ALPHA DEFENSINS IN VASCULAR INFLAMMATION

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TABLE OF CONTENTS

LIST OF ABBREVATIONS

Table of Contents

List of Abbrevations

111%	Percentage
µg	Microgram
µm	Micrometer
5-HAT	5-hydroxytryptamine
aa	Amino acid
ABCG5/8	ATP-binding cassette sub-family G member 5/8
ACAT2	Acyl-CoA-cholesterol-acyl-transferase 2
ADP	Adenosine diphosphate
APC	Antigen presenting cell
Apo	Apolipoprotein
approx.	Approximately
ATP	Adenosine triphosphate
CCL	CC-chemokine ligand
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CETP	Cholesteryl ester transfer protein
CLEC2	Calcium-dependent (C-type) lectin-like receptors 2
Cm	Centimeter
CRISP-3 (SGP-28)	Cysteine-rich secretory protein 3 (Specific granule protein of 28kDa)
CX3CL1	Chemokine (C-X3-C Motif) Ligand 1
CX3CR1	Chemokine (C-X3-C) Receptor 1
CXCR4	Chemokine (C-X-C Motif) Receptor 4
ECM	Extracellular matrix
FA	Fatty acids
FAT	Fatty acid translocase
FATP	Fatty acid transport proteins
FC	Free cholesterol
fMLP-R	N-formyl-leucyl-phenylalanine receptor
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GP	Glycoprotein
GPCR	G protein-coupled receptor
H_2O_2	Hydrogen peroxide
hBD	Human beta defensin
HD	Human defensin
HDL	High density lipoprotein
HLA-DR	Human Leukocyte Antigen – DR isotype

HMGB1	High mobility group box 1 protein
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HNP	Human neutrophil peptide
ICAM-1	Intercellular adhesion molecule 1
IDL	Intermediate density lipoprotein
Ig	Immunoglobulin
IL	Interleukin
kDa	Kilodaltons
LDL	Low density lipoprotein
LDL-R	Low density lipoprotein receptor
LFA-1	Lymphocyte function-associated antigen 1
LPL	Lipoprotein lipase
MAdCAM-1	Mucosal addressin cell adhesion molecule 1
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage colony-stimulating factor
MHC	Major Histocompatibility Complex
mL	Microliter
MLCK	Myosin light chain kinase
MMP	Matrix metalloproteinase
MPO	Myeloperoxidase
MPS	Monocytic phagocyte system, formerly: reticuloendothelial system, RES
NADP	Nicotinamide adenine dinucleotide phosphate
NE	Neutrophil elastase
ng	Nanogram
NO	Nitride oxide
NOX	NADPH oxidase
NPC1L1	Niemann-Pick-C1-like protein
NRAMP-1	Natural resistance-associated macrophage protein 1
oxLDL	oxidized LDL
PAI-1	Plasminogen activator inhibitor-1
PCSK9	Proprotein convertase subtilisin/kexin type 9
PDGF	Platelet-derived growth factor
PL	Phospholipid
PMN	Polymorphonuclear leukocytes
PPRs	Pattern recognition receptors
PSGL1	P-selectin glycoprotein ligand 1
Rap	Ras-related protein
ROS	Reactive oxygen species
SR	Scavenger receptor
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor

TNF-R	Tumor necrosis factor receptor
VCAM-1	Vascular cell adhesion molecule 1
VLA4	Very late antigen 4
VLDL	Very low density lipoprotein
VWF	von Willebrand factor
WHO	World Health Organization

1

INTRODUCTION

1. Introduction

Based on WHO statistics, 41 million people died of "non-communicable diseases" in 2016 [1]. Cardiovascular diseases cause more than three-quarters of those mortalities, a total of 17.9 million [1, 2]. In 2016 more than 300000 people in Germany died because of cardiovascular diseases. Of these, 72062 thousand people died of chronic ischaemic heart disease, 48669 thousand from acute myocardial infarction and 15823 from stroke [3, 4]. Most of the mentioned diseases are the result of chronic inflammatory disorders of the arterial endothelium leading progressively to a clinical condition generally referred to as atherosclerosis [5]. Accordingly, atherosclerosis is one of the most common reasons for cardiovascular diseases in industrialised and developing countries [6]. Over the last decades, a considerable number of risk factors have been identified including smoking [7–9], obesity [10–12], hypertension [13, 14], hypercholesterolemia [14–16], chronic kidney disease [17–19] and diabetes mellitus [20–22]. As a consequence, the damage and the resulting loss of function (e.g. loss of permeability) of the endothelium leads to an activation of the immune system [23, 24]. Such inflammatory processes, characterized mainly by the recruitment and migration of polymorphonuclear leukocytes (PMN), monocytes and lymphocytes, represent the central pathomechanism of atherosclerosis.

For a better understanding the following chapter 1 provides an overview of the immune system and its components, which play an essential role in the present work (Chapter 2 and 3). In addition, physiological and pathophysiological mechanisms that are important in this context will be discussed.

2. The Immune System

The human organism is exposed to a large number of pathogens each day. The immune system protects the body from infections by these pathogens. Two systems are essential to obtain adequate protection, the innate and the adaptive immunity [25]. The innate immunity comprises cells whose function is to recognize and eliminate potential pathogens immediately. For pattern recognition they have individual receptors on their surface (PPRs; pattern recognition receptors) [26]. The intracellular uptake of these pathogenic organisms occurs via a process known as phagocytosis [27, 28]. The most important cells of the innate immune system are granulocytes, monocytes, macrophages and dendritic cells. These cells are not only able to recognize and eliminate pathogens but also to produce and release immunomodulatory substances such as cytokines or chemokines [29, 30]. Such immunomodulatory substances can activate other immune cells and recruit them to the site of inflammation [30]. In this way, not only innate immune cells can be activated and recruited but also cells of the so-called adaptive immune system [30, 31]. Compared to the cells of innate immunity, these cells only recognize defined pathogens by their specific surface receptors [32, 33]. The most important cells here are B and T cells [33]. For recognition of these particular pathogens, antigen presenting cells (APC) such as dendritic cells, monocytes, macrophages and B cells are required [34]. The precise interaction of adaptive and innate immunity contributes tremendously to the health of the human organism. Interferences of this complex system can have considerable health consequences for the organism.

2.1 Components of the Immune System

All cells of the immune system originate from a common precursor cell (stem cell) [35]. Pluripotent stem cells have the ability to replicate, proliferate and differentiate themselves [35]. The differentiation enables the formation of blood cells such as thrombocytes, erythrocytes, granulocytes, monocytes and macrophages (myeloid progenitor cells) as well as B and T cells (lymphatic progenitor cells) [35, 36]. The proliferation and differentiation of the common precursor cells are triggered by a number of growth stimulating factors (Interleukin-6 (IL-6), IL-3, IL-7, GM-CSF) [37,

38]. In the following, immune cell types are described which play an important role in the context of this work.

2.1.1 Granulocytes

Granulocytes have a size of 7 – 10µm and have an irregularly shaped nucleus also known as polymorphonuclear leukocytes (PMN) [39, 40]. Based on their staining properties, they can be divided into eosinophilic, basophilic and neutrophilic granulocytes. With 40 – 75% in humans (20 – 30% in mice), neutrophil granulocytes represent the largest fraction of blood leukocytes [39, 41]. Neutrophil granulocytes originate from the bone marrow, where they are stored as a reserve population. From the bone marrow they migrate into the bloodstream, where they circulate for only a few hours before migrating into the respective tissues [39, 42]. Due to the very short lifespan, each day approximately 2×10^{11} new cells have to be produced in the bone marrow [39, 40, 42, 43].

2.1.1.1 Recruitment and Migration of Leukocytes after Endothelial Damage

PMNs are able to migrate from bloodstream into the affected tissue under inflammatory conditions. Recruitment and migration are usually initiated by pathological changes on the surface of the vascular endothelium. Multiple endogenous and exogenous stimuli can trigger endothelial damage. The resulting activation of the endothelial cells leads to the expression of different adhesion molecules on the endothelial surface [44]. The binding interaction of circulating neutrophil granulocytes with endothelially expressed adhesion molecules initiates the so-called adhesion cascade. Typically, this occurs sequentially in the following steps: capturing or thethering, rolling, adhesion, crawling and transmigration [42, 44]. Tethering describes the initial binding between adhesion molecules expressed by endothelial cells and recruited leukocytes. The binding is mainly mediated by a certain type of glycoproteins, the so-called selectins, which can be divided in L-selectin, P-selectin, and E-selectin [45]. L-selectin is presented by most of the leukocytes. In contrast, P-selectin is expressed by both platelets and endothelial

cells and E-selectin only by activated endothelial cells. L-selectin is primarily responsible for the recruitment of cells into inflammatory tissue [44-46]. It also mediates the binding of neutrophil granulocytes to already bound granulocytes ("secondary tethering") [45, 47]. P-selectin and E-selectin facilitate the primary contact between endothelium and leukocytes (tethering) and initiate the rolling along the endothelium [42, 44, 48]. In contrast to P-selectin, E-selectin is synthesised de novo; therefore it is not available within a few minutes but after approximately 90 minutes [44, 49, 50]. Selectins bind mainly to the P-selectin glycoprotein ligand 1 (PSGL1) [42, 44, 45, 51]. The binding to PSGL1, which is expressed by almost all leukocytes, ultimately leads to tethering and subsequent rolling, even under very high shear forces (1 – 10 dynes per cm^2) [42, 52]. Rolling along the endothelium under high shear forces requires a fast contrary binding as well as a rapid dissolution of receptor and ligand [53]. During rolling of the neutrophils, the binding between PSGL1 and selectin gradually dissolves until complete break-up. At the same time, a new binding of PSGL1 and P-selectin is initiated on the opposite side. During rolling recruited and activated leukocytes release multiple cytokines, chemokines and integrins, which enables subsequent binding ("arrest") [44, 54]. Integrins (VLA-4 and LFA-1) are transmembrane adhesion molecules that are present on leukocytes and platelets [42, 44]. These integrins bind to proteins of the immunoglobulin superfamily (MAdCAM-1, VCAM-1, ICAM-1) on the endothelium [42, 44, 51]. The binding can slow down the cell and eventually lead to arrest [51]. Due to their positive charge, chemokines are molecules that attach to negatively charged endothelial heparan sulfates. This consequently forms a concentration gradient which guides the cells to the site of the highest chemokine concentration (chemotaxis) [30]. The binding of chemokines to G-protein coupled receptors can lead to the activation of integrins (VLA-4, LFA-1) on the endothelial cell surface [44]. This process is also called "inside-out signalling" [44]. The "inside-out signalling" triggers conformational changes of the integrins resulting in the release of new binding domains. Conformational modification of of VLA-4 and LFA-1 leads to a more stable binding of VCAM-1 and ICAM-1 [44, 55]. This binding is essential for subsequent paracellular or transcellular migration of leukocytes [44, 55]. During migration through the vessel wall, the endothelial cell barrier and associated basement membrane are penetrated as well as the pericyte layer [42]. Formation of membrane protrusion of leukocytes ("crawling") that penetrate

intracellular ("transcellular") or paracellular is initiated by the binding of MAC-1 to ICAM-1 [42, 56, 57]. The binding of ICAM-1 subsequently activates various kinases (MAPK, GTPase), which in turn activate the myosin light chain kinase (MLCK) enzymatically, resulting in an increased contraction of the endothelial cells. This would consequently lead to the opening of interendothelial junctions. [44, 57]. Particularly at the membrane side, transcellular migration of the leukocytes is facilitated with a reduced content of extracellular matrix (< 60% collagen IV, laminin 10, nitrogen2) [42, 58]. Migration can further be mediated by α6β1 integrin and proteases such as matrix metalloproteinases (MMPs) and serine proteases (neutrophilic elastase (NE)), which enzymatically breakdown components of the extracellular matrix [42, 44, 59]. The following, adapted figure provides a general overview of leukocyte adhesion and migration [44].

