From the Department of Medicine, Solna
Cardiovascular Medicine Unit
Karolinska Institutet, Stockholm, Sweden

IMMUNOMETABOLIC REGULATION OF ATHEROSCLEROSIS

Martin Berg

Karolinska Institutet

Stockholm 2020

Published by Karolinska Institutet.
Printed by E-Print AB

ISBN 978-91-7831-917-6

IMMUNOMETABOLIC REGULATION OF ATHEROSCLEROSIS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Martin Berg

Principal Supervisor:
Associate Prof. Daniel FJ Ketelhuth
Karolinska Institutet
Department of Medicine, Solna
Division of Cardiovascular Medicine

Co-supervisor(s):
Prof. Göran K Hansson
Karolinska Institutet
Department of Medicine, Solna
Division of Cardiovascular Medicine

Associate Prof. Alice Assinger
Medical University of Vienna
Institute of Physiology
Centre of Physiology and Pharmacology

Opponent:
Associate Prof. Tracy McGaha
University of Toronto
Princess Margaret Cancer Center

Examination Board:
Associate Prof. Daniel C. Andersson
Karolinska Institutet
Department of Physiology and Pharmacology

Prof. Sophie Erhardt
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Neuropsychoimmunology

Associate Prof. Anja Meissner
Lund University
Department of Experimental Medical Science
Division of Vascular Biology

ABSTRACT

Ischaemic heart disease and stroke are the most common causes of death in the world, both diseases being manifestations of atherosclerotic cardiovascular disease. Atherosclerosis is a slow process initialized by the retention and accumulation of cholesterol rich lipoproteins in the innermost layer of the artery wall. Activation of an inflammatory response with the recruitment of immune cells lead to a buildup of plaques in the vessel. Both innate immune cells, most prominently macrophages, and adaptive immune cells play important roles in all stages of the development of atherosclerosis.

Metabolism is intimately linked with atherosclerosis development. Systemically increased levels of metabolites such as cholesterol and glucose are known risk factors for atherosclerosis. The metabolism in the microenvironment of the atherosclerotic plaque shape the immune response and influence disease progression. Immune cell metabolism of glucose and amino acids have been suggested as possible targets for future therapy. While modern therapies are effective at reducing known risk factors such as hyperlipidemia, considerable risk remains and few therapies for atherosclerosis target the underlying inflammatory mechanisms that drive the disease.

In **Paper I** the effect of indoleamine 2,3-dioxygenase (IDO) mediated tryptophan metabolism on atherosclerosis was investigated. Pharmacological inhibition of IDO with 1-methyl-tryptophan resulted in increased atherosclerotic burden in mice. Furthermore, *in vitro* data showed that the expression of pro-inflammatory molecules was increased on smooth muscle cells upon IDO inhibition. Treatment with the downstream tryptophan metabolite 3-hydroxyanthranilic acid (3-HAA) reversed both the *in vivo* and *in vitro* effects of IDO inhibition.

The effects of 3-HAA on lipoprotein metabolism was studied in **Paper II**. Activity of the transcription factor sterol response element binding protein 2 (SREBP2) was decreased when HepG2 hepatoma cells were treated with 3-HAA. Mice treated with the pharmacological inhibitor of the 3-HAA degrading enzyme 3-HAA oxygenase had less atherosclerotic plaque size and lower plasma lipids. *In vitro* experiments also showed 3-HAA to be a potent inhibitor of the inflammasome in macrophages.

In **Paper III** we showed that the metabolism of tryptophan is altered in human atherosclerotic disease. Patients with symptoms had an impaired metabolism of tryptophan to kynurenic acid, a metabolite that can induce anti-inflammatory responses, possibly via aryl hydrocarbon receptor activation. In **Paper IV** we demonstrate that the small molecule dichloroacetate (DCA), a known inhibitor of glycolysis, reduces atherosclerosis lesion size, plasma lipids and reprograms the immune system towards an anti-inflammatory phenotype. We also show that DCA is a potent inhibitor of inflammasome production of IL-1β.

LIST OF SCIENTIFIC PAPERS

I. Polyzos K, Ovchinnikova O, Berg M, Baumgartner R, Agardh H, Pirault J, Gisterå A, Assinger A, Laguna Fernandez A, Bäck M, Hansson GK, Ketelhuth DFJ.
Inhibition of indoleamine 2,3-dioxygenase promotes vascular inflammation and increases atherosclerosis in Apoe-/- mice.
Cardiovasc Res. 2015;106(2):295-302

II. Berg M*, Polyzos KA*, Agardh H, Baumgartner R, Forteza MJ, Kareinen I, Gisterå A, Bottcher G, Hurt-Camejo E, Hansson GK, Ketelhuth DFJ.
3-Hydroxyanthralinic acid Metabolism Controls the Hepatic Srebp/Lipoprotein axis, Inhibits Inflammasome Activation in Macrophages, and Decreases Atherosclerosis in LDLR-/- Mice.
Cardiovasc Res. 2019.

III. Baumgartner R*, Berg M*, Matic L, Polyzos KP, Forteza MJ, Hjorth SA, Schwartz TW, Paulson- Berne G, Hansson GK, Hedin U, Ketelhuth DFJ.
Evidence that deviation of the Kynurenine Pathway decreases kynurenic acid production and aryl hydrocarbon receptor signaling and aggravates atherosclerotic disease in humans.
J Intern Med. 2020 (in press)

IV. Forteza MJ, Berg M, Baumgartner R, Kareinen I, Beccaria Casagrande F, Hedin U, Zhang S, Vuckovic I, Dzeja PP, Polyzos KA, Trauelsen M, Schwartz TW, Dib L, Herrmann J, Matic L, Monaco C, Ketelhuth DFJ.
Pyruvate dehydrogenase kinase links glycolysis with succinate/GPR91 signalling to regulate vascular inflammation and atherosclerosis.
Manuscript.

*Equal contribution

OTHER RELATED PUBLICATIONS

I. Zhang L, Ovchinnikova O, Jonsson A, Lundberg AM, Berg M, Hansson GK, Ketelhuth DFJ.
The tryptophan metabolite 3-hydroxyanthranilic acid lowers plasma lipids and decreases atherosclerosis in hypercholesterolaemic mice.
Eur Heart J. 2012;33(16):2025-34.

II. Karadimou G, Folkersen L, Berg M, Perisic L, Discacciati A, Roy J, Hansson GK, Persson J, Paulsson-Berne G.
Low TLR7 gene expression in atherosclerotic plaques is associated with major adverse cardio- and cerebrovascular events.
Cardiovasc Res. 2017;113(1):30-39.

III. Perisic L, Iglesias MJ, Vesterlund M, Lengquist M, Hong MG, Saieed S, Sanchez-Rivera L, Berg M, Razuvaev A, Kronqvist M, Lund K, Caidahl K, Gillgren P, Pontén F, Uhlén M, Schwenk JM, Hansson GK, Paulsson-Berne G, Fagman E, Roy J, Hultgren R, Bergström G, Lehtiö J, Odeberg J, Hedin U.
Novel multiomics profiling of human carotid atherosclerotic plaques and plasma reveals biliverdin reductase B as a marker of intraplaque hemorrhage.
J Am Coll Cardiol Basic Trans Science. 2018;3:464-480

CONTENTS

LIST OF ABBREVIATIONS

1-MT	1-methyl-tryptophan
3-HAA	3-hydroxyanthranilic acid
5-HT	Serotonin
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
ACAT2	Acyltransferase isoform 2
ACC	Acetyl CoA carboxylase
ACMS	2-amino-3-carboxymuconic acid-6-semialdehyde
ACS	Acute coronary syndrome
AhR	Aryl hydrocarbon receptor
AMP	Enter the explanation
AMS	2-aminomuconic-6-semialdehyde
APC	Antigen presenting cell
Apo	Apolipoprotein
Apobec-1	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1
ASC	Associated speck-like protein containing CARD
ATP	Adenosine triphosphate
BCR	B-cell receptor
BiKE	Biobank of Karolinska Endarterectomies
BMDM	Bone marrow-derived macrophages
CAC	Coronary artery calcium
CAD	Coronary artery disease
CANTOS	Canakinumab Anti-Inflammatory Thrombosis Outcomes Study
CCL	C-C-motif-ligand
CCS	Chronic coronary syndrome
CD	Cluster of differentiation
cDC	Conventional dendritic cell
C-domain	Constant domain
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein

CNS	Central nervous system
CoA	Coenzyme A
CRP	C-reactive protein
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CVD	Cardiovascular disease
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DCA	Dichloroacetate
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DRE	Dioxin response element
eIF2α	Eukaryote initiation factor 2 subunit α
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
FABP	Fatty acid binding protein
FAS	Fatty acid synthase
FATP4	Fatty acid transport protein 4
FDG	18F-deoxyglucose
FFA	Free fatty acid
FH	Familiar hypercholesterolaemia
FOXP3	Forkhead box P3
FPLC	Fast protein liquid chromatography
GATA-3	GATA-binding protein 3
GCN2	General control nonderepressible 2
G-CSF	Granulocyte colony-stimulating factor
GLUT	Glucose transporter
GPR	G-protein coupled receptor
GSH	Glutathione
HAAO	3-hydroxyanthranilic acid 3,4-dioxygenase

HDL	High-density lipoprotein
HIF-1α	Hypoxia-induced factor 1α
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HMGCR	HMG-CoA reductase
hsCRP	High-sensitivity C-reactive protein
IDO	Indoleamine 2,3-dioxygenase
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
INF	Interferon
iNOS	Inducible nitric oxide synthase
INSIG	Insulin induced gene
ISR	Integrated stress response
iTReg	Inducible Treg
JAK/STAT1	Janus activated kinase / signal transducer activator of transcription 1
KMO	Kynurenine monooxygenase
KYAT	Kynurenine aminotransferase
Kyn	Kynurenine
KynA	Kynurenic acid
KYNU	Kynureninase
LCAT	Lecithin-cholesterol acyl-transfer
LDL	Low-density lipoprotein
LDLr	LDL-receptor
LN	Lymph node
LPL	Lipoprotein lipase
LRP1	LDL receptor-related protein 1
MAC	Membrane attack complex
MAG	Monoacylglycerol
MDSC	Myeloid-derived suppressor cell
MHC-I	Major histocompatibility complex class I
MHC-II	Major histocompatibility complex class II

mo-DC	Monocyte-derived dendritic cell
Mox	Macrophages induced by oxidized lipids
Mreg	Regulatory macrophages
mTOR	Mammalian target of Rapamycin
MTTP	Microsomal triglyceride transfer protein
NAD^+	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NASCET	North American Symptomatic Carotid Endarterectomy Trial
NET	Neutrophil extracellular trap
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK-cell	Natural killer cell
NKT-cell	Natural killer T-cell
NLR	Nucleotide-binding oligomerization domain-like receptor
NMDA	N-methyl-D-aspartate
NOD	Nucleotide-binding oligomerization domain
NPC1L1	Niemann-Pick C1 like 1
nSREBP-2	Nuclear SREBP-2
oxLDL	Oxidized LDL
OXPHOS	Oxidative phosphorylation
PA	Picolinic acid
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PCSK9	Proprotein convertase subtilisin/kexin type 9a
PD-1	Programmed cell-death protein 1
pDC	Plasmacytoid dendritic cell
PDH	Pyruvate dehydrogenase complex
PDK	Pyruvate dehydrogenase kinase
PDPK1	3-phosphoinositide-dependent protein kinase 1
PGC-1	Peroxisome proliferator-activated receptor γ coactivator 1
PKC-θ	Protein kinase C isoform θ
PPARγ	Peroxisome proliferator-activated receptor γ

pro-IL-1β	Immature form of IL-1β
PRR	Pattern recognition receptors
QA	Quinolinic acid
QPRT	Quinolinate phosphoribosyltransferase
RAG	Recombination activating gene
RORγt	RAR-related orphan receptor gamma isoform t
S1P	Site-1-protease
S2P	Site-2-protease
Scid	Severe combined immunodeficiency
SMC	Smooth muscle cell
SPM	Specialized pro-resolving mediators
SR-B1	Scavenger receptor B1
SRE	Sterol response element
SREBP	Sterol response element binding protein
TAG	Triacylglycerol
T-bet	T-box-containing protein expressed in T cells
TCA	Citric acid cycle
TCR	T-cell receptor
TDO	Tryptophan 2,3-dioxygenase
TFH	Follicular T-helper cell
TGF-β	Transforming growth factor β
T_H1	T-helper cell type 1
T_H17	T-helper cell type 17
T_H2	T-helper cell type 2
TLR	Toll-like receptor
TNF	Tumour necrosis factor
T_{Reg}	Regulatory T-helper cell
TRIF	TIR-domain-containing adapter-inducing interferon-β
Trp	Tryptophan
VCAM-1	Vascular adhesion molecule 1
WD	Western diet

V-domain	Variable domain
VLDL	Very low-density lipoprotein
XA	Xanthurenic acid

1 IMPACT OF ATHEROSCLEROTIC DISEASE

During the last century, diseases of the heart and blood vessels, known as cardiovascular disease (CVD), have replaced communicable diseases as the main cause of mortality in the world. Ischaemic heart disease is the single most common cause of death, accounting for 16.6% of total deaths globally in 2016, followed by stroke which caused 10.2% of deaths in the same year. The most common cause of death not related to CVD, chronic obstructive pulmonary disease, accounted for 5.4% of global mortality. A similar pattern is seen across all income levels, except in the countries with lowest income where communicable disease still drives mortality rates. While a small decrease in mortality rates can be seen in countries with the highest income, CVD deaths globally are on the rise (1).

Apart from being the principal cause of death and disability, CVD is also the most important cause of costs for developed healthcare systems. The total direct and indirect cost of CVD (including stroke) in the United States was estimated at an annual average of $329.7 billion for 2013 to 2014. That accounts for 14% of total health expenditures and is the highest cost of any diagnostic group. By 2035, 45.1% of the total population of the United States is predicted to suffer from at least one CVD, and total costs for CVD-related illness are predicted to rise to $749 billion (2).

Both of the main causes of death, ischaemic heart disease and stroke, are commonly caused by atherosclerosis. Atherosclerosis is recognized by the focal thickening of the inner most layer of the artery wall, the intima, a feature known as atheroma or atherosclerotic plaque. These atherosclerotic plaques contain lipids, several types of cells, connective tissue and debris. A premature form of the atherosclerotic plaque is the fatty streak, an aggregation of lipid-laden immune cells in the intima. Fatty streaks are seen in young patients and may recede or may develop into atherosclerotic plaques. The atherosclerotic plaques may mature over time and cause symptoms, either through the slow growth of a stable plaque obstructing arterial flow, or through rupture of the plaque and the sudden formation of an intra-arterial thrombus, the latter giving rise to acute onset end-organ ischaemia (3).

Through epidemiological studies, several risk factors for atherosclerosis have been found. The current concept of risk factors for atherosclerosis was first introduced as a result of the Framingham Heart Study (4). The risk factors discovered within the Framingham Heart Study include tobacco smoking, hypertension, hypercholesterolemia (specifically elevated low-density lipoprotein cholesterol [LDL-C] and decreased high-density lipoprotein cholesterol [HDL-C]), age, male sex and diabetes mellitus (5). Several of these risk factors are highly prevalent in the population; for example, 62% of Swedish men and 42% of women are expected to suffer from hypertension (6) and in 2015-2016, at least 12.4% of the adult U.S. population was estimated to suffer from hypercholesterolaemia (7). However, while conventional risk factors account for approximately 75% of CVD, substantial risk remains (8). Furthermore, even optimal treatment of risk factors in patients identified to be predisposed for CVD events does

not offer adequate protection, and considerable risk remains even with current preventive treatment (9). Thus a need for additional approaches in the treatment of CVD is apparent.

1.1 HUMAN ATHEROSCLEROSIS IS AN INFLAMMATORY DISEASE

The notion that atherosclerosis is an inflammatory disease was put forward in the 19th century by Rudolf Virchow (10), with Nikolai Anitschkow adding important insight into the role of cholesterol in vascular inflammation in the early 20th century. An important step in achieving acceptance of the hypothesis of inflammation as a main factor in atherosclerosis was taken with the discovery of an association between the acute phase reactant C-reactive protein (CRP) and atherosclerosis by Attilio Maseri et al. (11). In 1997, it was demonstrated that an elevated serum CRP level was associated with an increased risk of a vascular event later in life (12). The measurement of CRP is a common procedure to determine the level of inflammation in a patient, and the potential clinical applicability of this finding led to a wider spread of the notion that inflammation is intimately linked with the onset and progression of atherosclerosis.

However, the clinical findings of the 1990s were founded on several experimental observations relating CVD and atherosclerosis to inflammation made in the previous decade. Several inflammatory molecules such as cytokines and growth factors were previously associated with atherosclerosis in experimental studies (13). Other epidemiological evidence of the relationship between inflammation and atherosclerosis comes from the observation that patients suffering from autoimmune disease have an increased risk of CVD. The presence of rheumatoid arthritis, one of the most common autoimmune diseases, increases the risk of myocardial infarction by a magnitude approximately similar to that of the conventional risk factor diabetes mellitus (14).

The implication of inflammation and the immune system in the pathogenesis of atherosclerosis holds the promise of new therapeutic options targeting the cause of atherosclerosis. In 2017, the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) was the first randomized controlled trial to successfully show that an intervention directly targeting immune function could reduce CVD events and death independently from affecting conventional risk factors (15). The interleukin (IL) 1β targeting monoclonal antibody Canakinumab was used in patients with a previous myocardial infarction and an elevated level of high-sensitivity CRP (hsCRP $\geq$ 2 mg/l). While a decrease was seen in the primary endpoint (recurrent myocardial infarction, stroke or cardiovascular death), treatment with Canakinumab significantly increased the risk of mortality due to severe infection. This highlights both the potential of immunomodulatory treatment of CVD as well as it demonstrates the need for increased knowledge about the immune system and immune mechanisms in atherosclerosis to improve future therapies.

2 THE IMMUNE SYSTEM

Components of the immune systems are implicated in all stages of atherosclerotic disease. The immune system is a collective name for several cell types, tissues and organs dispersed throughout an organism tasked with defending the host against infection. To do so, an advanced

ability to discriminate between conditions that should give rise to a protective immune response, and conditions that should not, is needed so that an immune response is not developed against the organism's self. Apart from foreign microorganisms, the intricate immune system of a human must also clear away debris from normal cell turnover, remove mutated cells and plays an essential part in maintaining normal homeostasis. These diverse tasks call for extensive crosstalk between the immune system and other organ systems, and a great variety of available immune responses.

2.1 THE INNATE IMMUNE SYSTEM

The immune system ccn be divided into the innate immune system and the adaptive immune system. The innate immune system is the first line of defence against infection, specialized at recognizing invading microorganisms and alerting the slower but more specific adaptive immune system. Epithelial barriers, such as skin and mucosa, are part of the innate immune system and apart from physically hindering pathogens from entering the host, these organs normally secrete antimicrobial molecules. Once in the host, microorganisms are recognized by the innate immune system by receptors that bind molecular motifs specific for infectious agents. These molecular motifs are often referred to as pathogen-associated molecular patterns (PAMPs). Molecules associated with tissue damage also give rise to an innate immune response, and these molecules are usually seen as damage-associated molecular patterns (DAMPs). The receptors that recognize PAMPs and DAMPs are called pattern recognition receptors (PRRs)(16).

2.1.1 Innate immune receptors

There are several PRRs. A notable family of PRRs is the Toll-like receptors (TLRs). Located on the cell surface are TLRs recognizing components of bacteria. Examples of cell-surface TLRs are TLR4 which binds lipopolysaccharide, TLR5 which binds flagellin, TLR2 which binds peptidoglycan and, TLR1 and TLR6 that are involved in the recognition of bacterial lipopeptides. Endosomal TLRs recognize components of nucleic acids often seen during viral infections such as dsRNA (TLR3), ssRNA (TLR7 and TLR8) and CpG DNA (TLR9). Most often, TLRs function as homo- or heterodimers. Once activated, TLRs signal through the mediator protein MyD88 to activate the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Alternatively, some signals are transduced via TIR-domain-containing adapter-inducing interferon-β (TRIF), leading to the transcription of type 1 interferons (INF-I) (17, 18).

Another important family of PRRs is the nucleotide-binding oligomerization domain-like receptors (NOD-like receptors; NLRs). In contrast to membrane-bound TLRs, NLRs are cytosomal receptors and respond to a wide variety of PAMPs and DAMPs. There are at least 22 different NLRs that can be subdivided based on the N-terminal domain of the receptor. The N-terminal domain is responsible for receptor effector functions and interacts with other proteins. There are four main types of NLR N-terminal domains: the acidic transactivation

domain (NLRA), the baculoviral inhibitory repeat-like domain (NLRB), the caspase activation and recruitment domain (NLRC) and the pyrin domain (NLRP) (19). The C-terminus of the receptor is responsible for sensing and recognizing ligands. Several PAMPs and DAMPs are recognized by different NLRs, including components of bacteria and viruses; extracellular adenosine triphosphate (ATP); changes in intracellular ion concentration; monosodium urate-, calcium pyrophosphate- and cholesterol crystals; free fatty acids; and reactive oxygen species. Some NLRs, such as NLRC1 (also known as NOD-1) and NLRC2 (also known as NOD-2) function in a similar manner as TLRs in that when activated, the receptors stimulate transcription of pro-inflammatory genes through the activation of NF-κB (20). Other NLRs recruit other cytosolic proteins to form multimeric complexes called inflammasomes.

One of the most studied inflammasomes is the NLRP3 (also known as NALP3) inflammasome. Ligands for NLRP3 are structurally varied and include toxins or whole-pathogen components from microorganisms such as *Candida albicans* and *Staphylococcus aureus* as well as host-derived molecules such as extracellular ATP, and monosodium urate- or cholesterol-crystals. The activation of the inflammasome is tightly regulated, and during physiological conditions counteracting inhibitory mechanisms are believed to block aberrant inflammasome activation. The end product of inflammasome activity is the secretion of the mature forms of the cytokines IL-1β and IL-18. To achieve this several steps are required, of which the first is priming of the inflammasome.

During priming, pro-inflammatory signals such as TLR ligation and activation, or signals from pro-inflammatory cytokines such as tumour necrosis factor (TNF) and subsequent activation of NF-κB induce the transcription of components necessary for inflammasome function. Mainly the immature pro-form of IL-1β (pro-IL-1β) is needed as most cells do not have a ready supply of this cytokine during resting conditions. Additionally, components of the inflammasome machinery such as NLRP3 itself are increasingly transcribed upon pro-inflammatory signalling. Once primed, an activating signal is needed to initiate the assembly of the inflammasome proteins. The activation is typically ligation of NLRP3 with one of its agonists. Once activated, NLRP3 recruits the adaptor protein "associated speck-like protein containing CARD" (ASC). The final component in the complex is pro-caspase-1 and several complexes oligomerize to form the mature inflammasome. Pro-caspase-1 is cleaved and thus activated, and in turn caspase-1 cleaves pro-IL-1β into mature IL-1β that is secreted. Inflammasome activation is a potent pro-inflammatory mechanism and is known to induce pyroptosis, a form of cell death. Failed inhibition or overactivation of the inflammasome is associated with several autoimmune disorders such as periodic fever syndromes, but has also more recently been implicated in chronic diseases such as diabetes mellitus type 2 (21).

2.1.2 Monocytes and macrophages

Several cell types make up the innate immune system. The first cells to encounter a pathogen or initiate an inflammatory response are often mononuclear phagocytosing cells such as monocytes or macrophages. Monocytes derive from $CD34^+$ myeloid progenitor cells in the bone marrow. A supply of circulating monocytes is continuously released into the circulation.

Upon infection or inflammation, the bone marrow output of monocytes can be quickly increased, mainly as a response to cytokines such as granulocyte colony-stimulating factor (G-CSF), C-C-motif-ligand 2 (CCL2) and CCL5. The spleen also functions as a reservoir for monocytes to be recruited after inflammatory stimuli. Traditionally, two populations of monocytes have been described in human circulation: the classical monocytes, identified by $CD14^{++}CD16^{-}$ and non-classical monocytes, $CD14^{dim}CD16^{+}$. A similar division into two subsets can be made of murine monocytes, where the $Ly6C^{+}CCR2^{+}CX_3CR1^{low}$ cells are short lived and quickly recruited in to inflamed tissues while $Ly6C^{-}CCR2^{-}CX_3CR1^{high}$ cells migrate into non-inflamed tissues. However, with increasing ability to differentiate monocytes, the paradigm of two subsets has been challenged, and an intermediate subset of human monocytes has been proposed ($CD14^{+}CD16^{+}HLA^{-}DR^{+}CD86^{+}CD11c^{+}$). Classical monocytes would then gradually differentiate into non-classical monocytes by passing the intermediate stage. The function of the classical monocyte is to migrate to inflamed tissues, phagocytose antigens, and initiate the immune response. Intermediate monocytes display upregulation of several processes related to antigen presentation and are believed to be important for activation of the adaptive immune response as well as apoptosis control. Non-classical monocytes are important in antibody-mediated phagocytosis and in patrolling blood vessels. The transition between classical and non-classical monocytes is not clear-cut and with the advent of more advanced tools such as mass spectrometry coupled flow cytometry, an increasing number of further, partially overlapping, subdivisions of the monocyte population have been added. (22, 23)

Once migrated into an inflamed tissue, monocytes may differentiate into macrophages and dendritic cells (DCs); however, not all macrophages are derived from monocytes. A large portion of tissue resident macrophage populations are already established in foetal life and are not dependent on monocyte infiltration during adult life. Macrophages differentiated from monocytes are believed to have specific functions and phenotypes compared with tissue resident macrophages; however, the contribution of origin versus the contribution of local factors to macrophage function is still debated. Organs with self-renewing tissue resident macrophages include the brain, epidermis, lung and liver. The heart and pancreas have a slow renewal of their macrophage pool by infiltrating monocytes while the gut and dermis have a fast turnover of macrophages with a pronounced infiltration of circulating monocytes (24).

