Fakultät für Medizin

Linking inflammation and atherosclerosis

– novel mechanistic insights into how vascular inflammation fuels progression of atherosclerosis –

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List of abbreviations

ACTH	adrenocorticotropic hormone
Angpt1	angiopoietin 1
ANOVA	analysis of variance
Apo	apolipoprotein
ASC	apoptosis-associated speck-like protein
BCA	bicinchoninic acid
BM	bone marrow
BW	body weight
CAD	coronary artery disease
CANTOS	Canakinumab Anti-Inflammatory Thrombosis Outcomes Study
Casp1	caspase 1
CCL	CC-motif chemokine ligand
CD	cluster of differentiation
cDNA	complementary DNA
Col	collagen
CRP	C-reactive protein
CVD	cardiovascular disease
CXCL	CXC-motif chemokine ligand
EC	endothelial cell
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
Gapdh	glyceraldehyde 3-phosphate dehydrogenase
GFP	green fluorescent protein
HCD	high cholesterol diet
HPA	hypothalamus/pituitary/adrenal
HRP	horseradish peroxidase
HUVEC	human umbilical vein endothelial cell
ICAM1	intercellular adhesion molecule 1
Ig	immunoglobulin
IL	interleukin
i.p.	intraperitoneal
i.v.	intravenous
LDL	low-density lipoprotein
Ldlr	low-density lipoprotein receptor
mAoECs	mouse aortic endothelial cells
MI	myocardial infarction

Mmp	matrix metalloproteinase
mRNA	messenger RNA
NET	neutrophil extracellular trap
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NLRP3	NACHT, LRR and PYD domains-containing protein 3
Opn	osteopontin
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PE	phycoerythrin
pTH	phosphorylated tyrosine hydroxylase
PVDF	polyvinylidene difluoride
SAM	sympatho-adrenomedullary
Scf	stem cell factor
SMC	smooth muscle cell
(s)VCAM1	(soluble) vascular adhesion molecule 1
SNS	sympathetic nervous system
TH_1	T helper type 1
TNFα	tumor necrosis factor alpha
6-OHDA	6-hydroxydopamine

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Abstract

Cardiovascular disease and its complications, such as myocardial infarction, are the leading causes of death worldwide. Atherosclerosis, the underlying pathology, has long been considered a metabolic-driven disease, characterized by high plasma low-density lipoprotein levels and hence, passive lipid accumulation inside the arterial vessel wall. However, over the last decades, research has identified several inflammatory processes as major contributors to all stages of the disease. This is underlined by the fact that the incidence for cardiovascular events remains high, despite effective lipid-lowering therapies in patients at risk. Specifically, traditional and novel risk factors for cardiovascular disease were shown to convey their pathology via modifying both local and systemic inflammatory processes. Thus, there is a strong need for novel anti-atherosclerotic therapies targeting inflammation, especially in the context of specific risk factors in respective individuals.

Interleukin (IL)-1β is a prototypical pro-inflammatory cytokine and has been strongly correlated to atherosclerosis progression. Hence, neutralizing IL-1β in a large phase III clinical trial reduced secondary cardiovascular events in patients with previous myocardial infarction. The manuscript “Interleukin-1β suppression dampens inflammatory leukocyte production and uptake in atherosclerosis”, which is part of this paper-based dissertation, aimed to better investigate the biological basis of this beneficial effect as the exact mechanisms are not yet fully understood. In this context, it was shown that anti-IL-1β treatment mitigated vascular inflammation, and thus atherosclerosis progression, via affecting both inflammatory leukocyte supply from hematopoietic organs and uptake into atherosclerotic plaques.

Acute mental stress has been indicated a novel risk factor for atherosclerosis, specifically as a trigger for acute cardiovascular events. However, despite increasing evidence from epidemiological studies, it remains unclear how exactly acute mental stress rapidly drives plaque progression. In the second manuscript of this paper-based dissertation (“Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis”), it was demonstrated that acute mental stress increases vascular inflammation by increasing recruitment of inflammatory blood leukocytes. Mechanistically, this is driven by a local, norepinephrine-dependent activation of vascular endothelial cells. Further, it was shown that the process of stress-induced leukocyte recruitment can be pharmacologically targeted, e.g. via neutralizing the stress-related increase in specific chemoattractants.

Taken together, these data provide novel mechanistic cues which may aid to guide future anti-atherosclerotic therapies to reduce complications of atherosclerosis.

Zusammenfassung

Herz-Kreislauferkrankungen und daraus resultierende Komplikationen, wie z.B. Herzinfarkte, sind weltweit die häufigste Todesursache. Die zugrundeliegende Pathologie, die Atherosklerose, wurde lange Zeit als eine stoffwechselbedingte Erkrankung angesehen, bei der ein hoher Cholesterinspiegel im Blut zur passiven Akkumulation von Lipiden in der arteriellen Gefäßwand führt. In den letzten Jahrzehnten haben Forschungsergebnisse jedoch gezeigt, dass verschiedene Entzündungsprozesse in allen Stadien der Erkrankung eine wichtige Rolle spielen. Dies wird durch die Tatsache unterstrichen, dass die Inzidenz von kardiovaskulären Ereignissen trotz wirksamer lipidsenkender Therapien bei Risikopatienten weiterhin erhöht ist. Sowohl traditionelle als auch kürzlich entdeckte Risikofaktoren für Herz-Kreislauferkrankungen beeinflussen die Pathogenese der Atherosklerose durch die Veränderung lokaler und systemischer Entzündungsprozesse. Daher besteht ein dringender Bedarf an neuen anti-atherosklerotischen Therapien, die auf Entzündungsprozesse abzielen, insbesondere im Zusammenhang mit spezifischen Risikofaktoren für Herz-Kreislauferkrankungen bei betroffenen Personen.

Interleukin (IL)-1β ist ein prototypisches pro-inflammatorisches Zytokin, das stark mit dem Fortschreiten der Atherosklerose korreliert ist. Dementsprechend konnte die Neutralisierung von IL-1β in einer großen klinischen Phase-III-Studie die Zahl der sekundären kardiovaskulären Ereignisse bei Patienten mit vorangegangenem Myokardinfarkt verringern. Das Manuskript "Interleukin-1β suppression dampenens inflammatory leukocyte production and uptake in atherosclerosis" („Unterdrückung von Interleukin-1β dämpft die Produktion und Aufnahme entzündlicher Leukozyten in der Atherosklerose“), das Teil dieser kumulativen Dissertation ist, zielt darauf ab, die biologischen Grundlagen dieses präventiven Effekts besser zu untersuchen, da die genauen Mechanismen noch nicht vollständig verstanden sind. In diesem Zusammenhang konnte gezeigt werden, dass eine Anti-IL-1β-Behandlung die vaskuläre Entzündung und damit das Fortschreiten der Atherosklerose abschwächt, indem sie sowohl die Versorgung mit entzündlichen Leukozyten aus hämatopoetischen Organen als auch die Aufnahme in atherosklerotische Plaques beeinflusst.

Akuter psychischer Stress wurde als neuer Risikofaktor für Atherosklerose, insbesondere als Auslöser für akute kardiovaskuläre Ereignisse, identifiziert. Trotz zunehmender Anhaltspunkte aus epidemiologischen Studien ist jedoch nach wie vor unklar, wie genau akuter psychischer Stress die Plaqueprogression vorantreibt. Im zweiten Manuskript dieser kumulativen Dissertation (“Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis” / „Akuter psychischer Stress treibt die vaskuläre Entzündung an und fördert die Plaque-Destabilisierung in einem atherosklerotischen Mausmodell") konnte gezeigt werden, dass akuter psychischer Stress die vaskuläre Entzündung durch verstärkte Rekrutierung von entzündlichen Blutleukozyten

erhöht. Mechanistisch wird dies durch eine lokale, Noradrenalin-abhängige Aktivierung von Gefäßendothelzellen angetrieben. Darüber hinaus konnte demonstriert werden, dass der Prozess der stressinduzierten Leukozytenrekrutierung pharmakologisch beeinflusst werden kann, z. B. durch Neutralisierung des stressbedingten Anstiegs spezifischer Chemokine.

Insgesamt liefern die Daten dieser Arbeit neue mechanistische Hinweise, die bei der Entwicklung künftiger anti-atherosklerotischer Therapien zur Verringerung der Komplikationen der Atherosklerose hilfreich sein können.

1. Introduction

1.1 Atherosclerosis

1.1.1 Definition and epidemiology

Atherosclerosis is a chronic disease which is characterized by the accumulation of lipoprotein particles and inflammatory leukocytes inside the arterial vessel wall (Libby, Buring, et al., 2019). Over time, this leads to the build-up of atherosclerotic plaques. Advanced atherosclerotic lesions may ultimately cause flow-limiting obstruction of the arterial lumen, or plaque rupture/erosion may trigger thrombus formation, both leading to tissue ischemia (Libby, 2013). Consequently, atherosclerosis represents the underlying pathology of many cardiovascular complications such as coronary artery disease (CAD, e.g. acute coronary syndrome), cerebrovascular disease (e.g. stroke) and peripheral artery disease (Libby, Buring, et al., 2019).

Taken together, cardiovascular diseases (CVD) are the leading causes of death worldwide (G. A. Roth et al., 2020), with CAD being the most prevalent form of CVD (Townsend et al., 2016). Within the last decades, substantial progress has been made in the prevention and treatment of atherosclerosis, predominantly involving pharmacological and behavioral approaches (Ridker, 2016). Yet, residual risk remains high and the prevalence of CVD nearly doubled in the past three decades (Ridker, 2016; G. A. Roth et al., 2020).

1.1.2 Disease etiology of atherosclerosis

The development of atherosclerosis is characterized by different stages (initiation, progression and atherosclerotic plaque destabilization) involving metabolic and inflammatory components (Libby, 2002). Fig. 1 and the following paragraphs outline major steps in the disease etiology.

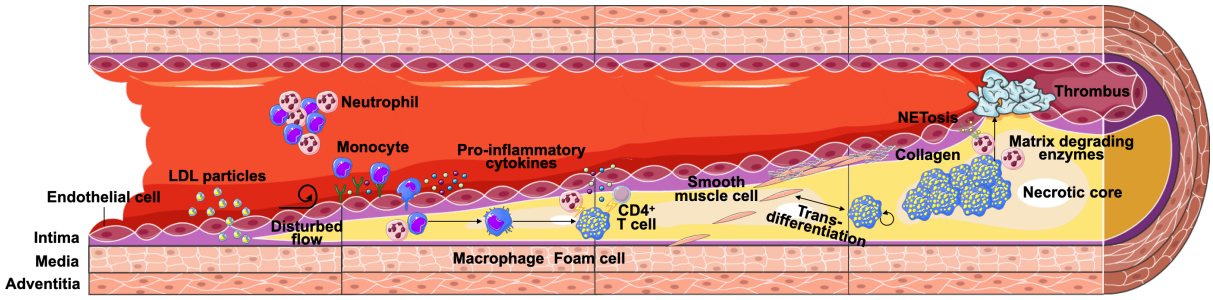

Fig. 1: Cellular processes involved in atherosclerosis development.
Initially, circulating low-density lipoprotein (LDL) particles are retained in the intimal space of the vessel wall. This is favored in regions of disturbed flow. The activated endothelium expresses adhesion molecules and pro-inflammatory cytokines, which leads to leukocyte uptake into the intima. Recruited monocytes differentiate into macrophages, engulf excess cholesterol and thus develop into lipid-loaded foam cells. The pro-inflammatory milieu is further enhanced by activated macrophages, neutrophils and cluster of differentiation (CD)4^+ T cells. To stabilize the lesion, smooth muscle cells migrate from the media to the intimal space where they produce collagen. Lesion

progression leads to the formation of a necrotic core that contains apoptotic foam cells and further promotes inflammation. Neutrophils contribute to lesion progression via the release of pro-inflammatory neutrophil extracellular traps (NETs) containing decondensed chromatin and granular content. Eventually, secreted matrix-degrading enzymes lead to fibrous cap breakdown and may cause thrombotic events. Figure modified from (Hinterdobler et al., 2021).

1.1.2.1 Initiation

Apolipoprotein (Apo)B-containing low-density lipoprotein (LDL) particles are dedicated to transport cholesterol from the liver to peripheral tissues via the bloodstream. In a setting of high plasma LDL levels, cholesterol is retained in the subendothelial (=intimal) space of the arterial wall. Uptake from the blood into arteries most likely happens by passive diffusion through endothelial cell (EC) junctions (Lusis, 2000). However, accumulation is promoted by endothelial dysfunction in atherosclerosis-prone regions with disturbed flow (Nigro et al., 2011; VanderLaan et al., 2004).

Additional to this metabolic trigger, extensive research over the past years has identified that inflammation contributes to all stages of atherosclerosis development (Libby, 2002). ECs upregulate adhesion molecule expression in response to cholesterol accumulation and oxidative modification as oxidation-specific epitopes resemble pathogen-associated molecular patterns (PAMPs, present in non-sterile inflammation) (Cybulsky & Gimbrone, 1991; Galkina & Ley, 2007; H. Li et al., 1993). Adhesion molecule expression is further favored by a systemic pro-inflammatory milieu and activation of ECs that are exposed to disturbed flow (Libby et al., 2011; Tabas et al., 2015). Additionally, ECs secrete pro-inflammatory chemokines such as CXC-motif chemokine ligand (CXCL)1 to attract leukocytes to the site of inflammation and to induce conformational changes of leukocyte integrins which allow the binding to endothelial adhesion molecules and subsequent leukocyte transmigration (Drechsler et al., 2015; Ley et al., 2007; Zernecke & Weber, 2014).