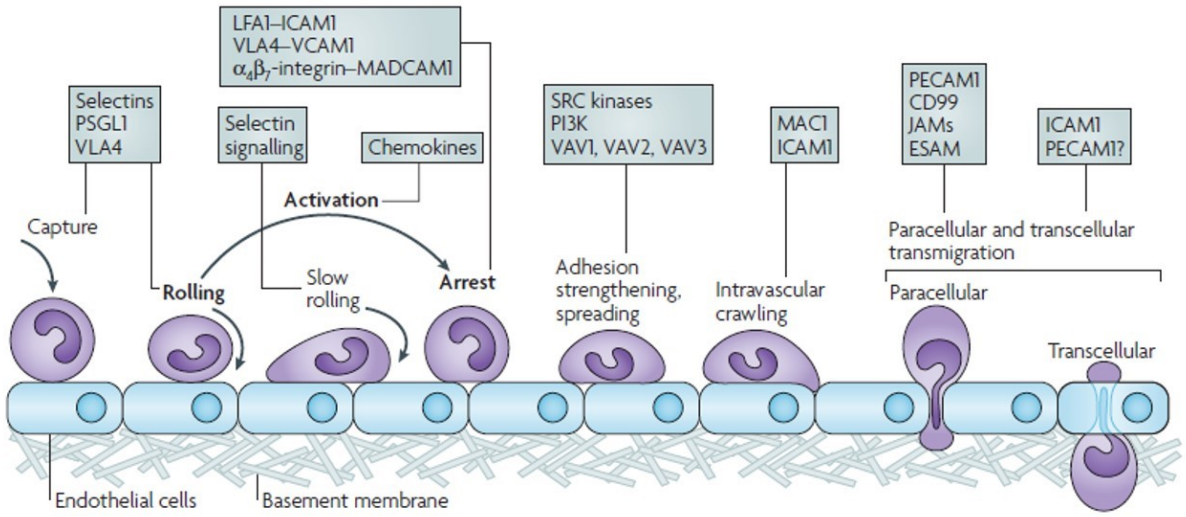


Figure 1. Overview of leucocyte adhesion and migration, adapted from Ley, K et al. [44].

2.1.1.2 Neutrophil Granule Proteins

Neutrophil granulocytes play a crucial role in the immune response. They have different mechanisms to protect the human body from pathogens. Once identified, neutrophils can absorb the pathogen intracellularly. After incorporation into the phagosome, it will be destroyed by NADPH oxygenase-dependent mechanisms (reactive oxygen species) or by antibacterial proteins (cathepsins, defensins, lactoferrin and lysozyme) [40]. Neutrophil granulocytes store these antibacterial

proteins and proteases in their granules. After the pathogen has been absorbed they fuse with the phagosome and the antibacterial proteins and proteases are released inside. Furthermore, the granules can also fuse with the plasma membrane leading to the extracellular release of its content [39]. Degranulation is initiated primarily after cell activation [39, 54]. There are two types of granules based on histological staining properties: peroxidase-positive and peroxidase-negative granules [38, 60]. The neutrophil granules are synthesised during myelopoiesis at the promyelocyte stage [54]. Due to their high content of myeloperoxidases (MPO) they are called "peroxidase-positive granules" (also "azurophilic granules" or "primary granules") [54]. The production of myeloperoxidases is terminated by differentiation into myelocytes. The granules formed from this point on are called "peroxidase-negative granules" [54]. Based on their content, they can then be further subdivided into secondary (specific granules) and tertiary granules (gelatinase granules) [38, 54, 61]. Secondary granules are formed during myelocyte and metamyelocyte formation and contain high levels of lactoferrin but only small amounts of gelatinase [54]. In contrast, tertiary granules are formed in already segmented neutrophils and are rich in gelatinase but low in lactoferrin [54]. A common structural feature of granules is the presence of a phospholipid bilayer and an intragranular matrix which provides storage of granule proteins. The calcium-dependent degranulation is performed sequentially [39, 54]. The first vesicles to be released are secretory vesicles, followed by gelatinase granules, specific granules and finally by azurophilic granules [62-64]. Azurophilic granules contain acid hydrolases and antimicrobial proteins [54, 65]. Many of these proteins are synthesised as proforms, which are proteolytically cleaved after contact with the granular membrane. Afterwards, they are stored in the active form [54, 66]. Two of the most important proteins of azurophil granules are myeloperoxidase and defensins. After neutrophil activation, myeloperoxidase (150kDa) is released into the phagosome or the environment by exocytosis [54, 67]. Myeloperoxidase reacts with H_2O_2 via NADPH oxidase (NOX), which enhances their activity. By oxidation of chloride, tyrosine and nitrite, reactive oxygen species are formed which can attack and destroy microorganism membrane surfaces [54, 67].

	MPO positive	MPO negative	
Localization	Azurophil (primary) granules	Specific (secondary) granules	Gelatinase (tertiary) granules
Membrane	CD68 CD63 Preseniline 1 Stomatin V-type H^+-ATPase	CD11b/CD18 CD15 CD66 CD67 Cytochrome b_{558} fMLP-R Rap1, Rap2 SCAMP Stomatin TNF-R VAMP-2 Vitronectin-R Laminin-R Leukolysin	CD11b/CD18 Cytochrome b_{558} fMLP-R Leukolysin NRAMP-1 SCAMP VAMP-2 SNAP-23, -25
Matrix	α_1-Antitrypsin α-Mannosidase Azurocidin β-Glucuronidase Cathepsins Defensins Elastase Lysozyme MPO N-acetyl-β-glucosaminidase Proteinase-3 Sialidase Ubiquitin-protein	Collagenase CRISP-3 (SGP-28) Gelatinase Histaminase Heparanase Lactoferrin Lysozyme	Acetyltransferase β_2-Microglobulin CRISP-3 Gelatinase Lysozyme

Table 1. Overview of the content of primary, secondary and tertiary neutrophil granules, adapted from Faurschou, M. et al. [54]

2.1.1.3 Immunomodulatory Role of Defensins

Along with cathelicidins, defensins are one of the two major groups of antimicrobial peptides and are found in every mammal [68]. They can be divided into three different groups based on structural characteristics: α-defensins, β-defensins and θ-defensins [68]. A common feature of these groups are six characteristic cysteine

residues, which are connected to 3 disulfide bridges to form the typical defensin structure [68, 69]. The first α-defensin was isolated from human PMNs [68, 70, 71]. The first human β-defensin (hBD) was extracted from plasma (hBD1) and subsequently from the skin of patients with psoriasis (hBD2, 3) [68, 70, 72]. α-defensins (human neutrophil peptides, HNP) are produced by neutrophil granulocytes and Paneth cells [73, 74]. Paneth cells are specialised immune cells of the small intestine and possess a high concentration of defensin-containing granules, which are released into the intestinal crypts after stimulation [69, 75]. β-defensins are mainly produced by epithelial cells of the lung, intestine and urogenital tract and prevent the colonisation of pathogens [75]. α-defensins which originate from β-defensins developed considerably later in mammals [68, 76]. A large number of mammals contain different β-defensins (opossum 32 β-defensins, mouse 40 β-defensins) and in contrast only a few α-defensins (Chimpanzees and macaques 6-8 α-defensins) [68, 77-83].

Alpha defensins are the most important component of neutrophile granules and account for 5% of the protein content (MPOs represent 3.5 - 7.0% in neutrophils, azuricidin + proteinase3 represents 1 - 3% and HNP1 - 3 represents 35 - 50%) [62, 84, 85]. Some species like mice and some ruminants do not contain α-defensins in their neutrophil granulocytes [68, 86]. In humans 6 different types of α-defensins (HNP1 - 6) have been identified located either in neutrophil granulocytes (HNP1 - 4) or Paneth cells (HD5 - 6) [70, 73, 82]. They are small (3.5kDa) cationic, antimicrobial and cytotoxic peptides consisting of approximately 35 - 50 amino acids [70]. α-defensins are synthesised during the late phase of myelopoiesis in the bone marrow in the progenitor cells of neutrophil granulocytes (promyelocytes, myelocytes) [87-89]. Defensins which are formed in promyelocytes are synthesised as 75 aa long proform, cleaved and stored as mature form in azurophilic granules [54, 90, 91]. Defensins that are synthesised in myelocytes are no longer stored in the azurophilic granules, but secreted directly as proform (75aa) [88]. Mature neutrophil granulocytes which are circulating in the blood do not produce α-defensins anymore [69]. The average plasma concentration is approximately 40ng/mL and can reach up to 0.9 - 170mg/mL when infections are present [92]. In general, a large number of immunomodulatory functions are associated with the defensins. They receive their name from their antimicrobial activity against a variety of bacteria, viruses, fungi and protozoa [70, 93, 94]. Antimicrobial activity

was observed in vitro at concentrations of 1 - 10µg/mL [69]. This can be experimentally inhibited by increasing or adding salts and plasma proteins [69, 95, 96]. As already mentioned, pathogens of neutrophil granulocytes are absorbed into the cell interior by phagocytosis and subsequently eliminated in the phagolysosome. Azurophilic granules fuse with the phagolysosome and release their contents into the interior [39]. Their antimicrobial activity is based, for instance, on the formation of pores in the cell membrane of pathogenic organisms. [70, 97-99]. In very high concentrations, especially in inflammatory tissue, some defensins also have cytotoxic functions [73, 82, 100-102]. Another important immunomodulatory function of defensins is the ability to activate and recruit other immune cells. They have chemotactic properties against monocytes and by binding G(ia) protein-coupled receptors also against $CD4^+$ and $CD8^+$ T cells [82, 103-105]. Also, HNP1 - 3 may increase the secretion of antibodies and cytokines from other immune cells [82, 106, 107] and are involved in wound healing by promoting cell proliferation [82, 108].

2.1.2 Monocytes

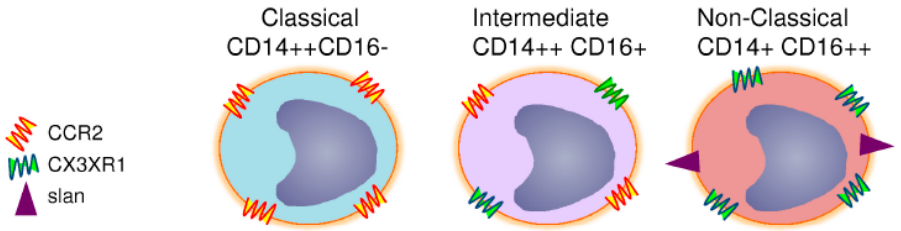
Monocytes originate from haematopoietic progenitor cells of the bone marrow [109-111]. Later on, they migrate from the bone marrow into the circulation, where they can be recruited into tissues in a very short time [112-115]. Monocytes have a size of 14 - 20µm and therefore represent the largest leukocyte population in the human organism [115]. They represent 10% of the total leukocytes in humans and up to 4% in mice [115, 116]. Important for the innate immunity, monocytes can kill bacteria, viruses, fungi and parasites in many different ways, for example via phagocytosis, secretion of myeloperoxidases, reactive oxygen species (ROS) or nitride oxide (NO) [117, 118]. In their entirety, monocytes and their derived phagocytic cells, as well as B cells and dendritic cells, form the so-called monocytic phagocyte system (MPS) (formerly: reticuloendothelial system, RES) [114]. These cells can take up and degrade antigens (antigen processing) by various mechanisms and present them as MHC class II complexes (Major Histocompatibility Complex II) on their surface [119]. The presence of these complexes leads to the activation of T cells [114, 119, 120]. Due to their diverse immunological functions, they play an important role especially in inflammatory diseases such as atherosclerosis [118, 121].