Another common way to subdivide macrophages other than being tissue resident or not, is based on their cytokine and receptor response to stimulation. Macrophages activated by ligation of TLRs as a response to invading foreign microorganisms primarily develop into a pro-inflammatory phenotype called M1 macrophages. Pro-inflammatory cytokines, particularly interferon-γ (INF-γ), also polarize macrophages into the M1 phenotype. Functionally, M1 macrophages specialize in phagocytosing foreign antigens and cell debris, killing and degrading phagocytosed material, and maintaining the inflammatory response. Upregulation of the transcription of pro-inflammatory cytokines such as TNF and IL-12b is a signature of M1 macrophages, as is a change in arginase metabolism with upregulation of inducible nitric oxide synthase (iNOS) to generate the free radical nitric oxide (NO). M2 macrophages are on the other end of the spectrum compared to pro-inflammatory M1 macrophages, as M2

macrophages play important roles in wound healing, resolution of inflammation and maintaining tissue homeostasis. Most of tissue resident macrophages are not activated during normal conditions and could be argued to be of the M2 phenotype. *In vitro*, a similar phenotype can be achieved by polarizing macrophages with the cytokines IL-4 and IL-13. Typical M2 macrophages express arginase instead of iNOS. M2 macrophages secrete the pro-tolerogenic cytokines IL-10 and transforming growth factor β (TGF-β), which are important in tissue repair. Components of the NLRP3 inflammasome also seem to be downregulated in M2 macrophages.

The dichotomy of dividing macrophages into M1 and M2 macrophages is, however, a simplification. For example, several subsets of M2 macrophages have been described (M2a, M2b and M2c), and each subset depends on different stimuli for polarization. Furthermore, other subsets of macrophages have been described, such as regulatory macrophages (Mregs), macrophages induced by oxidized lipids (Mox) and M4 macrophages. The applicability of the M1 and M2 classification, a nomenclature mainly sprung from the stimulation of macrophages *in vitro*, in the *in vivo* setting is also being questioned. It is clear that macrophages are a highly plastic cell type with the ability to change between several functions and roles depending on local stimuli and that classification should be done on a continuous spectrum rather than into clear-cut populations (25-28).

2.1.3 Dendritic cells

Dendritic cells (DCs) have traditionally been described to be of myeloid lineage like monocytes. Modern views on haematopoiesis have, however, given nuance to that belief, suggesting that myeloid and lymphoid potentials run together longer than previously suggested and explaining the observed lymphoid markers in some subsets of DCs. DCs originate in the bone marrow and are then released into the circulation. In the human setting, there are three main subsets of DCs: conventional DCs 1 and 2 (cDC1 and cDC2 respectively) and plasmacytoid DCs (pDCs). Finally, monocytes can differentiate into DCs under certain conditions, and are then called monocyte-derived DCs (mo-DC).

In the activation of the immune response, DCs have two functions. The first is to collect antigen and transport it to an appropriate lymphoid organ. To do so, DCs are spread throughout most tissues of the body but are particularly abundant at sites commonly exposed to foreign microorganisms, such as epithelial barriers. DCs form a cellular net throughout the tissues of the body where they sample their environment and take up antigens using a wide array of PRRs. Once an antigen is taken up and an inflammatory response is initiated, DCs lose adhesion to their environment and start to migrate along a chemokine gradient towards a lymph node (LN).

The DCs then begin to mature and to perform their second task in initiating an immune response, which is to present the antigen to the adaptive immune system and initiate an appropriate adaptive response. During the maturation process, the lysosomes of the DCs acidify to breakdown and process the antigen into small peptides. The antigen peptides are displayed on major histocompatibility complex class II (MHC-II) molecules that are trafficked to the cell

surface. To activate a robust immune response, DCs are also producers of cytokines and microbial activation induces the production of TNF, IL-12 and other pro-inflammatory cytokines. Dendritic cells that have not undergone maturation do not display antigen to the same extent. However, displaying the antigen is not enough to initiate an immune response. An additional signal is needed such as co-stimulation of the adaptive immune system by the DC, e. g., by CD80 or CD86. During resting conditions, DCs seem to play an important role in maintaining tolerance towards self-antigens while presenting endogenous peptides without co-stimulation.

The cDC2 subset seems to have the broadest ability to present foreign antigens and give rise to a wide variety of adaptive immune responses, even tolerogenic ones. In the case of cDC1, this subset is specialized in promoting a cellular immune response aimed at activating $CD8^+$ T cells and a T-helper cell type 1 (T_H1) response. pDCs are named for their morphological similarity with plasma cells, and the function of pDCs is particularly important in combatting viruses and neoplasia. TLR7 and TLR9, both receptors of nucleic acid elements are abundant in pDCs and upon activation, pDCs produce large quantities of INF-I. In certain autoimmune diseases and experimental settings, pDCs have been found to be tolerogenic and to induce regulatory cells of the adaptive immune response (29-31).

2.1.4 Granulocytes

Granulocytes, sometimes referred to as polymorphonuclear cells due to the multilobulated shape of their nuclei, are the most abundant immune cells in human blood. The most numerous granulocytes are neutrophils, numbering between approximately 1.6 and 5.9 million cells per milliliter of blood in healthy adults, constituting 50% to 70% of circulating immune cells (in mice, however, neutrophils constitute approximately 10% to 25% of circulating white cells) (32). At times of inflammation such as infection, the count of neutrophils quickly increases under the influence of G-CSF, sometimes doubling the count. Neutrophils are generated in the bone marrow. Once formed, they patrol the circulation, and during physiological conditions, they have limited survival in tissues. Exceptions are sites of reservoirs of mature neutrophils, such as the spleen and lungs, where mature neutrophils carry out surveillance and function as a pool to quickly recruit cells from in the event of inflammation. During inflammation the lifespan of neutrophils is substantially prolonged.

Neutrophils are the first responders at sites of inflammation. Local cells of the innate immune system are activated, secreting pro-inflammatory cytokines that activate the endothelium. By binding to selectins and subsequently to integrins, neutrophils first roll along and then attach to the activated endothelium. Once attached, the neutrophil moves across the endothelium and out into the inflamed tissue. Several effector functions are available to mature neutrophils. The neutrophils are adept phagocytes and can quickly phagocytose bacteria or sterile antigens. Additionally, as the name granulocyte implies, neutrophils are loaded with granules that can be released upon activation. The granules are filled with cytotoxic components such as myeloperoxidase or proteolytic enzymes. Finally, neutrophils may expel their DNA to form

net-like structures called neutrophil extracellular traps (NETs) coated with enzymes to trap and kill invading microorganisms (33).

Based on staining properties, two other forms of granulocytes exist, eosinophils and basophils. Eosinophils play an important role in defending the host against helminth and parasitic infection but are also implied in late phase hypersensitivity reactions. Basophils are rare white blood cells that also play a role in hypersensitivity reactions.

2.1.5 Additional innate immune cells

Mast cells are bone marrow derived innate immune cells. Typical mast cells have a strong affinity for immunoglobulin E (IgE) antibodies mainly through the FcεRI receptor. This affinity for IgE makes mast cells important effector cells in type 1 hypersensitivity reactions. Upon stimulation, mast cells release preformed granulae containing histamine, tryptase and chymase. Mast cells have been found in atherosclerotic plaques and have been argued to influence macrophage cholesterol uptake (34). In addition to mast cells, the innate immune system also includes natural killer cells (NK-cells) that are important in the defence against tumours and intracellular pathogens. Recently, innate cells from the lymphoid lineage called innate lymphoid cells (ILCs) have been discovered.

2.1.6 Complement

The innate immune system comprises a humoral arm made up of acute phase proteins and the complement system. The complement system is made up of several proteolytic proteins forming an enzymatic cascade, allowing for great amplification of the response upon activation. Three different pathways activate the complement system. The classical pathway is dependent on antibody coating of a pathogen to initiate the first signal. Certain molecular structures on microbes may directly activate the so called alternative pathway of complement activation. Third, mannose-binding lectin may also activate the complement cascade. Regardless of the mode of activation, the pathways join in the cleavage of complement factor C5 which initiates the assembly of a protein structure called the membrane attack complex (MAC). The MAC may then form a pore in the membrane of the microbe, killing it. However, the coating of microbes with complement, targeting them for phagocytosis by macrophages or neutrophils, is probably of greater functional importance than the direct bactericidal functions of the complement system. To not coat cells belonging to the self, an array of inhibitory molecules present at most cell membranes inhibit the binding of complement (35).

2.2 THE ADAPTIVE IMMUNE SYSTEM

While the innate immune system is tasked with detecting foreign microorganisms and alerting the organism of danger and infection, it falls on the adaptive immune system to mount an effective response. Furthermore, the adaptive immune system is capable of immunologic memory, which improves the response should the same antigen be encountered again. If the innate immune system is rigged for detecting common and repeating patterns found in

pathogens, the adaptive immune system excels at diversity. The main cells of the adaptive immune system are two types of lymphocytes, T cells and B cells.

2.2.1 T cells

While originating in the bone marrow, immature T cells migrate to the thymus to undergo maturation. The purpose of maturation is to generate T cells that are active enough to respond to pathological antigens but not activated when encountering host antigens. At the heart of this process is an antigen recognizing surface receptor called the T-cell receptor (TCR). The most common TCRs are heterodimers made up of one α chain and one β chain (36). The chains contain both constant domains (C) and variable domains (V) where the V-domains are responsible for antigen recognition.

The complete repertoire of antigens recognized by the pool of T cells is at least 2.5×10^7 but potentially as large as over 10^{20} antigens (37), and to achieve such diversity, genes encoded in the germline would not suffice. Instead, T cells have developed an advanced system to introduce variability in the peptide sequence of the antigen-sensing domain of the TCR. First, several variants of gene segments for the antigen-sensing domain exist that can be assembled in different combinations by lymphocyte-specific recombinases (recombination activating gene; RAG 1 and 2) to generate large variety. Second, in the recombination step, several changes to the nucleotide sequence are added, making the variation in the resulting sequence increase dramatically. This process does however allow for the generation of non-functioning sequences or even sequences that are not able to be translated into protein. Selection is therefore needed. In a process referred to as positive selection, only clones of T cells with intact TCRs that are able to bind antigen presenting receptors (MHC-II) are given stimuli for further maturation, and clones without functioning TCRs undergo apoptosis. Negative selection occurs when the maturing T cells that react strongly to self-antigens are removed. Conventional T cells can only detect peptide antigens presented by an antigen-presenting cell (APC) on an MHC-II molecule. The distribution of a specific TCR is clone specific, meaning that each T cell clone has a unique TCR that reacts to a specific antigen.

To become fully activated, T cells require two signals. The first signal (signal 1) is ligation of the TCR by an antigen displayed on MHC-II by an APC. A typical second signal (signal 2) is provided by costimulatory molecules on the APC, the most well-known of which are B7-1 (CD80) and B7-2 (CD86). Costimulatory molecules are upregulated on APCs by microorganisms or pro-inflammatory cytokines, thus ensuring that signal 2 is only present during an inflamed state. CD80 or CD86 binds to CD28 on T cells, which mediates the second signal to T cells. Another set of molecules important for co-stimulation of T cells are CD40 on APCs and CD40 ligand (CD40L) on activated T cells. Instead of functioning as co-stimulation per se, ligation of CD40 on APCs upregulates regular co-stimulatory molecules on the APC, thus allowing the activated T cell to aid in initiating an immune response. Other molecular interactions inhibit the immune response; for example cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell-death protein 1 (PD-1) bind ligands on APCs but inhibit the immune response.

T cells that have not yet been activated are referred to as naïve T cells. Once activated, most often by APCs that have migrated to a secondary lymphoid organ, the T cell starts to proliferate. As the specificity of the TCR is clonal, the number of T cells that recognize a particular antigen is greatly expanded. Of this expanded pool of antigen-specific T cells, most are recruited to carry out immune effector functions and are thus called effector cells, and when the stimulus of the particular antigen is cleared, these cells undergo apoptosis. A small fraction of cells, however, turn in to more long-lived memory cells, increasing immune response efficiency against future challenge by the same antigen.

2.2.2 T-cell types

Two types of conventional T cells exist, $CD4^+$ T cells and $CD8^+$ T cells. $CD8^+$ cells are often referred to as cytotoxic T-lymphocytes (CTLs) as they are mediators of cellular immunity. To be activated, CTLs are stimulated by antigens bound to major histocompatibility complex class I (MHC-I) molecules. MHC-I displays intracellular antigens from the cytosol and is present on all cells, while specialized APCs such as DCs may display phagocytosed antigens on MHC-I as well. This affinity for MHC-I makes CTLs important for immunity against intracellular pathogens such as viruses and intracellular bacteria. Once an activated CTL binds its corresponding antigen presented on MHC-I, it may secrete granule proteins such as granzymes and perforins that mediate cell death. An alternative effector function is to ligate the Fas receptor of an infected cell, causing apoptosis.

$CD4^+$ T cells have effector functions that enhance or fine tune other arms of the immune system, mainly by secreting cytokines or providing stimulatory cell-to-cell interactions, and are therefore referred to as T-helper (T_H) cells. There are several subsets of T_H cells based on the profile of their secreted cytokines and effector functions, where each T_H cell subset typically is dominant in a particular type of immune response (38). T_H cells that are activated in the presence of INF-γ and IL-12 activate the transcription factor T-box-containing protein expressed in T cells (T-bet) and become T_H1 cells. T_H1 cells are important for protection against intracellular bacteria and are adept at inducing bactericidal activity in phagocytosing cells of the innate immune system.

INF-γ is the most important cytokine secreted by T_H1 cells, not only activating macrophages and polarizing them towards M1, but also maintaining the T_H1 response as a positive feedback loop. If IL-4 instead is present during T_H cell activation, a T_H2 response is initiated. The main transcription factor governing T_H2 polarization is GATA-binding protein 3 (GATA-3). T_H2 cells are part of the immune response to parasites such as helminths and secrete IL-4 and IL-5, which potentiate eosinophil function. In addition, T_H2 cells secrete IL-10, which downregulates T_H1 responses and together with other T_H2 cytokines, promotes M2 macrophage polarization. A particular subset of T_H cells are T_H17 cells, named after their hallmark secreted cytokine, IL-17. T_H17 cells develop by the actions of pro-inflammatory cytokines such as IL-23, IL-6 and IL-1 as well as to some degree TGF-β. The main transcription factor driving the T_H17 response is RAR-related orphan receptor gamma isoform t (RORγt), and TH17 cells are important in fighting extracellular bacteria (36, 39).

Regulatory T_H cells (T_{Reg}) are important in maintaining immune tolerance against the self and for regulating pro-inflammatory immune responses. T_{Reg} cells comprise approximately 5-7% of $CD4^+$ T cells and are classically recognized as $CD4^+CD25^+$ cells with upregulation of the transcription factor forkhead box P3 (FOXP3). T_{Reg} cells can be generated already in the thymus or alternatively develop in the periphery as inducible T_{Reg} cells (iT_{Reg}), mainly in conjunction with gut epithelial barriers, under the influence of TGF-β. While thymic T_{Reg} cells display TCRs mainly recognizing self-antigens, iT_{Reg} cells have TCRs specific for a broad variety of substances, including innocuous foreign antigens. T_{Reg} cells maintain immunological tolerance during homeostasis and downregulate the immune response against their cognate antigen. To do this, T_{Reg} cells secrete regulating cytokines, such as IL-10 and TGF- β, as well as display regulatory molecules such as CTLA-4 (40).

Finally, a specific subset of T cells, called natural killer T cells (NKT-cells), may be generated in the thymus. NKT cells display a unique set of TCRs that recognize a variety of lipid antigens. The TCRs of NKT cells display less variety than regular T cells, and specific lipid components of bacteria activate several clones of NKT cells. Lipid antigens are presented to NKT cells on the CD1d molecule, expressed not only on APCs but also on several other tissue cells. Although often more sensitive to activation than conventional T cells, NKT cells require APC stimulation to fully activate, for example via CD80/CD86 ligation, and are sensitive to co-inhibitory pathways such as PD-1 ligation. Upon activation, NKT cells produce cytokines similar to a T_H1 response (41).

2.2.3 B cells

The other major lymphocyte population is, similarly as T cells, originated in the bone marrow from lymphoid progenitor cells. However, instead of migrating to the thymus for maturation, this lymphocyte population matures in the bone marrow and is thus referred to as B cells. B cells are responsible for the humoral arm of the adaptive immune system by differentiating into antibody-producing plasma cells. Similar to T cells, each B-cell clone has a unique affinity for a particular antigen. However, B cells do not have TCRs; instead they recognize antigens by membrane-bound antibodies, sometimes referred to as B-cell receptors (BCRs).

Antibodies, also known as immunoglobulins (Ig), contain four peptide chains, two heavy chains with a light chain each, connected by disulfide bonds to form a Y-shaped tetramer. The heavy chain contains 3 or 4 constant regions (C) and one variable region (V), whereas the light chain contains only one C and one V region. Variability in the V region is achieved through the action of recombinase analogous to the generation of the TCR. The V regions of one heavy and one light chain align to give each Ig two antigen binding sites. While the conventional TCR only recognizes peptide antigens displayed by MHC-II molecules, the tertiary structure of the antigen binding region of Ig allows binding of other types of antigens, as well as antigens not displayed by MHC molecules. There are two types of light chains, κ and λ, and each B cell is restricted to one type. Five types of heavy chains exist, α, γ, δ, ε, and μ, and Ig are classified by their heavy chain into IgA, IgG, IgD, IgE, or IgM respectively. BCRs are of IgD or IgM class, IgA is associated with mucosal immunity, IgE is implicated in parasite defence and eosinophil

activation as well as hypersensitivity, while IgG is the main class of a fully developed humoral adaptive immune response (36).

Specific signals are required to activate B cells. Several BCRs usually need to be ligated at once to activate a naïve B cell. Once activated, B cells may initiate the production of IgM and become short-lived plasma cells. To develop a more potent humoral response, T-cell interactions are necessary. The stimulated B cell endocytoses the bound antigen and may start to migrate towards the T-cell zone in a secondary lymphoid organ. B cells function as competent APCs for the antigen recognized by their respective BCR. In the borders between the follicle and T-cell zones, the B cell displaying peptide antigens of the endocytosed antigen may encounter a T_H cell with specificity for the displayed antigen. The T_H cell may then activate the B cell, for example, by CD40-CD40L interaction or by secreted cytokines. Activated B cells may then migrate to the follicle and proliferate rapidly, forming a germinal centre.

Through the interaction with specific follicular T-helper cells (T_{FH}), the type of heavy chain expressed by the B cell may change from μ to form other classes of Ig (e.g., IgG or IgE) in a process called class switching. In the DNA coding for Ig V regions, there is a highly increased rate of mutation during the germinal centre reaction. Through interaction with specialized follicular dendritic cells supplying pro-survival signals conditioned by antigen binding, B-cell clones producing Ig with the highest affinity are selected in a process called affinity maturation. Many of the high-affinity Ig expressing B cells develop into long-lasting plasma cells that produce large quantities of Ig. Some B cells however, develop into memory B cells that patrol tissues and the circulation for a long time, allowing for an efficient response upon challenge by the same antigen again (36, 42).

The largest population of B cells are the follicular B cells responsible for the above-described germinal centre formation. Another population, marginal zone B cells, is also found in secondary lymphoid organs. Both follicular B cells and marginal zone B cells come from the B-2 cell population. A third population of B cells, B-1 cells, is mainly found around mucosal interfaces, primarily in the gut and peritoneal cavity but also in the pleura. The B-1 cell population is believed to be foetal in origin and maintained by self-renewal. One of the major functions of B-1 cells is to secrete natural antibodies, mainly of the IgM or IgG3 type. Targets of natural antibodies are pathogen-associated structures such as cell wall components of bacteria, but B-1 cells seem to be specific for self-antigens to a larger extent than are other B cells, and natural antibodies also recognize antigens such as lipid molecules, which could be of particular interest in atherosclerosis. A recently discovered B-cell subset is B_{Reg} cells, which are induced by TLR9 stimulation and similarly to T_{Reg} cells have tolerogenic functions, for example, via the production of IL-10 and TGF-β (43, 44).

2.3 TOLERANCE AND RESOLUTION OF INFLAMMATION

Much research efforts has been made to map the intricate machinery that initiates an immune response against a foreign antigen. This is expected, as defending the host against foreign pathogens is arguably the main function for which the immune system evolved. Paul Ehrlich

observed however, by the beginning of the 20th century, the devastating effects of when this system fails and the immune system is turned on the self in what Ehrlich called *horror autotoxicus (45)*. During resting conditions, the immune system is not activated. From the earlier view of this merely being an absence of antigen, this state of tolerance is now determined to depend on several active functions. Furthermore, as inflammation is a central part of the pathophysiology in many modern diseases, the need for suppression of the inflammatory response is ever increasing. With chronic inflammatory diseases on the rise, interest in how the inflammatory response is resolved has seen a tremendous increase.

2.3.1 Tolerance

Immune tolerance is the absence of immune reactions to the self and the state in which the immune system is during homeostasis. Mechanisms of tolerance are sometimes divided into peripheral tolerance and central tolerance, where central tolerance is the process of negative selection in the generation of lymphocytes and peripheral tolerance is the generation of tolerance-maintaining cells in the tissues (such as iT_{Reg} cells). T_{Reg} cells are important effector cells for maintaining tolerance (36). Important T-cell dependent mechanisms of tolerance are the ligation of CTLA-4 and PD-1. Ligation of these co-inhibitory molecules to their receptors blocks T cell proliferation, induces anergy, downregulates the production of pro-inflammatory cytokines and promotes the production of tolerogenic cytokines such as IL-10 and TGF-β.

Tolerogenic signals can, however, already be provided by the innate immune system. Local factors in the microenvironment may push DCs to develop into tolerogenic DCs, often with a pDC-like morphology. Instead of activating naïve T cells, tolerogenic DCs cause T-cell anergy and secrete anti-inflammatory cytokines such as IL-10. The role of DCs in activating the adaptive immune system for a particular antigen makes tolerogenic DCs particularly interesting in autoimmune disease, given their ability to induce tolerance against a specific antigen (46, 47). Other innate cells important for immune tolerance are M2 macrophages that inhibit pro-inflammatory M1 responses. A less homogenous population of tolerance-induced innate cells are myeloid-derived suppressor cells (MDSCs). The MDSCs are not of clear lineage but comprise immature myeloid cells with features of both monocytes, DCs and granulocytes. During inflammation, particularly chronic inflammation, the population of MDSCs is increased, a focus of interest in tumour escape research (48).

Local tolerogenic conditions vary between tissues and anatomical sites. It has long been known that sites where an inflammatory reaction would have particularly negative consequences have strong mechanisms that inhibit local inflammatory reactions. These sites are said to be immune privileged, and examples include the central nervous system (CNS), the eye, the gravid uterus, testes, hair follicles, liver and the gut. From originally being seen as mostly an anatomical feature of barriers inhibiting the circulation of immune cells, such as the blood-brain barrier in the CNS and the unique circulation of the eye, immune privilege is now viewed as an active process that depends on local regulatory cells and the production of regulatory cytokines and cell ligands in the tissue. It has been proposed that the tunica media of arteries is a tissue with

increased immune privilege and that breakdown of this immune privilege is central in many CVDs (49, 50).

While mechanisms of tolerance are active processes that can be downregulated upon challenge with PAMPs or DAMPs, most pharmacological modulation of the immune system relies on immune suppression. The suppressive effects of anti-inflammatory and immunosuppressive agents remain the same regardless of the challenging antigen (e.g., an IL-1 targeting antibody will deplete IL-1 regardless of the physiological state of the organism or activation of the immune system). The unspecific nature of such treatments makes them prone to cause unwanted effects such as susceptibility to infection. The modulation of host tolerance and resolution pathways presents an attractive alternative approach for future treatment options (51).

2.3.2 Resolution of inflammation

Immune reactions and the inflammatory process are not only aimed at clearing pathogens. An integral part of the inflammatory response is tissue repair and a return to homeostasis, and every inflammatory reaction can be divided into an initiation phase and a resolution phase. While the predominant view for a long time was that with the removal of the causative antigen, the inflammatory response automatically diminished through the passive diffusion and dilution of pro-inflammatory mediators, it is now clear that resolution is an active process with a distinct cell and molecular machinery.

The process of resolution begins early in the inflammatory response, even before the causative antigen is removed (52). One hallmark feature of resolution is the clearance of dead cells, apoptotic bodies and other debris by macrophages. During the resolution process, macrophages are polarized to a more M2-like phenotype specialized at efferocytosing apoptotic bodies and debris. Signals promoting resolution include not only common tolerogenic mediators such as IL-10 and the effect of T_{Reg} cells but also specialized pro-resolving mediators (SPMs). SPMs are lipid mediators such as lipoxins, resolvins, maresins and protectins, which are derived from the ω-3 fatty acids eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and the ω-6 fatty acid arachidonic acid. Signalling pathways for the SPMs include specialized G-protein coupled receptors (GPR), mainly ALX/FPR2 and ChemR23. Apart from the SPMs, the protein annexin A1 is an important mediator of resolution, as are the gaseous mediators NO and H_2S (53).

3 METABOLISM

The reactions between molecules within an organism to generate energy or building blocks for new material constitute its metabolism. Metabolic processes are tightly regulated and variations in metabolism give important signals to the cell regarding both the extracellular and the intracellular environment. An ability to adapt metabolism to the needs of the current conditions is necessary for survival. While specific metabolic pathways exist for important classes of

nutrients such as lipids, amino acids and glucose, a tight linkage and overlap between metabolic pathways is common.