1.1.2.2 Progression

As a result of EC activation, inflammatory leukocytes, mainly bone marrow (BM)-derived monocytes, and T cells, are recruited to early atherosclerotic lesions (Galkina et al., 2006; Libby et al., 2011; Randolph, 2014). Although lesional neutrophil numbers are comparably low, there is growing evidence that neutrophils are also recruited to early atherosclerotic lesions, thereby contributing to subsequent inflammatory monocyte recruitment (Sager & Nahrendorf, 2016; Soehnlein, 2012). Once recruited, blood monocytes differentiate into macrophages which are dedicated to engulf both non-modified and oxidized LDL molecules via scavenger receptors (Ley et al., 2011; A. C. Li & Glass, 2002). Over time, these macrophages develop into lipid-rich foam cells due to cholesterol overloading (Libby, Buring, et al., 2019). While macrophages originating from recruited monocytes play a key role in early atherosclerosis,

recent studies have demonstrated that, in advanced lesions, foam cells are also derived from local proliferation and smooth muscle cell (SMC) transdifferentiation into macrophage-like cells (Allahverdian et al., 2014; Härdtner et al., 2020; Robbins et al., 2013). Cholesterol-overloaded cells further fuel inflammation by secreting inflammatory cytokines, thereby amplifying the local inflammatory response (Duewell et al., 2010; Libby, 2002). Ultimately, foam cells can undergo cell death as result of cell stress, which further exacerbates inflammation and accounts for the formation of necrotic cores inside the atheroma (Libby, Buring, et al., 2019; Moore & Tabas, 2011).

Neutrophils contribute to lesion progression both directly, via the release of pro-inflammatory neutrophil extracellular traps (NETs containing decondensed chromatin and granular content), and indirectly, via macrophage activation (Gaul et al., 2017; Silvestre-Roig et al., 2019, 2020). The adaptive immune system, mainly cluster of differentiation (CD)4^+ T lymphocytes, can also promote atherosclerosis progression. Polarization of naïve T cells occurs through the interaction with antigen-presenting cells (Wolf & Ley, 2019). These cells present, for example, peptides from ApoB which are recognized by specific T cell receptors on $CD4^+$ T cells. Depending on additional co-stimulatory molecules and secreted cytokines, naïve $CD4^+$ T cells can polarize into pro-atherogenic $CD4^+$ effector T helper type 1 (TH_1) cells which further aggravate inflammation by the secretion of various cytokines (Hedrick, 2018; Libby, 2002; Zhou et al., 2005).

The pro-inflammatory mechanisms described above are opposed by anti-inflammatory mechanisms that prevent or reverse plaque progression. Macrophages can polarize into both pro- and anti-inflammatory phenotypes, originally described as M1 and M2 macrophages (Barrett, 2018; Vergallo & Crea, 2020). Although this classification is nowadays considered an oversimplification, macrophages with an anti-atherogenic phenotype can contribute to plaque regression via emigration from the lesion, secretion of anti-inflammatory cytokines and increased cholesterol efflux (Barrett, 2018). Additionally, systemic cholesterol lowering can inhibit macrophage proliferation and thus promote plaque regression (Härdtner et al., 2020). Especially in early atherosclerosis, naïve $CD4^+$ T cells also differentiate into anti-atherogenic regulatory T cells which secrete, for example, the plaque stabilizing transforming growth factor-β (Robertson et al., 2003; Wolf & Ley, 2019). Smooth muscle cells (SMCs) can be recruited from the tunica media to the tunica intima where they produce extracellular matrix proteins (Tabas et al., 2015). These proteins form a fibrous cap to separate the pro-thrombotic plaque content from the vessel lumen and to stabilize the lesion.

Taken together, plaque progression depends on the ratio of pro-atherogenic and anti-atherogenic actions.

1.1.2.3 Destabilization

In case pro- and anti-atherogenic actions are balanced, atherosclerotic plaques remain stable. These are characterized by thick fibrous caps overlying the atheromatous material, low inflammatory activity and small necrotic cores (Silvestre-Roig et al., 2014). However, if atherosclerosis progresses further, plaques can acquire an unstable/vulnerable phenotype which ultimately leads to plaque rupture/erosion. Sustained inflammation interferes with extracellular matrix production by SMCs, and activated macrophages secrete different kinds of extracellular matrix degrading-proteinases, such as matrix metalloproteinases, all leading to fibrous cap thinning (Libby, Buring, et al., 2019; Moore & Tabas, 2011). Activated neutrophils amplify these processes by releasing various pro-inflammatory mediators and further matrix-degrading enzymes (Fernández-Ruiz, 2019; Franck et al., 2018; Mawhin et al., 2018). Ultimately, atherothrombosis, as a result of plaque rupture, can cause cardiovascular complications such as myocardial infarction (MI) or stroke.

Recently, a second mechanisms of plaque destabilization has been described. Plaque erosion, in contrast to plaque rupture, is caused by endothelial damage or denudation, causing thrombosis (Vergallo & Crea, 2020). Affected lesions often lack classical features of vulnerable plaques and rather possess a thick fibrous cap with a high content of extracellular matrix (Libby, Buring, et al., 2019). Further experimental data is needed to fully understand the mechanistic background of plaque erosion, yet recent evidence suggests a strong contribution of neutrophils and apoptotic ECs in the pathological process (Libby, Pasterkamp, et al., 2019).

1.1.3 Inflammation in atherosclerosis

Inflammatory leukocyte accumulation inside atherosclerotic plaques does not only occur through increased local recruitment but also as a result of increased systemic supply (Nahrendorf & Swirski, 2015; Poller et al., 2020). Initial evidence came from epidemiological studies which could show a correlation between total blood leukocyte numbers and CVD (Grau et al., 2004), specifically for myeloid (neutrophils and monocytes) subsets (Adamstein et al., 2021; Berg et al., 2012; Liu et al., 2020). Inflammatory leukocyte supply is controlled by hematopoiesis – the production of mature leukocytes from dedicated stem and progenitor cells – in the BM and under certain circumstances in the spleen (Nahrendorf & Swirski, 2015). Experimental studies show that hypercholesterolemia promotes the proliferation of hematopoietic stem cells with a bias towards the myeloid lineage, which results in high blood monocyte and neutrophils levels (Combadière et al., 2008; Drechsler et al., 2010; Silvestre-Roig et al., 2020; Soehnlein & Swirski, 2013; Yvan-Charvet et al., 2010). Additionally, circulating pro-inflammatory cytokines and chemokines prompt leukocyte production and release into the bloodstream (Drechsler et al., 2015; Poller et al., 2020; Tsou et al., 2007).

1.1.3.1 The inflammasome as part of the inflammatory response in atherosclerosis

As described above, activation of macrophages plays a key role in atherosclerotic disease progression. Among other mediators, macrophages release the prototypical pro-inflammatory cytokine interleukin (IL)-1β (Grebe et al., 2018; Libby, 2017). Local and systemic effects of IL-1β in atherosclerosis are numerous and include the expression of cytokines and leukocyte adhesion molecules, the activation of innate immune cells together with the induction of matrix-degrading enzymes, as well as the production of IL-6 (Libby, 2017). Consequently, neutralization of IL-1β reduced atheroma formation in mice (Vromman et al., 2019). Fig. 2 summarizes selected local effects of IL-1β on vascular cell types.

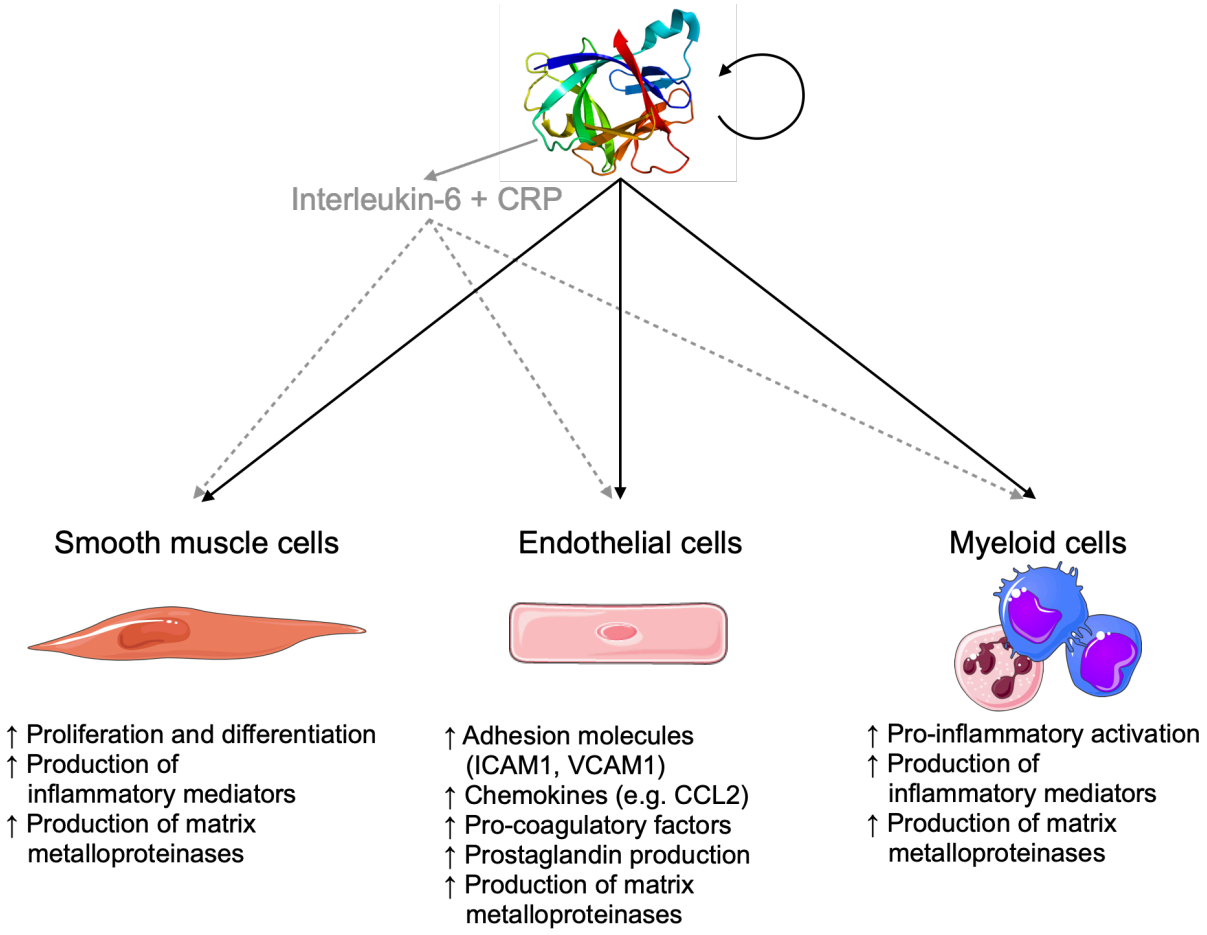

Fig. 2: Selected effects of IL-1β on vascular cell types

Interleukin (IL)-1β is produced by various cell types and leads to the overall pro-inflammatory activation of vascular cells including smooth muscle cells, endothelial cells and leukocytes of the myeloid lineage (monocytes, macrophages, neutrophils). Additionally, IL-1β can induce its own expression in an autocrine manner. Further, IL-1β induces the expression of IL-6 and systemic C-reactive protein (CRP), which further fuels pro-inflammatory processes in vascular cells. Modified from (Libby, 2017). CCL2 = CC-chemokine ligand 2, ICAM1 = intercellular adhesion molecule 1, VCAM1 = vascular adhesion molecule 1

Mature IL-1β is produced from pro-IL-1β mainly via proteolytic cleavage by caspase-1 (Afonina et al., 2015). Caspase-1 is part of the inflammasome, a multimeric protein complex consisting of the sensor protein NLRP3 (NACHT, LRR and PYD domains-containing protein 3), the adaptor protein ASC (apoptosis-associated speck-like protein) and procaspase-1 (Guo et al., 2015). In macrophages, the assembly of the inflammasome complex is dependent on two separate signals/hits: priming and NLRP3 activation (Abbate et al., 2020; Grebe et al., 2018). In the priming step, danger-associated molecular patterns (DAMPs) resulting from tissue damage promote transcription and translation of pro-IL-1β and NLRP3 inflammasome components via NF (nuclear factor)-κB. In atherosclerotic lesions, oxidized LDL can initiate inflammasome priming by binding to a complex including CD36, Toll-like receptor 4 (TLR4) and TLR6 (Sheedy et al., 2013). Further, intracellular cholesterol crystals formed by oxidized LDL can act as a trigger for NLRP3 activation (Duewell et al., 2010; Sheedy et al., 2013). This leads to the assembly of NLRP3 oligomers, ASC and procaspase-1, resulting in autocatalytic processing to active caspase-1 (Abbate et al., 2020). Fig. 3 displays a simplified summary of the intracellular processes that lead to the secretion of IL-1β in atherosclerosis.