For a long period, it was considered that monocytes are the precursor cells of macrophages and dendritic cells [116, 122]. Recent studies show that tissue-resident and tissue-specific macrophages, as well as some dendritic cells, already develop independently of monocytes during embryogenesis [110, 114, 123, 124].These precursor cells derive from the Yolk Sak or fetal liver and migrate into the corresponding tissue in which they start to proliferate [116, 124, 125]. According to the type of tissue they are named differently. Liver macrophages are named as Kupffer cells, epidermal cells as Langerhans cells, lung and brain-derived cells as alveolar macrophages and microglia, respectively [114, 123, 126–128]. Depending on the type of tissue, these specific tissue macrophages have a special transcriptional signature and thus different functions [129, 130]. In specific tissues different types of macrophages are present. So far three different types have been identified within the heart, skin, intestines and lungs [114, 131–134]. The ratio between the different types of macrophages varies according to tissue type and inflammation status [114].

2.1.2.1 Monocyte Subsets

Monocytes can be divided into subgroups based on their cell morphology and expressed surface markers [115, 135]. Human monocytes are divided into three different populations depending on the expression of CD14 and CD16 [115, 116, 136–138]. Classical monocytes ($CD14^{++}CD16^{-}$) represent about 80 – 90% of the monocytes in the plasma. The intermediate monocytes ($CD14^{++}CD16^{+}$) have a percentage of approx. 2 – 5% and the non-classical monocytes ($CD14^{+}CD16^{++}$) of approx. 2 – 10% [112, 115, 118, 137]. A classification of murine monocytes is also possible according to surface receptors depending on the expression of Ly6C, CD43 and CX3CR1. Three different populations can be classified into $Ly6C^{++}CD43^{+}CX3CR1$ and $Ly6C^{++}CD43^{++}CX3CR1^{+}$, $Ly6C^{+}CD43^{+}CX3CR1^{++}$ [115, 139, 140]. Based on the expression of different immune receptors, such as CCR2, CD62L, CD11b and TLR4, the classical monocytes ($CD14^{++}CD16^{-}$/$Ly6C^{++}CD43^{+}CX3CR1^{-}$) are considered to have high phagocytic and immunomodulatory activity [115, 141, 142]. Non-classical monocytes contain high levels of CX3CR1, CXCR4, LFA-1, TNFα which makes them highly migratory cells [137, 141, 143–145]. In contrast, they produce only a small amount of CCR2, CD11b. As mentioned above, under physiological

conditions non-classical monocytes represent a very small subpopulation (about 2 – 10%) of monocytes. However, their number increases enormously in the presence of inflammation or stress [142, 146]. The adopted and adjusted figure below gives an overview of different subgroups of human and mouse monocytes [115].



Chemotaxis	CCR2/CCL2	CCR2/CCL2 CX3CR1/CX3CL1	CX3CR1/CX3CL1
Surface markers	**CCR2**, CD62L, CD11b, TLR-4	CCR2, CX3CR1, HLA-DR, CD74, CD163,CLEC10A, GFRA2	**CX3CR1**, **slan**, CXCR4, HLA-DR, LFA-1
Cytokines	IL-10, IL-1	TNF-α, IL-10	⇧TNF-α
Frequency in Peripheral Blood	~80-90%	<15%	<15%
Mice equivalent	$Ly6C^{++}CD43^{+}$ $CX3CR1^{-}$	$Ly6C^{++}CD43^{++}$ $CX3CR1^{+}$	$Ly6C^{+}CD43^{++}$ $CX3CR1^{++}$

Figure 2. overview of different subgroups of human and mouse monocytes, adapted from Wacleche, V.S. et al. [115]

2.1.2.2 Characteristics of Monocyte Recruitment

The recruitment of monocytes takes place in the same sequential steps (tethering, rolling, attachment, transmigration) as described under 1.2.1.1 [140]. $Ly6C^{low}$ monocytes are recruited earlier to the site of inflammation than $Ly6C^{high}$ monocytes. Participating during the initial inflammatory reaction, they can release TNFα and chemokines [144]. However, the subsequent recruitment of $Ly6C^{high}$ monocytes is more advanced and robust being induced by CCR2, CCR5 and CX3CR1 [147]. So far, the recruitment of $Ly6C^{low}$ monocytes has been rarely investigated. It is known that they need CCR5, but not CX3CR1 for recruitment [147]. The rolling of the monocytes along the endothelium is mediated by P-selectin and E-selectin, whereas adhesion is mediated by VCAM-1 [148].

Monocyte-expressed molecules	Binding partners	Functions in monocyte recruitment
Chemokines and their receptors		
CCR2	CCL2, CCL7 and CCL12	Emigration of LY6C^{hi} monocytes from the bone marrow[18,26]
CX_3CR1	CX_3CL1	Patrolling; recruitment to splenic sites of bacterial infection[27]
CCR1 and CCR5	Various, including the shared ligands CCL3 and CCL5	Recruitment into or within inflamed tissues[41–43,125]
CCR6	CCL20	Possible role in the migration or function of LY6C^{hi} monocytes in inflamed tissues[52,53]
CCR7 and CCR8	CCL19 and CCL1, respectively	Migration of monocyte-derived DCs from the skin to lymph nodes[54]
CXCR2	MIF	Arrest in atherosclerotic arteries of mice[55]
Adhesion molecules		
L-selectin	Glycoproteins, including CD34, GLYCAM1 and MADCAM1	Tethering and rolling[56,57]; recruitment during thioglycollate-induced peritonitis[57]; migration to lymph nodes[58]
PSGL1	P-selectin and E-selectin	Migration through inflamed dermal venules[58]; tethering and rolling on atherosclerotic endothelium[61]
LFA1	ICAM1	Patrolling during the steady state; not involved in the early recruitment of inflammatory monocytes[11]
MAC1	ICAM1	Adhesion during acute inflammation[62,63]
VLA4	VCAM1	Adhesion to inflamed endothelium[60,61]
PECAM1	Endothelial PECAM1	Transendothelial migration[141,142]

CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CX_3CL1, CX_3C-chemokine ligand 1; CX_3CR1, CX_3C-chemokine receptor 1; CXCR2, CXC-chemokine receptor 2; DC, dendritic cell; GLYCAM1, glycosylation-dependent cell adhesion molecule 1; ICAM1, intercellular adhesion molecule 1; LFA1, lymphocyte function-associated antigen 1; MAC1, macrophage receptor 1; MADCAM1, mucosal addressin cell adhesion molecule 1; MIF, macrophage migration inhibitory factor; PECAM1, platelet endothelial cell adhesion molecule; PSGL1, P-selectin glycoprotein ligand 1; VCAM1, vascular cell adhesion molecule 1; VLA4, very late antigen 4.

Figure 3. Overview of chemokines and molecules involved in monocyte recruitment, adapted from Shi, C. et al. [140].

2.1.3 Thrombocytes

Thrombocytes are small anuclear cell fragments with a size of 2 - 3µm generated in the bone marrow (thrombopoiesis) [149, 150]. Fragmentation of polyploid megakaryocytes results in truncated cytoplasm fragments which are also known as proplatelets [151, 152]. A megakaryocyte produces about 6 - 8 proplatelets. Up to 1000 thrombocytes can be produced from one platelet resulting in approximately 5000 - 10000 per megakaryocytes [153, 154]. The initiation of proplatelet formation is stimulated by thrombopoietin, which is synthesised in the liver [152]. Thrombocytes have a lifespan of approximately 5 - 9 days in humans and up to 5 days in mice [155]. Ageing thrombocytes increasingly express phosphatidylserine on their surface, which leads to their absorption and degradation by phagocytic cells in the liver or spleen [154, 156]. One person can produce up to 10^{11} platelets per day. Approximately two-thirds of them are

circulating in the blood (1.5 to 4.5 x 10^8 platelets/mL in humans and 10^9 platelets/mL in mice) and one third is stored in the spleen [154, 157, 158].

2.1.3.1 Immunomodulatory Components of Platelets

In 1892 Giulio Bizzozero first described the important role of platelets in hemostasis [159, 160]. Thrombocytes also possess a variety of critical immunomodulatory properties [157, 161]. On the one hand they can express different immune receptors, and on the other hand, they contain a lot of immunomodulatory substances which are stored in their internal granules and which are secreted after stimulation [157]. Thrombocytes can release up to 300 different proteins [162]. There are three known types of granules: α-granula, δ-granula (dense granules) and lysosomes [161]. The degranulation is triggered by activation of thrombocyte receptors such as TLRs, G protein-coupled receptors (GPCRs) or various glycoprotein/glycoprotein complexes (GPVI and GPIb-IX-V [157, 163].

Platelet components	Superfamily	Molecule
α-granules	Adhesion molecules	P-selectin, Fibrinogen, VWF, Fibronectin
	Coagulation factors	Factor V, Factor XI, Factor XIII
	Mitogenic factors	PDGF, TGF-β
	Angiogenic factors	VEGF
	Igs	IgG, IgA, IgM
	Chemokines	CCL3 (MIP-1α), CCL5 (RANTES), CXCL1 (GROα), CXCL4 (PF4), CXCL5 (ENA78), CXCL7 (NAP2, β-thromboglobulin)
	Protease inhibitors	C1 inhibitor, α2-plasmin inhibitor, PAI-1
	Antimicrobial peptides	Thrombocidin 1 and 2
Dense granules	Amines	Serotonin (5-HT)
	Cations	Calcium, Magnesium
	Nucleotides	ATP, ADP
Lysosomes	Proteases	Cathepsin D, E, Collagenase
	Glyco-hydrolases	Heparinase, β-N-acetylglucosaminidase, β-glucuronidase, β-glycerophosphatase, β-galactosidase, α-D-glucosidase, α-L-fucosidase, β-D-fucosidase
Granule-independent soluble mediators	Cytoplasmic or membrane components	CCL7 (MCP3), IL-1β, HMGB1, β-defensin 1, 2, 3, Thromboxane A2, PAF, sCD40L
Surface adhesion molecules	Integrins	α5β1 (VLA-5), α6β1 (VLA-6), α2β1, α2bβ3 (GPIIb/IIIa)
	Adhesion receptors	GPIbα, ICAM-2, GPVI, CLEC2
Immune receptors	TLRs	TLR1, TLR2, TLR4, TLR6, TLR7, TLR9
	Co-stimulatory proteins (TNF and TNFR superfamily)	CD40, CD40L (CD154)
	Ig receptors	FcγRIIA (CD32), FcεRI, FcεRII (CD23), FcαRI
	Complement compoments	gC1qR, C5b-9

Table 2. summary of the surface receptors of thrombocytes as well as the content of different granules, adapted from Duerschied, D. et al. [161].