3.1 LIPID METABOLISM

Lipids are essential for all cells and tissues; as the main part of cell membranes, to store and distribute energy, for physical insulation and as part of signalling molecules. A particular class of lipids are sterols, most notably cholesterol. Lipids are acquired through the digestion of ingested nutrients and *de novo* synthesis.

3.1.1 Lipoprotein metabolism

The mean dietary intake of fat of the modern day population is between 30% and 40% of total energy intake, which is close to the nutritional recommendation for the Nordic countries of fat constituting 25 – 40% of total energy intake (54, 55). The majority of the ingested fat (approximately 95%) is esterified into triacylglycerols (TAGs). TAGs are hydrolysed by lipases in the gastrointestinal tract to free fatty acids (FFAs) and monoacylglycerols (MAGs) and, together with phospholipids and components of bile, emulsified into mixed micelles. Transport of the FFA into enterocytes across the brush border membrane occurs through both active protein receptor-mediated transport and passive processes, where long- and medium-length chain fatty acids are more dependent on transport, while short-chain fatty acids diffuse more easily. The most important transporters for fatty acids on the luminal side of enterocytes are CD36 and fatty acid transport protein 4 (FATP4), while fatty acid binding protein (FABP) is important for enriching FFAs at the cell membrane and organizing absorbed FFAs in enterocytes for further metabolism (56-58).

Cholesterol is a sterol that is an essential part of human cell membranes as well as the precursor for several bioactive molecules such as steroid hormones. Approximately 0.5% of dietary lipids are cholesterol. Cholesterol uptake over the brush border membrane in the intestine is primarily achieved by the Niemann-Pick C1 like 1 (NPC1L1) protein (59). Not only dietary cholesterol is absorbed, but also cholesterol secreted into the intestinal lumen with bile as part of the enterohepatic circulation.

Once absorbed, FFAs are conjugated to coenzyme A (CoA) to form acyl-CoA esters. In sequential steps, the acyl-CoA is re-esterified to MAGs to form diacylglycerols and finally new TAGs. Cholesterol is esterified with acyl-CoA to form cholesteryl esters (CE) by the enzyme acyl-CoA:cholesterol acyltransferase isoform 2 (ACAT2). While short-chain FFAs may diffuse through the enterocyte and enter the circulation, TAG and cholesterol need to be packaged into lipoprotein particles to be soluble enough to enter the circulation and to be directed for use by tissues throughout the organism. A key enzyme for packaging TAGs and CE into lipoprotein particles is microsomal triglyceride transfer protein (MTTP). Lipoprotein particles are divided based on density and size, where the chylomicrons produced by enterocytes have the lowest density. The protein moiety, made up of different apolipoproteins (Apo), also differs between the different lipoprotein particles. The other lipoprotein particles

apart from chylomicrons are very low-density lipoprotein (VLDL), low-density lipoprotein LDL and high-density lipoprotein (HDL) particles (58, 60).

Table 1: General features of apolipoprotein particles. TAG, triacylglycerol; CE, cholesteryl ester; C, cholesterol; PL, phospholipid (60).

Lipoprotein	Diameter (nm)	Apolipoprotein	TAG (%)	CE (%)	C (%)	PL (%)	Protein (%)
Chylomicrons	75 - 1200	B-48, C, E	86	3	1	8	2
VLDL	30 - 80	B-100, C, E	52	14	7	18	8
LDL	18 - 25	B-100	10	38	8	22	21
HDL	7.5 - 20	A	5 - 10	14 - 21	3 - 7	19 - 29	33 - 57

3.1.1.1 Chylomicrons

The main apolipoprotein of chylomicrons is ApoB-48. It is transcribed from the APOB gene, which encodes ApoB-100. In human enterocytes, the apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (Apobec-1) edits the APOB mRNA by changing a single nucleotide, which generates a stop codon that truncates the ApoB protein after 48% of the amino acids. ApoB-48 is lipidated in the endoplasmic reticulum (ER) by MTTP which loads nascent chylomicrons with TAG and CE as well as phospholipids. Unlipidated ApoB-48 is degraded. The lipid content of chylomicrons varies, and during the fasted state, chylomicrons with less lipids and thus smaller in diameter, are released. In the postprandial state, the TAG loading of chylomicrons increases manifold. Nascent chylomicrons then move to the Golgi to mature. Apart from the structurally necessary ApoB-48, chylomicrons may acquire a number of exchangeable apolipoproteins, such as ApoC. The mature chylomicron is released into the lymphatics and, via the thoracic duct, further into the circulation, where it may acquire ApoE from other lipoprotein particles such as HDL (61).

The TAGs of chylomicrons are used by tissues as fuel or for storage in adipocytes. To mobilize the lipids from the TAGs in the chylomicrons into the target cells, the fatty acids of the TAGs are hydrolysed to FFAs by lipoprotein lipase (LPL). LPL is expressed in cells that utilize FFAs as either fuel or storage such as myocytes, cardiomyocytes and adipocytes. Mature LPL is transported and attached to the glycocalyx of capillaries in the target tissues (62). Of the exchangeable apolipoproteins, Apo-CII is believed to facilitate LPL-mediated lipid uptake, while Apo-CI and Apo-CIII are believed to inhibit LPL-mediated uptake (63). The liver has a tissue-specific lipase called hepatic lipase that also hydrolyses TAG. As the chylomicron becomes increasingly poor in TAG, it is turned in to what is called a chylomicron remnant. The chylomicron remnants are cleared from the circulation by the liver, where ApoE binds to heparan sulfate proteoglycans to facilitate uptake of the chylomicron remnants by LDL receptors (LDLr) and LDL receptor-related protein 1 (LRP1) (61).

3.1.1.2 Very low-density lipoprotein particles

The second largest lipoprotein particle after chylomicrons is VLDL. VLDL is secreted by hepatocytes to recirculate TAGs and cholesterol to tissues. Sources of TAG include absorbed chylomicron remnants as well as FFAs taken up and re-esterified in the hepatocyte. The FFAs come via the portal circulation from the intestine or from FFAs released by adipocytes into the circulation during fasting. The majority of the *de novo* synthesis of cholesterol takes place in the liver, while peripheral tissues are mostly dependent on the uptake of cholesterol from recirculated lipoprotein particles. The structurally obligate apolipoprotein in VLDL is ApoB-100 in human as the human liver lacks Apobec-1 and only produces the full-length transcript of APOB. Several rodents, including mice and rats, do have active Apobec-1 analogues in liver tissue and thus synthesize VLDL containing either ApoB-100 or ApoB-48. The ApoB protein is lipidated mainly with TAG but also cholesterol and CE by MTTP in the ER, after which the forming VLDL particle is moved to the Golgi apparatus for maturation. VLDL is secreted as an approximately 35 to 100 nm diameter lipoprotein particle (64). Similar to chylomicrons, VLDL can acquire several different exchangeable apolipoproteins, most notably ApoE and species of ApoC (65).

The TAG in VLDL is used by peripheral tissues in a similar way as the TAG in chylomicrons, and LPL plays an important role in successively decreasing VLDL size and TAG content by hydrolysis, increasing the density and proportion of cholesterol in the lipoprotein particle. As the density increases and size decreases, the VLDL turns in to what is termed an intermediate density lipoprotein particle, and eventually, as the density increases, an LDL particle.

3.1.1.3 Low-density lipoprotein particles

As the VLDL particle is transformed into an LDL particle, it loses in most cases its exchangeable apolipoproteins, leaving the majority of LDL particles with only ApoB. The main lipid in LDL is CE, and, in humans, the majority of cholesterol in the circulation is found in the LDL fraction. The LDL particle is taken up via LDLr by receptor-mediated endocytosis. Mutations in the LDLr gene are the most common cause of familial hypercholesterolaemia (FH) (66), a class of conditions associated with greatly elevated serum cholesterol and increased CVD, and the study of FH has been pivotal in understanding the importance of LDL and LDLr in cholesterol metabolism and vascular health. The ApoB of LDL binds to LDLr and induces clathrin-dependent endocytosis. Acidification of the endosome causes a conformational change in the LDLr which releases the LDL particle. The LDL particle is degraded allowing for the use of carried cholesterol, and ApoB is broken down into amino acids. The LDLr is recycled and recirculated to the cell membrane. After mutations in proprotein convertase subtilisin/kexin type 9a (PCSK9) were proven to strongly influence plasma cholesterol (67), it was found that PCSK9 inhibits the recirculation of LDLr and instead targets the receptor for degradation, by inhibiting the conformational change in the endosome (60, 68).

3.1.1.4 High-density lipoprotein particles

Cholesterol transport out to peripheral tissues is managed by LDL. Transport of cholesterol from the periphery to the liver, so-called reverse cholesterol transport, is conducted by HDL. The major apolipoprotein of HDL is ApoA-I which is produced mainly by the liver and intestine (60, 69). In contrast to chylomicrons and VLDL, which are secreted as mature particles, ApoA-I is secreted not fully lipidated and mainly loaded with phospholipids as a nascent HDL, and maturation occurs in the circulation through interaction with other lipoproteins. The regulation of ApoA-I synthesis is mainly transcriptional, and several mediators have been shown to affect ApoA-I secretion such as dietary fat, niacin, alcohol, androgens, oestrogens, glucocorticoids and statins. While ApoA-I is the most abundant apolipoprotein in HDL, it has the ability to acquire several other exchangeable apolipoproteins, such as ApoA-II, ApoE, ApoC species and other less common proteins.

Several processes contribute to loading HDL with lipids to form mature HDL. The cholesterol efflux protein ATP-binding cassette transporter A1 (ABCA1) is one of the main exporters of cholesterol from peripheral cells, and ABCA1 binds ApoA-1 and allows for HDL uptake of cholesterol from tissues. Free cholesterol that is taken up from membranes and via ABCA1 is esterified by the enzyme lecithin-cholesterol acyl-transfer (LCAT) which is bound to HDL particles. Cholesteryl ester transfer protein (CETP) is a soluble enzyme present in human plasma secreted by the liver and the intestine. CETP mediates an exchange where HDL delivers CE to VLDL and LDL and gets TAG in return. This flux of CE back to VLDL and LDL caused high interest in CETP inhibition as a potential therapeutic avenue to lower the risk for CVD. However, clinical trials have failed to deliver positive results, and indeed, an increase in cardiovascular death is seen (70). The final uptake of HDL content in the circulation is mainly done in the liver, where HDL cholesterol is taken up via scavenger receptor class B type I (SR-BI). The receptor mediating complete particle uptake is still not known. SR-BI also seems to play a role in macrophage cholesterol efflux, together with ABCA1 and ATP-binding cassette transporter G1 (ABCG1) (69).

3.1.2 Fatty acid metabolism

Apart from being absorbed from the diet by the intestine, several cell types have the capability of synthesizing fatty acids, and most fatty acid synthesis takes place in the liver and adipocytes. The substrate for fatty acid synthesis is acetyl-CoA, generated by, for example, carbohydrate metabolism. Acetyl CoA carboxylase (ACC) catalyses the committing step of fatty acid synthesis by carboxylation of acetyl-CoA to malonyl-CoA (60). Subsequent elongation of the carbon chain is carried out by a multiunit enzyme in mammals called fatty acid synthase (FAS) restricted to the cytosol.

Fatty acid synthesis is regulated by the transcription factor sterol response element binding protein (SREBP) 1 (71). There are two genes encoding SREBPs: SREBF1 coding for the two isoforms SREBP-1a and SREBP-1c, and SREBF2 coding for SREBP-2. The isoform SREBP-1a is expressed at much lower levels, in the liver by almost a factor of 10 times less than isoform

1c, however, it is more widely distributed being the most prevalent form in certain metabolically inactive tissues. Being a much more potent transcription factor than 1c, isoform 1a upregulates FAS mRNA expression greater than 4 times more than 1c, and 1a also has a more pronounced effect on sterol synthesis than 1c (72, 73). SREBP-1c is mainly responsible for regulating fatty acid synthesis in liver and metabolically active tissue, while SREBP-2 regulates sterol metabolism.

To ensure separation of the synthetic and degrading pathways, degradation of fatty acids takes place in the mitochondrial matrix. Long-chain fatty acids are transported into the mitochondrion via carnitine shuttles, where they are degraded by β-oxidation (60), yielding acetyl-CoA that can be used for energy or in the generation of ketone bodies.

3.1.3 Cholesterol metabolism

Cholesterol is used by all cells in the body as part of the cell membrane. Specialized cells also use cholesterol for steroid hormone synthesis. A subject maintaining a normal Nordic diet absorbs approximately 250 – 350 mg of cholesterol from dietary intake per day. Endogenous synthesis of cholesterol however, normally supplies approximately 1 g of cholesterol per day (55). The balance of intake and synthesis is closely regulated, and increased intake results in lower synthesis. The substrate of cholesterol synthesis is acetyl-CoA, and the committed step of the pathway is the formation of mevalonate from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) catalysed by the enzyme HMG-CoA reductase (HMGCR), the inhibition of which is the pharmacological target of statins. Via the intermediates geranyl pyrophosphate, farnesyl pyrophosphate and squalene, cholesterol is formed (60).

The regulation of cell cholesterol homeostasis is carried out by SREBP-2. SREBP-2 acts as a sensor for cholesterol availability, and in conditions with low levels of cholesterol in the membrane, the basic-helix-loop-helix-leucine zipper protein SREBP-2 is cleaved by site-1-protease (S1P) and transported to the Golgi by a protein known as Scap. In the Golgi, site-2-protease (S2P) cleaves off the DNA binding domain of SREBP-2 that translocates to the nucleus and binds to sterol response elements (SREs), promoting gene transcription (74, 75). During periods of adequate sterol levels, membrane cholesterol causes Scap to undergo conformational change, allowing the binding of the protein “insulin induced gene” (INSIG) which in turn binds Scap to SREBP, inhibiting cleavage and translocation to the Golgi. Not only is the transcription of HMGCR and enzymes involved in cholesterol synthesis regulated by SREs, but several proteins regulating the cell sterol concentration are also induced by SREBP-2, such as LDLr. Curiously, even PCSK9, while normally causing a decrease in cholesterol influx by increasing LDLr degradation, is also upregulated by SREBP-2 activation.

Cholesterol cannot be degraded in the body but has to be excreted via bile, either as cholesterol or as part of bile acids and bile salts. The hepatocytes secrete bile into canaliculi, and free cholesterol can be secreted into the bile, a process that is regulated by the NPC1L1 receptor that mediates reabsorption to hepatocytes (76). Cholesterol and bile components may then be reabsorbed in the intestine as part of the enterohepatic circulation of sterols.

3.2 TRYPTOPHAN METABOLISM

Tryptophan (Trp) is the least abundant of all amino acids and a prerequisite for all lifeforms. While certain plants and prokaryotes are able to synthesize Trp *de novo*, mammals must acquire it through the diet (77). Apart from being used in protein synthesis, tryptophan is metabolized along two main pathways. Tryptophan hydroxylase carries out the reaction forming 5-hydroxytryptophan from Trp, which can then be further modified by aromatic amino acid decarboxylase to serotonine (5-hydroxytryptamine; 5-HT) (60). 5-HT is an important neurotransmitter in both the CNS and the enteric nervous system but also conveys signals in mast cells and platelets. Through the actions of serotonin-N-acetyl transferase and acetyl-serotonin O-methyltransferase, 5-HT can be metabolized to melatonin, primarily in parts of the CNS regulating sleep, such as the pineal gland. However, only approximately 1% of Trp is metabolized to 5-HT and melatonin, while 95% of Trp is metabolized along what is commonly called the kynurenine pathway.

3.2.1 The kynurenine pathway

The initial and rate-limiting step of the kynurenine pathway is the cleavage of Trp by one of two haem-dependent dioxygenases, tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), which exists in two isoforms, IDO1 and IDO2. TDO is mainly expressed in the liver with less pronounced expression in the gastrointestinal tract, brain and bone marrow (78-80). TDO in the liver metabolizes the quantitatively largest portion of Trp, approximately 90% of oxidized Trp (81), and liver TDO functions as a regulator of plasma Trp concentration, degrading excess ingested Trp that is neurotoxic in high concentrations (82). Of the dioxygenases, TDO has the highest specificity for Trp, is induced by glucocorticoids and stabilized by the availability of Trp (77) and is inhibited by feedback inhibition by protonated nicotinamide adenine dinucleotide phosphate (NADPH) (81).

While TDO is concentrated in the liver, IDO expression is more ubiquitous and expression is seen in the kidney, liver, both the male and female reproductive systems, and in immune cells and is likely to be inducible in several other tissues and cell types. In contrast to TDO, IDO has little influence on systemic Trp levels during basal conditions (81, 83). However, under inflammatory conditions, IDO transcription is greatly increased, the main inducer being INF-γ with INF-α exhibiting a less pronounced effect. Other pro-inflammatory cytokines such as TNF, IL-1β and IL-2, potentiate INF-γ-mediated IDO induction while mediators of resolution and tolerance, such as NO, TGF-β and IL-10, inhibit IDO induction by INF-γ (81).

Trp is cleaved by TDO or IDO into N-formylkynurenine, which is further metabolized to kynurenine (Kyn) by the enzyme N-formylkynurenine formamidase. Kyn can be modified to kynurenic acid (KynA) by one of four kynurenine aminotransferases (KYAT 1 - 4). KynA is an end product of the Kyn pathway, and its formation is irreversible. However, under normal conditions, the KYATs have a high K_M of approximately 4.7 mM for the reaction of Kyn to KynA, and other pathways of Kyn processing are favoured. In the main Kyn metabolizing pathway, Kyn can be metabolized to 3-hydroxykynurenine by kynurenine monooxygenase

(KMO) and then further to 3-hydroxyanthranilic acid (3-HAA) by the enzyme kynureninase (KYNU).

As an optional pathway to generate 3-HAA from Kyn, it may be transformed to anthranilic acid by KYNU, which can then be modified to 3-HAA by KMO. 3-HAA is then quickly degraded by the most active enzyme in the Kyn pathway, 3-hydroxyanthranilic acid 3,4-dioxygenase (HAAO), to form 2-amino-3-carboxymuconic acid-6-semialdehyde (ACMS), which is decarboxylated by ACMS decarboxylase into 2-aminomuconic-6-semialdehyde (AMS). In turn, AMS is metabolized by AMS dehydrogenase into 2-aminomuconic acid which can be further metabolized into acetyl-CoA. The Kyn pathway may also serve to generate substrate for nicotinamide metabolism. In this branch of the pathway, ACMS undergoes cyclization in a non-enzymatic process to form quinolinic acid (QA). QA can then serve as a substrate for further nicotinamide adenine dinucleotide (NAD^+) generation through the action of the enzyme quinolinate phosphoribosyltransferase (QPRT). Finally, the other possible end products of the Kyn pathway are picolinic acid (PA), formed from AMS through non-enzymatic cyclization, and xanthurenic acid (XA), formed from 3-hydroxykynurenine by KYAT (81) (Figure 1).

3.2.2 IDO as an immune modulator

When IDO was first described in the 1960s, the discoverers Hayaishi et al. connected IDO to immune function and soon discovered the strong upregulation by INF-γ (84, 85). However, it was not until Mellor and Munn et al. proved that IDO was integral for immune tolerance of allogeneic concepti in mice, and that inhibition of IDO with the pharmacological inhibitor 1-methyl-tryptophan (1-MT) caused rejection of the concepti, that research into the potential immunomodulatory properties of IDO received more widespread attention (86). The rejection of allogeneic concepti upon 1-MT treatment was already in the early experiments found to be T cell dependent. DCs, and in particular tolerogenic DCs expressing the marker CD123, were found to have high IDO expression and the capability of inducing T-cell anergy and T_{Reg} induction, a capability that was lost upon IDO inhibition with 1-MT (87, 88). Conversely, T_{Reg} cells strongly induce the expression of IDO in APCs in a CTLA-4 dependent manner, forming a positive feedback loop (89).

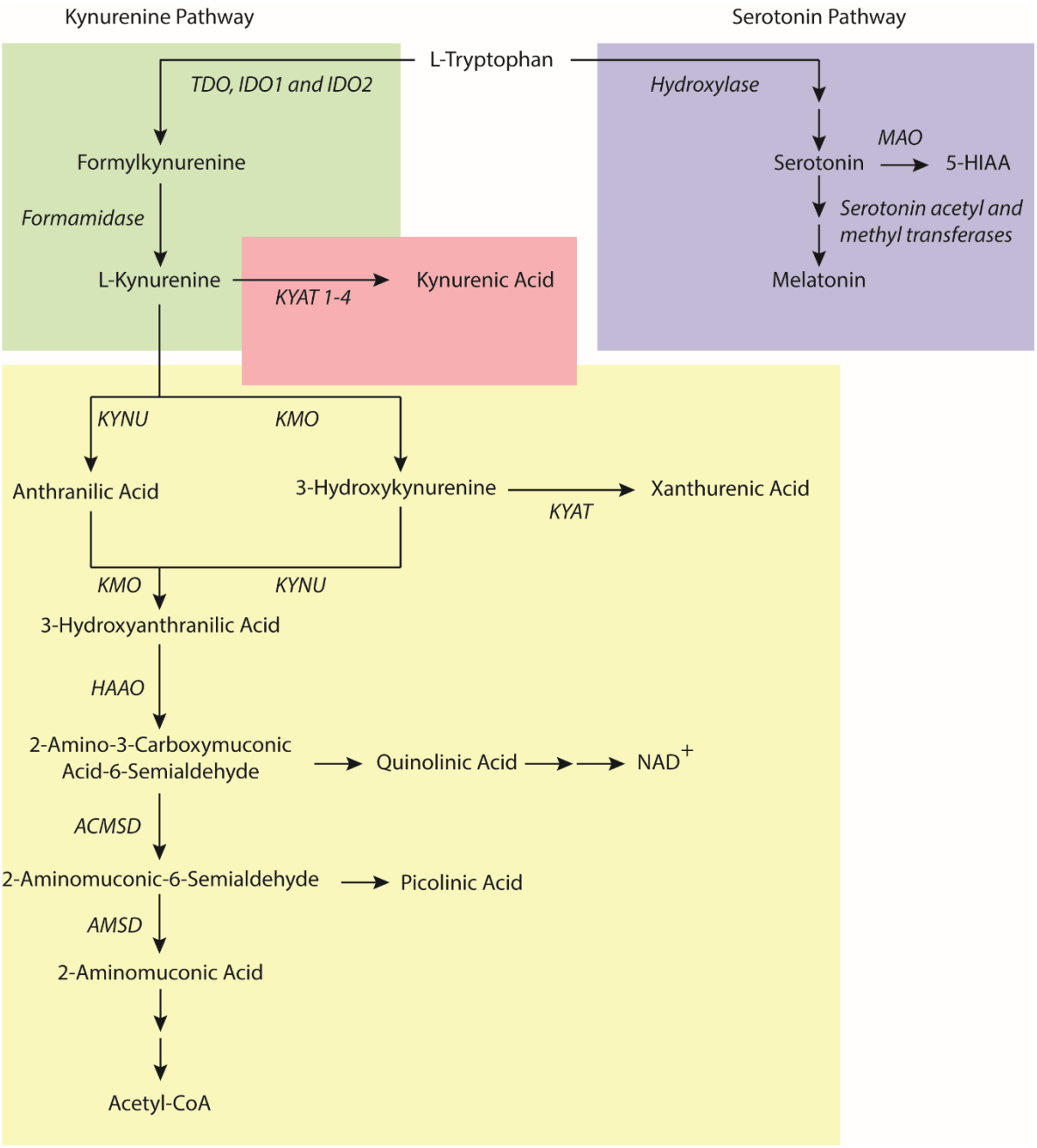

Figure 1. The metabolism of tryptophan. A small portion of tryptophan is metabolized to serotonin and melatonin (right), while the quantitatively largest portion of tryptophan in humans is metabolized along the kynurenine pathway (left). Enzymes are denoted in italics. Purple outlines the serotonin branch of metabolism and green the common part of the kynurenine pathway. The red box outlines the kynurenic acid branch of the kynurenine pathway while the yellow box outlines the quinolinic branch. MAO, monoamine oxidase; 5-HIAA, 5-hydroxyindoleacetic acid; TDO, tryptophan 2,3-dioxygenase; IDO, idoleamine 2,3-dioxygenase; KYAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KYAT, kynureninase; ACMSD, 2-amino-3-carboxymuconic acid-6-semialdehyde dehydrogenase; AMSD, 2-aminomuconic-6-semialdehyde dehydrogenase; NAD^+, nicotinamide adenine dinucleotide.

The kinase general control nonderepressible 2 (GCN2) is part of a cell protective system called the integrated stress response (ISR). In conditions of amino acid starvation, GCN2 is activated by empty tRNA, which leads to the phosphorylation of eukaryote initiation factor 2 subunit α (eIF2α) that in turn regulates protein synthesis and inhibits mRNA translation (90). Under inflammatory conditions, the induction of IDO by INF-γ and other cytokines causes a local

depletion of Trp. T cells are sensitive to GCN2 activation, responding with anergy. Activation of GCN2 by low Trp availability is believed to be a major mechanism by which IDO activity regulates T-cell responses (91). Interestingly, IDO consumption of Trp is mainly believed to occur in innate immune cells, e.g., plasmacytoid DCs.

The response to GCN2 activation in immune cells other than T cells is less well studied, and reports of GCN2 effects in macrophages point to an increased IL-6 response upon IDO-mediated GCN2 activation, and monocyte GCN2-depleted mice suffer increased lethality in experimental endotoxaemia (92). In addition to activating GCN2, Trp depletion may stimulate other metabolic sensors influencing the immune response, and it is known that IDO activation inhibits the mammalian target of Rapamycin (mTOR) and protein kinase C isoform θ (PKC-θ) by depleting Trp (93). The immune-modifying ability of IDO has been shown to be protective in several models of autoimmune disease, such as experimental autoimmune encephalitis (94), colitis (95), rheumatoid arthritis (96) and renal disease (97, 98). While beneficial in settings of autoimmunity and inflammatory disease, most human cancer types display an upregulation of IDO that mediates tumour immune escape (99). The IDO inhibitor 1-MT, and most often the D-isomer clinically named Indoximod, has been used in clinical trials as an adjuvant to cancer therapy with varying results (100).