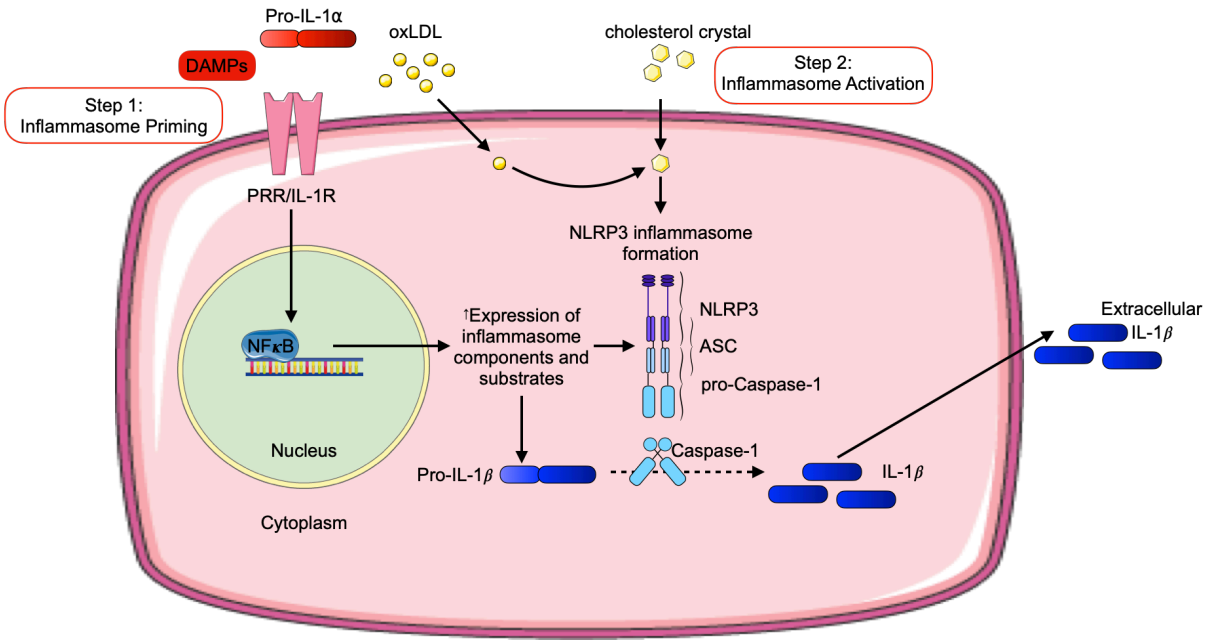

Fig. 3: Mechanisms leading to IL-1β secretion in atherosclerosis

Interleukin (IL)-1β secretion is predominantly mediated via the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome. Initially, this requires a priming step that leads to increased gene expression of inflammasome components and pro-IL-1β via the transcription factor NF (nuclear factor)-κB. In atherosclerotic plaques, this step is potentially mediated by various triggers including danger-associated molecular patterns (DAMPs), pro-IL-1α, but also oxidized LDL (oxLDL). A second trigger can then lead to inflammasome complex formation (=activation), which results in the cleavage of pro-IL-1β in mature IL-1β via Caspase-1. In atherosclerosis, detection of cholesterol crystals via the sensor protein NLRP3 can induce inflammasome activation. PRR = pattern

recognition receptor. IL-1R = Interleukin 1 receptor. ASC = apoptosis-associated speck-like protein. Modified from (Grebe et al., 2018).

Although macrophages may represent the most important source for IL-1β in atherosclerotic plaques, it is also expressed and secreted by other cell types such as neutrophils, ECs or SMCs (Abbate et al., 2020; Libby, 2017). Notably, extracellular pro-IL-1β can also be cleaved independent of caspase-1, by, for instance, caspase-8 or extracellular proteases from neutrophils (Afonina et al., 2015; Dinarello, 2019).

1.1.3.2 Targeting inflammation in clinical trials

As mentioned in 1.1.1, residual risk for CVD remains high despite successful lipid-lowering therapies, which is most likely attributed to the strong inflammatory component in disease etiology (Koenig, 2020; Ridker, 2016). Consequently, novel anti-atherosclerotic therapies aim to target various inflammatory pathways for the prevention of atherosclerotic complications (Libby & Everett, 2019). These include known anti-inflammatory drugs that are already approved for the treatment of other diseases such as methotrexate and colchicine. While methotrexate has failed to influence cardiovascular risk in the patients studied (Ridker, Everett, et al., 2019), colchicine has significantly reduced cardiovascular outcomes in several studies (Nidorf et al., 2013, 2020; Tardif et al., 2019).

As outlined above, the inflammasome – specifically its downstream mediator IL-1β – plays a key role in atherosclerosis. The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) is a pioneer study for targeting inflammation in atherosclerosis, and could show that administration of an antibody against IL-1β (canakinumab) reduced the risk for recurrent cardiovascular events in patients with previous MI (Ridker et al., 2017). Despite being the first clinical trial to show that anti-inflammatory therapies can reduce cardiovascular outcomes, canakinumab failed to get drug approval due to safety and cost issues after trial completion. Still, the results have inspired further preclinical and clinical studies to interfere with cascades upstream and downstream of IL-1β (Weber & Hundelshausen, 2017). MCC950 is a small molecule inhibitor of the NLRP3 inflammasome (Coll et al., 2015) which was shown to reduce atherosclerosis development in mice (Heijden et al., 2017) and by its mode of action also targets IL-18 (Ridker, MacFadyen, et al., 2019), another pro-inflammatory cytokine that is processed by the inflammasome complex (Abbate et al., 2020). Downstream of IL-1β, IL-6 is a potent pro-inflammatory cytokine. Thus, specifically targeting IL-6 might represent a further promising candidate for anti-atherosclerotic therapies (Ridker, MacFadyen, et al., 2019; Ridker, 2021).

Taken together, preclinical and clinical studies targeting inflammation in atherosclerosis demonstrate encouraging results but warrant further investigation to develop drugs that can be used in clinical practice in the future (Soehnlein & Libby, 2021).

1.2 Risk factors of atherosclerosis

In the past century, various risk factors for atherosclerosis and succeeding CVD have been discovered by epidemiological and experimental studies. Knowledge about these risk factors is necessary to identify susceptible individuals and to target prevention strategies (Lloyd-Jones, 2010). Recently, cardiovascular risk factors have been categorized into classical and non-classical risk factors. Classical risk factors have been known for a long time and have already been included in risk prediction models, prevention and treatment strategies (Levin & Rader, 2020; McGorrian et al., 2011; Piepoli et al., 2016). In addition, further lifestyle factors and pathologies have been identified recently and are thus termed non-classical or non-traditional risk factors. Most non-classical risk factors are to some extent modifiable, making them an interesting target for prevention and therapy (Schloss et al., 2020). As outlined in detail below, both classical and non-classical risk factors for atherosclerosis convey some of their pathology by modulating inflammatory mechanisms.

1.2.1 Classical risk factors

Being male is a classical risk factor for CVD and especially CAD (Mosca et al., 2011). This is, in part, attributed to sex differences in cardiovascular risk factors (Walli-Attaei et al., 2020). Still, recent studies indicate that male gender represents a cardiovascular risk factor *per se* by promoting vascular inflammatory processes (Man et al., 2020).

Aging and genetic predisposition are further classical, non-modifiable CVD risk factors. Age is the most important determinant of cardiovascular health (North & Sinclair, 2012). Additional to inherent complications of aging such as arterial stiffness and endothelial dysfunction (North & Sinclair, 2012; Paneni et al., 2017), biological aging induces inflammatory processes such as increased circulating cytokine levels or oxidative stress (Tyrrell & Goldstein, 2021; J. C. Wang & Bennett, 2012). Clonal hematopoiesis has been recently indicated to link aging with atherosclerosis (Jaiswal et al., 2017). In clonal hematopoiesis, increasing numbers of white blood cells are derived from single clones because of certain somatic mutations in hematopoiesis (Jaiswal & Ebert, 2019). Although the exact mechanisms remain unclear, clonal hematopoiesis promoted vascular inflammatory processes in an atherosclerotic mouse model (Fuster et al., 2017). Multiple genetic risk loci for CAD have been identified in large-cohort genome-wide association studies (Harst & Verweij, 2018; The CARDIoGRAMplusC4D Consortium et al., 2013). Although a single variant is associated with only moderate effects, certain combinations and high numbers of these common variants seem to account for a

significant proportion of CAD risk heritability (Mauersberger et al., 2021). Therefore, polygenetic risk scores try to predict individual CAD risk in mathematical models including numbers and weighted impacts of multiple variants (Levin & Rader, 2020). Not surprisingly, many CAD risk loci identified in GWAS or similar studies are associated with genes involved in inflammatory pathways such as the IL-6 receptor (Mauersberger et al., 2021).

Several metabolic factors such as hyperlipidemia and diabetes, but also arterial hypertension are well-known classical risk factors for CAD. Although these factors can be modified by lifestyle or medications, they all harbor some degree of genetic predisposition as well (Cai et al., 2020; Ma et al., 2010; Understanding Society Scientific Group et al., 2019). Hyperlipidemia – in particular hypercholesterolemia – is a key player in atherosclerotic lesion initiation as outlined above. In this context, it is now evident that atherosclerosis is not merely a metabolic disease caused by passive lipid accumulation but also entails a strong inflammatory component in which (modified) cholesterol represents an important pro-inflammatory trigger (Levitan et al., 2010; Sager & Nahrendorf, 2016). Unhealthy diet and obesity, additional to being classical CAD risk factors themselves (Ortega et al., 2016; Z. Wang et al., 2018), contribute substantially to elevated cholesterol levels (Carson et al., 2019; Mannu et al., 2013). Furthermore, obesity is strongly associated with chronic inflammation, thereby promoting atherosclerosis (Rocha & Libby, 2009). Likewise, diabetes progression is accompanied by a systemic pro-inflammatory and pro-oxidative milieu in patients, which is likely to aggravate atherosclerotic processes (Katakami, 2017; Pennathur & Heinecke, 2007; Yuan et al., 2018). Chronic arterial hypertension is another classical risk factor for CAD and strongly associates with endothelial dysfunction and inflammation although it is not yet clear if this is cause or consequence (Brandes, 2018; Dinh et al., 2014; Higashi et al., 2012).

In contrast to all conditions mentioned before, smoking by its nature is a modifiable, classical risk factor. Still, due to missing lifestyle changes, approximately 10 % of CVD deaths are attributable to smoking (Ezzati et al., 2005). This is caused by a multitude of pathophysiological mechanisms including circulating toxic compounds, oxidative stress, pro-coagulative priming, local vascular inflammation and dysfunction, and systemic inflammation (Messner & Bernhard, 2014; Münzel, Hahad, et al., 2020; Siggins et al., 2014).

1.2.2 Non-classical risk factors

A great number of epidemiological as well as experimental studies have shown that, apart from classical risk factors, additional lifestyle factors and comorbidities affect atherosclerosis development, especially via inflammatory processes.

Several communicable/infectious (=non-sterile) diseases, such as viral and bacterial infections (Kwong et al., 2018; Musher et al., 2019), and non-communicable/non-infectious (=sterile) pathologies, such as rheumatoid arthritis or prior MI or stroke, create a higher long-

term risk for CVD (Dutta et al., 2012; Skeoch & Bruce, 2015). Both non-sterile and sterile inflammatory diseases are likely to promote atherosclerosis progression via shared mechanisms (Sager & Koenig, 2017). Concretely, sterile inflammation results in increased circulating inflammatory leukocyte numbers through enhanced production in the BM, and increased recruitment via endothelial activation, ultimately aggravating vascular inflammation (Dutta et al., 2012, 2015; Sager et al., 2016; Sager & Koenig, 2017). Further studies, however, are needed to prove whether these findings can be extrapolated to non-sterile pathologies (Sager & Koenig, 2017).

An active lifestyle in general together with regular exercise was shown to modify CVD risk both through metabolic and anti-hypertensive actions (Dunstan et al., 2021; Lavie et al., 2019). Recently, a study in mice demonstrated that exercise attenuates atherosclerosis progression also by modifying local and systemic inflammation (Frodermann et al., 2019). Here, voluntary wheel running was shown to reduce leptin levels, which promotes long-lasting BM niche quiescence and retention factors via epigenetic modifications. Consequently, pro-inflammatory leukocyte supply and vascular inflammation is mitigated.

Enhanced production in the BM is a pro-inflammatory mechanism shared by sleep deprivation, a further novel risk factor for CAD (Bras, 2019; Tall & Jelic, 2019; Tobaldini et al., 2019). Mechanistically, sleep fragmentation/deprivation affects hypothalamic hypocretin production, a hormone that limits myeloid cell production in the BM under steady-state conditions (McAlpine et al., 2019). Additionally, sleep deprivation was shown to affect other biological mechanisms such as the autonomic nervous system, endothelial function and metabolic parameters (Tobaldini et al., 2019).

Air, light and noise pollution belong to another novel category of CVD risk factors (Daiber et al., 2019; Lelieveld et al., 2019; Münzel et al., 2021; Münzel, Miller, et al., 2020). Air pollution, specifically trough fine particulate matter (2.5 microns in diameter and less) and ozone gas, has been shown to affect cardiovascular risk by multiple mechanisms including hemostatic and homeostatic parameters, oxidative stress and, in particular, systemic inflammation (Al-Kindi et al., 2020; Brook et al., 2004). In this context, air pollution was shown to induce blood leukocytosis, leukocyte activation, endothelial dysfunction and increased circulating cytokine levels in humans (Münzel, Sørensen, et al., 2017; Münzel et al., 2018). In line, air pollution aggravated cardiovascular inflammation in atherosclerotic mouse models (M. R. Miller & Newby, 2019) although it is not yet clear if these are primary effects caused by fine particulate matter or secondary effects due to neuroendocrine activation and/or inflammation caused by lung injury. Furthermore, air together with light and noise pollution are likely to increase CVD risk indirectly by affecting other lifestyle factors such as sleep and chronic psychosocial stress (Kröller-Schön et al., 2018; Münzel, Daiber, et al., 2017; Münzel, Miller, et al., 2020).