2.1.3.2 Mechanisms of Thrombus Formation

Under non-inflammatory conditions, thrombocytes are patrolling along the vascular endothelium. By producing and releasing several mediators, endothelial cells ensure that platelets will not be activated under physiological conditions [154, 164]. Such mediators are for example diphosphohydrolases, amino oxidases, nitric oxide (NO) (maintains normal vascular tone), prostacyclin (inhibits platelet adhesion and aggregation) [48, 165]. These protective mechanisms are down-regulated after a vessel injury in order to initiate the required haemostasis [48]. Damage of the endothelium results in the release of subendothelial matrix proteins (collagen), inducing platelet adhesion and aggregation at the endothelium, which is mediated by the interaction between GPIb-V-IX and GPIa-IIa receptors, von Willebrand factor (VWF), collagen (primary hemostasis) [154, 166]. The binding of GPIa and VWf is particularly important at very high shear forces [155]. Furthermore, the interaction between endothelial cells and platelets is essential for initiation of the leukocyte adhesion cascade [48, 161]. The endothelial cells which are activated by the vascular injury express P-selectin on their surface, which induces a rolling of recruited thrombocytes along the vascular endothelium [48, 167]. The above-described binding of the ligands to the GP receptors induces a deformation of activated platelets (formation of pseudopodia) as well as the release of the granular content [154]. Released thrombocyte activators like thromboxane A2, ADP, serotonin and P-selectin enhance thrombocyte aggregation (formation of white thrombus), lead to vasoconstriction and recruitment of additional thrombocytes and leukocytes [150, 154, 168-170]. Simultaneously, various coagulation factors in plasma are activated because of the endothelial damage (factor XII, tissue thromboplastin (tissue factor), factor VII), initiating a cascade of successively activating coagulation factors [170, 171]. This ultimately leads to the production of thrombin in the presence of factor V, calcium and phospholipids [170, 172]. Thrombin is mainly responsible for the conversion of fibrinogen to fibrin, but is also able to activate other coagulation factors (Factors V, VIII, XIII) and promotes platelet aggregation [170]. The fibrin polymerises to an insoluble gel and is cross-linked under the influence of factor XIII. Cross-linking serves for additional stability of the thrombus [170, 173]. The incorporation of erythrocytes into the thrombus results in the so-called red thrombus [174].

2.2 Lipid Metabolism and Catabolism

Lipids are a group of substances consisting predominantly of hydrocarbon molecules. They can be divided into two groups, amphiphilic and hydrophobic lipids. Amphiphilic lipids include fatty acids (FA), phospholipids (PL) and free cholesterol (FC) [175]. Triglycerides and cholesterol esters responsible for the transport and storage of cholesterol are hydrophobic lipids. They have different functions in the organism acting mainly as energy sources and reservoirs. In addition, they are also essential for the synthesis of steroid hormones and bile acids [176]. Almost all cells in the body, except for erythrocytes, can produce cholesterol on their own (endogenous metabolism) [176, 177]. However, since this process is always accompanied by a loss of energy for the cell, an exogenous metabolism is prefered absorbing cholesterol from the circulation. The human organism can produce up to 1 gram cholesterol per day from acetic acid (Acetyl-CoA). The key enzyme is HMG-CoA reductase. Besides, there are approximately 0.2 - 0.4 grams which are absorbed from the food. So in total up to 1-2 grams of cholesterol are provided daily in the body. Non-required cholesterol is excreted via the bile acids [178]. In the circulation, cholesterol is predominantly transported by lipoproteins to the absorbing organs. There are three different transport pathways: the exogenous, the endogenous and the so-called reverse cholesterol transport [175, 179]. The exogenous pathway describes the uptake of lipids from food and their further transport to the corresponding organs. Shortly after the ingestion of food, the first triglycerides are hydrolysed and emulsified in the mouth and stomach by lipases and gastric acid. This results in the formation of small micelles. In the duodenum, the deposition of the lipase on the surface of the micelles and the influence of bile acids lead to a further emulsion of the lipids resulting in the formation of micelles which contain free fatty acids, cholesterol, bile acids and monoacylglycerols [180, 181]. The bile acids remain in the intestine and are absorbed in the ileum (enterohepatic circulation), whereas cholesterol, fatty acids and monoacylglycerols are absorbed together with fat-soluble vitamins in the duodenum and jejunum [182]. Cholesterol is taken up via a specific transporter, the so-called Niemann-Pick-C1-like protein (NPC1L1) [183]. Part of the absorbed cholesterol is released back into the intestinal lumen via the ABCG5 and ABCG8 receptors. Fee fatty acids and monoacylglycerols are transported into enterocytes via CD36 (Fatty acid translocase, FAT) or by fatty acid transport

proteins (FATP). After the uptake they are again esterified to triglycerides inside the cell [184, 185]. The intracellular esterification of cholesterol is mediated by acyl-CoA-cholesterol-acyl-transferase 2 (ACAT2). Subsequently, free cholesterol, as well as cholesteryl ester and triglycerides, are combined with apolipoproteins (ApoB-48, ApoA-I, A-IV, A-V) and phospholipids to form so-called chylomicrons (lipoprotein particles), which enables the transport in the blood despite the poor water solubility of lipids [181, 186, 187]. Based on their different densities, lipoproteins can be subdivided into chylomicrons, VLDL (Very low density lipoprotein), IDL (Intermediate density lipoprotein), LDL (Low density lipoprotein) and HDL (High density lipoprotein) [175]. For the stabilisation of the lipoproteins, different apolipoproteins are located in the surface membrane. The most important apolipoproteins are ApoA, ApoB, ApoC and ApoE [175]. Chylomicrons are not released directly into the circulation. Instead they first migrate into the lymph [188] avoiding the first-pass effect in the liver. Chylomicrons enter the bloodstream via the thoracic duct [181, 188]. Here they receive ApoC from HDL. In this process, ApoC-II plays an important role because it can activate endothelial lipoprotein lipase (LPL) [175]. LPL hydrolyses the triglycerides inside the chylomicrons into free fatty acids and monoglycerides, which are taken up by peripheral tissues (adipocytes, heart and skeletal muscle cells) [175]. By releasing fatty acids and monoglycerides and by absorbing additional cholesterol esters and ApoE from HDL, chylomicrons are converted into so-called remnants. The chylomicron remnants are then taken up by the liver by binding to the low density lipoprotein receptor-related protein 1 (LRP1) and low density lipoprotein receptor (LDL-R). After the receptor binding, hepatocytes absorb the triglycerides and cholesterol and form VLDL (endogenous transport pathway) for further transportation [175, 189]. The nascent VLDL particles are then released directly into the bloodstream. In contrast to chylomicrons, VLDLs contain ApoB-100 instead of ApoB-48 as the most important protein component. ApoC-I and ApoC-III directly inhibit the uptake into hepatocytes. ApoC-II activates the endothelial LPL, which again hydrolyses the triglycerides inside the VLDL [175]. The peripheral tissue and apolipoproteins absorb the fatty acids which are released during this reaction. As a result, IDLs (VLDL remnants) are generated from the VLDLs. IDLs can either be taken up into the hepatocytes by an interaction of ApoE and LDL-R or by binding to LRP1, or they can be converted into LDL by a further triglyceride loss. This occurs either by

the hepatic lipase or by the cholesteryl ester transfer protein (CETP) [190]. LDL contains only 20 - 30% of the original triglyceride content of the VLDL [186, 191]. ApoB-100 is the only apolipoprotein present in LDL [192]. Due to its low affinity for LDL-R, LDL is circulating much longer (up to two days) in the bloodstream than VLDL (2-4 hours) and chylomicrons (0.5 hours) [193, 194]. However, 90% of the circulating LDL is absorbed via the LDL-R. After the binding of LDL to LDL-R, the complex is concentrated at distinct membrane regions ("coated pits") and subsequently taken up by endocytosis ("coated vesicles") [186]. There, the so-called coated vesicles are transformed into endosomes by the loss of stabilising clathrin membrane, which then fuses with lysosomes [195]. Here the complex of ligand and receptor is degraded by different enzymes (lipases and proteases). In the endosomes LDL-R and LDL can also dissociate from each other due to the decreasing pH value so that the LDL-R can be transported back to the cell surface where it is again available for LDL uptake [195]. If the complex of LDL-R and LDL additionally binds PCSK9, dissociation is no longer possible in the endosomes, and the LDL-R will be degraded [196, 197]. This leads to a reduction of LDL-R at the membrane surface and consequently to an increase in the plasma concentration of LDL. The expression of LDL-R is regulated by the intracellular cholesterol concentration [186, 198]. Some LDLs in the plasma are not taken up by hepatocytes via LDL-R but via an alternative pathway, the so-called scavenger pathway. In contrast to LDL-R, this way is much slower but not saturable [190]. Macrophages predominantly express these receptors (e.g. SR-A) and play a key role especially in the context of atherogenesis and atheroprogression [199]. The very long circulation time of LDL makes them particularly susceptible to modifications and thus extremely atherogenic. In contrast to LDL, HDL is considered to have an atheroprotective function as they are mainly responsible for the transport of lipids from peripheral tissues and cells [186, 200]. HDL is produced extracellularly. ApoA-I which is synthesised in the liver and small intestine leads to the formation of disc-shaped, discoid nascent HDL by binding phospholipids and unesterified cholesterol [190, 201]. With increasing cholesterol uptake, mature and spherical HDL particles are formed over time. Further absorption of cholesterol and fusion with other HDL particles results in even larger HDL particles [175, 200]. In contrast to LDL particles or Remnants, the lifetime of HDL particles is much more variable and can be up to 4 days [202]. HDL particles can indirectly release cholesterol

esters to VLDL and LDL via cholesterol ester transfer protein (CETP)-mediated transfer or be directly absorbed into hepatocytes or steroid-producing cells via SR-B1 [179, 186, 190, 203]. As a result, ApoA-I will be released and can be used for the formation of new HDL or can be filtered glomerular and resorbed tubularly via the kidney [186].