3.2.3 Kynurenine metabolites

Several of the downstream metabolites of Trp have been shown to exert potent biological effects. KynA is a known agonist of the aryl hydrocarbon receptor (AhR) (101) and of GPR35 (102). AhR is a basic helix-loop-helix transcription factor that, during resting conditions, is bound to chaperones and inactive in the cytosol. Upon ligation of the receptor, AhR translocates to the nucleus and binds to dioxin response elements (DREs) in the DNA. The first known ligand of AhR was the irritant 2,3,7,8-tetrachlorodibenzo-p-dioxin, and AhR signalling was implicated in the response to noxious foreign substances (103). However, AhR is expressed at a particularly high level in the immune system and has been found to interact with several other pathways, including suppressing NF-κB action in macrophages (104).

The role of KynA in immune reactions is debated, and both suppressive and stimulatory effects are described. Treatment with KynA *in vitro* during unstimulated conditions has, for example, been shown to both increase (101, 105) and decrease (106) IL-6 secretion in different cell lines. KynA has also been implicated in the infiltration of neutrophils into the vascular wall (107). After stimulation, however, KynA has a more consistent immunosuppressive effect, for example, by inhibiting the secretion of pro-inflammatory cytokines such as TNF (108, 109).

In the CNS, KynA has long been known to have an effect on neurotransmission, particularly as an antagonist of the N-methyl-D-aspartate (NMDA) glutamate receptor (110). In a set of elegant experiments, Agudelo et al. demonstrated that exercise training induced KYAT activity and subsequent peripheral KynA synthesis, which led to a decreased expression of pro-inflammatory markers in the CNS and provided a molecular mechanism for the beneficial effects seen by exercise training in depression (111). In more recent work, the same research

group demonstrated that KynA, through the action of GPR35, increased the energy expenditure in mice, decreased white adipose tissue mass, and transformed adipose tissue into having a metabolically more favourable profile with increased expression of uncoupling protein 1. Furthermore, the adipose tissue of treated mice showed signs of decreased inflammation with the expression of genes connected to M2-type like macrophages and T_{Reg} cells (112).

Another Kyn metabolite with strong bioactivity is 3-HAA. Treatment with 3-HAA induces T-cell apoptosis in a caspase-8 dependent manner (113). The balance between T-cell subsets is seen to shift upon 3-HAA treatment with an increase in T_{Reg} cells (114). Although the exact mechanism of 3-HAA action is not fully known, it has been suggested that 3-HAA inhibits 3-phosphoinositide-dependent protein kinase 1 (PDPK1), thus inhibiting NF-κB activation and interfering with TCR activation, leading to T_H cell anergy and apoptosis (115). Other suggested mechanisms are related to redox balance, and 3-HAA has been suggested to deplete intracellular glutathione (GSH) in activated T cells and to induce the expression of haemoxygenase-1 (116, 117). Additionally, monocyte cell lines have been shown to respond to 3-HAA with apoptosis, although at higher treatment concentrations (118).

3.3 GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION

A steady supply of energy, carried in the form of ATP, is necessary for cell survival. Central to the generation of ATP is the metabolism of carbohydrates and the process of glucose degradation by glycolysis. The resulting metabolites may be used in a series of reactions known as the citric acid cycle (TCA), which provides fuel for mitochondrial oxidative phosphorylation, a process generating large amounts of ATP. However, several metabolites of glycolysis or TCA are necessary as materials for biomolecule synthesis while others have signalling functions. It is clear that both glycolysis and oxidative phosphorylation have several other functions beyond the generation of energy.

3.3.1 Glycolysis

Glycolysis is the generation of two molecules of pyruvate from one molecule of glucose through a series of reactions with a net result of two molecules of ATP generated in the process (60). The pathway is evolutionally conserved, and both prokaryotic and eukaryotic cells perform glycolysis. Glucose may enter the cell through glucose transporters (GLUTs), with different tissue distributions. Insulin sensitive GLUT-4 is abundant in muscle while a wide array of GLUTs are present on immune cells and, T_H cells are dependent on GLUT-1 function (119). Once in the cell, glucose is trapped after phosphorylation by hexokinase, isomerized to fructose and then phosphorylated again in the rate-limiting step of glycolysis by phosphofructokinase. The six-carbon sugar is then cleaved into two three-carbon compounds, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Thus far, glycolysis requires energy, and two molecules of ATP are spent. The two generated three-carbon compounds can be further metabolized to pyruvate, generating 2 molecules of ATP each, making the sum of ATP gained from running one glucose molecule through glycolysis 2 ATP. In tissues with a

high ratio of NADH to NAD^+, such as during hypoxic conditions, or in cells lacking mitochondria, pyruvate is reduced to lactate.

Glycolysis is tightly regulated and several metabolites function as negative feedback loops, such as phosphorylated glucose, which inhibits hexokinase and a high ATP to adenosine monophosphate (AMP) ratio, which inhibits phosphofructokinase. Hormonal regulation of glycolysis by insulin and glucagon also plays major regulatory roles. Interestingly, the transcription factor hypoxia-induced factor 1α (HIF-1α) has emerged as a potent inducer of glycolysis (120).

3.3.2 The citric acid cycle and oxidative phosphorylation

While the production of two molecules of lactate from one glucose molecule yields two ATP, an alternative path for the generation of large quantities of ATP would be for the pyruvate to enter the TCA and eventually be oxidized to CO_2 and water, fuelling ATP generation through oxidative phosphorylation (OXPHOS). The complete oxidation of glucose yields approximately 30 molecules of ATP, the majority of which, 26 molecules, are generated during OXPHOS (60). TCA takes place in the mitochondria, and pyruvate is transported into the mitochondrion by specific transporters. The committed and irreversible step of entry into the TCA cycle is the decarboxylation of pyruvate to acetyl-CoA by the pyruvate dehydrogenase (PDH) complex. PDH is tightly regulated, in part by feedback inhibition, and both acetyl-CoA and NADH inhibit PDH activity. However, the key regulatory process is inhibition of PDH due to phosphorylation by the enzyme pyruvate dehydrogenase kinase (PDK).

There are four isoforms of PDKs with slightly different tissue distributions where PDK2 and PDK4 are the most widely distributed and PDK1 and PDK3 are less common (121). PDK is an important switch, as PDH activity not only regulates the formation of lactate but also the shift between glycolysis and fatty acid oxidation as being the source for substrate in the TCA. Peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1 (PGC-1) and activation of the glucocorticoid receptor are both known to induce the expression of PDK4, while insulin seems to downregulate PDK4 expression (122, 123). Several pharmacological inhibitors of PDK exist, with one of the most thoroughly investigated being the small molecule dichloroacetate (DCA), which effectively inhibits PDK activity (124). Several studies have been conducted with DCA, mainly reprogramming the metabolic profile of cancer cells (124, 125).

TCA is a set of reactions where the two-carbon unit acetyl-CoA is completely oxidized to CO_2 and H_2O (Figure 2). Intermediate steps include the metabolism of citrate, isocitrate, α-ketoglutarate, succinyl-CoA, succinate, fumarate, malate and oxaloacetate. Several of the intermediates are important biomolecules. Succinate has been shown to have immune-activating properties by stimulating IL-1β release (120). GPR91 has been shown to be selectively ligated by succinate (126), and succinate signalling through GPR91 has been argued to promote pro-inflammatory mechanisms and activate innate immune responses (127, 128).

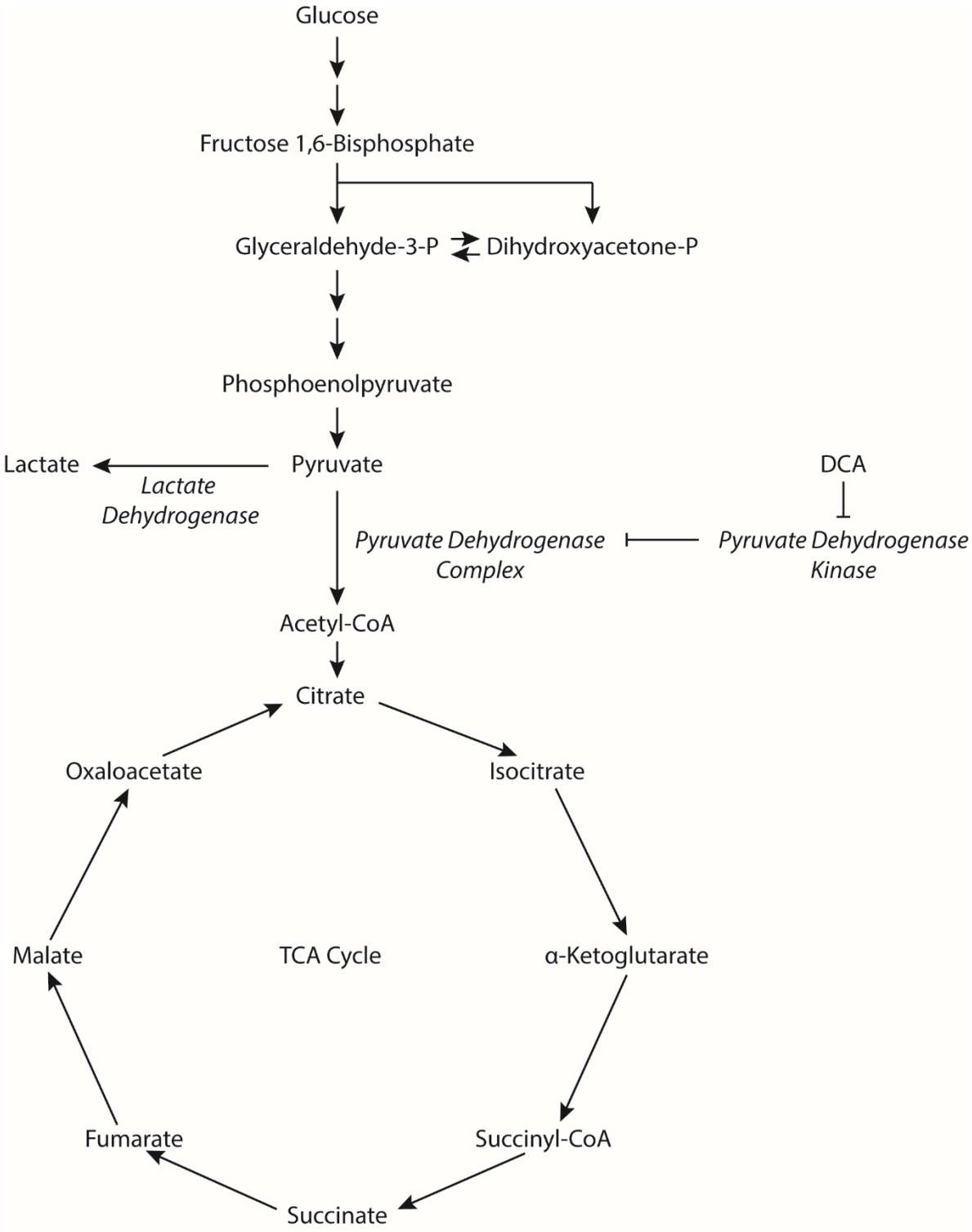

Figure 2. Glycolysis and the citric acid cycle. Glucose is metabolized in the glycolysis to pyruvate. Pyruvate may either enter the citric acid cycle or be used as substrate for lactate production. Dichloroacetate inhibits pyruvate dehydrogenase kinase, which in turn inhibits pyruvate dehydrogenase complex, the enzyme responsible for metabolizing pyruvate to acetyl-CoA. TCA, citric acid cycle; DCA, dichloroacetate; CoA, co-enzyme A.

3.3.3 The Warburg effect

The cellular metabolic switch to glycolysis and the formation of lactate during conditions of restricted oxygen is widely known and termed anaerobic glycolysis. However, in 1926 Otto Warburg et al. published the observation that tumours rely on glycolysis and lactate formation

even during normoxia (129). This phenomenon of a preference for glycolysis and lactate formation over OXPHOS during normoxia is referred to as aerobic glycolysis or the Warburg effect. It is prevalent in rapidly dividing and metabolically active cells, such as tumour cells or activated immune cells. The rationale for using the less efficient way of generating ATP, glycolysis, over OXPHOS is the need for biomolecules to form new nucleic acids, membranes and organelles (130). Active glycolysis favours the intake of glucose and increases the availability of intermediate metabolites. Indeed, a clear dichotomy can be seen in immune cells where naïve lymphocytes, T_{Reg} cells, and M2-like macrophages employ OXPHOS while activated T cells and M1-like macrophages activate glycolysis (131, 132). The metabolism of Trp by IDO has the ability to shift T-cell metabolism towards increased OXPHOS, possibly through the action of GCN2 (133, 134).

4 THE ATHEROSCLEROTIC PLAQUE

The most prominent feature of atherosclerosis and the feature that causes symptoms is the atherosclerotic plaque. The initial event of atherosclerotic plaque formation is the retention of LDL and ApoB-containing remnant lipoprotein particles in the intimal layer of large- and medium-sized arteries (135, 136), a process where proteoglycans seem to play a pivotal role (137, 138). Local factors predisposing towards lipoprotein retention and plaque formation are low shear stress and turbulent and oscillating flow (139). The mechanisms governing lipoprotein uptake in the endothelium are incompletely understood but involve, in part, specific receptors such as SR-BI and caveolin-1 (140, 141). Once retained, lipoprotein particles may undergo modifications such as aggregation or oxidation, generating highly immunogenic oxidized LDL particles (oxLDL) (142, 143). While oxidation improves macrophage uptake of the LDL particle and oxLDL is immunogenic, epitopes on native ApoB have also been shown to give rise to strong immune responses (144) and clinical trials of antioxidants in atherosclerosis have so far not yielded desired results (145-147).

Retention of lipoprotein particles in the intima activates the endothelium, induces expression of adhesion molecules such as vascular adhesion molecule 1 (VCAM-1), and leads to the recruitment of immune cells propagating an inflammatory and maladaptive response (148). Through the recruitment and proliferation of leukocytes by continuous pro-inflammatory processes, the plaque grows.

Symptoms of atherosclerotic disease arise either when the growing plaque obstructs the lumen of the artery to such a degree that distal blood flow is inadequate, as in chronic coronary syndromes (CCS; stable angina pectoris) and in peripheral arterial disease of the limbs. Alternatively, acute symptoms may arise from plaque rupture, with the exposure of pro-thrombotic material to the circulation causing an intraluminal thrombus formation that quickly obstructs blood flow causing end organ ischaemia. Examples of conditions caused by plaque rupture are acute coronary syndromes (ACS) including myocardial infarction in the case of plaque rupture in the coronary arteries, stroke when plaque rupture occurs in the carotid or intracranial arteries and bowel ischaemia with plaque rupture in mesenteric arteries.

Interestingly, the proportion of acute events due to plaque rupture is suggested to be declining, while other causes of thrombus formation on atherosclerotic plaques, such as endothelial erosion, are increasing, possibly due to increased statin use (149) (Figure 3).

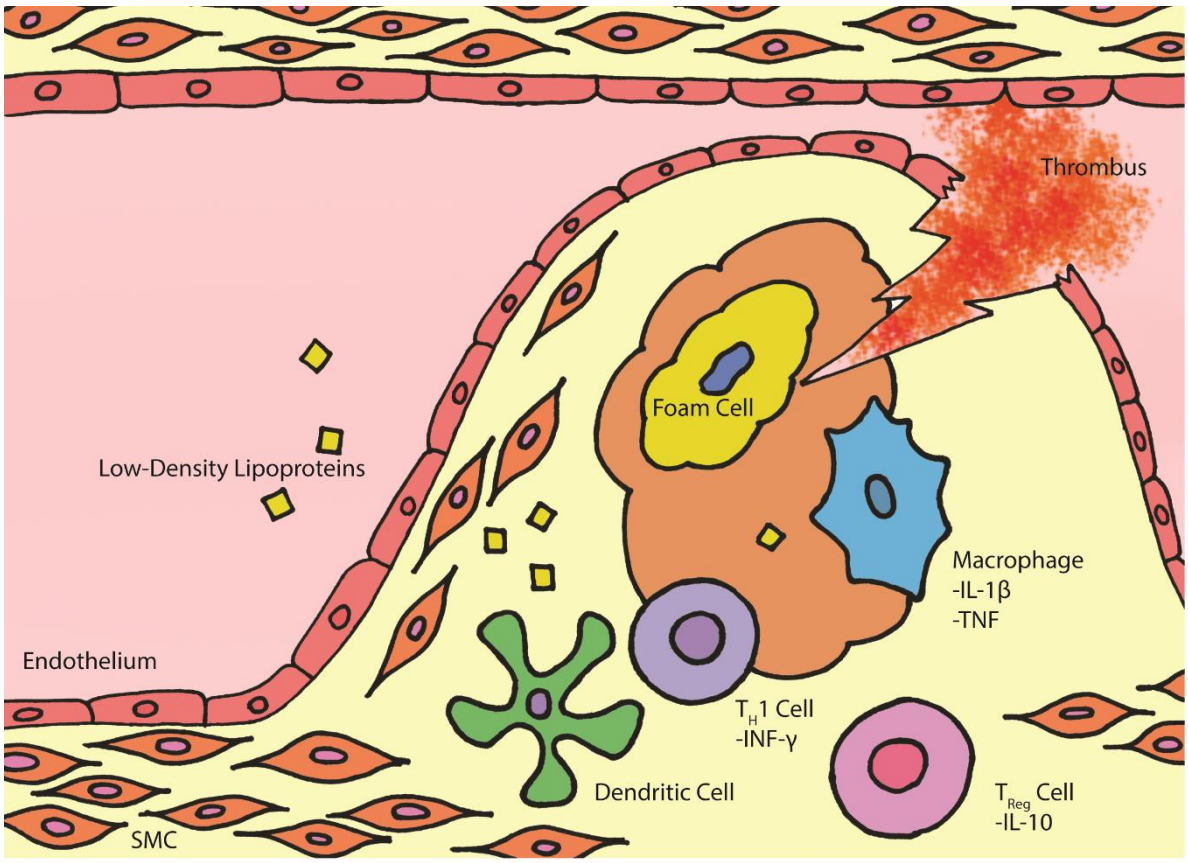

Figure 3. Overview of an atherosclerotic plaque. Low-density lipoproteins are retained in the intima. Immune cells such as monocytes, macrophages and T cells are recruited to the site. Macrophages may develop in to lipid laden foam cells. Depending on local environmental conditions, both pro-inflammatory cells such as T_H1 cells and tolerogenic cells such as T_{Reg} cells occur in plaques. Smooth muscle cells infiltrate the plaque and form a fibrous cap. If the fibrous cap ruptures, thrombogenic material is exposed to the bloodstream and a thrombus may form, causing end-organ ischaemia. INF, interferon; TNF, tumour necrosis factor; IL, interleukin; SMC, smooth muscle cell.

4.1 IMMUNE CELLS IN THE ATHEROSCLEROTIC PLAQUE

Atherosclerosis is a chronic inflammatory disease (3). The initial retention of cholesterol-rich lipoprotein particles and endothelial activation recruit mainly monocytes and macrophages which are the most prominent cells of the atherosclerotic plaque. Macrophages in atherosclerotic plaques originate both from differentiated monocytes recruited from the circulation and the proliferation of local macrophages, with the latter being the predominant factor (150). Plaque macrophages take up oxidized and native LDL, in part by scavenger receptors such as CD36 (151) and SR-A (152). The loading of macrophages with modified LDL saturates the cytoplasm with cholesterol, turning the macrophages into “foam cells”, hallmark cells of fatty streaks and atherosclerotic plaques (153). The importance of scavenger receptors such as CD36 *in vivo* has, however, been questioned, as results from experiments in mouse models are not uniform and it has been reported that *ApoE* $^{-/-}$ *CD36* $^{-/-}$ mice have larger

lesions than with *ApoE*$^{-/-}$ mice (154). Furthermore, patients with CD36 deficiency were demonstrated to have an increased risk of coronary artery disease (CAD) in a small study (155).

A factor other than uptake that influences macrophage lipid loading is macrophage cholesterol efflux. Important mediators of cholesterol efflux from macrophages to HDL particles are ABCA1 and ABCG1, and genetic ablation of these genes in macrophages leads to more severe atherosclerosis in mice (156, 157). Another possible mode for cholesterol efflux out of the atherosclerotic plaque is the migration of cholesterol-laden macrophages out of the intima, and it has been shown that local factors in the plaque prevent macrophages from migrating out, trapping them in the plaque (158). The uptake of oxLDL has been shown to induce several forms of cell death, including immunologically inert apoptosis, and necrosis and pyroptosis which generate immunogenic material (159-161). The accumulation of dead cells and debris, in conjunction with impaired macrophage efferocytosis observed in atherosclerotic plaques (162), leads to the formation of a necrotic core.

The loading of macrophages with oxLDL may also activate PRRs. Influx of oxLDL via CD36 is known to prime the NLRP3 inflammasome by inducing NF-κB (163, 164). Furthermore, the accumulation of cholesterol in the cytosol of macrophages leads to the precipitation of cholesterol crystals, which are well-known activators of the NLRP3 inflammasome (165). The role of the NLRP3 inflammasome in atherosclerosis is underscored by the finding that transfer of bone marrow cells genetically ablated for components of the NLRP3 inflammasome (either ASC, NLRP3 or IL-1α/β) abolishes plaque formation in *Ldlr*$^{-/-}$ mice (166). Curiously, the same strong effect was not observed in *ApoE*$^{-/-}$ mice (167). Studies in human cohorts show an increase in components of the NLRP3 inflammasome in atherosclerotic plaque material (168). The product of inflammasome activation, IL-1β, is also closely linked to atherosclerosis. IL-1β has been observed in human coronary atherosclerosis, and its expression correlates with the severity of disease (169). Mechanistic studies in *ApoE*$^{-/-}$ mice revealed that blockade or genetic ablation of IL-1β decreased fatty streak and plaque formation (170, 171). The final proof of concept that IL-1β is a valid target for atherosclerosis treatment came with the CANTOS trial in which inhibition of IL-1β by the monoclonal antibody Canakinumab decreased cardiovascular events (15).

Macrophages in atherosclerotic plaques display traits of several different subsets. Pro-inflammatory and matrix-degrading functions normally associated with an M1-like phenotype are associated with worsened disease, and M1-like macrophages have been linked to symptomatic plaques both in carotid and coronary arteries (172, 173). The distribution within the plaque also points towards a protective role of M2-like macrophages, which are often seen in the fibrous cap that prevents rupture, while M1-like macrophages are seen in the rupture-prone shoulder regions (174). M1-like and M2-like macrophages normally constitute 40% and 20%, respectively, of macrophages in mouse atherosclerotic lesions, thus allowing for a large proportion of less common and atherosclerosis-specific macrophages such as Mox macrophages (175).

The determining factor for macrophage polarization is the local plaque milieu, where T_H1 cytokines such as INF-γ promote an M1-like phenotype. The two polarizing cytokines M-CSF and GM-CSF have been described to play important and partly opposite roles in the vascular wall, where the latter is a known M1-like polarizing cytokine while the former is suggested to induce a more M2-like phenotype (176, 177). Interestingly, foam cells that normally display markers of both M1-like and M2-like phenotypes, are less prone to polarize towards M1-like functions compared to non-foamy macrophages, suggesting the uptake of lipids to be a protective response (178).

DCs are found in the vascular wall and more abundantly in atherosclerotic plaques where they play an important role (179). In an indirect manner, DCs may affect atherosclerotic plaques by lowering plasma cholesterol and increasing lipoprotein clearance via T-cell dependent antibody-mediated effects (180). As the main activators of the adaptive immune system, DCs are believed to be important in mobilizing T-cell responses in atherosclerotic plaques, and genetic ablation of the co-stimulatory molecules CD80/CD86 resulted in decreased early lesion formation in *Ldlr*$^{-/-}$ mice (181). As co-stimulatory molecules are found on several types of APCs, the contribution to the phenotype of DCs versus that of macrophages remains to be demonstrated.

A study targeting MyD88, the main adaptor protein involved in TLR activation, in DCs showed that the transfer of MyD88-deficient CD11c cells into *Ldlr*$^{-/-}$ mice resulted in more pronounced lesions (182). A likely mechanism causing the increased lesion development would be that while MyD88 deficiency inhibited T_H1 effector cells, it would also inhibit T_{Reg} cells, and indeed, a decreased T_{Reg} activity was seen in the plaques supporting the notion that T_{Reg} inhibiting functions outweigh the pro-inflammatory functions of effector T cells in this setting. The ability of DCs to shape immune responses has also been suggested as a potential therapeutic approach for atherosclerosis with the administration of tolerogenic DCs, for example, by taking advantage of the IDO-mediated induction of T_{Reg} cells (183).

In addition to myeloid cells such as macrophages and DCs being common in the plaque, the pro-inflammatory environment induces changes in vascular smooth muscle cells (SMCs). During the formation of the atherosclerotic plaque, media SMCs infiltrate the growing lesion, and are primarily found in the fibrous cap, where they have a stabilizing function. Collagen secreted by vascular SMCs improves the mechanical stability of the fibrous cap and the plaque while degradation of mature collagen increases vulnerability (184). The pro-inflammatory milieu of the atherosclerotic plaque induces vascular SMCs to change to a more myeloid phenotype, losing SMC markers and gaining phagocytic abilities (185).