For quite some time, chronic psychosocial stress itself has been suspected to influence CVD. However, it was not until the INTERHEART study that the contribution of psychosocial stress to CVD was robustly quantified in a large, world-wide case-control study (Yusuf et al., 2004). This study was designed to evaluate the relationships between major cardiovascular risk factors and the occurrence of MI. The results showed that long-term psychosocial stress is comparably associated with MI (odds ratio 2.67) as other classical risk factors such as hypertension (odds ratio 1.91) or diabetes (odds ratio 2.37). Further large-scale epidemiological studies analyzed the associations between individual long-term psychosocial stressors, for example social isolation, job strain or childhood abuse, and meta-analyses of the respective studies calculated odds ratios of up to 1.5 for the link between psychosocial stress and cardiovascular events (Kivimäki & Steptoe, 2018; Virtanen & Kivimäki, 2018). Additionally, chronic stress is likely to affect CVD risk by influencing lifestyle factors that evolved as non-classical risk factors as described above. Concretely, chronic stress was shown to promote smoking, sedentary lifestyle, disturbed sleep or an unhealthy diet (Kivimäki & Steptoe, 2018; Osborne et al., 2020). Epidemiological studies support the hypothesis that chronic stress conveys some of its pathology on CVD by modulating inflammatory processes. In this context, observational studies reported higher levels of circulating pro-inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor alpha (TNFα), IL-6 and soluble adhesion molecules in the plasma of stress-affected individuals (Känel et al., 2008; Kiecolt-Glaser et al., 2003; Lagraauw et al., 2015). In line, blood inflammatory leukocytes are higher in medical residents on an intensive care unit on duty compared to off duty, reflecting the stress status (Heidt et al., 2014). Further, studies using state-of-the-art imaging techniques found an association between brain amygdala activity (corresponding to the degree of perceived stress), BM hematopoiesis, arterial inflammation, and cardiovascular events (Kang et al., 2021; Tawakol et al., 2017). Similar to observations in stressed humans, experimental animal studies report higher levels of systemic inflammatory markers and blood leukocyte levels in response to chronic psychosocial stress (Barnum et al., 2012; Heidt et al., 2014; E. S. Miller et al., 2019). Mechanistically, the sympathetic nervous system (SNS) is assumably responsible for higher leukocyte levels due to increased hematopoietic stem cell proliferation, and a concomitant bias towards myelopoiesis and an inflammatory gene signature in circulating leukocytes (Heidt et al., 2014; Powell et al., 2013). Additional to circulating leukocytes, chronic stress was also shown to affect ECs resulting in increased adhesion molecule expression and endothelial dysfunction, most likely caused by systemic low-grade inflammation and activation of the renin-angiotensin-aldosterone system (Bevilacqua et al., 1985; Deng et al., 2019; Groeschel & Braam, 2011; Mackay et al., 1993; Mateo et al., 2006; Puhlmann et al., 2005; Sher et al., 2020). Consequently, atherosclerosis is accelerated in animal models of chronic stress

(Lagraauw et al., 2015) and characterized by features of vulnerable plaques (Heidt et al., 2014; Najafi et al., 2013; L. Roth et al., 2015; T. Zhang et al., 2011; Zheng et al., 2014).

1.3 Acute psychosocial stress as novel risk factor for atherosclerosis

In addition to the above-mentioned risk factors, acute psychosocial stress is a novel independent risk factors for atherosclerosis (Hinterdobler et al., 2021). Stress is defined as the body´s response to external or internal challenges that disturb homeostasis and require adaptation beyond an individual´s perceived ability to cope (Glaser & Kiecolt-Glaser, 2005). In detail, an environmental stimulus (=stressor) triggers a reaction in the brain (=stress perception) where the information is integrated, and activates physiologic fight-or-flight responses in the body (Buijs & Eden, 2000; Viswanathan & Dhabhar, 2005). Although strict discrimination criteria are missing, psychosocial stressors are classified based on exposure duration and severity (Dimsdale, 2008). In this context, chronic stress, as described above, refers to consistent or repetitive stressors with rather low intensity such as work stress, social isolation, caring responsibilities or marital unhappiness (Kivimäki & Steptoe, 2018). Acute stress, by contrast, is considered short-term and drastic stressors such as anxiety, grief, natural disasters or acts of war (Mittleman & Mostofsky, 2011; Mostofsky et al., 2014). As the stress stimulus is integrated by the brain, perceived severity of the same stressor strongly depends on individual stress susceptibility (Ebner & Singewald, 2017). Notably, acute stress, compared to chronic stress, associates with an even greater incidence for CVD in people with manifest atherosclerosis (Kivimäki & Steptoe, 2018).

1.3.1 Physiological stress axes and neuroimmune interactions

As mentioned above, stress stimuli are perceived by the brain and promote the activation of a physiologic fight-or-flight response, a mechanism essential to ensure survival by reacting to threats and enduring potential injuries (Nahrendorf, 2020). In detail, neuronal networks activate three major pathways in the body: the hypothalamus-pituitary-adrenal (HPA) axis, the sympatho-adrenomedullary (SAM) axis and local activation of the SNS (Lagraauw et al., 2015). Activation of the HPA axis leads the hypothalamus to release the corticotropin releasing hormone, which results in subsequent adrenocorticotropic hormone (ACTH) secretion into the blood stream via the pituitary gland. In the adrenal cortex, ACTH triggers the systemic release of cortisol and other glucocorticoid hormones. The SAM axis acts via sympathetic innervation of the adrenal medulla resulting in systemic catecholamine (epinephrine and norepinephrine) release. Further, the SNS can also activate tissues via the local release of norepinephrine by projecting nerve endings.

Experimental evidence suggests three distinct physiological stress reactions with a potential influence on CVD. The fight-or-flight response modifies hemodynamic parameters,

for example cardiac output, blood pressure and heart rate (Rozanski et al., 1999), which prepares the body for potential physical exertion. Further, stress affects hemostasis including platelet activation (Känel, 2015; Koudouovoh-Tripp et al., 2020), most likely in preparation for potential injuries that require blood clotting. Finally, stress was shown to strongly promote pro-inflammatory processes (Hinterdobler et al., 2021).

Neuroimmunology describes the interactions between the nervous and the immune system, two systems that operate together to adapt to potential threats (Salvador et al., 2021). However, neuroimmune interaction also occur under steady-state conditions, for example in circadian leukocyte trafficking which is partly controlled by the SNS (Ince et al., 2019; Juan et al., 2019; Scheiermann et al., 2012). Interestingly, nervous system activity is involved in the regulation of hematopoiesis, cell fate decisions and the release of mature leukocytes (Carnevale & Lembo, 2020; Katayama et al., 2006; Pierce et al., 2017; Vasamsetti et al., 2018). Further, the nervous system was also shown to influence inflammatory processes locally inside the vessel, e.g. via modulating adhesion molecule expression by aortic ECs in a mouse model of MI (Sager et al., 2016).

Although interactions between the nervous and the immune system can be bidirectional, signaling from the nervous to the immune system seems to be responsible for the detrimental effects of stress on human health (Glaser & Kiecolt-Glaser, 2005). While some studies suggest a short-term immune-enhancing effect of stress (Dhabhar et al., 2012; Viswanathan & Dhabhar, 2005), continuous stress exposure is thought to compromise the body´s immune system as energy resources are re-distributed to systems immediately necessary for the fight-or-flight response (Ince et al., 2019; Qing et al., 2020; Wieduwild et al., 2020). In the context of acute psychosocial stress, vascular inflammation, atherosclerosis and CVD, research has just started to investigate neuroimmune interactions as a potential link.

1.3.2 Epidemiological evidence

Observational studies showed that, in contrast to chronic stress which gradually increases the risk for CVD, acute psychosocial stress can act as an acute trigger for cardiovascular events in high-risk people (Kivimäki & Steptoe, 2018; Mittleman & Mostofsky, 2011; Steptoe & Kivimäki, 2012). On a population-level, natural catastrophes (such as earthquakes) or war and terrorist attacks associate with higher incidences for CVD compared to control periods (Leor et al., 1996; Meisel et al., 1991). For example, a recent study examining the occurrences of acute cardiovascular events during the Great East Japan Earthquake in 2011 compared to previous years found that the incidence of acute coronary syndromes, heart failure and stroke significantly increased directly after the earthquake (Aoki et al., 2012). Major sporting events, such as world cup soccer, or sociopolitical events, such as presidential elections, represent

more moderate forms of acute psychosocial stress. Still, they have also been associated with increased CVD risk (Leeka et al., 2010; Mefford et al., 2020; Wilbert-Lampen et al., 2008).

Several individual-level studies provide further evidence for acute psychosocial stress being a trigger of acute cardiac events (Edmondson et al., 2012). For example, the risk for MI or acute coronary syndromes is 4.74-fold higher immediately after outbursts of anger as calculated by a recent meta-analysis (Mostofsky et al., 2014). Likewise, data from the INTERHEART study shows an odds ratio of 2.44 for acute MI in the context of emotional upset (Smyth et al., 2016). Additional to linking acute psychosocial stress to a higher risk for CVD, epidemiological studies provided implications for mechanistic causalities. Beyond changes in hemodynamics and hemostasis, observational studies suggest a strong link between acute stress, inflammation and CVD (Lagraauw et al., 2015). A study investigating the effect of a major sporting event reported a higher incidence of cardiac emergencies on days of matches involving the local national team (Wilbert-Lampen et al., 2008). In subjects suffering from a stress-associated cardiovascular event, this was paralleled by higher plasma levels of classical inflammatory mediators such as soluble vascular adhesion molecule 1 (sVCAM1), monocyte chemoattractant protein 1 (MCP-1, also known as CC-motif chemokine ligand (CCL)2) and TNFα compared to control patients (Wilbert-Lampen et al., 2010). In line, higher circulating inflammatory cytokines were found in patients experiencing an acute stress-associated form of cardiomyopathy called Takotsubo cardiomyopathy (Scally et al., 2019).

1.3.3 Experimental evidence

Further evidence that acute stress promotes pro-inflammatory processes comes from experimental human data. In experiments using arithmetic tasks, public speaking tests or similar stressors, researchers found that acute stress influences several inflammation-associated parameters: plasma levels of pro-inflammatory mediators such as IL-6, IL-1β and soluble endothelial adhesion molecules (Heinz et al., 2003; Kim et al., 2019; Koelsch et al., 2016; Kuebler et al., 2015) but also microvascular dysfunction (Vaccarino et al., 2018). Of note, elevated levels of circulating IL-6 and norepinephrine in response to acute stress exposure were associated with peripheral vasoconstriction and a higher risk for future cardiovascular events in participants with pre-existing atherosclerosis (Kim et al., 2019). Additionally, one study demonstrated that the rise in inflammatory cytokines is paralleled by a pro-inflammatory gene expression profile in blood leukocytes, most likely mediated by the transcription factor NF-κB (Kuebler et al., 2015).

In experimental human studies, blood leukocytes are often analyzed together with circulating mediators in order to check the inflammation status. As mentioned above, chronic stress generally raises circulating total leukocyte and inflammatory subset levels. By contrast, acute psychosocial stress seems to affect leukocyte subsets divergently. While neutrophils

and natural killer cells were elevated in a study using skydiving to simulate acute stress, monocytes and lymphocytes decreased immediately before the challenge (Breen et al., 2016). This is contrasted by a study reporting a general increase of all major leukocyte subsets in response to a public speaking test (Goebel et al., 2000). The conflicting results might be explained by different leukocyte kinetics, varying stress stimuli between studies and differences in the inter-individual stress susceptibility, but also by the complex redistribution of leukocytes within hematopoietic organs and tissues (Ince et al., 2019). In any case, leukocyte dynamics in response to acute psychosocial stress warrant further investigation, especially under controlled settings.

Although highly relevant, experimental studies in humans naturally possess limited power to investigate disease mechanisms. Thus, research requires proper animal models to reveal mechanisms linking acute psychosocial stress with cardiovascular events (=atherosclerosis progression). Atherosclerosis is typically studied in animals with specific genetic knockouts leading to hypercholesterolemia and lesion development similar to human atherosclerosis (Getz & Reardon, 2018). For example, *ApoE* or *Ldlr* knockout mice are widely used animal models as they develop high cholesterol levels and atherosclerotic lesions upon a high-cholesterol diet (HCD). Established acute stress models have been adopted from psychological studies and include severe stressors such as restraint stress (Qing et al., 2020; Viswanathan & Dhabhar, 2005; Xu et al., 2020), in which animals are immobilized for minutes up to several hours, pain stress models such as eye bleeding (Qing et al., 2020) or social defeat (Qing et al., 2020; Xu et al., 2020).

Such studies demonstrated that acute stress strongly increases circulating cytokine and chemokine levels (Cheng et al., 2015; Qing et al., 2020). Regarding circulating blood leukocyte levels, animal studies – in contrast to human studies – consistently report decreasing monocyte and lymphocyte levels, while neutrophil levels are unchanged or even increase (Viswanathan & Dhabhar, 2005; Wouw et al., 2020; Xu et al., 2020). Although massively mobilized from reservoirs, blood leukocytes rapidly traffic to sites of inflammation and immune activation, which overshoots supply (Dhabhar et al., 2012). However, how exactly acute mental stress affects blood leukocytes is poorly understood. As generally known, leukocyte recruitment also requires activation of the endothelial compartment in respective tissues (Marchini et al., 2021). Still, very little is known about the inflammatory effects of acute stress on the vasculature. The same applies to the process of plaque destabilization. Since plaque rupture does not occur spontaneously in classical, atherosclerotic animal models, research predominantly focused on surrogate markers of plaque stability phenotypes (Lagraauw et al., 2015, 2019).

Taken together, experimental human and animal data strongly link acute psychosocial stress to increased vascular inflammation (Fig. 4). However, several questions, specifically

regarding the effect of acute stress on circulating leukocytes and ECs, and on how acute mental stress rapidly drives plaque rupture, remain unanswered.