2.3 Pathology of Atherosclerosis

Atherosclerosis is a chronic inflammatory process that can persist over several decades [204]. At a very young age first atherosclerotic lesions can occur, which lead later on to clinical manifestations such as angina pectoris, myocardial infarction and stroke [24]. Many different risk factors trigger the development of atherosclerosis. This includes genetic predisposition, age and the presence of cardiovascular risk factors such as smoking, obesity, high blood pressure, hypercholesterolemia and diabetes mellitus [205]. Atherosclerosis is clinically mainly located at so-called prediction sites including coronary vessels and the carotid artery [204]. The onset of the disease is assoctiated with initial damage of the arterial endothelium, which can be caused by the above-mentioned risk factors. R. Ross already discovered with his "Response to Injury" theory that this initial damage to the endothelium is significantly responsible for the development of atherosclerosis [206-208]. Under physiological conditions endothelial cells produce a variety of substances that protect the vascular wall from inflammation or damage. For example, the endothelial production of nitric oxide (NO) not only leads to vasodilatation, but also inhibits the activation of thrombocytes and reduces the adhesion of leukocytes to the endothelium [186]. Damage to endothelium can lead to the loss of anti-inflammatory and antithrombotic activity of endothelial NO secretion [186, 209]. As a consequence, the loss of function of the endothelium may facilitate migration and accumulation of LDL into the subendothelial tissue layer of the vascular intima [210]. Here LDL is modified oxidatively by binding ApoB-100 to proteoglycans to produce the so-called oxLDL [186, 209-211]. While this stimulus acts as a strong inflammatory agent on endothelial cells, it is also able to recruit immune cells from the blood into the intima [198, 212]. Endothelial cells activated this way express different adhesion molecules (E- and P-selectin, VCAM-1 and ICAM-1) on their surface [186, 213]. In addition,

cytokines, chemokines such as MCP-1 ("monocyte chemoattractant protein-1"), and growth factors such as M-CSF ("macrophage colony-stimulating factor") and G-CSF ("granulocyte-macrophage-colony-stimulating factor") are released [214]. As a result, leukocytes are recruited from the bloodstream, can attach to the inflammatory endothelium and migrate into the intima [215]. In the first phase of atherogenesis, phagocytic cells of innate immunity play a very important role (neutrophil granulocytes, monocytes) [216]. Activated neutrophils can release proteins such as antimicrobial peptides (cathelicidins and defensins) and serine proteases during adhesion to the vascular endothelium and migration into the intima [54]. These granules proteins offer numerous immunomodulatory functions, which enables the recruitment of further immune cells (e.g. monocytes) from the circulation [217]. The growth factors that are produced by endothelium (M-CSF and G-CSF) interact with surface receptors of the monocytes to induce their migration and subsequent differentiation into macrophages and dendritic cells [240]. In the intima, macrophages then bind oxLDL via scavenger receptors (e.g. SRA-1, SRA-2, MARCO, CD36, SR-B1) and take it up intracellularly [185, 211, 218]. Since this form of lipid uptake does not have a negative feedback in contrast to LDL-R uptake, it results in a massive overloading of macrophages and as a consequence to the formation of foam cells [219]. The formation, proliferation and especially the accumulation of these cells lead to the production of fatty streaks, which are a reversible form of atherosclerotic plaques [24]. During further progression of atherosclerosis, secretion of PDGF and TGF-β from activated macrophages and foam cells causes proliferation of smooth muscle cells leading to an increase in the extracellular matrix (ECM) [24]. The resulting and growing plaque comprises a necrotic core, ECM and smooth muscle cells surrounded by a stabilising fibrous cap. Progression of the plaque causes a pathological thickening of the intima, which reduces the vascular vessel diameter. Macrophages which are stimulated by inflammation mediators are synthesising matrix metalloproteases which degrade the components of the extracellular matrix [220]. The degradation and subsequent thinning of the ECM results in unstable plagues susceptible to rupture [186, 220, 221]. Plaque rupture promotes the contact between blood and the thrombotic contents located in the necrotic core of the plaque. This process can lead to a rapid, complete thrombotic occlusion of the artery with the consequence

of acute ischemia syndromes (myocardial infarction, sudden cardiac death and stroke) [221].

2.4 Outline of this Thesis

The reduced quality of life, the high incidence of fatalities and incurring costs for the public health system due to atherosclerotic lesions and their consequences necessitate further investigations and better understanding of the pathogenesis of atherosclerosis. A better understanding of the underlying vascular inflammatory processes and associated molecular mechanisms are essential for the development of new preventive strategies and therapeutic approaches. Atherosclerosis is defined as a chronic inflammatory disease of the vascular system in which the immune system with its different immune cells plays an essential role [204]. Especially neutrophils have an important function in this context, as they account for 40 - 75% of all leukocytes in the blood and are considerably involved in the inflammatory process [39, 222, 223]. Activated neutrophil granulocytes are able to release immunomodulatory granule proteins to modify the progression of inflammation [39, 54]. HNP1 is the most abundant peptide of neutrophil granulocytes [224]. Because of their numerous immunomodulatory functions, the question arises to how much human neutrophil peptides may influence vascular inflammation. The aim of the thesis was to investigate the influence of the immunomodulating human neutrophil peptide 1 (HNP1, α-defensin) on vascular inflammation, to analyse underlying molecular mechanisms and to examine the resulting consequences for the pathogenesis of atherosclerosis in order to develop new preventive strategies and therapeutic approaches (Chapter 2 and 3).

Chapter 2

The study described in Chapter 2 focused on the analysis of the impact of neutrophil and platelet-derived granule proteins on endothelial monocyte adhesion. It was shown that HNP1, secreted by neutrophil granulocytes, forms a heteromer with CCL5 that is secreted by platelets. This heteromer subsequently promotes monocyte adhesion to the endothelium. Based on binding analyses, it was possible to develop a synthetic peptide that interrupts heteromer formation and reduces endothelial monocyte adhesion. This effect was further investigated in situ in an animal model of cardiac ischemia and reperfusion, where consistent results could be demonstrated.
I performed and analysed the experiments shown in Figure 1 under the supervision of J.-E.A..

Chapter 3

The work described in Chapter 3 represents an ongoing study based on the preliminary findings of the study presented in Chapter 2. Since endothelial monocyte adhesion and its subsequent migration and transformation into tissue macrophages is an essential pathophysiological process in the development and progression of atherosclerosis, the aim was to investigate the influence and role of HNP1 in this context. Unexpectedly, initial study results showed a very different function of HNP1 in the diet-induced Apolipoprotein E (ApoE) knockout model. We were able to show that the peptide secreted by neutrophil granulocytes forms a complex with apolipoproteins, mainly with ApoC, in the circulatory system. This complex formation subsequently led to more rapid excretion of low density lipoprotein (LDL) from the blood via the low density lipoprotein receptor (LDL-R) in the liver. As a result, atherosclerotic endothelial lesions were reduced in the atherosclerotic mouse model. In addition, we were able to reduce the size of existing advanced lesions by therapeutic intravenous administration of HNP1.
I performed and analyzed experiments shown in Figure 1 – 4 and Supplementary Data and contributed to manuscript writing. Y.D. and M.D. contributed to study design, supervision, data analysis, and funding.

References

1. WHO. World Health Statistics 2016. Available from: http://www.who.int/gho/publications/world_health_statistics/2016/en/.

2. WHO. World Health Statistics 2018. Available from: http://www.who.int/gho/publications/world_health_statistics/2018/EN_WHS2018_Part2.pdf?ua=1.

3. Bundesamt, S. Todesursachenstatistik 2016. Available from: https://www-genesis.destatis.de/genesis/online/link/tabelleErgebnis/23211-0002.

4. Bundesamt, S. Sterbefälle insgesamt nach der ICD-10 im Jahr 2016. Available from: https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Gesundheit/Todesursachen/Tabellen/sterbefaelle-herz-kreislauf-erkrankungen-insgesamt.html.

5. WHO. Classification of atherosclerotic lesions. 1958; Available from: http://apps.who.int/iris/bitstream/handle/10665/40402/WHO_TRS_143.pdf;jsessionid=95D4514BB280CB413058EF1EC6C517EB?sequence=1.

6. Herrington, W., et al., Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circ Res, 2016. 118(4): p. 535-46.

7. Di Cicco, M.E., V. Ragazzo, and T. Jacinto, Mortality in relation to smoking: the British Doctors Study. Breathe (Sheff), 2016. 12(3): p. 275-276.

8. Lu, L., D.F. Mackay, and J.P. Pell, Meta-analysis of the association between cigarette smoking and peripheral arterial disease. Heart, 2014. 100(5): p. 414-23.

9. Million Women Study Collaborative, G., The Million Women Study: design and characteristics of the study population. The Million Women Study Collaborative Group. Breast Cancer Res, 1999. 1(1): p. 73-80.

10. Prospective Studies, C., et al., Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet, 2009. 373(9669): p. 1083-96.

11. Fall, T., et al., The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis. PLoS Med, 2013. 10(6): p. e1001474.

12. Holmes, M.V., et al., Causal effects of body mass index on cardiometabolic traits and events: a Mendelian randomization analysis. Am J Hum Genet, 2014. 94(2): p. 198-208.

13. Lewington, S., et al., Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet, 2002. 360(9349): p. 1903-13.

14. Prospective Studies, C., et al., Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet, 2007. 370(9602): p. 1829-39.

15. Cholesterol Treatment Trialists, C., et al., Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet, 2010. 376(9753): p. 1670-81.

16. Emerging Risk Factors, C., et al., Major lipids, apolipoproteins, and risk of vascular disease. JAMA, 2009. 302(18): p. 1993-2000.

17. Chronic Kidney Disease Prognosis, C., et al., Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet, 2010. 375(9731): p. 2073-81.

18. Schiffrin, E.L., M.L. Lipman, and J.F. Mann, Chronic kidney disease: effects on the cardiovascular system. Circulation, 2007. 116(1): p. 85-97.

19. Palmer, S.C., et al., Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. JAMA, 2011. 305(11): p. 1119-27.

20. Beckman, J.A., M.A. Creager, and P. Libby, Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA, 2002. 287(19): p. 2570-81.

21. Rao Kondapally Seshasai, S., et al., Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med, 2011. 364(9): p. 829-841.

22. Gerstein, H.C., et al., Effects of intensive glycaemic control on ischaemic heart disease: analysis of data from the randomised, controlled ACCORD trial. Lancet, 2014. 384(9958): p. 1936-41.

23. Ross, R., Atherosclerosis--an inflammatory disease. N Engl J Med, 1999. 340(2): p. 115-26.

24. Lusis, A.J., Atherosclerosis. Nature, 2000. 407(6801): p. 233-41.

25. Parkin, J. and B. Cohen, An overview of the immune system. Lancet, 2001. 357(9270): p. 1777-89.

26. Riera Romo, M., D. Perez-Martinez, and C. Castillo Ferrer, Innate immunity in vertebrates: an overview. Immunology, 2016. 148(2): p. 125-39.

27. Gordon, S., Phagocytosis: An Immunobiologic Process. Immunity, 2016. 44(3): p. 463-475.

28. Flannagan, R.S., V. Jaumouille, and S. Grinstein, The cell biology of phagocytosis. Annu Rev Pathol, 2012. 7: p. 61-98.

29. Rot, A. and U.H. von Andrian, Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu Rev Immunol, 2004. 22: p. 891-928.

30. Turner, M.D., et al., Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta, 2014. 1843(11): p. 2563-2582.

31. Esche, C., C. Stellato, and L.A. Beck, Chemokines: key players in innate and adaptive immunity. J Invest Dermatol, 2005. 125(4): p. 615-28.

32. Bonilla, F.A. and H.C. Oettgen, Adaptive immunity. J Allergy Clin Immunol, 2010. 125(2 Suppl 2): p. S33-40.

33. Iwasaki, A. and R. Medzhitov, Control of adaptive immunity by the innate immune system. Nat Immunol, 2015. 16(4): p. 343-53.

34. Kashem, S.W., M. Haniffa, and D.H. Kaplan, Antigen-Presenting Cells in the Skin. Annu Rev Immunol, 2017. 35: p. 469-499.

35. Laurenti, E. and B. Gottgens, From haematopoietic stem cells to complex differentiation landscapes. Nature, 2018. 553(7689): p. 418-426.

36. Woolthuis, C.M. and C.Y. Park, Hematopoietic stem/progenitor cell commitment to the megakaryocyte lineage. Blood, 2016. 127(10): p. 1242-8.