Adaptive immune responses are highly active in atherosclerotic plaques. T cells are found in high proportion, particularly in the fibrous cap and the shoulder region of the plaque constituting 22% and 18% of cells at each location (186). An active T-cell response is seen in plaque rupture (187). Immunodeficient mice without lymphocytes (scid/scid mice) display a dramatic decrease in atherosclerotic plaque formation (188). Depletion of $CD4^+$ cells leads to less lesion formation, and the pro-atherogenic effect of $CD4^+$ cells is dependent on the T_H1

subset (189). Both oxLDL and native LDL are potential sources of epitopes for T cells, and while increasing oxidation leads to decreased T-cell reactivity, this may be due to degradation of ApoB epitopes by excessive oxidation (143, 190). Aggregation of LDL particles correlates with T-cell activation *in vitro* and is associated with cardiovascular events in humans (191).

The main cytokine of the T_H1 response is INF-γ, which has been demonstrated to aggravate atherosclerosis in numerous studies. Deletion of the INF-γ receptor causes a decrease in atherosclerosis in both *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice (192, 193), and deletion of the T_H1-inducing transcription factor Tbet also induces less INF-γ production and less plaque formation (194). INF-γ signals mainly via the Janus activated kinase / signal transducer activator of transcription 1 (JAK/STAT1) pathway, and transfer of STAT1 deficient bone marrow results in reduced atherosclerosis in mouse models (195).

Congruent with the notion that atherosclerosis is a T_H1-mediated disease, signs of T_H2 activity, such as IL-4 and IL-5 production, are found to a much lesser extent than T_H1 in atherosclerotic plaques (196). The role of T_H2 responses in atherosclerosis is debated. While a skewing of the immune response towards T_H2 could limit detrimental T_H1 responses, individual T_H2 cytokines are shown to have both pro- and anti-atherogenic effects (197-199). Some of the protective effects of T_H2 cytokines are likely mediated through the generation of a beneficial B cell response.

T_H17 cells have been shown to have a highly pro-inflammatory role in several autoimmune diseases. The role of T_H17 in atherosclerosis is less clear. Hypercholesterolaemic atherosclerosis-prone *ApoE*$^{-/-}$ mice were found to have increased IL-17A, and developed less plaque upon treatment with anti-IL-17A antibodies (200). However, IL-17A is important for collagen synthesis and confers a TGF-β mediated plaque-stabilizing effect, and blockade of IL-17A leads to increased plaque vulnerability (201).

T_{Reg} cells are found in small numbers in atherosclerotic plaques but have a potent anti-atherogenic effect. The number of lesional T_{Reg} cells fluctuates with plasma cholesterol in a mouse model of atherosclerosis, where prolonged hypercholesterolaemia attenuates T_{Reg} infiltration (202). The depletion of T_{Reg} cells increases atherosclerosis (203), as does depletion of the T_{Reg} signature cytokine IL-10 in *ApoE*$^{-/-}$ mice (204). Genetic ablation of the T_{Reg}-secreted cytokine TGF-β also leads to larger and more inflamed plaques in mice, while TGF-β has a complex role also promoting plaque cellular proliferation (205). In human atherosclerosis, there is evidence that T_{Reg} cell numbers and functionality are reduced in patients with symptomatic coronary atherosclerosis (206), and low $CD4^+FoxP3^+$ counts increase the risk of myocardial infarction (207).

B cells were not considered to be of importance in atherosclerosis for a long time. Detection of B cells in atherosclerotic plaques is scarce (186); however in advanced plaques follicle-like structures, i.e., tertiary lymphoid organs, with aggregations of B cells, develop in the adventitia (208). In a set of elegant experiments, Caligiuri et al. demonstrated that splenectomy of *ApoE*$^{-/-}$ mice aggravated atherosclerosis but that this outcome could be reversed by adoptive transfer

of B cells (209). In seemingly conflicting results however, pharmacological deletion of B cells led to a decrease in atherosclerosis (210). In part, contradicting results in the field of B cells in atherosclerosis can be attributed to separate B cell subsets having different effects, for example, the recently discovered B_{Reg} cells have been shown to be protective in atherosclerosis in an IL-10 dependent manner (211).

Antibodies against oxLDL have been found in both human and experimental atherosclerotic plaques (212) and are thought to clear oxLDL and reduce atherosclerosis. Of particular importance for the atheroprotective effect of antibodies are naturally produced IgMs against oxLDL produced by B-1 cells and *Ldlr*$^{-/-}$ mice depleted of IgM show aggravated atherosclerotic disease (213). B-cell depletion with anti-CD20 antibody treatment, however, decreased atherosclerotic burden in mice, believed to be partially due to a preferential deletion of B-2 cells, and indeed reconstitution with B-2 but not B-1 abolished the effect of anti-CD20 treatment (214). In a recent study, the effect of germinal centre derived IgG was studied. With abolition of germinal centre IgG production, a decrease in plaque size was observed while SMC content was decreased and plaques were more prone to rupture, which further highlights the complex role of B cells in atherosclerosis (215).

4.2 TRYPTOPHAN METABOLISM IN ATHEROSCLEROTIC DISEASE

As a strong modulator of inflammatory responses, implications of Trp metabolism exist at several stages of atherosclerotic disease. The expression and activity of IDO is strongly upregulated by INF-γ, a key cytokine in atherosclerosis, and thus, kynurenines have been proposed as tools in the diagnosis and prognosis of atherosclerosis. Dysregulation of Trp metabolism and the Kyn pathway has been linked to several risk factors for CVD.

In a Finnish cohort, IDO activity, as measured by the Kyn/Trp ratio, had a positive correlation with risk factors for atherosclerosis, such as CRP and parameters of plasma cholesterol (216). A univariate correlation with intima-media thickness, an early marker of atherosclerosis, was also seen in females; however, when corrected for other risk factors the association was lost. A connection has also been made between kynurenine metabolites and type II diabetes mellitus. Plasma levels of Kyn, KynA and 3-hydroxykynurenine were elevated in patients with diabetic retinopathy (217). In hepatitis C infection, kynurenine metabolism has been suggested to correlate with insulin resistance, although a positive correlation was seen with both Trp and Kyn, and no correlation was seen with the Kyn/Trp ratio (218). The metabolite XA has been suggested to interfere with insulin signalling and synthesis (219) and has been found in elevated concentrations in urine in a small study of patients with type II diabetes (220). Additionally, KynA has been suggested to inhibit pro-insulin synthesis by islet cells *in vitro* (221). In the setting of type I diabetes mellitus, a disease marked by autoimmune destruction of pancreatic beta cells, the metabolism of Trp seems to have a protective effect. In streptozotosin-induced experimental type I diabetes, inhibition of IDO aggravated disease (222). IDO induction has been observed in human islets as a protective response to INF-γ (223). Taken together, while increasing levels are present in the pro-inflammatory setting of type II diabetes, the protective

role in type I diabetes speaks for IDO induction being a protective consequence rather than a cause of diabetes.

Kynurenines have been studied in relation to hypertension. Treatment with Kyn relaxed smooth muscle tension in porcine arterial rings. Furthermore, after induction of IDO with INF-γ, the same effect was observed with Trp, an effect believed to be endothelium dependent but lost with concomitant treatment with 1-MT (224). Kyn also reduced blood pressure in hypertensive rats. Hypotension is a fatal complication of sepsis, and increased IDO activity was observed in septic patients compared to healthy subjects, while IDO activity was also correlated to the need for inotrope medication in septic shock (225). In a mouse model of endotoxaemia, IDO knockout mice ($IDO^{-/-}$) were observed to have higher survival, and in human experimental endotoxaemia, IDO activity correlated with hypotension (226, 227). KynA has also been implicated in the regulation of blood pressure, where injections of KynA in hypertensive rats reduced blood pressure, and mutations in the genes encoding KYATs are seen in spontaneously hypertensive rats (228, 229).

Modulation of IDO has been implicated in the regulation of plasma lipids. In an early exploratory study of $IDO^{-/-}$ on a BALBc background, a dramatic increase in plasma TAG was noted (230). We have shown that treatment of *Ldlr*$^{-/-}$ mice with the metabolite 3-HAA decreases both plasma cholesterol and TAG and provides a more favourable lipoprotein profile (231). The mechanism behind this effect is explored in **Paper II,** while **Paper I** demonstrates effects on plasma lipoproteins by pharmacological IDO inhibition.

Epidemiological studies have linked IDO activation to atherosclerotic disease. A decrease in Trp and an increase in the Kyn/Trp ratio were observed in patients with CAD compared with healthy controls (232). In patients with CAD, manifested as CCS, a high Kyn/Trp ratio was associated with an elevated risk of cardiovascular events and all-cause mortality, particularly in the quartile of patients with the highest Kyn/Trp ratio (233). Furthermore, the same research group demonstrated that the Kyn metabolites KynA, 3-hydroxykynurenine, AA and 3-HAA correlated with an increased risk of myocardial infarction in patients with CCS, a risk that was even more pronounced in those with concomitant diabetes or pre-diabetes (234). The urinary Kyn/Trp ratio was found to correlate strongly with both myocardial infarction, major coronary events and all-cause mortality, while surprisingly, no correlation was seen with ischaemic stroke (235). Additionally, in previously healthy elderly subjects, an elevated Kyn/Trp ratio, level of Kyn or level of 3-hydroxykynurenine at baseline predicted future coronary events (236, 237). KynA has been reported to increase the risk of all-cause mortality or recurrent myocardial infarction in a cohort of patients admitted for myocardial infarction (238). One large clinical trial connected to Kyn metabolites in CAD has been conducted. Oral treatment with Tranilast, a 3-HAA synthetic analogue, was used to prevent restenosis after percutaneous coronary intervention. No effect was seen in the primary outcome of death, myocardial infarction or revascularization, although a non-significant decrease in myocardial infarction was seen (239). While data on IDO activity in relation to stroke development are less clear than coronary

atherosclerosis, the Kyn/Trp ratio has been reported to correlate with stroke severity and infarct volume (240).

In an alternative approach by Li et al., the relationship between the Kyn pathway activity and CVD was investigated by means of Mendelian randomization. By this approach, IDO activity has a protective effect on the development of ischaemic heart disease, and a similar trend was seen for stroke (241). Interestingly, a similar trend towards protection was also observed for KYAT3. While seemingly contradictory to other epidemiological studies, the result of the Mendelian randomization approach strengthens the notion that IDO activity and Kyn metabolite levels are elevated in patients with CVD as a consequence rather than a cause of disease. Apart from experimental and clinical studies from other fields, a plethora of experimental investigations of IDO and Kyn in the vascular system support this notion. Indeed, the strong upregulation of IDO and Kyn metabolites upon inflammation as a feedback response highlights the need to differentiate the aim of individual studies. Studies aimed at discovering biomarkers may not be suitable for drawing conclusions regarding disease cause or mechanisms, and vice versa.

In **Paper I**, we were the first to describe the effect of pharmacological IDO inhibition on atherosclerosis. Shortly after, Cole et al. used genetic ablation of IDO in *ApoE*$^{-/-}$ mice to show similar results of increased atherosclerosis formation and aggravated plaque inflammation (242). Treatment of *Ldlr*$^{-/-}$ mice with 3-HAA reduced atherosclerotic plaque formation, reduced both T-cell and macrophage contents in plaques and downregulated T-cell markers in the spleen (231). The interplay between IDO-expressing DCs and T_{Reg} cell generation has been investigated in several publications. IDO-expressing pDCs are responsible for controlling T-cell proliferation and infiltration into atherosclerotic plaques, an effect that is abrogated by 1-MT, and inducing T_{Reg} cells that inhibit plaque inflammation (243, 244). Treatment of high fat diet fed mice expressing human ApoB with DCs loaded with ApoB and polarized with TGF-β to induce a tolerogenic phenotype resulted in an increase in arterial IDO, increased T_{Reg} cell numbers and reduced atherosclerosis and vascular inflammation, possibly through CTLA-4-mediated mechanisms (183). Surprisingly, one study reported increased IL-10 production and decreased atherosclerosis in IDO$^{-/-}$ mice (238). The same research group, however, recently published a report investigating the strong effect of gut microbiota on the IDO effect, which could provide one possible explanation for the differing results from different laboratories (245).

The effect of Trp metabolism on non-immune vascular cells is important for IDO effects in atherosclerosis. Cuffy et al. demonstrated that vascular SMC IDO activity is integral for maintaining tunica media immunoprivilege (246). In a model of allografting, they showed that during intact IDO function, vascular SMCs prevent lymphocyte infiltration of the media; however, this effect is lost upon 1-MT treatment. 3-HAA is suggested to act by inducing haemoxygenase-1 expression in endothelial cells by activating the transcription factor NF-E2 related factor 2, leading to decreased activation of NF-κB and CCL2 secretion by endothelial cells (117).

4.3 METABOLISM IN ATHEROSCLEROTIC DISEASE

The fact that cancer cells and activated immune cells such as M1-like macrophages and T effector cells have an increased rate of glycolysis and thereby increased glucose consumption has routinely been used for the discovery and monitoring of neoplastic and infectious diseases by means of positron emission-tomography for a long time. It was noted early that atherosclerotic plaques display a positive signal when labelled glucose is used, most commonly ^{18}F-deoxyglucose (FDG). Support for the inflammatory component of atherosclerotic disease and the connection to modified immune metabolism come from FdG imaging studies showing that increased glycolytic activity in the spleen and bone marrow is associated with an increased risk of future cardiovascular events (247). Investigating the arterial wall and atherosclerotic plaque specifically also shows that increased FdG uptake in the carotid artery is associated with increased ipsilateral stroke recurrence in patients with stroke, and increased FdG uptake in the ascending aorta is associated with subsequent CVD events in a cohort without previous CVD (248, 249). These studies demonstrate that not only is the atherosclerotic plaque detectable by its specific metabolism but also that the extent of the change to increased glucose metabolism is tied to plaque vulnerability.

Further evidence of an important metabolic component of atherosclerotic disease comes from metabolomic profiling of carotid artery plaques. Cluster analysis of metabolomics data separated plaques into one cluster containing highly glycolysing, reduced lipid oxidation group of plaques and a second group of plaques without increased glycolysis that maintained lipid oxidation (250). These two groups were shown to highly correspond to symptomatic and asymptomatic plaques, with the symptomatic plaques being the group with increased glycolysis. Analysis of individual metabolites revealed a decreased hexose concentration and increased lactate in the symptomatic plaques, and increased expression of glycolytic enzymes, both pointing towards a shifted metabolism in vulnerable plaques. Experimental data support this notion, where the blocking of pathways necessary for glycolysis, such as GLUT1 glucose transport, demonstrates a decrease in atherosclerotic burden in *ApoE*$^{-/-}$ mice (251).

Metabolic pathways other than glucose metabolism, such as lipid metabolism and lipoproteins, are also connected to modulation of the immune response. It is known that a high-cholesterol western diet (WD) induces inflammation in *Ldlr*$^{-/-}$ mice. Through elegant work, Christ et al. demonstrated that WD feeding and hyperlipidaemia metabolically reprograms the immune system in *Ldlr*$^{-/-}$ mice, making myeloid cells from WD-treated mice more reactive even long after hypercholesterolaemia has subsided (252). This metabolic reprogramming was dependent on NLRP3 function. Interestingly, the sterol metabolism regulator SREBP-2 has been shown to regulate IL-1β release (253), and SREBP-2 induced in the endothelium has experimentally been linked to NLRP3 activation and plaque formation in mice (254), and SREBP-1 has been shown to induce NLRP3 in the aorta of atherosclerotic pigs (255).

5 AIMS

The aim of this thesis is to study immunometabolic mechanisms in atherosclerosis, including the kynurenine pathway and glucose metabolism.

The specific aims for the included papers were as follows:

Paper I: To determine the role of IDO-mediated Trp metabolism in atherosclerosis.

Paper II: To clarify the mechanism by which 3-HAA affects vascular inflammation and lipoprotein metabolism.

Paper III: To characterize and understand the role of the kynurenine pathway in human atherosclerosis.

Paper IV: To investigate the effect of the metabolic reprogramming of immune cells through the inhibition of PDK in atherosclerosis.

6 METHODOLOGICAL CONSIDERATIONS

6.1 MOUSE MODELS IN ATHEROSCLEROSIS

Mice are widely used in research, and the characteristics of common strains, such as C57Bl/6 mice are well known and the whole genome has been sequenced. Wild-type mice do not develop atherosclerosis, mainly due to their lipoprotein profile being different from humans. Mice circulate a majority of their plasma cholesterol in the HDL fraction, while in humans, most cholesterol is found in the LDL fraction. The progression in humans from fatty streak to complex atherosclerotic plaque takes several decades, much longer than the lifespan of a mouse. To develop atherosclerosis, genetic manipulation and modification of the mouse lipoprotein profile is necessary. Two knockout mouse strains, *ApoE*$^{-/-}$ mice (256) and *Ldlr*$^{-/-}$ mice (257), are the most widely used models for atherosclerosis. The use of mouse models offers several advantages to mechanistic research, including well-established inbreeding, which reduces experimental variability, fast reproductive cycles and the option to perform mechanistic experiments that would not be technically possible or ethically feasible in humans.

ApoE$^{-/-}$ mice develop hypercholesterolaemia, mainly due to inhibited clearance of chylomicrons, VLDL and intermediate lipoprotein particles. Thus, a majority of cholesterol in *ApoE*$^{-/-}$ mice is found in these lipoprotein fractions. Plaque development in *ApoE-/-* mice occurs even on a chow diet; however, atherogenesis can be accelerated by feeding a high-cholesterol diet. The distribution of plaque formation follows the distribution seen in humans, with sites of turbulent flow such as the minor curvature of the aorta being more affected. The development of atherosclerotic plaques in *ApoE*$^{-/-}$ mice goes through all stages of development found in humans, from fatty streak to fibrous or complex plaques with necrotic cores. The most prominent difference between rodent disease and human atherosclerosis is that plaques in mice do not rupture spontaneously. Another drawback of the *ApoE*$^{-/-}$ mouse model is that myeloid cells with intact ApoE synthesis secrete enough ApoE to mitigate hypercholesterolaemia, thus limiting the use of *ApoE*$^{-/-}$ mice in bone marrow transfer experiments.

Deletion of *Ldlr* in mice also induces hypercholesterolaemia, due to reduced clearance of VLDL and LDL from the circulation. The hypercholesterolaemia of *Ldlr*$^{-/-}$ mice is, however, not as pronounced as that in *ApoE*$^{-/-}$ mice, and *Ldlr*$^{-/-}$ mice display an approximate doubling of plasma cholesterol compared to wild-type mice. Lipoprotein profiles of *Ldlr*$^{-/-}$ mice are more similar to the situation seen in most humans, with more cholesterol found in the LDL fraction. Fed a chow diet, *Ldlr*$^{-/-}$ mice do only develop fatty streaks and occasionally very early atherosclerotic lesions. On a high-fat and high-cholesterol diet, *Ldlr*$^{-/-}$ mice develop plaques at sites similar to human atherosclerosis, and while complex plaques are seen, progression of lesions is not as pronounced as that seen in *ApoE*$^{-/-}$ mice. Similar to *ApoE*$^{-/-}$ mice, the plaques do not rupture spontaneously. The deletion of Ldlr complicates the study of metabolic processes that potentially involve the uptake and binding of LDL particles.

In **Paper I**, *ApoE*$^{-/-}$ mice were used to study the role of IDO in vascular inflammation and atherosclerotic plaque development. For **Paper II**, *Ldlr*$^{-/-}$ mice were used. In **Paper IV**, *ApoE*$^{-/-}$ mice were used, and in **Paper III**, only wild type C57Bl/6 mice were used.

6.2 PATIENT COHORT

Human blood, tissues, plaque mRNA data and proteomics data from the Biobank of Karolinska Endarterectomies (BiKE) were used in **Paper III** and **Paper IV**. The biobank enrols patients who undergo endarterectomy of atherosclerotic plaques in the carotid artery due to symptomatic (minor stroke, transient ischaemic attack or amaurosis fugax) and asymptomatic high-grade stenosis according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) (258). BiKE encompasses plaque material, plasma samples both locally from the plaque and from peripheral sites, and patient clinical data. Snap-frozen plaque material that was processed and then purified for mRNA assessed by chip array was evaluated for gene expression in **Paper III**, along with plasma and sections of paraffin embedded plaque tissue. In **Paper IV**, plaque material retrieved from BiKE was used for proteomic evaluation, and fresh plaque tissue was cultured for *in vitro* assays.

6.3 EVALUATION OF EXPERIMENTAL ATHEROSCLEROSIS

Experimental atherosclerosis was quantified using two methods. In **Paper I**, **Paper II** and **Paper IV** *en face* staining of thoracic aorta with Sudan IV was used, with subsequent quantification of the stained area by manual measurement using the ImageJ software. Plaque quantification was also carried out in the aortic root, the most proximal part of the ascending aorta. Frozen sagittal sections of the first 800 µm of the aorta were stained for lipid content with Oil Red O, and the stained area was quantified manually on digital micrograph images for each level of the aorta. Evaluation of plaque composition, stability and inflammation was performed by immunohistochemistry staining of frozen aortic root sections for vascular and immune cells, such as $CD68^+$ macrophages, $CD4^+$ T cells, alpha smooth muscle actin-expressing SMCs, and VCAM-1.

6.4 LIPOPROTEIN PROFILES

Lipoprotein profiles were determined from mouse plasma after fractionation by size exclusion fast protein liquid chromatography (FPLC). Detection of total cholesterol and TAG in the fractionated material was performed by colourimetric reactions from commercially available kits. Profiles of ApoB-containing lipoproteins secreted by HepG2 cells were analysed in cell supernatant. The collected supernatant was concentrated by centrifugation in filter centrifuge units before fractionation by FPLC. Detection of ApoB-containing lipoprotein particles was carried out by enzyme-linked immunosorbent assay (ELISA).

6.5 STATISTICAL ANALYSIS

To analyse data where a Gaussian normal distribution could not be assumed, the Mann-Whitney U-test was carried out to determine the statistical significance of differences between two groups. In instances with more than two groups, Kruskal-Wallis one-way analysis of

variance with Dunn's multiple comparisons post hoc test was performed. Correlations between two variables that could not be assumed to have a normal distribution were assessed by Spearman's rank correlation coefficient (**Paper I** and **Paper II**). The statistical significance of differences between two groups with a normal distribution was calculated by paired or unpaired Student's t-test. Linear correlation between two normally distributed variables was presented as the Pearson linear correlation coefficient. In **Paper III**, a binary logistic regression model was produced to evaluate the effect of gene expression on symptoms from carotid atherosclerotic plaques. Where correction for multiple comparisons was indicated, the Bonferroni method of correction was used. Acceptable type I error was set at 0.05 (5%), and in the planning of animal experiments, the type II error was set to 0.8 (80%). All statistical calculations were performed with either GraphPad Prism (GraphPad Software Inc., USA) or SPSS (version 26.0.0, IBM Corporation, USA).

7 RESULTS AND DISCUSSION

7.1 IDO-MEDIATED TRYPTOPHAN METABOLISM REGULATES ATHEROSCLEROSIS FORMATION

In **Paper I**, we investigated the effects of IDO activity on atherosclerosis formation. Ample evidence existed, both from experimental studies and other inflammatory diseases where IDO had a protective role, as well as epidemiological data that linked IDO to CVD events. A pharmacological approach was used in which the IDO inhibitor 1-MT was administered in drinking water to *ApoE*$^{-/-}$ mice fed a western diet.

Inhibition of IDO aggravated atherosclerotic disease and plaque formation in *ApoE*$^{-/-}$ mice both in the aortic arch and the aortic root. Mice treated with 1-MT had more CD68^{+} macrophage infiltration into the plaques and expressed more VCAM-1. Expression of the pro-inflammatory cytokines CCL2, CXCL10 and TNF was increased in the aortas of treated mice. Surprisingly, a strong upregulation of VCAM-1 was seen not only in the plaque but also in the tunica media of treated mice, and medial VCAM-1 expression correlated strongly with plaque size. Only modest changes in plasma lipids were seen, particularly a decrease in HDL cholesterol.

We had previously shown that administration of the Kyn metabolite 3-HAA protected against atherosclerosis and reduced inflammation (231). In an attempt to reverse the effects seen with 1-MT treatment, a second experiment was run in which 1-MT-treated mice were given back 3-HAA or vehicle via intraperitoneal injection. Simultaneous treatment with 3-HAA completely reversed the effect of 1-MT treatment on lesion size, CD68^{+} macrophage infiltration and VCAM-1 expression both in plaques and in the media.

As a result of the observed increase in VCAM-1 in the media in IDO-inhibited mice, an *in vitro* system with human coronary SMCs was set up to further investigate the effects of IDO inhibition on SMCs. The cultured human SMCs displayed a similar upregulation of the VCAM-1 response to INF-γ stimulation with 1-MT treatment as that observed *in vivo*. Treatment of the cultured cells with 3-HAA inhibited the effect of 1-MT.

Activation of the endothelium and expression of cellular adhesion molecules such as VCAM-1 is an early step in atherosclerosis development and is necessary for the recruitment of immune cells into the forming plaque (148, 259). Not only VCAM-1 expressed on endothelial cells, but also VCAM-1 on SMCs are suggested to influence atherosclerotic plaque formation (260). While the observed VCAM-1 expression could be a result of increased inflammatory burden, it could also be implicated in the formation of the atherosclerotic lesion. Trapping of macrophages in the vascular wall has been suggested to maintain a pro-inflammatory environment and to inhibit cholesterol efflux from the vasculature, and the expression of VCAM-1 by SMCs is proposed to be one mechanism retaining macrophages in the plaque (261).

IDO is a known regulator of T-cell function *in vitro* and *in vivo*. In **Paper I**, however, mostly innate immune cells such as macrophages, and vascular cells were affected by 1-MT treatment.

Staining of T cells in plaques, expression of T-cell markers in spleen and splenocyte proliferation rates were not affected by 1-MT treatment. As mice were sacrificed at the end of the experiment at 20 weeks of age after 8 weeks of treatment, it is possible that changes in the T-cell response were in effect earlier during the treatment. Changes in adaptive immune responses could modulate innate immune responses and lesion formation, thus having an impact on the results seen; however no certain effects on adaptive immunity could be seen in **Paper I**.