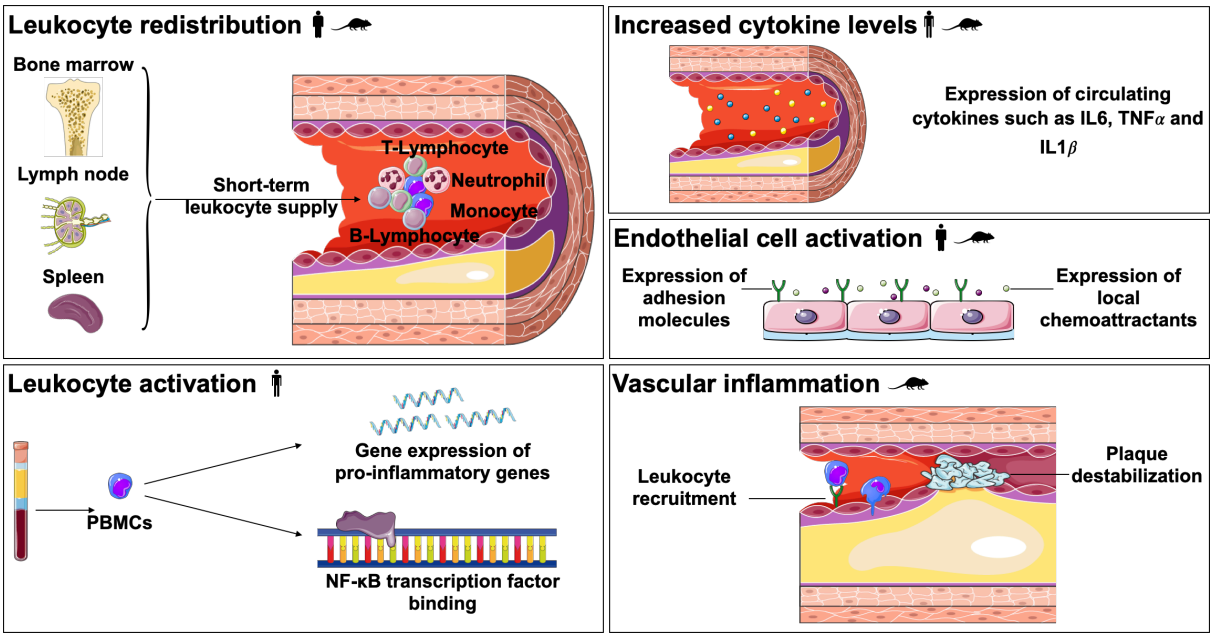

Fig. 4: Mechanisms linking acute psychosocial stress with vascular inflammation

Acute psychosocial stress affects inflammatory processes along the whole cascade of atherosclerotic disease etiology. This figure summarizes current experimental evidence on how acute psychosocial influences specifically leukocyte distribution, pro-inflammatory signaling in the circulation, leukocyte and endothelial cell activation, and inflammation in the atherosclerotic vessel wall. = evidence from human experimental data, = evidence from animal experimental data, IL = interleukin, PBMC = peripheral blood mononuclear cell, TNF = tumor necrosis factor. Figure modified from (Hinterdobler et al., 2021).

1.4 Aim

It is now well established that inflammation plays a key role in the etiology of atherosclerosis. However, to better exploit the therapeutic potential of anti-inflammatory treatment, further research on exact mechanisms in distinct settings is needed. This thesis aimed to investigate two specific questions:

1) What are the exact mechanisms that underlie the beneficial effects of pharmacological IL-1β suppression?
2) How can acute mental stress rapidly trigger plaque destabilization in patients with pre-existing atherosclerosis?

2. Key methods

Some experiments for the manuscripts included in this paper-based dissertation were done by other authors, cooperation partners or commercial companies. Therefore, the respective experimental procedures are not outlined in this methodology section. Apart from that, this paragraph describes in detail all key methods that were used in the publications.

2.1 Animal experiments

2.1.1 Mice

C57BL/6J mice (wild-type, JAX stock #000664), UBC-GFP mice (C57BL/6-Tg(UBC-GFP)30Scha/J, JAX stock #004353), *ApoE*$^{-/-}$ mice (B6.129P2-*Apoe*^{tm1Unc}/J, JAX stock #002052), *Ldlr*$^{-/-}$ mice (B6.129S7-*Ldlr*^{tm1Her}/J, JAX stock #002207) and *Casp1*$^{-/-}$ mice (B6N.129S2-*Casp1*^{tm1Flv}/J, JAX stock #016621, on a mixed C57BL/6J;C57BL/6N genetic background) were purchased from the Jackson Laboratory.

In standard atherosclerosis experiments, eight- to twelve-week-old *ApoE*$^{-/-}$ mice from both sexes were fed a HCD (21.2 % fat by weight and 0.2 % cholesterol, TD.88137, Envigo) for six to eight weeks. Male and female *ApoE*$^{-/-}$ mice that underwent MI surgery were on HCD for a total of ten weeks. Female *Ldlr*$^{-/-}$ mice for BM chimera generation were put on a HCD for twelve weeks following a recovery period of six weeks after BM irradiation.

In all experiments, age- and sex-matched littermates were randomly assigned to treatment or control groups. At the end of experiments, mice were euthanized under isoflurane anesthesia by cervical dislocation or exsanguination.

2.1.2 IL-1β suppression

For anti-IL-1β treatment, 01BSUR (10 mg/kg body weight (BW), donated from Novartis, Basel, Switzerland), a murine analog of canakinumab, or isotype-matched control IgG2a (10 mg/kg BW) were injected subcutaneously once a week (Sager et al., 2015). For NLRP3-inflammasome inhibition, MCC950 (10 mg/kg BW, AG-CR1-3615, AdipoGen) or vehicle (PBS) were injected intraperitoneally (i.p.) every 48 h for three weeks (Coll et al., 2015).

2.1.3 Generation of bone marrow chimeras

To investigate the cellular contributions to IL-1β production, BM chimeras with hematopoietic cell-specific caspase-1 knockout were generated. For that, *Ldlr*$^{-/-}$ mice at the age of eight weeks underwent lethal BM irradiation at the Institute for Cardiovascular Prevention (IPEK, Munich). BM for reconstitution was obtained from femurs of wild-type or *Casp1*$^{-/-}$ donor animals. After flushing the bone and filtering the cells through a 40 µm cell strainer, cell

numbers were manually determined using a hemocytometer. Around 6 h after irradiation, *Ldlr*$^{-/-}$ mice were injected intravenously (i.v.) with 3x10^{6} BM cells from respective donor animals.

2.1.4 Acute stress simulation

Acute mental stress was simulated using immobilization, a common method to apply psychological stress due to perceived confinement (Glavin et al., 1994). For that, animals were placed in mouse holders/restrainers (HLD-MM, Kent Scientific), without squeezing or compression, for up to 3 h (as either a single session or once daily for three consecutive days). Mice were sacrificed immediately or until up to 24 h after the final stress episode.

2.1.5 Adoptive transfer of green fluorescent protein (GFP)-labelled neutrophils and monocytes

Purified neutrophils and monocytes from UBC-GFP donor animals were obtained as described in 2.4. Respective experimental groups received i.v. injections with equal amounts of cells and organs were harvested 24 h later. In respective organs, the number of CD11b^{high}GFP^{high} cells was quantified using flow cytometry.

2.1.6 Chemical depletion of sympathetic nervous system

6-hydroxydopamine (6-OHDA) is a neurotoxin and regularly used to deplete sympathetic nervous fibers (Sager et al., 2016). In the respective experiments using 6-OHDA (162957, Sigma-Aldrich), mice were injected i.p. with a dose of 100 mg/kg BW six days and another dose of 250 mg/kg BW four days before stress exposure. Control animals received the corresponding vehicle injection (0.1 % ascorbic acid (3149.1, Roth) in 0.9 % sodium chloride (NaCl) solution (Fresenius)).

2.1.7 Intervention with anti-CXCL1 and anti-CCL7

To neutralize circulating chemokine levels, animals received i.p. injections with either anti-CXCL1 and anti-CCL7 (AF-456-NA and AF-453-NA, 20 µg each, R&D) or an Immunoglobulin G (IgG) control (AB-108-C, R&D) under isoflurane anesthesia 30 min prior to stress exposure.

2.2. Tissue processing

Peripheral blood samples were subjected to red blood cell lysis in 1X RBC lysis buffer (420302, BioLegend). After 5 min, the reaction was stopped with 1X PBS, centrifuged at 800 xg for 10 min at 4 °C and resuspended in FACS buffer (PBS containing 0.5 % bovine serum albumin, A2153, Sigma). To generate BM single cell suspensions, femurs were flushed, and the resulting suspension was filtered through a 40 µm cell strainer and collected in FACS buffer.

Similarly, excised spleens and inguinal lymph nodes were plunged through a 40 µm cell strainer. After centrifugation, cells were resuspended in FACS buffer.

Tissues from aorta, lung, skin, heart, kidney, and liver were digested prior to the flow cytometric analysis of leukocytes. For that, organs were minced and incubated at 37 °C at 750 rpm for 1 h on a thermoshaker in digestion buffer containing collagenase I (450 U/ml, C0130), collagenase XI (125 U/ml, C7657), DNase I (60 U/ml, D5319), hyaluronidase (60 U/ml, H3506) and HEPES buffer solution (83264, all Sigma-Aldrich) in 1X PBS. All digested organs were processed through a 40 µm cell strainer, centrifuged, and resuspended to generate single cell suspensions. After that, a 10 µl aliquot of each sample was obtained to determine total numbers of leukocytes using a hemocytometer.

For the flow cytometric analysis of aortic ECs, aortas were digested at 37 °C at 750 rpm for 40 min in 1X PBS containing DNase I (250 U/ml) and collagenase IV (10 mg/ml, LS004212, CellSystems). For fluorescence-activated sorting of different aortic cell populations, aortas were digested in an optimized digestion buffer ((DNase I (250 U/ml), collagenase II (280 U/ml, LS004177, Worthington Biochemical Corporation), collagenase IV (215 U/ml, LS004189, Worthington Biochemical Corporation) and dispase (1.2 U/ml, 17105-041, Gibco)) at 37 °C at 750 rpm for 1 h.

2.3 Flow cytometry and fluorescence-activated cell sorting

Flow cytometric staining was performed on single cell suspensions from processed tissue or cell culture cells, and conducted at 4 °C in FACS buffer. In every analysis, cells were first gated on viable (FSC-A vs. SSC-A) and single (FSC-A vs. FSC-W and SSC-A vs. SSC-W) cells.

To compensate, all antibodies were conjugated to OneComp eBeads (01-1111-42, Thermo Fisher). GFP-positive samples were used to compensate for GFP fluorescence. Flow cytometry data were acquired on an LSRFortessa (BD Bioscience) and corresponding data were analyzed using FlowJo software (version 9 or 10).

2.3.1 Murine cells

For myeloid cell analysis of tissues, cells were stained with anti-mouse hematopoietic lineage markers and leukocyte subset markers as listed in table 1. Neutrophils were identified as lineage^{high}CD45.2^{high}CD11b^{high}CD115^{low}Ly6C^{intermediate}. In hematopoietic organs (blood, BM and spleen), monocytes were identified as lineage^{low}CD45.2^{high}CD11b^{high}CD115^{high}Ly6C$^{high/low}$, while in the remaining organs, monocytes were identified as lineage^{low}CD45.2^{high}CD11b^{high}F4/80^{low}Ly6C$^{high/low}$ and macrophages as lineage^{low}CD45.2^{high}CD11b^{high}F4/80^{high}Ly6C$^{low/intermediate}$.

Tab. 1: Flow cytometry antibodies used for mouse myeloid cell analysis

Target	Conjugate	Manufacturer	Catalogue#	Clone	Dilution
B220	PE	Biolegend	103208	RA3-6B2	1:600
CD90.2	PE	Biolegend	140308	53-2.1	1:3000
CD49b	PE	Biolegend	108908	DX5	1:1200
NK1.1	PE	Biolegend	108708	PK136	1:600
Ter-119	PE	Biolegend	116208	TER-119	1:600
Ly6G	PE	Biolegend	127608	1A8	1:600
CD45.2	PerCP/Cy5.5	Biolegend	109828	104	1:300
CD11b	APC/Cy7	Biolegend	101226	M1/70	1:600
CD115	BV711 or biotin	Biolegend	135515 or 135508	AFS98	1:600
F4/80	PE/Cy7	Biolegend	123114	BM8	1:600
Ly6C	FITC or BV421	Biolegend	128006 or 128032	HK1.4	1:600
Streptavidin	BV510	Biolegend	405234	-	1:300

To stain blood lymphoid cells, cells were incubated with antibodies listed in table 2. B cells were identified as $CD19^{high}CD3^{low}$ and T cells as $CD19^{low}CD3^{high}$.

Tab. 2: Flow cytometry antibodies used for mouse blood lymphoid cell analysis

Target	Conjugate	Manufacturer	Catalogue#	Clone	Dilution
CD3	BV421	Biolegend	100227	17A2	1:600
CD19	BV605	Biolegend	115539	6D5	1:600
Ly6C	FITC	Biolegend	128006	HK1.4	1:600
CD115	PerCP/Cy5.5	Biolegend	135525	AFS98	1:600

For aortic EC analysis and sorting, staining was performed with antibodies listed in table 3. ECs were identified as $CD45.2^{low}$, $CD31^{high}$, and $CD107a^{intermediate/high}$. For cultivated aortic EC staining, the antibody against CD45.2 was omitted.

Tab. 3: Flow cytometry antibodies used for mouse aortic EC analysis and sorting

Target	Conjugate	Manufacturer	Catalogue#	Clone	Dilution
CD45.2	PerCP/Cy5.5	Biolegend	109828	104	1:300
CD31	BV421	Biolegend	102424	390	1:600
CD107a (LAMP1)	APC/Cy7	Biolegend	121616	1D4B	1:300
CD54 (ICAM1)	APC	Biolegend	116120	YN1/1.7.4	1:600
CD102 (ICAM2)	Biotin	Biolegend	105604	3C4	1:600
CD106 (VCAM1)	PE/Cy7	Biolegend	105720	429	1:300
CD62E (E-Selectin)	PE	BD Bioscience	553751	10E9.6	1:300
CD62P (P-Selectin)	FITC	BD Bioscience	553744	RB40.34	1:300
Streptavidin	BV510	Biolegend	405234	-	1:300

For sorting different aortic cell types, antibodies as listed in table 4 were used. Fibroblasts were identified as $CD45^{low}CD140^{high}$, ECs as $CD45^{low}CD31^{high}$, macrophages as $CD45^{high}CD11b^{high}lineage^{low}$, and neutrophils as $CD45^{high}CD11b^{high}lineage^{high}$.

