Johannes, N. Morone, R. G. Parton, G. Raposo, P. Sens, C. Lamaze, and P. Nassoy, "Cells respond to mechanical stress by rapid disassembly of caveolae," *Cell*, vol. 144, pp. 402–413, 2011.

[134] C. Lamaze, A. Dujeancourt, T. Baba, C. G. Lo, A. Benmerah, and A. Dautry-Varsat, "Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway," *Molecular Cell*, vol. 7, no. 3, pp. 661–671, 2001.

[135] H. Radhakrishna, "ADP-ribosylation factor 6 regulates a novel plasma membrane recycling pathway," *The Journal of Cell Biology*, vol. 139, no. 1, pp. 49–61, 1997.

[136] S. Mayor, R. G. Parton, and J. G. Donaldson, "Clathrin-independent pathways of endocytosis.," *Cold Spring Harbor Laboratiry Press*, vol. 6, pp. 1–20, 2014.

[137] E. Metschnikoff, "Lecture on phagocytosis and immunity," *The Britsh Medical Journal*, vol. 1, no. 1570, pp. 213–217, 1891.

[138] R. S. Flannagan, V. Jaumouillé, and S. Grinstein, "The cell biology of phagocytosis," *Annual Review of Pathology: Mechanisms of Disease*, vol. 7, no. 1, pp. 61–98, 2012.

[139] E. Groves, A. E. Dart, V. Covarelli, and E. Caron, "Molecular mechanisms of phagocytic uptake in mammalian cells," *Cellular and Molecular Life Sciences*, vol. 65, no. 13, pp. 1957–1976, 2008.

[140] R. S. Flannagan, G. Cosío, and S. Grinstein, "Antimicrobial mechanisms of phagocytes and bacterial evasion strategies," *Nature Reviews Microbiology*, vol. 7, no. 5, pp. 355–366, 2009.

[141] M. D. Woolard and J. A. Frelinger, "Outsmarting the host: Bacteria modulating the immune response," *Immunological Research*, vol. 41, pp. 188–202, 2008.

[142] H. Sarantis and S. Grinstein, "Subversion of phagocytosis for pathogen survival," *Cell Host and Microbe*, vol. 12, no. 4, pp. 419–431, 2012.

[143] N. V. Serbina, T. Jia, T. M. Hohl, and E. G. Pamer, "Monocyte-mediated defense against microbial pathogens," *Annual Review of Immunology*, vol. 26, no. 1, pp. 421–452, 2008.

[144] M. Guha and N. Mackman, "LPS induction of gene expression in human monocytes," *Cell Signal*, vol. 13, no. 2, pp. 85–94., 2001.

[145] F. C. Hansen, M. Kalle-Brune, M. J. A. van der Plas, A.-C. Stromdahl, M. Malmsten, M. Morgelin, and A. Schmidtchen, "The thrombin-derived host defense peptide GKY25 inhibits endotoxin-induced responses through interactions with lipopolysaccharide and macrophages/monocytes," *The Journal of Immunology*, vol. 194, no. 11, pp. 5397–5406, 2015.

[146] X. Tang, D. Basavarajappa, J. Z. Haeggström, and M. Wan, "P2X7 receptor regulates internalization of antimicrobial peptide LL-37 by human macrophages that promotes intracellular pathogen clearance," *The Journal of Immunology*, vol. 195, no. 3, pp. 1191–1201, 2015.

[147] Y. Kawaguchi, T. Takeuchi, K. Kuwata, J. Chiba, Y. Hatanaka, I. Nakase, and S. Futaki, "Syndecan-4 is a receptor for clathrin-mediated endocytosis of arginine-rich cell-penetrating peptides," *Bioconjugate Chemistry*, vol. 27, no. 4, pp. 1119–1130, 2016.

[148] T. Matsubara, R. Otani, M. Yamashita, H. Maeno, H. Nodono, and T. Sato, "Selective intracellular delivery of ganglioside GM3-binding peptide through caveolae/raft-mediated endocytosis," *Biomacromolecules*, vol. 18, no. 2, pp. 355–362, 2017.