Inhibition of IDO was achieved with the pharmacological inhibitor 1-MT. A mix of D and L isomers was used after initial pilot studies revealed the mix to be a more potent inhibitor of IDO in peripheral blood mononuclear cells (PBMCs) than the individual isomers. Effective systemic inhibition of IDO activity was confirmed by measuring the Kyn/Trp ratio in the spleen and duodenum. Although observations exist that would argue for specific roles of the different isomers where the L stereoisomer inhibits IDO1 (262) and only the D isomer inhibits IDO2 (263), more recent work has cast doubt over this assumption (264). It is also known that 1-MT may have effects not related to its inhibition of IDO (265). Shortly after the publication of **Paper I**, Cole et al. published a study using IDO$^{-/-}$ *ApoE*$^{-/-}$ mice where deletion of IDO had a similar effect, i.e., aggravating plaque formation, as was observed in **Paper I**. This strengthens the hypothesis that the observed results were due to IDO inhibition. Furthermore, while not mechanistically proving the involvement of IDO, the observation that replenishment of the Kyn pathway by treatment with 3-HAA completely reversed the effect of 1-MT also supports the involvement of the Kyn pathway in the regulation of atherosclerosis.

7.2 THE KYN METABOLITE 3-HAA ACTS BY INHIBITING SREBP-2 AND THE NLRP3 INFLAMMASOME

We previously demonstrated the atheroprotective effect of the Kyn metabolite 3-HAA (231). However, the mechanisms by which 3-HAA reduces vascular inflammation and lowers plasma lipids were not known. In **Paper II**, we investigated the metabolic and immunological pathways affected by 3-HAA. *In vitro* systems using the human hepatoma cell line HepG2 were set up to explore the metabolic effects of the metabolite, and mouse bone marrow-derived macrophages (BMDMs) were used to explore the mechanisms involved in innate immunity. Furthermore, a pharmacological inhibitor of the 3-HAA-degrading enzyme HAAO was used to explore the effects of endogenously produced 3-HAA in *Ldlr*$^{-/-}$ mice.

When treated with 3-HAA, HepG2 cells exhibited a dose dependent reduction in SREBP-2 transcription. Nuclear translocation of SREBP-2 (nSREBP-2) was also reduced. The expression of SREBP-1 was not affected; however, when stimulated with insulin, a known inducer of SREBP-1, treatment with 3-HAA also reduced SREBP-1 expression. Treatment with 3-HAA also reduced HepG2 secretion of ApoB-containing lipoproteins. Mouse BMDMs treated with 3-HAA showed a drastic inhibition of NLRP3 inflammasome activation, and the supernatant concentration of IL-1β was dose-dependently decreased. Curiously, the effect was present both when treatment with 3-HAA was carried out before LPS priming and when cells were treated only 30 minutes before activation with ATP. Investigation of cleaved caspase 1

by western blot showed a robust reduction in cleaved protein with 3-HAA treatment. To raise the level of endogenously produced 3-HAA, *Ldlr*$^{-/-}$ mice were treated with NCR-631, an HAAO inhibitor. Elevated endogenous 3-HAA levels resulted in reduced atherosclerosis in the aorta and lowered plasma lipids comparable to 3-HAA treatment. NCR-631 treatment did not affect plaque composition with regard to CD68^{+} macrophages or CD4^{+} T cells but did reduce aortic caspase 1, mainly uncleaved pro-caspase 1. In line with our *in vitro* results, NCR-631 treatment reduced hepatic SREBP-2 and HMGCR expression, while SREBP-1 expression was unchanged. NCR-631 treatment reduced the lipid content in the liver, but did not affect LDL clearance from the plasma.

SREBP-2 is a transcription factor that regulates cell sterol content, and increased activity corresponds to an increase in cell actions to acquire more sterols, both through synthesis and uptake. The increase in cholesterol uptake is utilized in statin treatment of hypercholesterolaemia, where inhibition of HMGCR induces the activation of SREBP-2 in hepatocytes, and the SREBP-2 induced upregulation of LDLR results in higher hepatic uptake of plasma lipoprotein particles (266). Treatment with 3-HAA also reduced the plasma lipoprotein content, but decreased SREBP-2 activity which suggests a different mode of action compared to statins. One plausible hypothesis is that 3-HAA reduces the synthesis of sterols by inhibiting SREBP-2. This hypothesis is supported by the observation that there was no increase in LDL clearance from plasma, while a lower cholesterol content was seen in the faeces of treated animals, although the observations were too few to draw robust conclusions. Furthermore, the reduced lipoprotein secretion in HepG2 cells could also be explained by reduced substrate synthesis. In **Paper II**, as in our earlier work with 3-HAA in *Ldlr*$^{-/-}$ mice (231), we see a clear effect on plasma lipids, while the effects of IDO inhibition on lipids in *ApoE*$^{-/-}$ mice in **Paper I** are only modest. Additionally, in models using genetic inhibition of IDO, mice with IDO$^{-/-}$ on an *Ldlr*$^{-/-}$ background displayed an increase in plasma lipids, mainly TAG (230), while IDO$^{-/-}$ on an *ApoE*$^{-/-}$ background was not reported to show major changes in plasma lipids (242). LDLR is a direct target regulated by SREBP-2, and it is possible that if the lipid-lowering effect of 3-HAA is mediated by SREBP-2 inhibition, a parallel inhibition of Ldlr transcription would counteract the lipid-lowering effect in *ApoE*$^{-/-}$ mice.

The regulation of SREBP-2 activity is active on several levels. As SREBP-2 transcription is partly regulated by a feed-forward mechanism (267), decreased nuclear translocation may further decrease the transcription of new SREBP-2. Interestingly, we found a similar effect on SREBP-2 expression in HepG2 cells when treated with a PDPK1 inhibitor as when treated with 3-HAA. One suggested target of 3-HAA is PDPK1 (115). The PDPK1 – AKT – mTOR pathway is also implicated in SREBP regulation, where activation of the pathway stimulates SREBP activation (268). Intriguingly, the NLRP3 inflammasome has also been proposed to be regulated by the AKT – mTOR pathway, possibly linking our findings of 3-HAA action on both SREBP-2 and NLRP3 inflammasome (269). Alternatively, SREBP-2 is suggested to have a direct effect on NLRP3 activity (254).The finding that 3-HAA inhibited inflammasomes both when treatment was given during priming, but also shortly before assembly induced by ATP would suggest, at least in part, a direct effect of 3-HAA on assembly of the inflammasome, as

there would be insufficient time for effects mediated by gene transcription. The inhibitor NCR-631 increases the endogenous levels of 3-HAA (270). As a decrease was seen in pro-caspase 1 expression in the aortas of NCR-631 treated mice, it is possible that 3-HAA affects both the priming and assembly of the inflammasome.

7.3 DEVIATION OF THE KYN PATHWAY AWAY FROM KYNA FORMATION AGGRAVATES HUMAN ATHEROSCLEROSIS

The role of the Kyn pathway and the balance of different branches within the pathway, in human atherosclerosis were explored in **Paper III**. Transcriptomic data from human atherosclerotic plaques available through a collaboration with the BiKE biobank were analysed for the expression of Kyn pathway enzymes. We found that the first common steps of the pathway, and the branch of the pathway leading to the formation of QA (the "QA branch") were upregulated in atherosclerotic plaques compared to healthy arteries, while the branch of the pathway leading to KynA (the "KynA branch") was downregulated. Correlations were made between the expression of Kyn pathway enzymes and the expression of pro- and anti-inflammatory genes. The main enzymes of the QA branch were found to have a positive correlation with pro-inflammatory and plaque-destabilizing genes, whereas enzymes of the KynA branch were found to have an inverse correlation with such genes and instead correlated with the expression of stabilizing and tolerogenic factors. Sections of plaque material were stained for the expression of Kyn pathway enzymes, and while a clear upregulation in sites of macrophage activity near the necrotic core could be seen for IDO and KMO from the common and QA branch of the pathway, respectively, KYAT1 from the KynA branch did not have the same staining pattern. Expression patterns of plaques from symptomatic and asymptomatic patients were compared, both at the mRNA and protein levels, and plasma was analysed for Kyn pathway metabolites. Symptomatic plaques showed a decrease in the expression of KYAT enzymes, both in mRNA and protein. Enzyme activity of the KynA branch was decreased in symptomatic patients, as seen by the lower KynA/3-HAA ratio. Interestingly, activity of the common branch, which includes IDO, was also lower in symptomatic patients as seen by the lower Kyn/Trp ratio.

Clinical parameters were retrieved, and a logistic regression model was set up to describe the effect of KYAT expression on the odds of being symptomatic or asymptomatic. A significant decrease in the odds of having presented with symptoms was seen with increasing expression of KYAT1. The molecular mechanism by which the Kyn pathway metabolite KynA acts was further explored *in vitro*. Treatment of macrophages generated from human PBMCs indicated that KynA has anti-inflammatory properties that are mediated via activation of the AhR. The anti-inflammatory properties of KynA were confirmed in a mouse model of peritonitis.

Activation of the Kyn pathway has previously been described in CVD in epidemiological studies (233-237). The expression of IDO has been shown in advanced atherosclerotic plaques (271). However, **Paper III** explores the specific intermediate steps of the Kyn pathway in atherosclerosis and how changes within the pathway affect the atherosclerotic plaque. One advantage of using material from the BiKE biobank is that when comparing symptomatic and

asymptomatic plaques, both groups have advanced disease that requires surgery. When studying the Kyn pathway, which is strongly upregulated by inflammation, there is always a question of whether observed differences between groups could be the result of underlying differences in inflammation. By comparing two groups that both have advanced disease, such as symptomatic and asymptomatic patients in BiKE, this problem could be partially mitigated. It is therefore interesting that we observed increased IDO activity, demonstrated by an increased Kyn/Trp ratio, in plasma from asymptomatic patients. This observation goes well with the finding of a recent Mendelian randomization study that found genetically instrumented IDO activity to be protective in ischaemic heart disease (241).

In **Paper III**, we demonstrate that a deviation from the KynA branch of the Kyn pathway, to the QA branch of the pathway, is associated with a more inflammatory plaque phenotype and higher odds of being a symptomatic patient. In **Paper I** and **Paper II**, and in earlier work (231), we demonstrated that 3-HAA, a metabolite within the QA branch of the Kyn pathway, is atheroprotective. As we did not evaluate all individual metabolites in **Paper III**, the properties of the particular metabolite 3-HAA could still be atheroprotective. Furthermore, the common branch of the Kyn pathway is upregulated in both asymptomatic and symptomatic patients. In **Paper I** and **Paper II,** we did not study how 3-HAA treatment affected the Kyn pathway, and it is possible that administration of a QA branch metabolite also changed the flux in the KynA branch of the pathway. In the study by Zhang et al. (231), however, we showed that 3-HAA treatment downregulated hepatic KYNU and HAAO expression, suggesting a possible role of 3-HAA in modulating Kyn pathway flux.

In immunohistochemistry stainings, we show that IDO and KMO are most upregulated in areas of plaques rich in macrophages and immune cells. This raises the question of in which cells of the atherosclerotic plaque does the observed deviation of the Kyn pathway take place. Using transcriptomic data from PBMCs from BiKE patients, we observed a weak trend towards a similar deviation of the Kyn pathway away from KynA formation in PBMCs from symptomatic patients, as was seen in plaque tissue (Figure 4).

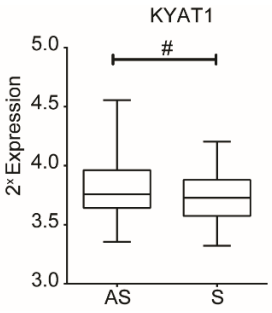

Figure 4: Expression of KYAT1 in PBMCs from BiKE. A trend towards decreased expression is seen. AS; asymptomatic. S; symptomatic. # $p<0.1$

To further investigate the deviation of the Kyn pathway in circulating myeloid cells, a publicly available data set, consisting of transcriptomic data from $CD14^+$ cells from a population study, with measurement of the coronary artery calcium (CAC) score (available through NCBI-GEO, http://www.ncbi.nlm.nih.gov/geo, GSE56045, deposited by Yongmei Liu, previously published (272)) was used. Subjects were stratified based on CAC-score as previously shown (273). In subjects with above median expression of KYAT1, there was a clearly reduced odds

ratio of having the highest category of CAC-score compared to subjects with below median KYAT1 expression (Table 2). However, the same pattern was not observed with KYAT2 (data not shown). These findings raise the question of whether deviation of the Kyn pathway is already present in circulating immune cells, in addition to locally in the atherosclerotic plaque, something that warrants future studies.

Table 2: High or low KYAT1 expression in a population study. "Median Low" denotes KYAT1 expression below the median, "Median High" denotes KYAT1 expression above the median. OR refers to the odds of a patient having the specified CAC-score comparing above the median expression level to below the median expression level.* $p<0.05$.

	No CAC	Low CAC	Intermediate CAC	High CAC	Total	Missing
Total	337	324	187	293	1141	61
Median Low	166	163	87	171	587	
Median High	171	161	100	122	554	
OR (CI95%)	1,132 (0,878-1,460)	1,066 (0,824-1,378)	1,266 (0,925-1,733)	0,687 (0,525-0,899)		
χ^2-test	0,338	0,628	0,141	0,006*		

7.4 METABOLIC REPROGRAMMING VIA PDK1 INHIBITION REGULATES VASCULAR INFLAMMATION

In **Paper IV**, we explored the role of metabolic reprogramming by targeting PDKs with the inhibitor DCA in atherosclerosis. We show that treatment of *ApoE*$^{-/-}$ mice on a western-type diet with DCA drastically reduces atherosclerotic burden. Furthermore, treatment with DCA reduced plaque inflammation, as demonstrated by a reduction in infiltrating CD68$^+$ macrophages and CD4$^+$ T cells while increasing plaque stability by increasing alpha smooth muscle actin and collagen in the plaque. A distinct change in immunological phenotype was observed upon DCA treatment, with a reduction in M1-like macrophage markers, upregulation of M2-like macrophage markers and downregulation of markers of T_H1-type responses in both spleen and aorta. Interestingly, we demonstrate that DCA has the ability to block NLRP3 inflammasome activation and IL-1β secretion from macrophages.

Metabolomic studies revealed, apart from an expected shift away from lactate production, that DCA treatment reduces cell accumulation of succinate. The addition of succinate abrogated the effects of DCA on NLRP3 inhibition. The reversal of the DCA effect on NLRP3 inflammasome by succinate was not seen when treated with a GPR91 antagonist, implicating the succinate receptor GPR91 signalling as a potential mechanism of succinate to increase inflammasome activity. Furthermore, we showed that DCA treatment decreased the expression of PDK in the spleen and aorta, in the latter particularly isoforms PDK1 and PDK4, but not in liver. In human carotid artery tissue, PDK1 protein expression was increased in plaque

compared to adjacent tissue, and DCA had a reducing effect on the secretion of pro-inflammatory cytokines by plaque cells cultured *in vitro*.

Similar to our study, a recently published study also found that DCA reduces the atherosclerotic burden in *ApoE*$^{-/-}$ mice (274). DCA was found to reduce white adipose tissue and induce thermogenesis and increase the expression of the brown adipose tissue marker uncoupling protein 1. The study suggested that the effect of DCA was at least partly mediated by hepatic expression of fibroblast growth factor 21. Additionally, in our study we observed changes not only in glucose metabolism but also in lipid metabolism, with a decrease in plasma cholesterol at the end of the study. The observed metabolic changes in lipid metabolism, both in the study of Min et al. (274) and in our study, are surprising considering that the activity of PDK has been argued to promote lipolysis (121). The observed ability of DCA to promote glucose oxidation without apparent accumulation of lipids warrants further investigation.

The connection between aberrant immune activation and increased lactate production is clinically well recognized, particularly in sepsis and septic shock. While there have been clinical trials of DCA treatment in septic patients, the most complete studies failed to show an effect on mortality and haemodynamic function (275). It is worth noting however, that the study of Stacpoole et al. included not only septic patients but all patients with severe lactic acidosis and that the inclusion criteria favoured the recruitment of patients with advanced disease and a poor prognosis. Experimental data argue for the ability of DCA to reprogram the immune response, inhibiting the maladaptive immune response in sepsis and improving the survival of mice undergoing caecal ligation and puncture, an experimental sepsis model (276). The reprogramming of the immune system observed in Paper IV could thus be applied in several other clinical settings beyond atherosclerosis.

7.5 CONCLUDING REMARKS

The idea that atherosclerosis is a chronic inflammatory disease is widely accepted today. Yet, no therapies routinely used in the clinical setting specifically target the underlying inflammatory responses driving atherosclerosis. The close link between metabolism and the immune response offers a new approach to investigate important mechanisms in the pathophysiology of atherosclerosis, and to discover new and better avenues for treatment.

Paper I, **II** and **III** of this thesis focus on the strong immune regulatory potential of Trp metabolism through the Kyn pathway. Inhibition of Trp metabolism results in increased inflammation and atherosclerosis. The Trp metabolite 3-HAA is shown to regulate sterol metabolism by inhibiting SREBP2 activity, and a deviation away from KynA production is seen in symptomatic carotid atherosclerosis.

In **Paper IV** the PDK inhibitor DCA was investigated in the context of atherosclerosis. Active immune cells, both T cells and macrophages, favours aerobic glycolysis and lactate production over TCA cycle, and treatment with DCA reduced atherosclerotic burden in mice. A summary of the findings in this thesis can be seen in Figure 5.

The field of immunometabolism, the study of the interplay between the traditional science disciplines of metabolism and immunology, is an emerging field of research. The present thesis highlights the potent immune-modulating potential that can be achieved from manipulation of one metabolic pathway, the Kyn pathway of Trp metabolism. The Kyn pathway has several features that makes it promising as a therapeutic target. The metabolites in the pathway are small molecules and as such more convenient as analogues for pharmacological agents than, for example, large proteins such as antibodies which are complicated to produce and to administer. For several enzymes in the Kyn pathway there are well-established pharmacological inhibitors that already are used *in vivo*, as seen in **Paper I** and **II**. The use of 1-MT in cancer therapy will hopefully add knowledge of the clinical implication of Kyn pathway modulation over the coming years. The present thesis highlights the need for careful cardiovascular surveillance in patients receiving IDO inhibitors. Furthermore, several metabolites of the Kyn pathway are quickly degraded and have short half-lives in plasma, which necessitates further research before they can effectively be used as pharmacological agents.

The reprogramming of the immune response in atherosclerosis after modulation of glucose metabolism with DCA observed in **Paper IV** can be generalized to several other settings outside of the field of CVD. Regulation of glucose metabolism, succinate availability and TCA flux is of interest in any inflammatory response. It is the hope and belief of the author that the insights gained from the studies in the present thesis, together with other work in the field of immunometabolism, will provide new approaches to treatment and prevention of not only atherosclerosis, but all inflammatory diseases.

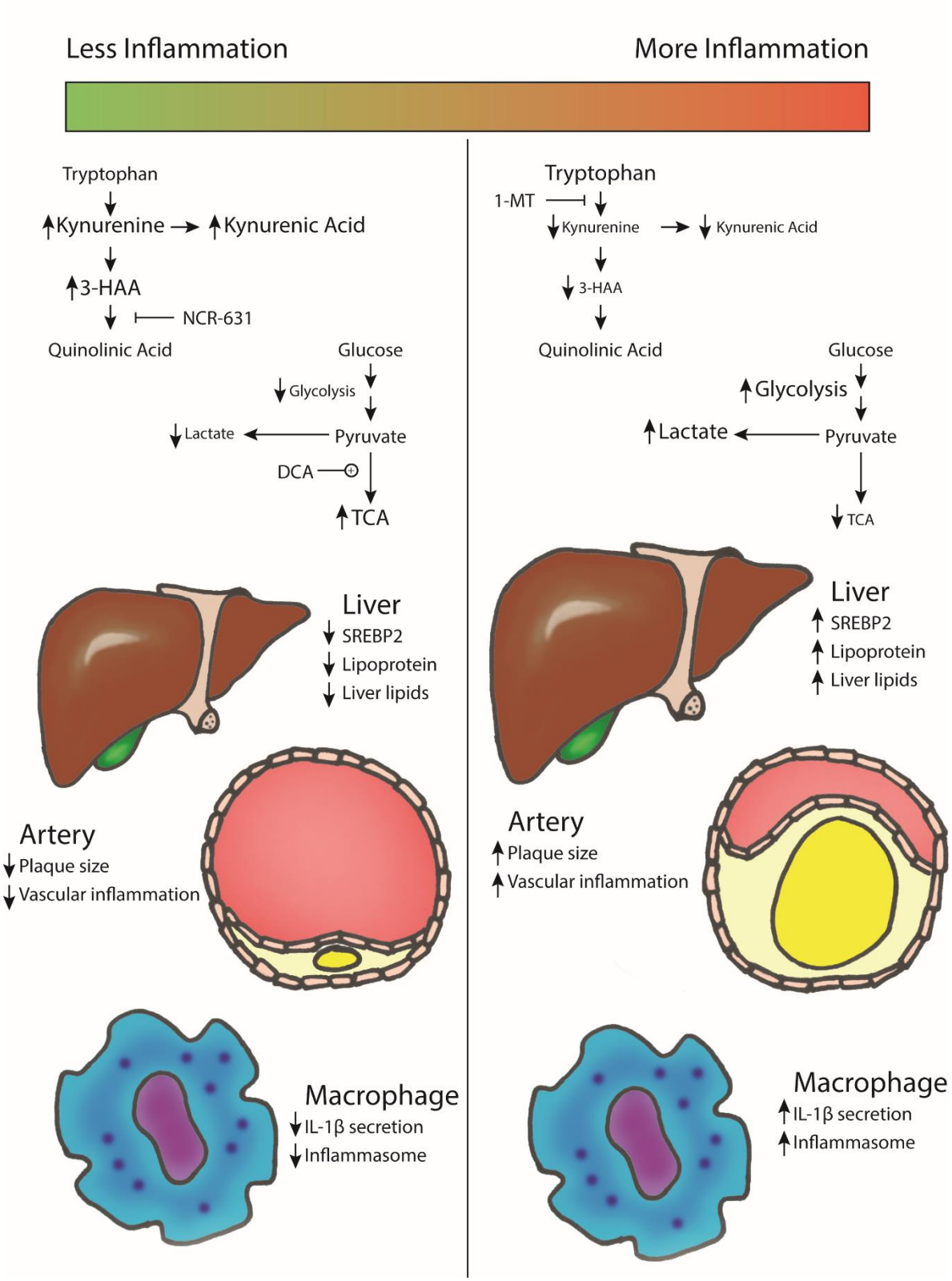

Figure 5. A summary of findings in the current thesis. Modulation of metabolism of tryptophan or metabolic flow through the glycolytic pathway is associated with the level of inflammation, and can be observed both in liver, arteries and at the level of immune cells. 3-HAA, 3-hydroxyanthranilic acid; DCA, dichloroacetate; TCA, citric acid cycle; SREBP2, sterol response element binding protein 2; IL, interleukin.

8 ACKNOWLEDGEMENTS

During my years as a PhD-student I have received enormous support from so many intelligent and co-workers and friends. I would in particular like to thank:

My main supervisor, **Daniel Ketelhuth** for taking me in and guiding me on my scientific path all these years. Your warm and kind personality together with your knowledge and skill as a researcher make for a wonderful supervisor and I feel very privileged to have had the opportunity to learn from you.

My co-supervisor **Göran Hansson** who made my recruitment to the lab possible and who has shared so much good advice in the making of this thesis. Without your expertise in the field, this thesis would not have been possible.

My co-supervisor **Alice Assinger**. While time was not enough for some of the studies we had planned, you always supported me and made sure to give me some kind words when you were in Stockholm.

All past and present members of the Ketelhuth team; **Konstantinos Polyzos**, **Maria-José Forteza**, **Roland Baumgartner**, **Ilona Kareinen**, **Aastha Arora**, **Gustavo Tripodi** and **Felipe Beccaria Casagrande**. Thank you for all the hours of hard work we shared collaborating in the lab, and thank you for the wonderful time we have shared outside the lab, the beers, showing me your hometowns and discussing life's big questions in between the science. I am glad to have friends such as you.

My mentor **Sigrid Lundberg**, for your long term commitment to me, for always being a support to count on in both good and bad times.

A very warm thank you to **Anneli Olsson**, **Linda Haglund** and **Ingrid Törnberg**. Without your technical skills the studies in this thesis would not have been made. In addition to being excellent professionals, you made me feel at home in the lab and were always a joy to be around.

Thanks **Gabrielle Paulsson-Berne** for always looking out for the rest of us, and occasionally giving me a nudge in the right direction, and all other principal investigators, **Magnus Bäck**, **Zhong-Qun Yan**, **Peder Olofsson** and **Stephen Malin** for the fruitful discussions over the years.

I would like to thank all my fellow PhD-students, **Glykeria Karadimou**, **Miguel Carracedo**, **Gonzalo Artiach Castañon**, **Marcelo Petri**, **Nikolaos Skenteris**, **Xintong Jiang**, **Xiao-Ying Zhang**, **Yajuan Wang**, **April Caravaca**, **Alessandro Gallina**, **Monica Centa**, **Katrin Habir**, **Tinna Christersdottir**, **Daniela Strodthoff**, **Bianca Suur**, **Samuel Röhl**, for all the laughs and good times we have shared. You are inspiring persons and I hope to continue sharing both science and laughs with you over the coming years. A special thanks to **Leif Söderström** for bringing me in to the lab.