Tab. 4: Flow cytometry antibodies used for mouse aortic cell sorting

Target	Conjugate	Manufacturer	Catalogue#	Clone	Dilution
B220	PE	Biolegend	103208	RA3-6B2	1:600
CD90.2	PE	Biolegend	140308	53-2.1	1:3000
CD49b	PE	Biolegend	108908	DX5	1:1200
NK1.1	PE	Biolegend	108708	PK136	1:600
Ter-119	PE	Biolegend	116208	TER-119	1:600
Ly6G	PE	Biolegend	127608	1A8	1:600
CD31	BV421	Biolegend	102424	390	1:600
CD45	BV605	Biolegend	103140	30-F11	1:600
CD140a	PerCP/Cy5.5	Biolegend	135914	APA5	1:600
CD11b	APC/Cy7	Biolegend	101226	M1/70	1:600

2.3.2 Human cells

For the analysis of blood leukocytes, cells were stained with hematopoietic lineage markers and leukocyte subset markers as listed in table 5. Monocytes were identified using forward and side scatter as well as CD11b. Within this population, numbers of monocyte subsets $CD14^{high}CD16^{low}$, $CD14^{low}CD16^{high}$, and $CD14^{high}CD16^{high}$ were quantified. Neutrophils and lymphocytes were identified using forward and side scatter.

Tab. 5: Flow cytometry antibodies used for human blood leukocyte analysis

Target	Conjugate	Manufacturer	Catalogue#	Clone	Dilution
CD3	Biotin	Biolegend	300404	UCHT1	1:600
CD19	Biotin	Biolegend	302204	HIB19	1:600
CD20	Biotin	Biolegend	302350	2H7	1:600
CD56	Biotin	Biolegend	362536	5.1H11	1:600
CD11b	APC	Biolegend	301310	ICRF44	1:600
CD16	BV711	Biolegend	302044	3G8	1:600
CD14	BV421	Biolegend	325628	HCD14	1:600
Streptavidin	FITC	Biolegend	405202	-	1:600

2.4 Cell sorting

Purified neutrophils and monocytes for adoptive transfer experiments were obtained by isolating cells from the BM of UBC-GFP donor animals and incubating them with the antibodies Ly6G-PE (127608, clone 1A8) and CD115-biotin (135508, clone AFS98, both BioLegend). This allowed subsequent coupling to magnetic beads (anti-PE and streptavidin microbeads, 130-048-801 and 130-048-101, Miltenyi Biotec) and separation of cells via magnetic-activated cell separation columns (130-042-401, Miltenyi Biotec).

2.5 Histology

Aortic roots were dissected, embedded in molds containing OCT compound (SA62550-01, Tissue Tek, Science Services) and frozen over liquid nitrogen. Using a cryotome, 5-10 µm cross-sections around the aortic valve region were obtained.

Prior to immunohistochemical staining, sections were fixed in ice-cold acetone for 10 min and treated with peroxidase block (S202386-2, Agilent). Incubation of the sections with primary antibodies (table 6) was performed over night at 4 °C.

Tab. 6: Primary antibodies used for immunohistochemistry on mouse tissue

Target	Host species	Manufacturer	Catalogue#
CD11b	rat	Biolegend	101202
Ly6G	rat	Biolegend	127602
CD19	rat	Abcam	ab25232
CD3	rabbit	Abcam	ab16669
Collagen I	rabbit	Abcam	ab21286
Alpha smooth muscle actin (SMA)	rabbit	Abcam	ab5694

Incubation with primary antibodies was followed by a respective biotinylated secondary antibody staining (BA-4001 (anti-rat) or B-1000 (anti-rabbit), Vector Laboratories). In a third step, the VECTASTAIN ABC kit (PK-4000, Vector Laboratories) was used to couple the enzyme horse radish peroxidase to avidin, which is then able to bind the biotinylated secondary antibody. By applying AEC substrate (K3461, Dako), areas with coupled antibody complexes displayed red color development. Additionally, cell nuclei were counterstained with Gill´s hematoxylin solution II (1051752500, Merck Millipore). Stainings were evaluated by analyzing either the positive signal area per total plaque area or, if possible, the number of individual cells per total plaque area.

2.6 Real-time quantitative polymerase chain reaction (qPCR)

Aortic arches were excised, stored in 500 µl Qiazol (79306, Qiagen) at -80 °C, and minced using a mechanical disruptor (TH220, Omni) with soft-tissue tips (32750, Omni). RNA extraction from aortic arches was performed with the RNeasy Mini kit (74104, Qiagen). An additional genomic DNA removal step was conducted using the RNase-free DNase set (79254, Qiagen). After RNA extraction, quality of the sample was assessed with a NanoQuant Plate on an Infinite M200 PRO plate reader (both TECAN) and first-strand complementary DNA (cDNA) was generated with the High-Capacity RNA-to-cDNA kit (4388950, Applied Biosystems). Real-time qPCR was performed using TaqMan probes (all Thermo Fisher) as listed in table 7 and TaqMan Fast Universal Master Mix (4366073, Thermo Fisher). All reactions were performed in a total volume of 10 µl and over 40 cycles on a ViiA 7 system. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) was used as a housekeeping gene. To quantify mRNA levels, $2^{-\Delta Ct}$ values were used.

Tab. 7: TaqMan probes used for qPCR

Target	Catalogue#
Angpt1 (Angiopoietin 1)	Mm00456503_m1
Ccl2	Mm00441242_m1
Ccl7	Mm00443113_m1
Col1a2 (Collagen type 1 alpha 2)	Mm00483888_m1
Col3a1	Mm01254476_m1
Cxcl1	Mm04207460_m1
Cxcl12	Mm00445553_m1
Cx3cl1	Mm00436454_m1
Gapdh	Mm99999915_g1
Il6	Mm00446190_m1
Il10	Mm01288386_m1
Mmp3 (Matrix metalloproteinase)	Mm00440295_m1
Mmp9	Mm00442991_m1
Mmp10	Mm01168399_m1
Opn (osteopontin)	Mm00436767_m1
Scf (Stem cell factor)	Mm00442972_m1
Tgfb1	Mm01178820_m1
Tnf	Mm00443258_m1
Vcam1	Mm01320970_m1

2.7 Protein biochemistry

2.7.1 Commercially available kits and enzyme-linked immunosorbent assays (ELISAs)

Mouse aortic arches were excised, stored in catecholamine stabilizing buffer (0.01 N hydrochloric acid, 0.15 mM ethylenediaminetetraacetic acid (EDTA), 4 mM sodium metabisulfite in H_2O dest.; adjusted to pH 7) at -80 °C for up to one week, and minced as described in 2.6. Mouse peripheral blood samples were collected in EDTA-coated tubes. For

plasma extraction, blood was centrifuged at 2000 x g for 15 min at 4 °C. Cell culture supernatant was collected directly and frozen at -80 °C until measurements.

An EA633/96 ELISA (DLD Diagnostika) was performed for norepinephrine quantification in aortic arches. In plasma, the chemokine & cytokine assay (36-Plex Mouse ProcartaPlex™ Panel 1A, EPX360-26092-90, Thermo Fisher) was used for detecting 36 different chemokines and cytokines in a multiplex fashion. Additionally, EA633/96, EA632/96 (both DLD Diagnostika), ab108821, ab216951, ab205571 (all Abcam), and MJE00B (R&D) were used in plasma for norepinephrine, epinephrine, corticosterone (1:100 sample dilution), CXCL1, CCL7, and CCL2 measurements, respectively. In cell culture supernatant, ab216951, ab205571, ab100712, ab229440, ab229393 (all Abcam) were used to determine CXCL1, CCL7, IL-6, IL-1β, and TNFα levels. All measurements were performed according to the manufacturers' instructions.

2.7.2 Western blot

Aortic arches were excised and stored at -80 °C. For tissue disruption, aortic arches were minced with scissors in 200 µl RIPA buffer (9806, Cell Signaling Technology, supplemented with 1 % proteinase (Halt™ Protease Inhibitor Cocktail, 78429, Thermo Fisher) and phosphatase inhibitor cocktail (Halt™ Phosphatase Inhibitor Cocktail, 78420, Thermo Fisher)) and homogenized by mechanical disruption with soft-tissue tips as described above. Cell lysates were generated by sonicating 3 times for 30 s with intermitting 30 s on ice and centrifuging at 21,000 g for 5 min at 4 °C to remove cell debris.

Protein concentrations were determined with a bicinchoninic acid (BCA) assay (23225, ThermoFisher) according to the manufacturer´s protocol and samples were adjusted with RIPA buffer to contain 35 µg total protein. After supplementation with 4X Laemmli samples buffer (1610747, Bio-Rad), they were heated for 5 min at 95 °C. To separate proteins, SDS-gel electrophoresis was performed using 4-20 % Mini-PROTEAN® TGX™ precast gradient gels (4561094, Bio-Rad) at 300 V for 18 min in 1X Tris/glycine/SDS buffer (1610732, Bio-Rad). Subsequent wet blotting (25 mM Tris, 192 mM glycine, 20 % v/v methanol, pH 8.3) was carried out at 100 V for 90 min using methanol-activated polyvinylidene difluoride (PVDF) membranes (1704157, Merck Millipore).

All buffers for antibody staining were based on TBS-T (20 mM Tris, 150 mM NaCL, 0.1 % Tween, pH 7.6) and contained 5 % BSA. First, the PVDF membrane was blocked for 1 h at room temperature with the aforementioned buffer. Anti-pTH (phosphorylated tyrosine hydroxylase, ab5935, 1:500, Merck Millipore) was incubated overnight at 4 °C and the appropriate HRP (horse radish peroxidase)-conjugated secondary antibody (anti-rabbit, 7074, 1:100,000, Cell Signaling) was incubated for 1 h at room temperature. For signal detection of pTH, membranes were incubated using SuperSignal™ West Dura Extended Duration

Substrate (37076, Thermo Fisher) and imaged with an ImageQuant LAS 4000 imaging system (GE Healthcare Life Sciences). Subsequently, blots were washed and incubated for 1 h at room temperature with anti-GAPDH (5174P, 1:50000, Cell Signaling), followed by the incubation with HRP-conjugated secondary antibody for 1 h at room temperature. Signal detection was performed as described above.

2.8 Cell culture

BM-derived macrophages were obtained by flushing one femur of a mouse with 20 ml PBS (0.5 % BSA). The resulting cell suspension was centrifuged at 400 g for 5 min at 4 °C and treated with RBC lysis as described above. In a 6-well plate, 1,000,000 cells were plated in 1 ml of RPMI medium (A1049101, Gibco) containing 10 % fetal calf serum (S0615, Sigma), 1 % Penicillin/Streptomycin (15070-063, Gibco), and 100 ng/ml M-CSF (416-ML-050, R&D). Additionally, 1 ml of fresh medium was added the day after. Following a wash step with PBS to remove non-adherent cells and debris, medium was completely replaced at day 3 and 5. On day 5, norepinephrine (1 µM, A7257-1G, Sigma) or HCl vehicle was added repetitively every 8 h for 24 h.

Primary murine aortic endothelial (mAoECs, C57-6052, CellBiologics), aortic smooth muscle (C57-6080, CellBiologics), and aortic fibroblast cells (C57-6075, CellBiologics) were cultured in corresponding cell culture medium (PB-M1168, PB-M2268, PB-M1167, PeloBiotech) in a humidified incubator with 5 % CO_2 at 37 °C. In static experiments, 50,000 cells were seeded into a 24-well plate, cultivated for 24 h, and stimulated with norepinephrine (1 µM) or HCl vehicle repetitively every 8 h for another 24 h. In flow experiments, 200,000 mAoECs were plated into channel slides with 0.2 mm (high shear stress) and 0.8 mm (low shear stress) chamber height (80166 and 80196, Ibidi). They were cultivated under flow conditions in an Ibidi pump system (flow profile: 1 h at 7 dyn/cm^2, 1 h at 10 dyn/cm^2, 1 h at 15 dyn/cm^2, and 30 dyn/cm^2 until harvest for 0.2 mm slides; 1 h at 0.5 dyn/cm^2, 1 h at 0.7 dyn/cm^2, 1 h at 1 dyn/cm^2, and 2 dyn/cm^2 until harvest for 0.8 mm slides) for 24 h and stimulated with hormones (1 µM norepinephrine repetitively every 8 h, 1 µM epinephrine E-4250-1G repetitively every 8 h, 1 µM corticosterone 27840-100MG once; all Sigma) for another 24 h. For flow cytometry experiments, cells were harvested using Accutase solution (A6964-100ML, Sigma). Neutrophils and monocytes used in leukocyte adhesion assays were isolated and purified from BM as described in 2.4. The purified cells were stained with Calcein-AM (C3100MP, Invitrogen) for 1 h and stimulated mAoECs were incubated with the leukocytes under flow conditions. After 1 h, cells were washed off and slides were imaged at 10x magnification using a fluorescence microscope with a green filter set (DMRB, Leica). Adherent leukocytes were quantified with ImageJ.

For adrenoreceptor-blocker experiments, phentolamine hydrochloride (P7547, 1 µM, Sigma), propranolol hydrochloride (P0884, 1 µM, Sigma) or respective vehicle were added to the pump system once a day. Anti-CXCL1, anti-CCL7 (AF-456-NA and AF-453-NA, 0.1 µg/ml, R&D) or an IgG control (AB-108-C, 0.1 µg/ml, R&D) were added to the culture medium at the same time as purified neutrophils and monocytes, and incubated for 1 h. Slides were processed and imaged as described above.

Human umbilical vein endothelial cells (HUVECs, C-12200, PromoCell) were cultured in complete endothelial cell growth medium (C-22011, PromoCell). For flow experiments, they were seeded into channel slides with 0.8 mm chamber height, cultivated under flow conditions for 24 h (flow profile: 1 h at 1 dyn/cm2, 1 h at 3 dyn/cm2, 1 h at 5 dyn/cm2, and 10 dyn/cm2 until harvest) and incubated with norepinephrine (1 µM, repetitively every 8 h) or HCl vehicle for another 24 h. For subsequent monocyte adhesion assays, THP-1 cells (ATCC TIB-202, ATCC, cultured in RPMI medium containing 10 % fetal calf serum) were stained with Calcein-AM for 1 h, and stimulated HUVECs were incubated with THP-1 cells under flow conditions for 1 h. Slides were imaged and analyzed as described above.