37. Crane, G.M., E. Jeffery, and S.J. Morrison, Adult haematopoietic stem cell niches. Nat Rev Immunol, 2017. 17(9): p. 573-590.

38. Cowland, J.B. and N. Borregaard, Granulopoiesis and granules of human neutrophils. Immunol Rev, 2016. 273(1): p. 11-28.

39. Mayadas, T.N., X. Cullere, and C.A. Lowell, The multifaceted functions of neutrophils. Annu Rev Pathol, 2014. 9: p. 181-218.

40. Borregaard, N., Neutrophils, from marrow to microbes. Immunity, 2010. 33(5): p. 657-70.

41. O'Connell, K.E., et al., Practical murine hematopathology: a comparative review and implications for research. Comp Med, 2015. 65(2): p. 96-113.

42. Kolaczkowska, E. and P. Kubes, Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol, 2013. 13(3): p. 159-75.

43. Summers, C., et al., Neutrophil kinetics in health and disease. Trends Immunol, 2010. 31(8): p. 318-24.

44. Ley, K., et al., Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol, 2007. 7(9): p. 678-89.

45. McEver, R.P., Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. Cardiovasc Res, 2015. 107(3): p. 331-9.

46. Arbones, M.L., et al., Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. Immunity, 1994. 1(4): p. 247-60.

47. Bargatze, R.F., et al., Neutrophils roll on adherent neutrophils bound to cytokine-induced endothelial cells via L-selectin on the rolling cells. J Exp Med, 1994. 180(5): p. 1785-92.

48. Herter, J.M., J. Rossaint, and A. Zarbock, Platelets in inflammation and immunity. J Thromb Haemost, 2014. 12(11): p. 1764-75.

49. Petri, B., M. Phillipson, and P. Kubes, The physiology of leukocyte recruitment: an in vivo perspective. J Immunol, 2008. 180(10): p. 6439-46.

50. Bevilacqua, M.P., et al., Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci U S A, 1987. 84(24): p. 9238-42.

51. Zarbock, A., et al., Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. Blood, 2011. 118(26): p. 6743-51.

52. Sundd, P., et al., 'Slings' enable neutrophil rolling at high shear. Nature, 2012. 488(7411): p. 399-403.

53. Ramachandran, V., et al., Dynamic alterations of membrane tethers stabilize leukocyte rolling on P-selectin. Proc Natl Acad Sci U S A, 2004. 101(37): p. 13519-24.

54. Faurschou, M. and N. Borregaard, Neutrophil granules and secretory vesicles in inflammation. Microbes Infect, 2003. 5(14): p. 1317-27.

55. Sigal, A., et al., The LFA-1 integrin supports rolling adhesions on ICAM-1 under physiological shear flow in a permissive cellular environment. J Immunol, 2000. 165(1): p. 442-52.

56. Petri, B., et al., Endothelial LSP1 is involved in endothelial dome formation, minimizing vascular permeability changes during neutrophil transmigration in vivo. Blood, 2011. 117(3): p. 942-52.

57. Phillipson, M., et al., Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. J Exp Med, 2006. 203(12): p. 2569-75.

58. Wang, S., et al., Venular basement membranes contain specific matrix protein low expression regions that act as exit points for emigrating neutrophils. J Exp Med, 2006. 203(6): p. 1519-32.

59. Kolaczkowska, E., et al., Neutrophil elastase activity compensates for a genetic lack of matrix metalloproteinase-9 (MMP-9) in leukocyte infiltration in a model of experimental peritonitis. J Leukoc Biol, 2009. 85(3): p. 374-81.

60. Spicer, S.S. and J.H. Hardin, Ultrastructure, cytochemistry, and function of neutrophil leukocyte granules. A review. Lab Invest, 1969. 20(5): p. 488-97.

61. Kjeldsen, L., et al., Subcellular localization and release of human neutrophil gelatinase, confirming the existence of separate gelatinase-containing granules. Biochem J, 1992. 287 (Pt 2): p. 603-10.

62. Rice, W.G., et al., Defensin-rich dense granules of human neutrophils. Blood, 1987. 70(3): p. 757-65.

63. Sengelov, H., et al., Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. J Immunol, 1995. 154(8): p. 4157-65.

64. Borregaard, N. and J.B. Cowland, Granules of the human neutrophilic polymorphonuclear leukocyte. Blood, 1997. 89(10): p. 3503-21.

65. Cham, B.P., J.M. Gerrard, and D.F. Bainton, Granulophysin is located in the membrane of azurophilic granules in human neutrophils and mobilizes to the plasma membrane following cell stimulation. Am J Pathol, 1994. 144(6): p. 1369-80.

66. Wu, Z., et al., From pro defensins to defensins: synthesis and characterization of human neutrophil pro alpha-defensin-1 and its mature domain. J Pept Res, 2003. 62(2): p. 53-62.

67. Aratani, Y., Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys, 2018. 640: p. 47-52.

68. Lehrer, R.I. and W. Lu, alpha-Defensins in human innate immunity. Immunol Rev, 2012. 245(1): p. 84-112.

69. Ganz, T., Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol, 2003. 3(9): p. 710-20.

70. Ganz, T., et al., Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest, 1985. 76(4): p. 1427-35.

71. Tang, Y.Q., et al., A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. Science, 1999. 286(5439): p. 498-502.

72. Bensch, K.W., et al., hBD-1: a novel beta-defensin from human plasma. FEBS Lett, 1995. 368(2): p. 331-5.

73. Lehrer, R.I., A.K. Lichtenstein, and T. Ganz, Defensins: antimicrobial and cytotoxic peptides of mammalian cells. Annu Rev Immunol, 1993. 11: p. 105-28.

74. Agerberth, B., et al., The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood, 2000. 96(9): p. 3086-93.

75. Ayabe, T., et al., Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. Nat Immunol, 2000. 1(2): p. 113-8.

76. Liu, L., et al., The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. Genomics, 1997. 43(3): p. 316-20.

77. Lynn, D.J. and D.G. Bradley, Discovery of alpha-defensins in basal mammals. Dev Comp Immunol, 2007. 31(10): p. 963-7.

78. Schutte, B.C., et al., Discovery of five conserved beta -defensin gene clusters using a computational search strategy. Proc Natl Acad Sci U S A, 2002. 99(4): p. 2129-33.

79. Scheetz, T., et al., Genomics-based approaches to gene discovery in innate immunity. Immunol Rev, 2002. 190: p. 137-45.

80. Das, S., et al., Comparative genomics and evolution of the alpha-defensin multigene family in primates. Mol Biol Evol, 2010. 27(10): p. 2333-43.

81. Bruhn, O., et al., The repertoire of equine intestinal alpha-defensins. BMC Genomics, 2009. 10: p. 631.

82. Kim, C. and S.H. Kaufmann, Defensin: a multifunctional molecule lives up to its versatile name. Trends Microbiol, 2006. 14(10): p. 428-31.

83. Belov, K., et al., Characterization of the opossum immune genome provides insights into the evolution of the mammalian immune system. Genome Res, 2007. 17(7): p. 982-91.

84. Wilde, C.G., et al., Characterization of two azurphil granule proteases with active-site homology to neutrophil elastase. J Biol Chem, 1990. 265(4): p. 2038-41.

85. Ganz, T., M.E. Selsted, and R.I. Lehrer, Defensins. Eur J Haematol, 1990. 44(1): p. 1-8.

86. Eisenhauer, P.B. and R.I. Lehrer, Mouse neutrophils lack defensins. Infect Immun, 1992. 60(8): p. 3446-7.

87. Cowland, J.B. and N. Borregaard, The individual regulation of granule protein mRNA levels during neutrophil maturation explains the heterogeneity of neutrophil granules. J Leukoc Biol, 1999. 66(6): p. 989-95.

88. Arnljots, K., et al., Timing, targeting and sorting of azurophil granule proteins in human myeloid cells. Leukemia, 1998. 12(11): p. 1789-95.

89. Yount, N.Y., et al., Rat neutrophil defensins. Precursor structures and expression during neutrophilic myelopoiesis. J Immunol, 1995. 155(9): p. 4476-84.

90. Valore, E.V. and T. Ganz, Posttranslational processing of defensins in immature human myeloid cells. Blood, 1992. 79(6): p. 1538-44.

91. Liu, L. and T. Ganz, The pro region of human neutrophil defensin contains a motif that is essential for normal subcellular sorting. Blood, 1995. 85(4): p. 1095-103.

92. Panyutich, A.V., et al., Plasma defensin concentrations are elevated in patients with septicemia or bacterial meningitis. J Lab Clin Med, 1993. 122(2): p. 202-7.

93. Daher, K.A., M.E. Selsted, and R.I. Lehrer, Direct inactivation of viruses by human granulocyte defensins. J Virol, 1986. 60(3): p. 1068-74.

94. Lehrer, R.I., et al., Modulation of the in vitro candidacidal activity of human neutrophil defensins by target cell metabolism and divalent cations. J Clin Invest, 1988. 81(6): p. 1829-35.

95. Bowdish, D.M., D.J. Davidson, and R.E. Hancock, A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci, 2005. 6(1): p. 35-51.

96. Yang, D., et al., Mammalian defensins in immunity: more than just microbicidal. Trends Immunol, 2002. 23(6): p. 291-6.

97. Lichtenstein, A., Mechanism of mammalian cell lysis mediated by peptide defensins. Evidence for an initial alteration of the plasma membrane. J Clin Invest, 1991. 88(1): p. 93-100.

98. Lehrer, R.I., et al., Interaction of human defensins with Escherichia coli. Mechanism of bactericidal activity. J Clin Invest, 1989. 84(2): p. 553-61.

99. Kagan, B.L., et al., Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc Natl Acad Sci U S A, 1990. 87(1): p. 210-4.

100. Lichtenstein, A., et al., In vitro tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocytes. Blood, 1986. 68(6): p. 1407-10.

101. Lichtenstein, A.K., et al., Mechanism of target cytolysis by peptide defensins. Target cell metabolic activities, possibly involving endocytosis, are crucial for expression of cytotoxicity. J Immunol, 1988. 140(8): p. 2686-94.

102. Van Wetering, S., et al., Effect of defensins on interleukin-8 synthesis in airway epithelial cells. Am J Physiol, 1997. 272(5 Pt 1): p. L888-96.

103. Territo, M.C., et al., Monocyte-chemotactic activity of defensins from human neutrophils. J Clin Invest, 1989. 84(6): p. 2017-20.

104. Chertov, O., et al., Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. J Biol Chem, 1996. 271(6): p. 2935-40.

105. Yang, D., et al., Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. J Leukoc Biol, 2000. 68(1): p. 9-14.

106. Lillard, J.W., Jr., et al., Mechanisms for induction of acquired host immunity by neutrophil peptide defensins. Proc Natl Acad Sci U S A, 1999. 96(2): p. 651-6.

107. Tani, K., et al., Defensins act as potent adjuvants that promote cellular and humoral immune responses in mice to a lymphoma idiotype and carrier antigens. Int Immunol, 2000. 12(5): p. 691-700.

108. Aarbiou, J., et al., Neutrophil defensins enhance lung epithelial wound closure and mucin gene expression in vitro. Am J Respir Cell Mol Biol, 2004. 30(2): p. 193-201.