To all the postdoctoral fellows, **Anton Gisterå**, **Silke Thul**, **Andrés Laguna Fernandéz**, **Silke Thul**, **Hildur Arnardottir**, **John Pirault**, **Reiner Mailer**, **Sanna Hellberg**, **Laura Tarnawski**, **Daniel Johansson**, **Hanna Agardh**, **Vladimir Shavva**, **Sven Pawelzik**, **Lasse Folkersen**, **Olga Ovchinnikova**, **Maria Klement**, **Albert Dahdah** and **Kajsa Prokopec**, who have guided me both amid the pipettes and outside of the lab.

Thanks also to **Selam Assefa** for excellent help at AKM.

For much-appreciated guidance and collaboration, particularly towards the end of my studies, I would like to thank **Per Eriksson**. Thank you also **Ulf Hedin** and **Ljubica Matic** for good collaborations.

Thanks to all my friends, particularly **KB** for technical support with making figures and also for supporting me in preparing a dinner together with **David Stigson**.

Finally, I would like to thank my family, my parents **Sören** and **Katarina**, and my brother **Henrik** for all their support and input, and my partner **Sebastian** for invaluable support and encouragement.

9 REFERENCES

1. Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva: World Health Organization; 2018.

2. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation. 2018;137(12):e67-e492.

3. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685-95.

4. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J, 3rd. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med. 1961;55:33-50.

5. Pencina MJ, D'Agostino RB, Sr., Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the framingham heart study. Circulation. 2009;119(24):3078-84.

6. Carlsson AC, Wandell PE, de Faire U, Hellenius ML. Prevalence of hypertension in immigrants and Swedish-born individuals, a cross-sectional study of 60-year-old men and women in Sweden. J Hypertens. 2008;26(12):2295-302.

7. Carroll MD, Fryar CD, Nguyen DT. Total and High-density Lipoprotein Cholesterol in Adults: United States, 2015–2016. NCHS data brief. 2017(290).

8. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The Lancet. 2004;364(9438):937-52.

9. Robinson JG, Huijgen R, Ray K, Persons J, Kastelein JJ, Pencina MJ. Determining When to Add Nonstatin Therapy: A Quantitative Approach. J Am Coll Cardiol. 2016;68(22):2412-21.

10. Mayerl C, Lukasser M, Sedivy R, Niederegger H, Seiler R, Wick G. Atherosclerosis research from past to present--on the track of two pathologists with opposing views, Carl von Rokitansky and Rudolf Virchow. Virchows Arch. 2006;449(1):96-103.

11. Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, et al. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N Engl J Med. 1994;331(7):417-24.

12. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997;336(14):973-9.

13. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362(6423):801-9.

14. Crowson CS, Liao KP, Davis JM, 3rd, Solomon DH, Matteson EL, Knutson KL, et al. Rheumatoid arthritis and cardiovascular disease. Am Heart J. 2013;166(4):622-8 e1.

15. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017;377(12):1119-31.

16. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. Immunol Rev. 2012;249(1):158-75.

17. Beutler BA. TLRs and innate immunity. Blood. 2009;113(7):1399-407.

18. Ullah MO, Sweet MJ, Mansell A, Kellie S, Kobe B. TRIF-dependent TLR signaling, its functions in host defense and inflammation, and its potential as a therapeutic target. J Leukoc Biol. 2016;100(1):27-45.

19. Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, et al. The NLR gene family: a standard nomenclature. Immunity. 2008;28(3):285-7.

20. Kim YK, Shin JS, Nahm MH. NOD-Like Receptors in Infection, Immunity, and Diseases. Yonsei Med J. 2016;57(1):5-14.

21. Schroder K, Tschopp J. The inflammasomes. Cell. 2010;140(6):821-32.

22. Kapellos TS, Bonaguro L, Gemund I, Reusch N, Saglam A, Hinkley ER, et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol. 2019;10:2035.

23. Geissmann F, Jung S, Littman DR. Blood Monocytes Consist of Two Principal Subsets with Distinct Migratory Properties. Immunity. 2003;19(1):71-82.

24. Ginhoux F, Guilliams M. Tissue-Resident Macrophage Ontogeny and Homeostasis. Immunity. 2016;44(3):439-49.

25. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. Front Immunol. 2019;10:1084.

26. Awad F, Assrawi E, Jumeau C, Georgin-Lavialle S, Cobret L, Duquesnoy P, et al. Impact of human monocyte and macrophage polarization on NLR expression and NLRP3 inflammasome activation. PLoS One. 2017;12(4):e0175336.

27. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol. 2011;11(11):723-37.

28. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol. 2006;177(10):7303-11.

29. Collin M, Bigley V. Human dendritic cell subsets: an update. Immunology. 2018;154(1):3-20.

30. Steinman RM. Decisions about dendritic cells: past, present, and future. Annu Rev Immunol. 2012;30:1-22.

31. Reizis B. Plasmacytoid Dendritic Cells: Development, Regulation, and Function. Immunity. 2019;50(1):37-50.

32. Karolinska Universitetslaboratoriet Stockholm, Sweden [updated 2018-01-19Cited 2020-01-16]. Available from: https://www.karolinska.se/for-vardgivare/karolinska-universitetslaboratoriet/.

33. Liew PX, Kubes P. The Neutrophil's Role During Health and Disease. Physiol Rev. 2019;99(2):1223-48.

34. Kovanen PT, Bot I. Mast cells in atherosclerotic cardiovascular disease - Activators and actions. Eur J Pharmacol. 2017;816:37-46.

35. Sarma JV, Ward PA. The complement system. Cell Tissue Res. 2011;343(1):227-35.

36. Abbas AK, Lichtman AH, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. Philadelphia, PA: Saunders; 2012.

37. Zarnitsyna VI, Evavold BD, Schoettle LN, Blattman JN, Antia R. Estimating the diversity, completeness, and cross-reactivity of the T cell repertoire. Front Immunol. 2013;4:485.

38. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol. 2002;2(12):933-44.

39. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature. 2006;441(7090):231-4.

40. Raffin C, Vo LT, Bluestone JA. Treg cell-based therapies: challenges and perspectives. Nat Rev Immunol. 2019.

41. van Puijvelde GHM, Kuiper J. NKT cells in cardiovascular diseases. Eur J Pharmacol. 2017;816:47-57.

42. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. Nat Rev Immunol. 2015;15(3):160-71.

43. Baumgarth N. A Hard(y) Look at B-1 Cell Development and Function. J Immunol. 2017;199(10):3387-94.

44. Sage AP, Tsiantoulas D, Binder CJ, Mallat Z. The role of B cells in atherosclerosis. Nat Rev Cardiol. 2019;16(3):180-96.

45. Margo CE, Harman LE. Autoimmune disease: Conceptual history and contributions of ocular immunology. Surv Ophthalmol. 2016;61(5):680-8.

46. Svensson M, Maroof A, Ato M, Kaye PM. Stromal cells direct local differentiation of regulatory dendritic cells. Immunity. 2004;21(6):805-16.

47. Wakkach A, Fournier N, Brun V, Breittmayer J-P, Cottrez F, Groux H. Characterization of Dendritic Cells that Induce Tolerance and T Regulatory 1 Cell Differentiation In Vivo. Immunity. 2003;18(5):605-17.

48. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9(3):162-74.

49. Forrester JV, Xu H, Lambe T, Cornall R. Immune privilege or privileged immunity? Mucosal Immunol. 2008;1(5):372-81.

50. Tellides G, Pober JS. Inflammatory and immune responses in the arterial media. Circ Res. 2015;116(2):312-22.

51. Rang HP, Dale MM, Ritter JM, Flower RJ. Pharmacology. Philadelphia: Churchill Livingstone; 2007.

52. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005;6(12):1191-7.

53. Back M, Yurdagul A, Jr., Tabas I, Oorni K, Kovanen PT. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. Nat Rev Cardiol. 2019;16(7):389-406.

54. Gray-Donald K, Jacobs-Starkey L, Johnson-Down L. Food habits of Canadians: reduction in fat intake over a generation. Can J Public Health. 2000;91(5):381-5.

55. Nordic Nutrition Recommendations 2012. Copenhagen: Nordisk Ministerråd; 2014.

56. Gimeno RE, Hirsch DJ, Punreddy S, Sun Y, Ortegon AM, Wu H, et al. Targeted deletion of fatty acid transport protein-4 results in early embryonic lethality. J Biol Chem. 2003;278(49):49512-6.

57. Abumrad N, Harmon C, Ibrahimi A. Membrane transport of long-chain fatty acids: evidence for a facilitated process. J Lipid Res. 1998;39(12):2309-18.

58. Wang TY, Liu M, Portincasa P, Wang DQ. New insights into the molecular mechanism of intestinal fatty acid absorption. Eur J Clin Invest. 2013;43(11):1203-23.

59. Altmann SW, Davis HR, Jr., Zhu LJ, Yao X, Hoos LM, Tetzloff G, et al. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science. 2004;303(5661):1201-4.

60. Berg JM, Tymoczko JL, Gatto Jr. GJ, Stryer L. Biochemistry. New York: Macmillan International; 2019.

61. Dash S, Xiao C, Morgantini C, Lewis GF. New Insights into the Regulation of Chylomicron Production. Annu Rev Nutr. 2015;35:265-94.

62. Davies BS, Beigneux AP, Barnes RH, 2nd, Tu Y, Gin P, Weinstein MM, et al. GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. Cell Metab. 2010;12(1):42-52.

63. Kersten S. Physiological regulation of lipoprotein lipase. Biochim Biophys Acta. 2014;1841(7):919-33.

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

65. Mendivil CO, Rimm EB, Furtado J, Sacks FM. Apolipoprotein E in VLDL and LDL with apolipoprotein C-III is associated with a lower risk of coronary heart disease. J Am Heart Assoc. 2013;2(3):e000130.

66. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat. 1992;1(6):445-66.

67. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003;34(2):154-6.

68. Park SW, Moon YA, Horton JD. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. J Biol Chem. 2004;279(48):50630-8.

69. Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ Res. 2005;96(12):1221-32.

70. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007;357(21):2109-22.

71. Shao W, Espenshade PJ. Expanding roles for SREBP in metabolism. Cell Metab. 2012;16(4):414-9.

72. Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest. 1997;99(5):846-54.

73. Oberkofler H, Schraml E, Krempler F, Patsch W. Restoration of sterol-regulatory-element-binding protein-1c gene expression in HepG2 cells by peroxisome-proliferator-activated receptor-gamma co-activator-1alpha. Biochem J. 2004;381(Pt 2):357-63.

74. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell. 2006;124(1):35-46.

75. Espenshade PJ, Cheng D, Goldstein JL, Brown MS. Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. J Biol Chem. 1999;274(32):22795-804.

76. Temel RE, Tang W, Ma Y, Rudel LL, Willingham MC, Ioannou YA, et al. Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. J Clin Invest. 2007;117(7):1968-78.

77. Moffett JR, Namboodiri MA. Tryptophan and the immune response. Immunol Cell Biol. 2003;81(4):247-65.

78. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.

79. TDO2 Stockholm: The Human Protein Atlas; [Available from: http://www.proteinatlas.org.

80. Salter M, Knowles RG, Pogson CI. Quantification of the importance of individual steps in the control of aromatic amino acid metabolism. Biochem J. 1986;234(3):635-47.

81. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017;10:1178646917691938.

82. Badawy AA, Evans M. Animal liver tryptophan pyrrolases: Absence of apoenzyme and of hormonal induction mechanism from species sensitive to tryptophan toxicity. Biochem J. 1976;158(1):79-88.

83. Too LK, Li KM, Suarna C, Maghzal GJ, Stocker R, McGregor IS, et al. Deletion of TDO2, IDO-1 and IDO-2 differentially affects mouse behavior and cognitive function. Behav Brain Res. 2016;312:102-17.

84. Yamamoto S, Hayaishi O. Tryptophan pyrrolase of rabbit intestine. D- and L-tryptophan-cleaving enzyme or enzymes. J Biol Chem. 1967;242(22):5260-6.

85. Hayaishi O. My life with tryptophan--never a dull moment. Protein Sci. 1993;2(3):472-5.

86. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998;281(5380):1191-3.

87. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science. 2002;297(5588):1867-70.

88. Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA. Indoleamine 2,3-Dioxygenase Production by Human Dendritic Cells Results in the Inhibition of T Cell Proliferation. The Journal of Immunology. 2000;164(7):3596-9.

89. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, et al. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol. 2003;4(12):1206-12.

90. Wek SA, Zhu S, Wek RC. The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. Mol Cell Biol. 1995;15(8):4497-506.

91. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity. 2005;22(5):633-42.

92. Liu H, Huang L, Bradley J, Liu K, Bardhan K, Ron D, et al. GCN2-dependent metabolic stress is essential for endotoxemic cytokine induction and pathology. Mol Cell Biol. 2014;34(3):428-38.

93. Metz R, Rust S, Duhadaway JB, Mautino MR, Munn DH, Vahanian NN, et al. IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: A novel IDO effector pathway targeted by D-1-methyl-tryptophan. Oncoimmunology. 2012;1(9):1460-8.

94. Yan Y, Zhang GX, Gran B, Fallarino F, Yu S, Li H, et al. IDO upregulates regulatory T cells via tryptophan catabolite and suppresses encephalitogenic T cell responses in experimental autoimmune encephalomyelitis. J Immunol. 2010;185(10):5953-61.

95. Gurtner GJ, Newberry RD, Schloemann SR, McDonald KG, Stenson WF. Inhibition of indoleamine 2,3-dioxygenase augments trinitrobenzene sulfonic acid colitis in mice. Gastroenterology. 2003;125(6):1762-73.

96. Szanto S, Koreny T, Mikecz K, Glant TT, Szekanecz Z, Varga J. Inhibition of indoleamine 2,3-dioxygenase-mediated tryptophan catabolism accelerates collagen-induced arthritis in mice. Arthritis Res Ther. 2007;9(3):R50.

97. Hou W, Huang G, Cao X, Zhang Y, Zhang J, Li Y. Suppression of experimental autoimmune glomerulonephritis by tryptophan. J Nephrol. 2014;27(1):19-28.

98. Chaudhary K, Shinde R, Liu H, Gnana-Prakasam JP, Veeranan-Karmegam R, Huang L, et al. Amino acid metabolism inhibits antibody-driven kidney injury by inducing autophagy. J Immunol. 2015;194(12):5713-24.

99. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9(10):1269-74.

100. Fox E, Oliver T, Rowe M, Thomas S, Zakharia Y, Gilman PB, et al. Indoximod: An Immunometabolic Adjuvant That Empowers T Cell Activity in Cancer. Front Oncol. 2018;8:370.

101. DiNatale BC, Murray IA, Schroeder JC, Flaveny CA, Lahoti TS, Laurenzana EM, et al. Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that

synergistically induces interleukin-6 in the presence of inflammatory signaling. Toxicol Sci. 2010;115(1):89-97.

102. Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem. 2006;281(31):22021-8.

103. Stockinger B, Di Meglio P, Gialitakis M, Duarte JH. The aryl hydrocarbon receptor: multitasking in the immune system. Annu Rev Immunol. 2014;32:403-32.

104. Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, et al. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. J Exp Med. 2009;206(9):2027-35.

105. Malaczewska J, Siwicki AK, Wojcik RM, Turski WA, Kaczorek E. The effect of kynurenic acid on the synthesis of selected cytokines by murine splenocytes - in vitro and ex vivo studies. Cent Eur J Immunol. 2016;41(1):39-46.

106. Maaetoft-Udsen K, Shimoda LM, Frokiaer H, Turner H. Aryl hydrocarbon receptor ligand effects in RBL2H3 cells. J Immunotoxicol. 2012;9(3):327-37.

107. Barth MC, Ahluwalia N, Anderson TJ, Hardy GJ, Sinha S, Alvarez-Cardona JA, et al. Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. J Biol Chem. 2009;284(29):19189-95.

108. Kiank C, Zeden JP, Drude S, Domanska G, Fusch G, Otten W, et al. Psychological stress-induced, IDO1-dependent tryptophan catabolism: implications on immunosuppression in mice and humans. PLoS One. 2010;5(7):e11825.

109. Tiszlavicz Z, Nemeth B, Fulop F, Vecsei L, Tapai K, Ocsovszky I, et al. Different inhibitory effects of kynurenic acid and a novel kynurenic acid analogue on tumour necrosis factor-alpha (TNF-alpha) production by mononuclear cells, HMGB1 production by monocytes and HNP1-3 secretion by neutrophils. Naunyn Schmiedebergs Arch Pharmacol. 2011;383(5):447-55.

110. Birch PJ, Grossman CJ, Hayes AG. Kynurenate and FG9041 have both competitive and non-competitive antagonist actions at excitatory amino acid receptors. European Journal of Pharmacology. 1988;151(2):313-5.

111. Agudelo LZ, Femenia T, Orhan F, Porsmyr-Palmertz M, Goiny M, Martinez-Redondo V, et al. Skeletal Muscle PGC-1alpha1 Modulates Kynurenine Metabolism and Mediates Resilience to Stress-Induced Depression. Cell. 2014;159(1):33-45.

112. Agudelo LZ, Ferreira DMS, Cervenka I, Bryzgalova G, Dadvar S, Jannig PR, et al. Kynurenic Acid and Gpr35 Regulate Adipose Tissue Energy Homeostasis and Inflammation. Cell Metabolism. 2018;27(2):378-92.e5.

113. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, et al. T cell apoptosis by tryptophan catabolism. Cell Death Differ. 2002;9(10):1069-77.

114. Favre D, Mold J, Hunt PW, Kanwar B, Loke P, Seu L, et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. Sci Transl Med. 2010;2(32):32ra6.

115. Hayashi T, Mo JH, Gong X, Rossetto C, Jang A, Beck L, et al. 3-Hydroxyanthranilic acid inhibits PDK1 activation and suppresses experimental asthma by inducing T cell apoptosis. Proc Natl Acad Sci U S A. 2007;104(47):18619-24.

116. Lee SM, Lee YS, Choi JH, Park SG, Choi IW, Joo YD, et al. Tryptophan metabolite 3-hydroxyanthranilic acid selectively induces activated T cell death via intracellular GSH depletion. Immunol Lett. 2010;132(1-2):53-60.

117. Pae HO, Oh GS, Lee BS, Rim JS, Kim YM, Chung HT. 3-Hydroxyanthranilic acid, one of L-tryptophan metabolites, inhibits monocyte chemoattractant protein-1 secretion and vascular cell adhesion molecule-1 expression via heme oxygenase-1 induction in human umbilical vein endothelial cells. Atherosclerosis. 2006;187(2):274-84.

118. Morita T, Saito K, Takemura M, Maekawa N, Fujigaki S, Fujii H, et al. 3-Hydroxyanthranilic acid, an L-tryptophan metabolite, induces apoptosis in monocyte-derived cells stimulated by interferon-gamma. Ann Clin Biochem. 2001;38(Pt 3):242-51.

119. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. Cell Metab. 2014;20(1):61-72.

120. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature. 2013;496(7444):238-42.

121. Zhang S, Hulver MW, McMillan RP, Cline MA, Gilbert ER. The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. Nutr Metab (Lond). 2014;11(1):10.

122. Wende AR, Huss JM, Schaeffer PJ, Giguere V, Kelly DP. PGC-1alpha coactivates PDK4 gene expression via the orphan nuclear receptor ERRalpha: a mechanism for transcriptional control of muscle glucose metabolism. Mol Cell Biol. 2005;25(24):10684-94.

123. Connaughton S, Chowdhury F, Attia RR, Song S, Zhang Y, Elam MB, et al. Regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) gene expression by glucocorticoids and insulin. Mol Cell Endocrinol. 2010;315(1-2):159-67.

124. Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, et al. Metabolic modulation of glioblastoma with dichloroacetate. Sci Transl Med. 2010;2(31):31ra4.

125. Sun RC, Fadia M, Dahlstrom JE, Parish CR, Board PG, Blackburn AC. Reversal of the glycolytic phenotype by dichloroacetate inhibits metastatic breast cancer cell growth in vitro and in vivo. Breast Cancer Res Treat. 2010;120(1):253-60.

126. He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature. 2004;429(6988):188-93.

127. Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, et al. Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat Immunol. 2008;9(11):1261-9.

128. Littlewood-Evans A, Sarret S, Apfel V, Loesle P, Dawson J, Zhang J, et al. GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. J Exp Med. 2016;213(9):1655-62.

129. Warburg O, Wind F, Negelein E. Über den Stoffwechsel von Tumoren im Körper. Klinische Wochenschrift. 1926;5(19):829-32.

130. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029-33.

131. Marelli-Berg FM, Fu H, Mauro C. Molecular mechanisms of metabolic reprogramming in proliferating cells: implications for T-cell-mediated immunity. Immunology. 2012;136(4):363-9.

132. Rodriguez-Prados JC, Traves PG, Cuenca J, Rico D, Aragones J, Martin-Sanz P, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. J Immunol. 2010;185(1):605-14.

133. Eleftheriadis T, Pissas G, Antoniadi G, Spanoulis A, Liakopoulos V, Stefanidis I. Indoleamine 2,3-dioxygenase increases p53 levels in alloreactive human T cells, and both indoleamine 2,3-dioxygenase and p53 suppress glucose uptake, glycolysis and proliferation. Int Immunol. 2014;26(12):673-84.

134. Eleftheriadis T, Pissas G, Yiannaki E, Markala D, Arampatzis S, Antoniadi G, et al. Inhibition of indoleamine 2,3-dioxygenase in mixed lymphocyte reaction affects glucose influx and enzymes involved in aerobic glycolysis and glutaminolysis in alloreactive T-cells. Hum Immunol. 2013;74(12):1501-9.

135. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol. 1995;15(5):551-61.

136. Boren J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. Curr Opin Lipidol. 2016;27(5):473-83.

137. Flood C, Gustafsson M, Richardson PE, Harvey SC, Segrest JP, Boren J. Identification of the proteoglycan binding site in apolipoprotein B48. J Biol Chem. 2002;277(35):32228-33.

138. Tran-Lundmark K, Tran PK, Paulsson-Berne G, Friden V, Soininen R, Tryggvason K, et al. Heparan sulfate in perlecan promotes mouse atherosclerosis: roles in lipid permeability, lipid retention, and smooth muscle cell proliferation. Circ Res. 2008;103(1):43-52.

139. Dai G, Kaazempur-Mofrad MR, Natarajan S, Zhang Y, Vaughn S, Blackman BR, et al. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. Proc Natl Acad Sci U S A. 2004;101(41):14871-6.

140. Armstrong SM, Sugiyama MG, Fung KY, Gao Y, Wang C, Levy AS, et al. A novel assay uncovers an unexpected role for SR-BI in LDL transcytosis. Cardiovasc Res. 2015;108(2):268-77.

141. Fernandez-Hernando C, Yu J, Suarez Y, Rahner C, Davalos A, Lasuncion MA, et al. Genetic evidence supporting a critical role of endothelial caveolin-1 during the progression of atherosclerosis. Cell Metab. 2009;10(1):48-54.

142. Witztum JL. The oxidation hypothesis of atherosclerosis. The Lancet. 1994;344(8925):793-5.

143. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci U S A. 1995;92(9):3893-7.

144. Ketelhuth DF, Rios FJ, Wang Y, Liu H, Johansson ME, Fredrikson GN, et al. Identification of a danger-associated peptide from apolipoprotein B100 (ApoBDS-1) that triggers innate proatherogenic responses. Circulation. 2011;124(22):2433-43, 1-7.

145. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med. 1996;334(18):1145-9.

146. Hodis HN, Mack WJ, LaBree L, Mahrer PR, Sevanian A, Liu CR, et al. Alpha-tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). Circulation. 2002;106(12):1453-9.

147. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA. 2005;294(1):56-65.

148. Cybulsky MI, Gimbrone MA, Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991;251(4995):788-91.

149. Libby P, Pasterkamp G. Requiem for the 'vulnerable plaque'. Eur Heart J. 2015;36(43):2984-7.

150. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med. 2013;19(9):1166-72.

151. Boullier A, Gillotte KL, Horkko S, Green SR, Friedman P, Dennis EA, et al. The binding of oxidized low density lipoprotein to mouse CD36 is mediated in part by oxidized phospholipids that are associated with both the lipid and protein moieties of the lipoprotein. J Biol Chem. 2000;275(13):9163-9.

152. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, et al. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. J Biol Chem. 2002;277(51):49982-8.

153. Gerrity RG. The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions. Am J Pathol. 1981;103:181-90.

154. Moore KJ, Kunjathoor VV, Koehn SL, Manning JJ, Tseng AA, Silver JM, et al. Loss of receptor-mediated lipid uptake via scavenger receptor A or CD36 pathways does not ameliorate atherosclerosis in hyperlipidemic mice. J Clin Invest. 2005;115(8):2192-201.

155. Yuasa-Kawase M, Masuda D, Yamashita T, Kawase R, Nakaoka H, Inagaki M, et al. Patients with CD36 deficiency are associated with enhanced atherosclerotic cardiovascular diseases. J Atheroscler Thromb. 2012;19(3):263-75.

156. van Eck M, Bos IS, Kaminski WE, Orso E, Rothe G, Twisk J, et al. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. Proc Natl Acad Sci U S A. 2002;99(9):6298-303.

157. Westerterp M, Murphy AJ, Wang M, Pagler TA, Vengrenyuk Y, Kappus MS, et al. Deficiency of ATP-binding cassette transporters A1 and G1 in macrophages increases inflammation and accelerates atherosclerosis in mice. Circ Res. 2013;112(11):1456-65.

158. van Gils JM, Derby MC, Fernandes LR, Ramkhelawon B, Ray TD, Rayner KJ, et al. The neuroimmune guidance cue netrin-1 promotes atherosclerosis by inhibiting the emigration of macrophages from plaques. Nat Immunol. 2012;13(2):136-43.

159. Andres V, Pello OM, Silvestre-Roig C. Macrophage proliferation and apoptosis in atherosclerosis. Curr Opin Lipidol. 2012;23(5):429-38.