2.9 Data analysis

All statistical analysis was performed using GraphPad Prism version 8. Prior to each analysis, data were tested for normality distribution. If the sample size was large enough ($n \geq 8$), normal distribution of data was assessed using the D'Agostino-Pearson omnibus normality test. If sample sizes were $n<8$, data were analyzed using the Shapiro-Wilk test. Further, a two-sided ROUT´s test was used to determine statistical outliers. If appropriate, absolute values were normalized to one representative experiment to adjust for inter-experimental variations.

For comparisons between two groups, data were analyzed using two-tailed Student´s unpaired/Student´s paired *t*-test (for normally distributed data, according to study design) or Mann-Whitney/Wilcoxon test (for non-normally distributed data) as appropriate. When comparing more groups, a one-way analysis of variance (ANOVA, for normally distributed data) followed by a Holm-Sidak test for multiple comparisons or a Kruskal-Wallis test (for non-normally distributed data) followed by a Dunn´s test for multiple comparisons was performed. For multiple comparisons with paired designs, repeated measures one-way ANOVA or mixed-effects analysis were used. Contingency analysis for plaque rupture experiments was performed using Fisher´s exact test.

3. Discussion

3.1 Interleukin-1β suppression dampens inflammatory leukocyte production and uptake in atherosclerosis

IL-1β is a potent pro-inflammatory cytokine and its blood levels strongly associate with CAD processes including plaque progression and destabilization (Abbate et al., 2020). Consequently, pharmacological neutralization of IL-1β via a monoclonal antibody reduced recurrent cardiovascular events in patients with previous MI in CANTOS, a large phase III clinical trial (Ridker et al., 2017). However, the biological basis of this beneficial effect is not yet fully understood. As canakinumab, the neutralizing antibody used in CANTOS, failed to get drug approval due to safety (and cost) issues, further knowledge on how IL-1β suppression dampens atherosclerosis progression is needed to guide future anti-inflammatory therapies.

This study explored two different pharmacological therapies to deplete bioactive IL-1β in the context of atherosclerosis. MCC950 is a small molecule inhibitor that interferes with upstream NLRP3 inflammasome formation and thus pro-IL-1β processing (Coll et al., 2015), while canakinumab (or its murine equivalent) neutralizes downstream IL-1β (Ridker et al., 2011). In line with other publications (Heijden et al., 2017; Vromman et al., 2019), this study found that IL-1β suppression (with both therapeutic strategies) attenuates atherosclerosis progression as reflected by reduced plaque size and decreased inflammatory leukocyte accumulation in an *ApoE*$^{-/-}$ mouse model. Additionally, it was shown that the overall transcriptional profile of the atherosclerotic aortas was shifted towards attenuated extracellular matrix breakdown and inflammation in general. Previous publications reported decreased expression levels of endothelial adhesion molecules and chemoattractants upon IL-1β suppression (Denes et al., 2012; Heijden et al., 2017; Shemesh et al., 2012), surrogate markers of dampened leukocyte recruitment. Indeed, this study directly proves that IL-1β suppression reduces leukocyte recruitment using adoptive transfer experiments.

Upstream of the leukocyte recruitment process, this study indicates that IL-1β suppression affects leukocyte supply as indicated by reduced circulating inflammatory leukocyte numbers. In the underlying analysis, it was found that this is caused by decreased proliferation of hematopoietic stem and progenitor cells in the BM. This is in line with previous findings that demonstrated the importance of IL-1β in emergency hematopoiesis (Dinarello, 2005, 2017; Garlanda et al., 2013; Pietras et al., 2016; Sager et al., 2015). However, none of the previous publications has shown that proliferation of hematopoietic stem cells in atherosclerosis is IL-1β-dependent. The notion that IL-1β suppression leads to reduced blood monocyte levels in mice is consistent with human data from the CANTOS trial. Further, a recent sub-analysis of the CANTOS trial showed that blood neutrophil numbers are modulated by canakinumab (Adamstein et al., 2021), which aligns with this study´s blood neutrophil data in mice.

This indicates that IL-1β suppression may reduce production of BM-derived pro-inflammatory leukocytes also in humans. Further, circulating monocyte numbers may serve as a readily available biomarker to identify responders in anti-inflammatory treatments.

To better investigate the effects of anti-IL-1β treatment as seen in the CANTOS trial, an experiment in atherosclerotic, post-MI mice was performed. Here, it could be shown that IL-1β suppression mitigates vascular inflammation not only in primary prevention (=early atherosclerosis) but also secondary prevention (=post-MI). Mechanistically, this is achieved by similar mechanisms, namely reduced supply and uptake of inflammatory leukocytes into atherosclerotic plaques.

As depicted in Fig. 2+3 (1.1.3.1), IL-1β is produced at inflammatory sites in response to various external stimuli. In atherosclerosis, the major source of IL-1β is likely comprised of plaque macrophages, although other cell types like ECs and SMCs may also contribute to IL-1β release under inflammatory conditions (Ridker et al., 2011). To better investigate the cellular source of IL-1β production in atherosclerosis, mice with a hematopoietic cell deficiency in Caspase-1 on an atherosclerotic background were generated. Thus, all cells of the hematopoietic system fail to cleave pro-IL-1β via the NLRP3 inflammasome. The obtained results indicate that hematopoietic cell-derived IL-1β contributes substantially to the processes that fuel vascular inflammation.

Taken together, these data provide complementary mechanistic data on how IL-1β suppression dampens atherosclerosis progression via affecting both inflammatory leukocyte supply from hematopoietic organs and uptake into atherosclerotic plaques. This knowledge may help to develop future anti-inflammatory therapies in patients with CVD.

3.2 Acute mental stress drives vascular inflammation and promotes plaque destabilization

Acute mental stress has been identified as a novel trigger for the onset of acute coronary syndrome (Kivimäki & Steptoe, 2018; Yusuf et al., 2004). However, despite numerous epidemiological and pre-clinical studies, it is not yet exactly known how acute mental stress drives plaque destabilization so rapidly (Hinterdobler et al., 2021). As a matter of fact, current treatment does not address CVD risk associated with acute mental stress. This study aimed to better understand the mechanisms that drive vascular inflammation and consequently promote plaque rupture in response to acute psychological stress.

In this manuscript, evidence is provided that acute stress exposure leads to rapid expansion of inflammatory leukocytes in distinct tissue, including atherosclerotic plaques if present. This is primarily driven via rapid, stress-induced blood leukocyte recruitment. Consequently, enhanced vascular inflammation drives plaque rupture by inducing plaque-destabilizing processes. Mechanistically, activation of the SNS modulates vascular endothelial

cells, which promotes recruitment. Among other effects, stress exposure increases circulating chemokine levels such as CXCL1 and CCL7. Further, this study provides evidence that acute stress-induced leukocyte recruitment can be therapeutically targeted by neutralizing these two chemokines.

Fig. 4 (see 1.3.3) summarizes previous experimental evidence on how acute mental stress affects inflammation in atherosclerosis in five potential categories: leukocyte redistribution, leukocyte activation, modulation of circulating cytokine levels, EC activation and inflammatory processes inside the vascular wall. This study could replicate some of the already known mechanisms but also expanded the knowledge on how acute mental stress promotes atherosclerosis progression via inflammatory mechanisms. Regarding blood leukocyte dynamics, it was shown in mice and humans that inflammatory leukocytes rapidly deplete from the blood in response to acute stress exposure. This is in line with recent animal studies that report decreasing monocyte and lymphocyte levels after stress exposure (Viswanathan & Dhabhar, 2005; Wouw et al., 2020; Xu et al., 2020). A study by Dhabhar et al. shows that leukocytes are massively mobilized from reservoirs and subsequently traffic to sites of inflammation and immune activation upon stress exposure (Dhabhar et al., 2012; Viswanathan & Dhabhar, 2005). Accordingly, cell tracking experiments demonstrated that blood leukocytes are recruited to specific organs including plaques if underlying atherosclerosis is present. Leukocyte recruitment is a process that involves complex interactions between two major players: the leukocyte itself but also the endothelium at sites of transmigration (Ley et al., 2007). Specifically, leukocyte chemotaxis, as well as rolling and firm adhesion of leukocytes on endothelial selectins and adhesion molecules are key steps in the leukocyte adhesion cascade in vessels (Gerhardt & Ley, 2015). The data from this thesis demonstrate that acute stress activates ECs, which leads to an increase in adhesion molecule expression and chemokine release. This is in line with studies that report higher circulating adhesion molecule levels (Heinz et al., 2003; Wilbert-Lampen et al., 2010), serving as surrogate markers for EC adhesion molecule expression, in response to acute stress. Further, several publications have previously reported higher levels of circulating chemokines upon acute stress exposure in mice (Cheng et al., 2015; Qing et al., 2020). Still, these studies have not addressed in detail which cells produce and release the respective chemokines. Although research in this thesis primarily focused on the local effects of acute stress on endothelial cells, other tissue-resident cells might be involved in the enhanced chemokine release into the circulation. Further, as mentioned above, acute stress leads to increased blood cytokine levels (Cheng et al., 2015; Heinz et al., 2003; Koelsch et al., 2016; Kuebler et al., 2015; Wilbert-Lampen et al., 2010). Thus, future research is needed to identify the systemic effects of circulating factors, such as IL-6, on both endothelial cells and blood leukocytes. Compared to the other categories, relatively little has been known on how acute mental stress affects inflammatory processes

inside the vascular wall and thus promotes plaque destabilization. Using different stressors, previous studies demonstrated that acute mental stress promotes surrogate markers of plaque destabilization (Lagraauw et al., 2019), and can lead to MI in hypercholesterolemic mice (Caligiuri et al., 1999). Beyond that, data from this thesis provide evidence that acute mental stress directly promotes plaque rupture in a mouse plaque rupture model. Mechanistically, it was demonstrated that acute stress leads to an expansion of plaque inflammatory leukocytes, a decrease in plaque SMC content and a shift towards enhanced extracellular matrix breakdown, all features of vulnerable plaques (Silvestre-Roig et al., 2014).

Stress perception activates three major neuroendocrine axes as outlined above. This leads to both systemic (epinephrine and glucocorticoids from the adrenal glands) as well a local (norepinephrine from sympathetic tissue innervation) stress hormone release (Glaser & Kiecolt-Glaser, 2005). Thus, it was addressed if one of these hormones is responsible for the observed EC activation. In cell culture experiments, only norepinephrine exposure, but not epinephrine or corticosterone, increased EC adhesion molecule expression and adhesion of leukocytes to an EC layer. This aligns with previous reports that suggest a prominent role of the SNS in stress-induced phenotypes (Heidt et al., 2014; Qing et al., 2020; B. Zhang et al., 2020). However, future research is needed to investigate potential effects of systemic hormones on circulating leukocytes.

These findings should also be interpreted in the context of other studies that have recently provided experimental evidence for the link between non-classical risk factors and CVD. Similar to acute mental stress, studies on sedentary lifestyle (Frodermann et al., 2019), sleep deprivation (McAlpine et al., 2019), and air and noise pollution (Lelieveld et al., 2019; Münzel, Sørensen, et al., 2017) have all identified inflammatory processes in the respective pathophysiological mechanisms (Schloss et al., 2020). This is also the case for chronic mental stress (Heidt et al., 2014). However, in contrast to acute mental stress, where leukocyte levels decrease from blood as a result of increased recruitment, chronic mental stress activates hematopoietic stem cells in the BM leading to overall higher leukocyte production and hence supply.

Taken together, this study provides novel mechanistic insights into how acute mental stress promotes vascular inflammation and hence, plaque rupture. These findings substantiate associations from various epidemiological studies which have previously linked acute mental stress to the onset of cardiovascular complications (Aoki et al., 2012; Edmondson et al., 2012; Kivimäki & Steptoe, 2018; Leor et al., 1996; Meisel et al., 1991; Smyth et al., 2016; Wilbert-Lampen et al., 2008). From a clinical perspective, this study highlights the need to identify people at risk that are both stress-susceptible and have pre-existing atherosclerosis. In future research, targeting the stress-induced uptake of blood leukocytes, as was done by neutralizing specific chemoattractants, may present a novel therapeutic avenue.

3.3 Discussion across dissertation topics

Despite effective lipid-lowering therapies, residual risk for CVD remains high. This is mostly attributed to inflammatory mechanisms in the disease pathology (Koenig, 2020; Ridker, MacFadyen, et al., 2019). Thus, there is a strong need for novel anti-atherosclerotic therapies targeting inflammation in general (Libby & Everett, 2019). Additionally, non-classical risk factors have been strongly associated with CVD (Yusuf et al., 2004). Although most of these risk factors are potentially modifiable, they still contribute considerably to atherosclerosis and its complications, mainly conveyed via the modulation of inflammatory processes (Schloss et al., 2020). Thus, there is also a strong need to pharmacologically alter inflammatory processes in the context of lifestyle-related risk factors, such as acute mental stress. Concomitantly, in the wake of personalized medicine, it is necessary to identify people at risk for CVD in general but also people at risk due to their risk factor profile. However, this requires specific and established biomarkers (Ruparelia et al., 2017; Soehnlein & Libby, 2021).

Small molecule inhibitors and antibodies against various inflammation-related targets have been tested as novel anti-atherosclerotic therapies in pre-clinical and clinical trials (Libby & Everett, 2019; Ruparelia et al., 2017). However, despite some encouraging results, none of these anti-inflammatory therapies has made it into clinical practice yet. Nonetheless, the CANTOS trial using canakinumab, a neutralizing antibody against IL-1β, has convincingly demonstrated that targeting inflammation reduces recurrent cardiovascular events (Ridker et al., 2017). Thus, the failure of canakinumab to get drug approval should not be seen as disappointing but rather fuel future research to gain more in-deep knowledge on how inflammation promotes atherosclerosis and its complications. The results from this thesis provided further mechanistic insights into links between hematopoiesis and atherogenesis, and into the beneficial effects of IL-1β-targeted therapies. This knowledge can potentially be exploited in future anti-inflammatory interventions in atherosclerosis.