109. Geissmann, F., The origin of dendritic cells. Nat Immunol, 2007. 8(6): p. 558-60.

110. Auffray, C., M.H. Sieweke, and F. Geissmann, Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu Rev Immunol, 2009. 27: p. 669-92.

111. Alvarez-Errico, D., et al., Epigenetic control of myeloid cell differentiation, identity and function. Nat Rev Immunol, 2015. 15(1): p. 7-17.

112. Ziegler-Heitbrock, L., Reprint of: Monocyte subsets in man and other species. Cell Immunol, 2014. 291(1-2): p. 11-5.

113. Patel, A.A., et al., The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J Exp Med, 2017. 214(7): p. 1913-1923.

114. Jakubzick, C.V., G.J. Randolph, and P.M. Henson, Monocyte differentiation and antigen-presenting functions. Nat Rev Immunol, 2017. 17(6): p. 349-362.

115. Wacleche, V.S., et al., The Biology of Monocytes and Dendritic Cells: Contribution to HIV Pathogenesis. Viruses, 2018. 10(2).

116. Ginhoux, F. and S. Jung, Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol, 2014. 14(6): p. 392-404.

117. Saha, P. and F. Geissmann, Toward a functional characterization of blood monocytes. Immunol Cell Biol, 2011. 89(1): p. 2-4.

118. Serbina, N.V., et al., Monocyte-mediated defense against microbial pathogens. Annu Rev Immunol, 2008. 26: p. 421-52.

119. Jakubzick, C., et al., Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. Immunity, 2013. 39(3): p. 599-610.

120. Kim, T.S. and T.J. Braciale, Respiratory dendritic cell subsets differ in their capacity to support the induction of virus-specific cytotoxic CD8+ T cell responses. PLoS One, 2009. 4(1): p. e4204.

121. Woollard, K.J. and F. Geissmann, Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol, 2010. 7(2): p. 77-86.

122. Geissmann, F., et al., Development of monocytes, macrophages, and dendritic cells. Science, 2010. 327(5966): p. 656-61.

123. Hoeffel, G., et al., Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. J Exp Med, 2012. 209(6): p. 1167-81.

124. Epelman, S., K.J. Lavine, and G.J. Randolph, Origin and functions of tissue macrophages. Immunity, 2014. 41(1): p. 21-35.

125. Dey, A., J. Allen, and P.A. Hankey-Giblin, Ontogeny and polarization of macrophages in inflammation: blood monocytes versus tissue macrophages. Front Immunol, 2014. 5: p. 683.

126. Chorro, L. and F. Geissmann, Development and homeostasis of 'resident' myeloid cells: the case of the Langerhans cell. Trends Immunol, 2010. 31(12): p. 438-45.

127. Krenkel, O. and F. Tacke, Liver macrophages in tissue homeostasis and disease. Nat Rev Immunol, 2017. 17(5): p. 306-321.

128. Goldmann, T., et al., Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat Immunol, 2016. 17(7): p. 797-805.

129. Gautier, E.L., et al., Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol, 2012. 13(11): p. 1118-28.

130. Scott, C.L., S. Henri, and M. Guilliams, Mononuclear phagocytes of the intestine, the skin, and the lung. Immunol Rev, 2014. 262(1): p. 9-24.

131. Epelman, S., et al., Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. Immunity, 2014. 40(1): p. 91-104.

132. Tamoutounour, S., et al., Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. Immunity, 2013. 39(5): p. 925-38.

133. Tamoutounour, S., et al., CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. Eur J Immunol, 2012. 42(12): p. 3150-66.

134. Gibbings, S.L., et al., Three Unique Interstitial Macrophages in the Murine Lung at Steady State. Am J Respir Cell Mol Biol, 2017. 57(1): p. 66-76.

135. Passlick, B., D. Flieger, and H.W. Ziegler-Heitbrock, Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood, 1989. 74(7): p. 2527-34.

136. Geissmann, F., S. Jung, and D.R. Littman, Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity, 2003. 19(1): p. 71-82.

137. Ziegler-Heitbrock, L., et al., Nomenclature of monocytes and dendritic cells in blood. Blood, 2010. 116(16): p. e74-80.

138. Lauvau, G., et al., Inflammatory monocyte effector mechanisms. Cell Immunol, 2014. 291(1-2): p. 32-40.

139. Cros, J., et al., Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity, 2010. 33(3): p. 375-86.

140. Shi, C. and E.G. Pamer, Monocyte recruitment during infection and inflammation. Nat Rev Immunol, 2011. 11(11): p. 762-74.

141. Anbazhagan, K., et al., Transcriptomic network support distinct roles of classical and non-classical monocytes in human. Int Rev Immunol, 2014. 33(6): p. 470-89.

142. Qu, C., et al., Monocyte-derived dendritic cells: targets as potent antigen-presenting cells for the design of vaccines against infectious diseases. Int J Infect Dis, 2014. 19: p. 1-5.

143. Weber, C., et al., Differential chemokine receptor expression and function in human monocyte subpopulations. J Leukoc Biol, 2000. 67(5): p. 699-704.

144. Auffray, C., et al., Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science, 2007. 317(5838): p. 666-70.

145. Belge, K.U., et al., The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. J Immunol, 2002. 168(7): p. 3536-42.

146. Steppich, B., et al., Selective mobilization of CD14(+)CD16(+) monocytes by exercise. Am J Physiol Cell Physiol, 2000. 279(3): p. C578-86.

147. Tacke, F., et al., Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest, 2007. 117(1): p. 185-94.

148. Galkina, E. and K. Ley, Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol, 2007. 27(11): p. 2292-301.

149. Junt, T., et al., Dynamic visualization of thrombopoiesis within bone marrow. Science, 2007. 317(5845): p. 1767-70.

150. Karshovska, E., C. Weber, and P. von Hundelshausen, Platelet chemokines in health and disease. Thromb Haemost, 2013. 110(5): p. 894-902.

151. Patel, S.R., J.H. Hartwig, and J.E. Italiano, Jr., The biogenesis of platelets from megakaryocyte proplatelets. J Clin Invest, 2005. 115(12): p. 3348-54.

152. Kaushansky, K., Historical review: megakaryopoiesis and thrombopoiesis. Blood, 2008. 111(3): p. 981-6.

153. Stenberg, P.E. and J. Levin, Mechanisms of platelet production. Blood Cells, 1989. 15(1): p. 23-47.

154. Ghoshal, K. and M. Bhattacharyya, Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis. ScientificWorldJournal, 2014. 2014: p. 781857.

155. Hou, Y., et al., Platelets in hemostasis and thrombosis: Novel mechanisms of fibrinogen-independent platelet aggregation and fibronectin-mediated protein wave of hemostasis. J Biomed Res, 2015. 29.

156. Zhao, J., et al., Cryopreserved platelets augment the inflammatory response: role of phosphatidylserine- and P-selectin-mediated platelet phagocytosis in macrophages. Transfusion, 2019.

157. Kim, S.J., R.P. Davis, and C.N. Jenne, Platelets as Modulators of Inflammation. Semin Thromb Hemost, 2018. 44(2): p. 91-101.

158. Levin, J. and S. Ebbe, Why are recently published platelet counts in normal mice so low? Blood, 1994. 83(12): p. 3829-31.

159. Bizzozero, G., Su di un nuovo elemento morfologico del sangue dei mammiferi e della sua importanza nella trombosi e nella coagulazione[J]. in L'Osservatore. 1881. p. 785-787.

160. Bizzozero, G., Uber einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und Blutgrinnung [J]. Virchows Archiv, 1882: p. 261-332.

161. Duerschmied, D., C. Bode, and I. Ahrens, Immune functions of platelets. Thromb Haemost, 2014. 112(4): p. 678-91.

162. Morrell, C.N., et al., Emerging roles for platelets as immune and inflammatory cells. Blood, 2014. 123(18): p. 2759-67.

163. Chen, W., et al., Inhibiting GPIbalpha Shedding Preserves Post-Transfusion Recovery and Hemostatic Function of Platelets After Prolonged Storage. Arterioscler Thromb Vasc Biol, 2016. 36(9): p. 1821-8.

164. Gryglewski, R.J., R.M. Botting, and J.R. Vane, Mediators produced by the endothelial cell. Hypertension, 1988. 12(6): p. 530-48.

165. van Gils, J.M., J.J. Zwaginga, and P.L. Hordijk, Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. J Leukoc Biol, 2009. 85(2): p. 195-204.

166. Ruggeri, Z.M., Mechanisms initiating platelet thrombus formation. Thromb Haemost, 1997. 78(1): p. 611-6.

167. Ruggeri, Z.M. and G.L. Mendolicchio, Adhesion mechanisms in platelet function. Circ Res, 2007. 100(12): p. 1673-85.

168. Flaumenhaft, R., Molecular basis of platelet granule secretion. Arterioscler Thromb Vasc Biol, 2003. 23(7): p. 1152-60.

169. Yngen, M., et al., Acute hyperglycemia increases soluble P-selectin in male patients with mild diabetes mellitus. Blood Coagul Fibrinolysis, 2001. 12(2): p. 109-16.

170. Palta, S., R. Saroa, and A. Palta, Overview of the coagulation system. Indian J Anaesth, 2014. 58(5): p. 515-23.

171. Owens, A.P., 3rd and N. Mackman, Tissue factor and thrombosis: The clot starts here. Thromb Haemost, 2010. 104(3): p. 432-9.

172. Wolberg, A.S. and R.A. Campbell, Thrombin generation, fibrin clot formation and hemostasis. Transfus Apher Sci, 2008. 38(1): p. 15-23.

173. Lasne, D., B. Jude, and S. Susen, From normal to pathological hemostasis. Can J Anaesth, 2006. 53(6 Suppl): p. S2-11.

174. Munnix, I.C., et al., Platelet response heterogeneity in thrombus formation. Thromb Haemost, 2009. 102(6): p. 1149-56.

175. Kenneth R Feingold, M.a.C.G., MD, PhD. Introduction to Lipids and Lipoproteins. 2015; Available from: https://www.ncbi.nlm.nih.gov/books/NBK305896/.

176. Cerqueira, N.M., et al., Cholesterol Biosynthesis: A Mechanistic Overview. Biochemistry, 2016. 55(39): p. 5483-5506.

177. Jeney, V., G. Balla, and J. Balla, Red blood cell, hemoglobin and heme in the progression of atherosclerosis. Front Physiol, 2014. 5: p. 379.

178. Cohen, D.E., Balancing cholesterol synthesis and absorption in the gastrointestinal tract. J Clin Lipidol, 2008. 2(2): p. S1-3.

179. Lund-Katz, S. and M.C. Phillips, High density lipoprotein structure-function and role in reverse cholesterol transport. Subcell Biochem, 2010. 51: p. 183-227.

180. Mansbach, C.M., 2nd and F. Gorelick, Development and physiological regulation of intestinal lipid absorption. II. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and secretion of chylomicrons. Am J Physiol Gastrointest Liver Physiol, 2007. 293(4): p. G645-50.

181. Giammanco, A., et al., The pathophysiology of intestinal lipoprotein production. Front Physiol, 2015. 6: p. 61.

182. Hamilton, R.L., Synthesis and secretion of plasma lipoproteins. Adv Exp Med Biol, 1972. 26(0): p. 7-24.

183. Altmann, S.W., et al., Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science, 2004. 303(5661): p. 1201-4.

184. Storch, J. and A.E. Thumser, Tissue-specific functions in the fatty acid-binding protein family. J Biol Chem, 2010. 285(43): p. 32679-83.