160. Karunakaran D, Geoffrion M, Wei L, Gan W, Richards L, Shangari P, et al. Targeting macrophage necroptosis for therapeutic and diagnostic interventions in atherosclerosis. Sci Adv. 2016;2(7):e1600224.

161. Grebe A, Hoss F, Latz E. NLRP3 Inflammasome and the IL-1 Pathway in Atherosclerosis. Circ Res. 2018;122(12):1722-40.

162. Thorp E, Subramanian M, Tabas I. The role of macrophages and dendritic cells in the clearance of apoptotic cells in advanced atherosclerosis. Eur J Immunol. 2011;41(9):2515-8.

163. Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhelawon B, Carpenter SB, et al. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. Nat Immunol. 2013;14(8):812-20.

164. Rhoads JP, Lukens JR, Wilhelm AJ, Moore JL, Mendez-Fernandez Y, Kanneganti TD, et al. Oxidized Low-Density Lipoprotein Immune Complex Priming of the Nlrp3 Inflammasome Involves TLR and FcgammaR Cooperation and Is Dependent on CARD9. J Immunol. 2017;198(5):2105-14.

165. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. PLoS One. 2010;5(7):e11765.

166. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010;464(7293):1357-61.

167. Menu P, Pellegrin M, Aubert JF, Bouzourene K, Tardivel A, Mazzolai L, et al. Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome. Cell Death Dis. 2011;2:e137.

168. Paramel Varghese G, Folkersen L, Strawbridge RJ, Halvorsen B, Yndestad A, Ranheim T, et al. NLRP3 Inflammasome Expression and Activation in Human Atherosclerosis. J Am Heart Assoc. 2016;5(5).

169. Galea J, Armstrong J, Gadsdon P, Holden H, Francis SE, Holt CM. Interleukin-1 beta in coronary arteries of patients with ischemic heart disease. Arterioscler Thromb Vasc Biol. 1996;16(8):1000-6.

170. Elhage R, Maret A, Pieraggi MT, Thiers JC, Arnal JF, Bayard F. Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice. Circulation. 1998;97(3):242-4.

171. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, et al. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol. 2003;23(4):656-60.

172. Cho KY, Miyoshi H, Kuroda S, Yasuda H, Kamiyama K, Nakagawara J, et al. The Phenotype of Infiltrating Macrophages Influences Arteriosclerotic Plaque Vulnerability in the Carotid Artery. Journal of Stroke and Cerebrovascular Diseases. 2013;22(7):910-8.

173. Lee CW, Hwang I, Park CS, Lee H, Park DW, Kang SJ, et al. Macrophage heterogeneity of culprit coronary plaques in patients with acute myocardial infarction or stable angina. Am J Clin Pathol. 2013;139(3):317-22.

174. Stoger JL, Gijbels MJ, van der Velden S, Manca M, van der Loos CM, Biessen EA, et al. Distribution of macrophage polarization markers in human atherosclerosis. Atherosclerosis. 2012;225(2):461-8.

175. Kadl A, Meher AK, Sharma PR, Lee MY, Doran AC, Johnstone SR, et al. Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. Circ Res. 2010;107(6):737-46.

176. Singhal A, Subramanian M. Colony stimulating factors (CSFs): Complex roles in atherosclerosis. Cytokine. 2019;122:154190.

177. Di Gregoli K, Johnson JL. Role of colony-stimulating factors in atherosclerosis. Curr Opin Lipidol. 2012;23(5):412-21.

178. da Silva RF, Lappalainen J, Lee-Rueckert M, Kovanen PT. Conversion of human M-CSF macrophages into foam cells reduces their proinflammatory responses to classical M1-polarizing activation. Atherosclerosis. 2016;248:170-8.

179. Bobryshev Y, Lord RS. Mapping of vascular dendritic cells in atherosclerotic arteries suggests their involvement in local immune-inflammatory reactions. Cardiovascular Research. 1998;37(3):799-810.

180. Gautier EL, Huby T, Saint-Charles F, Ouzilleau B, Pirault J, Deswaerte V, et al. Conventional dendritic cells at the crossroads between immunity and cholesterol homeostasis in atherosclerosis. Circulation. 2009;119(17):2367-75.

181. Buono C, Pang H, Uchida Y, Libby P, Sharpe AH, Lichtman AH. B7-1/B7-2 costimulation regulates plaque antigen-specific T-cell responses and atherogenesis in low-density lipoprotein receptor-deficient mice. Circulation. 2004;109(16):2009-15.

182. Subramanian M, Thorp E, Hansson GK, Tabas I. Treg-mediated suppression of atherosclerosis requires MYD88 signaling in DCs. J Clin Invest. 2013;123(1):179-88.

183. Forteza MJ, Polyzos KA, Baumgartner R, Suur BE, Mussbacher M, Johansson DK, et al. Activation of the Regulatory T-Cell/Indoleamine 2,3-Dioxygenase Axis Reduces Vascular Inflammation and Atherosclerosis in Hyperlipidemic Mice. Front Immunol. 2018;9:950.

184. Ovchinnikova OA, Folkersen L, Persson J, Lindeman JH, Ueland T, Aukrust P, et al. The collagen cross-linking enzyme lysyl oxidase is associated with the healing of human atherosclerotic lesions. J Intern Med. 2014;276(5):525-36.

185. Feil S, Fehrenbacher B, Lukowski R, Essmann F, Schulze-Osthoff K, Schaller M, et al. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells during atherogenesis. Circ Res. 2014;115(7):662-7.

186. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis. 1986;6(2):131-8.

187. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89(1):36-44.

188. Zhou X, Nicoletti A, Elhage R, Hansson GK. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. Circulation. 2000;102(24):2919-22.

189. Huber SA, Sakkinen P, David C, Newell MK, Tracy RP. T helper-cell phenotype regulates atherosclerosis in mice under conditions of mild hypercholesterolemia. Circulation. 2001;103(21):2610-6.

190. Hermansson A, Ketelhuth DF, Strodthoff D, Wurm M, Hansson EM, Nicoletti A, et al. Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. J Exp Med. 2010;207(5):1081-93.

191. Ruuth M, Nguyen SD, Vihervaara T, Hilvo M, Laajala TD, Kondadi PK, et al. Susceptibility of low-density lipoprotein particles to aggregate depends on particle lipidome, is modifiable, and associates with future cardiovascular deaths. Eur Heart J. 2018;39(27):2562-73.

192. Gupta S, Pablo AM, Jiang X, Wang N, Tall AR, Schindler C. IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. J Clin Invest. 1997;99(11):2752-61.

193. Buono C, Come CE, Stavrakis G, Maguire GF, Connelly PW, Lichtman AH. Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLR-deficient mouse. Arterioscler Thromb Vasc Biol. 2003;23(3):454-60.

194. Buono C, Binder CJ, Stavrakis G, Witztum JL, Glimcher LH, Lichtman AH. T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. Proc Natl Acad Sci U S A. 2005;102(5):1596-601.

195. Agrawal S, Febbraio M, Podrez E, Cathcart MK, Stark GR, Chisolm GM. Signal transducer and activator of transcription 1 is required for optimal foam cell formation and atherosclerotic lesion development. Circulation. 2007;115(23):2939-47.

196. Frostegård J, Ulfgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, et al. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis. 1999;145(1):33-43.

197. King VL, Szilvassy SJ, Daugherty A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor-/- mice. Arterioscler Thromb Vasc Biol. 2002;22(3):456-61.

198. Binder CJ, Hartvigsen K, Chang MK, Miller M, Broide D, Palinski W, et al. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. J Clin Invest. 2004;114(3):427-37.

199. Cardilo-Reis L, Gruber S, Schreier SM, Drechsler M, Papac-Milicevic N, Weber C, et al. Interleukin-13 protects from atherosclerosis and modulates plaque composition by skewing the macrophage phenotype. EMBO Mol Med. 2012;4(10):1072-86.

200. Smith E, Prasad KM, Butcher M, Dobrian A, Kolls JK, Ley K, et al. Blockade of interleukin-17A results in reduced atherosclerosis in apolipoprotein E-deficient mice. Circulation. 2010;121(15):1746-55.

201. Gistera A, Robertson AK, Andersson J, Ketelhuth DF, Ovchinnikova O, Nilsson SK, et al. Transforming growth factor-beta signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17-dependent pathway. Sci Transl Med. 2013;5(196):196ra00.

202. Maganto-Garcia E, Tarrio ML, Grabie N, Bu DX, Lichtman AH. Dynamic changes in regulatory T cells are linked to levels of diet-induced hypercholesterolemia. Circulation. 2011;124(2):185-95.

203. Klingenberg R, Gerdes N, Badeau RM, Gistera A, Strodthoff D, Ketelhuth DF, et al. Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. J Clin Invest. 2013;123(3):1323-34.

204. Caligiuri G, Rudling M, Ollivier V, Jacob MP, Michel JB, Hansson GK, et al. Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice. Mol Med. 2003;9(1-2):10-7.

205. Robertson AK, Rudling M, Zhou X, Gorelik L, Flavell RA, Hansson GK. Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. J Clin Invest. 2003;112(9):1342-50.

206. Mor A, Luboshits G, Planer D, Keren G, George J. Altered status of CD4(+)CD25(+) regulatory T cells in patients with acute coronary syndromes. Eur Heart J. 2006;27(21):2530-7.

207. Wigren M, Bjorkbacka H, Andersson L, Ljungcrantz I, Fredrikson GN, Persson M, et al. Low levels of circulating CD4+FoxP3+ T cells are associated with an increased risk for development of myocardial infarction but not for stroke. Arterioscler Thromb Vasc Biol. 2012;32(8):2000-4.

208. Houtkamp MA, de Boer OJ, van der Loos CM, van der Wal AC, Becker AE. Adventitial infiltrates associated with advanced atherosclerotic plaques: structural organization suggests generation of local humoral immune responses. The Journal of Pathology. 2001;193(2):263-9.

209. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J Clin Invest. 2002;109(6):745-53.

210. Ait-Oufella H, Herbin O, Bouaziz JD, Binder CJ, Uyttenhove C, Laurans L, et al. B cell depletion reduces the development of atherosclerosis in mice. J Exp Med. 2010;207(8):1579-87.

211. Strom AC, Cross AJ, Cole JE, Blair PA, Leib C, Goddard ME, et al. B regulatory cells are increased in hypercholesterolaemic mice and protect from lesion development via IL-10. Thromb Haemost. 2015;114(4):835-47.

212. Yla-Herttuala S, Palinski W, Butler SW, Picard S, Steinberg D, Witztum JL. Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. Arterioscler Thromb. 1994;14(1):32-40.

213. Lewis MJ, Malik TH, Ehrenstein MR, Boyle JJ, Botto M, Haskard DO. Immunoglobulin M is required for protection against atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2009;120(5):417-26.

214. Kyaw T, Tay C, Khan A, Dumouchel V, Cao A, To K, et al. Conventional B2 B cell depletion ameliorates whereas its adoptive transfer aggravates atherosclerosis. J Immunol. 2010;185(7):4410-9.

215. Centa M, Jin H, Hofste L, Hellberg S, Busch A, Baumgartner R, et al. Germinal Center-Derived Antibodies Promote Atherosclerosis Plaque Size and Stability. Circulation. 2019;139(21):2466-82.

216. Pertovaara M, Raitala A, Juonala M, Lehtimaki T, Huhtala H, Oja SS, et al. Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study. Clin Exp Immunol. 2007;148(1):106-11.

217. Munipally PK, Agraharm SG, Valavala VK, Gundae S, Turlapati NR. Evaluation of indoleamine 2,3-dioxygenase expression and kynurenine pathway metabolites levels in serum samples of diabetic retinopathy patients. Arch Physiol Biochem. 2011;117(5):254-8.

218. Oxenkrug GF, Turski WA, Zgrajka W, Weinstock JV, Summergrad P. Tryptophan-kynurenine metabolism and insulin resistance in hepatitis C patients. Hepat Res Treat. 2013;2013:149247.

219. Kotake Y, Ueda T, Mori T, Igaki S, Hattori M. Abnormal tryptophan metabolism and experimental diabetes by xanthurenic acid (XA). Acta Vitaminol Enzymol. 1975;29(1-6):236-9.

220. Hattori M, Kotake Y, Kotake Y. Studies on the urinary excretion of xanthurenic acid in diabetics. Acta Vitaminol Enzymol. 1984;6(3):221-8.

221. Okamoto H. Regulation of proinsulin synthesis in pancreatic islets and a new aspect to insulin-dependent diabetes. Mol Cell Biochem. 1981;37(1):43-61.

222. Fallarino F, Volpi C, Zelante T, Vacca C, Calvitti M, Fioretti MC, et al. IDO mediates TLR9-driven protection from experimental autoimmune diabetes. J Immunol. 2009;183(10):6303-12.

223. Sarkar SA, Wong R, Hackl SI, Moua O, Gill RG, Wiseman A, et al. Induction of indoleamine 2,3-dioxygenase by interferon-gamma in human islets. Diabetes. 2007;56(1):72-9.

224. Wang Y, Liu H, McKenzie G, Witting PK, Stasch JP, Hahn M, et al. Kynurenine is an endothelium-derived relaxing factor produced during inflammation. Nat Med. 2010;16(3):279-85.

225. Changsirivathanathamrong D, Wang Y, Rajbhandari D, Maghzal GJ, Mak WM, Woolfe C, et al. Tryptophan metabolism to kynurenine is a potential novel contributor to hypotension in human sepsis. Crit Care Med. 2011;39(12):2678-83.

226. Jung ID, Lee MG, Chang JH, Lee JS, Jeong YI, Lee CM, et al. Blockade of indoleamine 2,3-dioxygenase protects mice against lipopolysaccharide-induced endotoxin shock. J Immunol. 2009;182(5):3146-54.

227. Padberg JS, Van Meurs M, Kielstein JT, Martens-Lobenhoffer J, Bode-Boger SM, Zijlstra JG, et al. Indoleamine-2,3-dioxygenase activity in experimental human endotoxemia. Exp Transl Stroke Med. 2012;4(1):24.

228. Ito S, Komatsu K, Tsukamoto K, Sved AF. Excitatory amino acids in the rostral ventrolateral medulla support blood pressure in spontaneously hypertensive rats. Hypertension. 2000;35(1 Pt 2):413-7.

229. Kwok JB, Kapoor R, Gotoda T, Iwamoto Y, Iizuka Y, Yamada N, et al. A missense mutation in kynurenine aminotransferase-1 in spontaneously hypertensive rats. J Biol Chem. 2002;277(39):35779-82.

230. Chang MY, Smith C, DuHadaway JB, Pyle JR, Boulden J, Soler AP, et al. Cardiac and gastrointestinal liabilities caused by deficiency in the immune modulatory enzyme indoleamine 2,3-dioxygenase. Cancer Biol Ther. 2011;12(12):1050-8.

231. Zhang L, Ovchinnikova O, Jonsson A, Lundberg AM, Berg M, Hansson GK, et al. The tryptophan metabolite 3-hydroxyanthranilic acid lowers plasma lipids and decreases atherosclerosis in hypercholesterolaemic mice. Eur Heart J. 2012;33(16):2025-34.

232. Wirleitner B, Rudzite V, Neurauter G, Murr C, Kalnins U, Erglis A, et al. Immune activation and degradation of tryptophan in coronary heart disease. Eur J Clin Invest. 2003;33(7):550-4.

233. Pedersen ER, Midttun O, Ueland PM, Schartum-Hansen H, Seifert R, Igland J, et al. Systemic markers of interferon-gamma-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. Arterioscler Thromb Vasc Biol. 2011;31(3):698-704.

234. Pedersen ER, Tuseth N, Eussen SJ, Ueland PM, Strand E, Svingen GF, et al. Associations of plasma kynurenines with risk of acute myocardial infarction in patients with stable angina pectoris. Arterioscler Thromb Vasc Biol. 2015;35(2):455-62.

235. Pedersen ER, Svingen GF, Schartum-Hansen H, Ueland PM, Ebbing M, Nordrehaug JE, et al. Urinary excretion of kynurenine and tryptophan, cardiovascular events, and mortality after elective coronary angiography. Eur Heart J. 2013;34(34):2689-96.

236. Sulo G, Vollset SE, Nygard O, Midttun O, Ueland PM, Eussen SJ, et al. Neopterin and kynurenine-tryptophan ratio as predictors of coronary events in older adults, the Hordaland Health Study. Int J Cardiol. 2013;168(2):1435-40.

237. Eussen SJ, Ueland PM, Vollset SE, Nygard O, Midttun O, Sulo G, et al. Kynurenines as predictors of acute coronary events in the Hordaland Health Study. Int J Cardiol. 2015;189:18-24.

238. Metghalchi S, Ponnuswamy P, Simon T, Haddad Y, Laurans L, Clement M, et al. Indoleamine 2,3-Dioxygenase Fine-Tunes Immune Homeostasis in Atherosclerosis and Colitis through Repression of Interleukin-10 Production. Cell Metab. 2015;22(3):460-71.

239. Holmes DR, Jr., Savage M, LaBlanche JM, Grip L, Serruys PW, Fitzgerald P, et al. Results of Prevention of REStenosis with Tranilast and its Outcomes (PRESTO) trial. Circulation. 2002;106(10):1243-50.

240. Brouns R, Verkerk R, Aerts T, De Surgeloose D, Wauters A, Scharpe S, et al. The role of tryptophan catabolism along the kynurenine pathway in acute ischemic stroke. Neurochem Res. 2010;35(9):1315-22.

241. Li M, Kwok MK, Fong SSM, Schooling CM. Indoleamine 2,3-dioxygenase and ischemic heart disease: a Mendelian Randomization study. Sci Rep. 2019;9(1):8491.

242. Cole JE, Astola N, Cribbs AP, Goddard ME, Park I, Green P, et al. Indoleamine 2,3-dioxygenase-1 is protective in atherosclerosis and its metabolites provide new opportunities for drug development. Proc Natl Acad Sci U S A. 2015;112(42):13033-8.

243. Daissormont IT, Christ A, Temmerman L, Sampedro Millares S, Seijkens T, Manca M, et al. Plasmacytoid dendritic cells protect against atherosclerosis by tuning T-cell proliferation and activity. Circ Res. 2011;109(12):1387-95.

244. Yun TJ, Lee JS, Machmach K, Shim D, Choi J, Wi YJ, et al. Indoleamine 2,3-Dioxygenase-Expressing Aortic Plasmacytoid Dendritic Cells Protect against Atherosclerosis by Induction of Regulatory T Cells. Cell Metab. 2016;23(5):852-66.

245. Laurans L, Venteclef N, Haddad Y, Chajadine M, Alzaid F, Metghalchi S, et al. Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health. Nat Med. 2018;24(8):1113-20.

246. Cuffy MC, Silverio AM, Qin L, Wang Y, Eid R, Brandacher G, et al. Induction of Indoleamine 2,3-Dioxygenase in Vascular Smooth Muscle Cells by Interferon- Contributes to Medial Immunoprivilege. The Journal of Immunology. 2007;179(8):5246-54.

247. Emami H, Singh P, MacNabb M, Vucic E, Lavender Z, Rudd JH, et al. Splenic metabolic activity predicts risk of future cardiovascular events: demonstration of a cardiosplenic axis in humans. JACC Cardiovasc Imaging. 2015;8(2):121-30.

248. Marnane M, Merwick A, Sheehan OC, Hannon N, Foran P, Grant T, et al. Carotid plaque inflammation on 18F-fluorodeoxyglucose positron emission tomography predicts early stroke recurrence. Ann Neurol. 2012;71(5):709-18.

249. Figueroa AL, Abdelbaky A, Truong QA, Corsini E, MacNabb MH, Lavender ZR, et al. Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events. JACC Cardiovasc Imaging. 2013;6(12):1250-9.

250. Tomas L, Edsfeldt A, Mollet IG, Perisic Matic L, Prehn C, Adamski J, et al. Altered metabolism distinguishes high-risk from stable carotid atherosclerotic plaques. Eur Heart J. 2018;39(24):2301-10.

251. Sarrazy V, Viaud M, Westerterp M, Ivanov S, Giorgetti-Peraldi S, Guinamard R, et al. Disruption of Glut1 in Hematopoietic Stem Cells Prevents Myelopoiesis and Enhanced Glucose Flux in Atheromatous Plaques of ApoE(-/-) Mice. Circ Res. 2016;118(7):1062-77.

252. Christ A, Gunther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, et al. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. Cell. 2018;172(1-2):162-75 e14.

253. Reboldi A, Dang EV, McDonald JG, Liang G, Russell DW, Cyster JG. 25-Hydroxycholesterol suppresses interleukin 1 driven inflammation downstream of type I interferon. Science. 2014;345(6197).

254. Xiao H, Lu M, Lin TY, Chen Z, Chen G, Wang WC, et al. Sterol regulatory element binding protein 2 activation of NLRP3 inflammasome in endothelium mediates hemodynamic-induced atherosclerosis susceptibility. Circulation. 2013;128(6):632-42.

255. Li Y, Xu S, Jiang B, Cohen RA, Zang M. Activation of sterol regulatory element binding protein and NLRP3 inflammasome in atherosclerotic lesion development in diabetic pigs. PLoS One. 2013;8(6):e67532.

256. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci U S A. 1992;89(10):4471-5.

257. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest. 1993;92(2):883-93.

258. Rothwell PM, Pendlebury ST, Wardlaw J, Warlow CP. Critical appraisal of the design and reporting of studies of imaging and measurement of carotid stenosis. Stroke. 2000;31(6):1444-50.

259. O'Brien KD, Allen MD, McDonald TO, Chait A, Harlan JM, Fishbein D, et al. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. J Clin Invest. 1993;92(2):945-51.

260. Braun M. Cellular adhesion molecules on vascular smooth muscle cells. Cardiovascular Research. 1999;41(2):395-401.

261. Cai Q, Lanting L, Natarajan R. Growth factors induce monocyte binding to vascular smooth muscle cells: implications for monocyte retention in atherosclerosis. Am J Physiol Cell Physiol. 2004;287(3):C707-14.

262. Lob S, Konigsrainer A, Schafer R, Rammensee HG, Opelz G, Terness P. Levo- but not dextro-1-methyl tryptophan abrogates the IDO activity of human dendritic cells. Blood. 2008;111(4):2152-4.

263. Metz R, Duhadaway JB, Kamasani U, Laury-Kleintop L, Muller AJ, Prendergast GC. Novel tryptophan catabolic enzyme IDO2 is the preferred biochemical target of the antitumor indoleamine 2,3-dioxygenase inhibitory compound D-1-methyl-tryptophan. Cancer Res. 2007;67(15):7082-7.

264. Qian F, Liao J, Villella J, Edwards R, Kalinski P, Lele S, et al. Effects of 1-methyltryptophan stereoisomers on IDO2 enzyme activity and IDO2-mediated arrest of human T cell proliferation. Cancer Immunol Immunother. 2012;61(11):2013-20.

265. Agaugue S, Perrin-Cocon L, Coutant F, Andre P, Lotteau V. 1-Methyl-tryptophan can interfere with TLR signaling in dendritic cells independently of IDO activity. J Immunol. 2006;177(4):2061-71.

266. Schonewille M, de Boer JF, Mele L, Wolters H, Bloks VW, Wolters JC, et al. Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice. J Lipid Res. 2016;57(8):1455-64.

267. Tang JJ, Li JG, Qi W, Qiu WW, Li PS, Li BL, et al. Inhibition of SREBP by a small molecule, betulin, improves hyperlipidemia and insulin resistance and reduces atherosclerotic plaques. Cell Metab. 2011;13(1):44-56.

268. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 2008;8(3):224-36.

269. Li X, Zhang X, Pan Y, Shi G, Ren J, Fan H, et al. mTOR regulates NLRP3 inflammasome activation via reactive oxygen species in murine lupus. Acta Biochim Biophys Sin (Shanghai). 2018;50(9):888-96.

270. Fornstedt-Wallin B LJ, Fredriksson G, Schwarcz R, Luthman J. 3-Hydroxyanthranilic acid accumulation following administration of the 3-hydroxyanthranilic acid 3,4-dioxygenase inhibitor NCR-631. Eur J Pharmacol. 1999;10;386(1):15-24.

271. Niinisalo P, Oksala N, Levula M, Pelto-Huikko M, Jarvinen O, Salenius JP, et al. Activation of indoleamine 2,3-dioxygenase-induced tryptophan degradation in advanced atherosclerotic plaques: Tampere vascular study. Ann Med. 2010;42(1):55-63.

272. Reynolds LM, Ding J, Taylor JR, Lohman K, Soranzo N, de la Fuente A, et al. Transcriptomic profiles of aging in purified human immune cells. BMC Genomics. 2015;16:333.

273. Lakoski SG, Greenland P, Wong ND, Schreiner PJ, Herrington DM, Kronmal RA, et al. Coronary artery calcium scores and risk for cardiovascular events in women classified as "low risk" based on Framingham risk score: the multi-ethnic study of atherosclerosis (MESA). Arch Intern Med. 2007;167(22):2437-42.

274. Min BK, Oh CJ, Park S, Lee JM, Go Y, Park BY, et al. Therapeutic effect of dichloroacetate against atherosclerosis via hepatic FGF21 induction mediated by acute AMPK activation. Exp Mol Med. 2019;51(10):1-12.

275. Stacpoole PW, Wright EC, Baumgartner TG, Bersin RM, Buchalter S, Curry SH, et al. A controlled clinical trial of dichloroacetate for treatment of lactic acidosis in adults. The Dichloroacetate-Lactic Acidosis Study Group. N Engl J Med. 1992;327(22):1564-9.

276. McCall CE, Zabalawi M, Liu T, Martin A, Long DL, Buechler NL, et al. Pyruvate dehydrogenase complex stimulation promotes immunometabolic homeostasis and sepsis survival. JCI Insight. 2018;3(15).