185. Endemann, G., et al., CD36 is a receptor for oxidized low density lipoprotein. J Biol Chem, 1993. 268(16): p. 11811-6.

186. Helkin, A., et al., Dyslipidemia Part 1--Review of Lipid Metabolism and Vascular Cell Physiology. Vasc Endovascular Surg, 2016. 50(2): p. 107-18.

187. Davidson, N.O. and G.S. Shelness, APOLIPOPROTEIN B: mRNA editing, lipoprotein assembly, and presecretory degradation. Annu Rev Nutr, 2000. 20: p. 169-93.

188. Mansbach, C.M. and S.A. Siddiqi, The biogenesis of chylomicrons. Annu Rev Physiol, 2010. 72: p. 315-33.

189. Tiwari, S. and S.A. Siddiqi, Intracellular trafficking and secretion of VLDL. Arterioscler Thromb Vasc Biol, 2012. 32(5): p. 1079-86.

190. Kwiterovich, P.O., Jr., The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review. Am J Cardiol, 2000. 86(12A): p. 5L-10L.

191. Badimon, L., G. Vilahur, and T. Padro, Lipoproteins, platelets and atherothrombosis. Rev Esp Cardiol, 2009. 62(10): p. 1161-78.

192. Prassl, R., Human low density lipoprotein: the mystery of core lipid packing. J Lipid Res, 2011. 52(2): p. 187-8.

193. Grundy, S.M. and H.Y. Mok, Chylomicron clearance in normal and hyperlipidemic man. Metabolism, 1976. 25(11): p. 1225-39.

194. Ernst J Schaefer. The Measurement of Lipids, Lipoproteins, Apolipoproteins, Fatty Acids, and Sterols, and Next Generation Sequencing for the Diagnosis and Treatment of Lipid Disorders. 2016; Available from: https://www.ncbi.nlm.nih.gov/books/NBK355892/.

195. Jeon, H. and S.C. Blacklow, Structure and physiologic function of the low-density lipoprotein receptor. Annu Rev Biochem, 2005. 74: p. 535-62.

196. Go, G.W. and A. Mani, Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis. Yale J Biol Med, 2012. 85(1): p. 19-28.

197. Poirier, S., et al., The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. J Biol Chem, 2008. 283(4): p. 2363-72.

198. Galeano, N.F., et al., Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. J Lipid Res, 1998. 39(6): p. 1263-73.

199. Dhaliwal, B.S. and U.P. Steinbrecher, Scavenger receptors and oxidized low density lipoproteins. Clin Chim Acta, 1999. 286(1-2): p. 191-205.

200. Marz, W., et al., HDL cholesterol: reappraisal of its clinical relevance. Clin Res Cardiol, 2017. 106(9): p. 663-675.

201. Riwanto, M. and U. Landmesser, High density lipoproteins and endothelial functions: mechanistic insights and alterations in cardiovascular disease. J Lipid Res, 2013. 54(12): p. 3227-43.

202. Kuai, R., et al., High-Density Lipoproteins: Nature's Multifunctional Nanoparticles. ACS Nano, 2016. 10(3): p. 3015-41.

203. Medh, J.D., et al., Hepatic triglyceride lipase promotes low density lipoprotein receptor-mediated catabolism of very low density lipoproteins in vitro. J Lipid Res, 1999. 40(7): p. 1263-75.

204. Weber, C. and H. Noels, Atherosclerosis: current pathogenesis and therapeutic options. Nat Med, 2011. 17(11): p. 1410-22.

205. Rafieian-Kopaei, M., et al., Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med, 2014. 5(8): p. 927-46.

206. Ross, R., J. Glomset, and L. Harker, Response to injury and atherogenesis. Am J Pathol, 1977. 86(3): p. 675-84.

207. Ross, R. and J.A. Glomset, The pathogenesis of atherosclerosis (first of two parts). N Engl J Med, 1976. 295(7): p. 369-77.

208. Ross, R. and J.A. Glomset, The pathogenesis of atherosclerosis (second of two parts). N Engl J Med, 1976. 295(8): p. 420-5.

209. Pirillo, A., G.D. Norata, and A.L. Catapano, LOX-1, OxLDL, and atherosclerosis. Mediators Inflamm, 2013. 2013: p. 152786.

210. Tabas, I., K.J. Williams, and J. Boren, Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation, 2007. 116(16): p. 1832-44.

211. Levitan, I., S. Volkov, and P.V. Subbaiah, Oxidized LDL: diversity, patterns of recognition, and pathophysiology. Antioxid Redox Signal, 2010. 13(1): p. 39-75.

212. Li, D. and J.L. Mehta, Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. Circulation, 2000. 101(25): p. 2889-95.

213. Li, D., et al., Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: role of LOX-1. J Pharmacol Exp Ther, 2002. 302(2): p. 601-5.

214. Johnson, J.L. and A.C. Newby, Macrophage heterogeneity in atherosclerotic plaques. Curr Opin Lipidol, 2009. 20(5): p. 370-8.

215. Green, M. and M.A. Harrington, A comparison of macrophage colony-stimulating factor (M-CSF) gene expression in primary and immortalized endothelial cells. J Hematother Stem Cell Res, 2000. 9(2): p. 237-46.

216. Hansson, G.K. and A. Hermansson, The immune system in atherosclerosis. Nat Immunol, 2011. 12(3): p. 204-12.

217. Soehnlein, O., Multiple roles for neutrophils in atherosclerosis. Circ Res, 2012. 110(6): p. 875-88.

218. Collot-Teixeira, S., et al., CD36 and macrophages in atherosclerosis. Cardiovasc Res, 2007. 75(3): p. 468-77.

219. McLaren, J.E., et al., Cytokines, macrophage lipid metabolism and foam cells: implications for cardiovascular disease therapy. Prog Lipid Res, 2011. 50(4): p. 331-47.

220. Moore, K.J. and I. Tabas, Macrophages in the pathogenesis of atherosclerosis. Cell, 2011. 145(3): p. 341-55.

221. Virmani, R., et al., Vulnerable plaque: the pathology of unstable coronary lesions. J Interv Cardiol, 2002. 15(6): p. 439-46.

222. Brook, M., et al., Neutrophil-derived alpha defensins control inflammation by inhibiting macrophage mRNA translation. Proc Natl Acad Sci U S A, 2016. 113(16): p. 4350-5.

223. Nathan, C., Points of control in inflammation. Nature, 2002. 420(6917): p. 846-52.

224. Ganz, T. and R.I. Lehrer, Antimicrobial peptides of leukocytes. Curr Opin Hematol, 1997. 4(1): p. 53-8.

2

Recruitment of classical monocytes in inflammation can be inhibited by disturbing heteromers of neutrophil HNP1 and platelet CCL5

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3

Human neutrophil peptide 1 limits hypercholesterolemia-induced atherosclerosis by increasing hepatic LDL clearance

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4

LIST OF PUBLICATIONS

List of Publications

Pro-Angiogenic Macrophage Phenotype to Promote Myocardial Repair.
Ferraro B, Leoni G, Hinkel R, Ormanns S, Paulin N, Ortega-Gomez A, Viola JR, de Jong R, Bongiovanni D, Bozoglu T, Maas SL, D'Amico M, Kessler T, Zeller T, Hristov M, Reutelingsperger C, Sager HB, Döring Y, Nahrendorf M, Kupatt C, Soehnlein O.
J Am Coll Cardiol. 2019 Jun 18;73(23):2990-3002. doi: 10.1016/j.jacc.2019.03.503.

Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death.
Silvestre-Roig C, Braster Q, Wichapong K, Lee EY, Teulon JM, Berrebeh N, Winter J, Adrover JM, Santos GS, Froese A, Lemnitzer P, Ortega-Gómez A, Chevre R, Marschner J, Schumski A, Winter C, Perez-Olivares L, Pan C, Paulin N, Schoufour T, Hartwig H, González-Ramos S, Kamp F, Megens RTA, Mowen KA, Gunzer M, Maegdefessel L, Hackeng T, Lutgens E, Daemen M, von Blume J, Anders HJ, Nikolaev VO, Pellequer JL, Weber C, Hidalgo A, Nicolaes GAF, Wong GCL, Soehnlein O.
Nature. 2019 May;569(7755):236-240. doi: 10.1038/s41586-019-1167-6. Epub 2019 May 1.

Double-Strand DNA Sensing Aim2 Inflammasome Regulates Atherosclerotic Plaque Vulnerability.
Paulin N, Viola JR, Maas SL, de Jong R, Fernandes-Alnemri T, Weber C, Drechsler M, Döring Y, Soehnlein O.
Circulation. 2018 Jul 17;138(3):321-323. doi: 10.1161/CIRCULATIONAHA.117.033098.

Deletion of MFGE8 Inhibits Neointima Formation upon Arterial Damage.
Viola JR, Lemnitzer P, Paulin N, Drechsler M, Nazari-Jahantigh M, Maas S, De Jong RJ, Winter J, Schober A, Weber C, Atabai K, Soehnlein O.
Thromb Haemost. 2018 Jul;118(7):1340-1342. doi: 10.1055/s-0038-1649522. Epub 2018 Jun 4. No abstract available.

Human Neutrophil Peptide 1 Limits Hypercholesterolemia-induced Atherosclerosis by Increasing Hepatic LDL Clearance.
Paulin N, Döring Y, Kooijman S, Blanchet X, Viola JR, de Jong R, Mandl M, Hendrikse J, Schiener M, von Hundelshausen P, Vogt A, Weber C, Bdeir K, Hofmann SM, Rensen PCN, Drechsler M, Soehnlein O.
EBioMedicine. 2017 Feb;16:204-211. doi: 10.1016/j.ebiom.2017.01.006. Epub 2017 Jan 7.

Protective Aptitude of Annexin A1 in Arterial Neointima Formation in Atherosclerosis-Prone Mice-Brief Report.
de Jong RJ, Paulin N, Lemnitzer P, Viola JR, Winter C, Ferraro B, Grommes J, Weber C, Reutelingsperger C, Drechsler M, Soehnlein O.
Arterioscler Thromb Vasc Biol. 2017 Feb;37(2):312-315. doi: 10.1161/ATVBAHA.116.308744. Epub 2016 Dec 29

Recruitment of classical monocytes can be inhibited by disturbing heteromers of neutrophil HNP1 and platelet CCL5.
Alard JE, Ortega-Gomez A, Wichapong K, Bongiovanni D, Horckmans M, Megens RT, Leoni G, Ferraro B, Rossaint J, Paulin N, Ng J, Ippel H, Suylen D, Hinkel R, Blanchet X, Gaillard F, D'Amico M, von Hundelshausen P, Zarbock A, Scheiermann C, Hackeng TM, Steffens S, Kupatt C, Nicolaes GA, Weber C, Soehnlein O.
Sci Transl Med. 2015 Dec 9;7(317):317ra196. doi: 10.1126/scitranslmed.aad5330.

Atherosclerotic Plaque Destabilization in Mice: A Comparative Study.
Hartwig H, Silvestre-Roig C, Hendrikse J, Beckers L, Paulin N, Van der Heiden K, Braster Q, Drechsler M, Daemen MJ, Lutgens E, Soehnlein O.
PLoS One. 2015 Oct 22;10(10):e0141019. doi: 10.1371/journal.pone.0141019. eCollection 2015.