Although canakinumab is the most prominent one, other neutralizing antibodies against cytokines and chemokines have been successfully tested in pre-clinical and clinical studies on atherosclerosis (Libby & Everett, 2019; Soehnlein & Libby, 2021). These include antibodies against the CXCL1/CXCR (CXC chemokine receptor)2 axis (Soehnlein et al., 2013) and the CCL2/CCR (CC chemokine receptor)2 axis (Gilbert et al., 2011), both of which are involved in myeloid cell mobilization and recruitment to sites of inflammation (Soehnlein & Libby, 2021). Interestingly, experiments from this thesis revealed an increase in circulating CXCL1 and CCL7 (another ligand for CCR2), and neutralization of these chemokines resulted in attenuated stress-induced leukocyte recruitment. Future studies are needed to determine the link between CXCL1- and CCL7-mediated leukocyte recruitment and stress-induced plaque rupture. However, targeting stress-induced uptake of inflammatory leukocytes, possibly via

neutralizing chemoattractants such as CXCL1 and CCL7, may provide a novel therapeutic option to prevent cardiovascular complications in response to acute stress.

Given the numerous pathways on how inflammation affects atherosclerosis, there is a multitude of promising future treatment options. A key challenge will be to tailor pharmacological interventions in order to reduce pro-atherosclerotic inflammation without affecting host defense against infections (Baylis et al., 2018; Hinterdobler et al., 2021; Schloss et al., 2020).

Taken together, the observed findings may guide future trials and aid to identify patients that respond best to anti-inflammatory treatment.

4. Manuscript “Interleukin-1β suppression dampens inflammatory leukocyte production and uptake in atherosclerosis”

4.1 Summary

Targeting IL-1β is a promising therapeutic approach to reduce cardiovascular events. Neutralization with a monoclonal antibody (canakinumab) in a human trial reduced the CVD risk in secondary prevention after MI. However, the exact mechanisms for these beneficial effects remain incompletely understood. Additionally, canakinumab failed to get drug approval after the clinical trial and little is known about potential benefits of IL-1β suppression in primary prevention. Therefore, the aim of this thesis was to further investigate the beneficial effects of IL-1β inhibition to provide stronger mechanistic data for future anti-inflammatory therapies in patients.

It could be shown in pre-clinical experiments that, in line with already published literature, anti-IL-1β treatment reduced the accumulation of leukocytes in atherosclerotic aortas both in primary (no previous MI) and secondary (previous MI) prevention. Mechanistically, IL-1β suppression modulated ECs towards a less inflammatory phenotype, characterized by lower expression levels of adhesion molecules and leukocyte attracting phenotypes. Further, anti-IL-1β treatment affects inflammatory leukocyte supply by decreasing proliferation of BM hematopoietic stem and progenitor cells. Additionally, these data indicate that specifically hematopoietic cell-derived IL-1β contributes to the adverse effects in atherosclerosis. In line with the findings from animal experiments, patients treated with canakinumab showed reduced blood monocyte numbers.

Taken together, the results demonstrate that anti-IL-1β treatment beneficially alters both blood inflammatory leukocyte supply and uptake in the context of atherosclerosis. This provides novel mechanistic insights for potential future anti-inflammatory therapies in CVD.

4.2 Individual contribution

This project has been carried out in joint cooperation with a medical student, Jan Hettwer. Together with Prof. Sager, we conceptualized the work. As Jan Hettwer´s supervisor in the lab, I assisted in the hands-on work and functioned as the main coordinator of the individual experiments. Additionally, I analyzed and interpreted results specifically from *in vivo* experiments (e.g. flow cytometry). Regarding the publication, both Jan Hettwer and me designed the figures. Additionally, I assisted in writing and editing the manuscript.

5. Manuscript “Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis”

5.1 Summary

Acute mental stress exposure is known to be a major trigger for the onset of acute coronary syndromes, even in patients with state-of-the-art medical treatment. However, how acute mental stress rapidly drives plaque destabilization causing acute coronary syndrome is poorly understood. Therefore, the aim of this project was to investigate the underlying mechanisms.

Both in a human study and in animal experiments, leukocytes rapidly deplete from the circulation in response to acute mental stress. Cell-tracking experiments revealed that these leukocytes, specifically monocytes and neutrophils, are recruited to distinct organs and, if present, atherosclerotic plaques. As a consequence, acute mental stress promotes vascular inflammation and hence, plaque rupture by enhancing plaque-destabilizing processes. In line with their pivotal role in leukocyte recruitment, data from this thesis indicate that acute mental stress strongly activates ECs. Mechanistically, using both *in vitro* and *in vivo* experiments, it was found that specifically the SNS (via locally released norepinephrine) plays a key role in the modulation of ECs and in the recruitment process in response to acute mental stress. Further, the results demonstrate that targeting CXCL1 and CCL7, important chemokines in leukocyte recruitment, dampens uptake of monocytes and neutrophils to atherosclerotic plaques under acute stress conditions.

Taken together, this work provides novel mechanistic insight into how acute mental stress affects vascular inflammation and potentially plaque rupture. Targeting stress-induced recruitment of inflammatory leukocytes may provide novel therapeutic avenues for future research.

5.2 Individual contribution

After joining the lab of Prof. Sager in October 2017, I have gradually taken over the major responsibility for the entire project management. Concretely, I designed the individual experiments in agreement with Prof. Sager, performed most of the hands-on work *in vitro* and *in vivo*, analyzed the results and interpreted the data. Additionally, I supervised two medical students, Almut Meesmann and Simin Schott, in the context of this project. Together, we specifically established setups for *in vitro* experiments under flow conditions. Regarding the publication, I designed the figures and wrote the manuscript together with Prof. Sager.

Bibliography

Abbate, A., Toldo, S., Marchetti, C., Kron, J., Tassell, B. W. V., & Dinarello, C. A. (2020). Interleukin-1 and the Inflammasome as Therapeutic Targets in Cardiovascular Disease. *Circulation Research*, *126*(9), 1260–1280. https://doi.org/10.1161/circresaha.120.315937

Adamstein, N. H., MacFadyen, J. G., Rose, L. M., Glynn, R. J., Dey, A. K., Libby, P., Tabas, I. A., Mehta, N. N., & Ridker, P. M. (2021). The neutrophil–lymphocyte ratio and incident atherosclerotic events: analyses from five contemporary randomized trials. *European Heart Journal*, *42*(9), 896–903. https://doi.org/10.1093/eurheartj/ehaa1034

Afonina, I. S., Müller, C., Martin, S. J., & Beyaert, R. (2015). Proteolytic Processing of Interleukin-1 Family Cytokines: Variations on a Common Theme. *Immunity*, *42*(6), 991–1004. https://doi.org/10.1016/j.immuni.2015.06.003

Al-Kindi, S. G., Brook, R. D., Biswal, S., & Rajagopalan, S. (2020). Environmental determinants of cardiovascular disease: lessons learned from air pollution. *Nature Reviews Cardiology*, *17*(10), 656–672. https://doi.org/10.1038/s41569-020-0371-2

Allahverdian, S., Chehroudi, A. C., McManus, B. M., Abraham, T., & Francis, G. A. (2014). Contribution of Intimal Smooth Muscle Cells to Cholesterol Accumulation and Macrophage-Like Cells in Human Atherosclerosis. *Circulation*, *129*(15), 1551–1559. https://doi.org/10.1161/circulationaha.113.005015

Aoki, T., Fukumoto, Y., Yasuda, S., Sakata, Y., Ito, K., Takahashi, J., Miyata, S., Tsuji, I., & Shimokawa, H. (2012). The Great East Japan Earthquake Disaster and cardiovascular diseases. *European Heart Journal*, *33*(22), 2796–2803. https://doi.org/10.1093/eurheartj/ehs288

Barnum, C. J., Page, T. W. W., F., H., Neigh, G. N., & Tansey, M. G. (2012). Psychological stress in adolescent and adult mice increases neuroinflammation and attenuates the response to LPS challenge. *Journal of Neuroinflammation*, *9*(1), 15. https://doi.org/10.1186/1742-2094-9-9

Barrett, T. J. (2018). Macrophages in Atherosclerosis Regression. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *40*(1), 20–33. https://doi.org/10.1161/atvbaha.119.312802

Baylis, R. A., Gomez, D., Mallat, Z., Pasterkamp, G., & Owens, G. K. (2018). The CANTOS Trial. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *37*(11), e174–e177. https://doi.org/10.1161/atvbaha.117.310097

Berg, K. E., Ljungcrantz, I., Andersson, L., Bryngelsson, C., Hedblad, B., Fredrikson, G. N., Nilsson, J., & Björkbacka, H. (2012). Elevated CD14++CD16− Monocytes Predict Cardiovascular Events. *Circulation: Cardiovascular Genetics*, *5*(1), 122–131. https://doi.org/10.1161/circgenetics.111.960385

Bevilacqua, M. P., Pober, J. S., Wheeler, M. E., Cotran, R. S., & Gimbrone, M. A. (1985). Interleukin-1 activation of vascular endothelium. Effects on procoagulant activity and leukocyte adhesion. *The American Journal of Pathology*, *121*(3), 394–403.

Brandes, R. P. (2018). Endothelial Dysfunction and Hypertension. *Hypertension*, *64*(5), 924–928. https://doi.org/10.1161/hypertensionaha.114.03575

Bras, A. L. (2019). Poor sleep linked to atherosclerosis. *Nature Reviews Cardiology*, *16*(3), 132–132. https://doi.org/10.1038/s41569-019-0162-9

Breen, M. S., Beliakova-Bethell, N., Mujica-Parodi, L. R., Carlson, J. M., Ensign, W. Y., Woelk, C. H., & Rana, B. K. (2016). Acute psychological stress induces short-term variable immune response. *Brain, Behavior, and Immunity*, *53*, 172–182. https://doi.org/10.1016/j.bbi.2015.10.008

Brook, R. D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S. C., Tager, I., & Expert Panel on Population and Prevention Science of the American Heart Association. (2004). Air Pollution and Cardiovascular Disease. *Circulation*, *109*(21), 2655–2671. https://doi.org/10.1161/01.cir.0000128587.30041.c8

Buijs, R. M., & Eden, C. G. V. (2000). The integration of stress by the hypothalamus, amygdala and prefrontal cortex: balance between the autonomic nervous system and the neuroendocrine system. *Progress in Brain Research*, *126*, 117–132. https://doi.org/10.1016/s0079-6123(00)26011-1

Cai, L., Wheeler, E., Kerrison, N. D., Luan, J., Deloukas, P., Franks, P. W., Amiano, P., Ardanaz, E., Bonet, C., Fagherazzi, G., Groop, L. C., Kaaks, R., Huerta, J. M., Masala, G., Nilsson, P. M., Overvad, K., Pala, V., Panico, S., Rodriguez-Barranco, M., … Wareham, N. J. (2020). Genome-wide association analysis of type 2 diabetes in the EPIC-InterAct study. *Scientific Data*, *7*(1), 393. https://doi.org/10.1038/s41597-020-00716-7

Caligiuri, G., Levy, B., Pernow, J., Thorén, P., & Hansson, G. K. (1999). Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. *Proceedings of the National Academy of Sciences*, *96*(12), 6920–6924. https://doi.org/10.1073/pnas.96.12.6920

Carnevale, D., & Lembo, G. (2020). Neuroimmune interactions in cardiovascular diseases. *Cardiovascular Research*, *117*(2), 402–410. https://doi.org/10.1093/cvr/cvaa151

Carson, J. A. S., Lichtenstein, A. H., Anderson, C. A. M., Appel, L. J., Kris-Etherton, P. M., Meyer, K. A., Petersen, K., Polonsky, T., Horn, L. V., & On behalf of the American Heart Association Nutrition Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Arteriosclerosis Thrombosis and Vascular Biology; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Peripheral Vascular Disease; and Stroke Council. (2019). Dietary Cholesterol and Cardiovascular Risk: A Science Advisory From the American Heart Association. *Circulation*, *141*(3), e39–e53. https://doi.org/10.1161/cir.0000000000000743

Cheng, Y., Jope, R. S., & Beurel, E. (2015). A pre-conditioning stress accelerates increases in mouse plasma inflammatory cytokines induced by stress. *BMC Neuroscience*, *16*(1), 31. https://doi.org/10.1186/s12868-015-0169-z

Coll, R. C., Robertson, A. A. B., Chae, J. J., Higgins, S. C., Muñoz-Planillo, R., Inserra, M. C., Vetter, I., Dungan, L. S., Monks, B. G., Stutz, A., Croker, D. E., Butler, M. S., Haneklaus, M., Sutton, C. E., Núñez, G., Latz, E., Kastner, D. L., Mills, K. H. G., Masters, S. L., … O'Neill, L. A. J. (2015). A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nature Medicine*, *21*(3), 248–255. https://doi.org/10.1038/nm.3806

Combadière, C., Potteaux, S., Rodero, M., Simon, T., Pezard, A., Esposito, B., Merval, R., Proudfoot, A., Tedgui, A., & Mallat, Z. (2008). Combined Inhibition of CCL2, CX3CR1, and CCR5 Abrogates Ly6Chi and Ly6Clo Monocytosis and Almost Abolishes Atherosclerosis in Hypercholesterolemic Mice. *Circulation*, *117*(13), 1649–1657. https://doi.org/10.1161/circulationaha.107.745091

Cybulsky, M., & Gimbrone, M. (1991). Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science*, *251*(4995), 788–791. https://doi.org/10.1126/science.1990440

Daiber, A., Lelieveld, J., Steven, S., Oelze, M., Kröller-Schön, S., Sørensen, M., & Münzel, T. (2019). The “exposome” concept – how environmental risk factors influence cardiovascular health. *Acta Biochimica Polonica*. https://doi.org/10.18388/abp.2019_2853

Denes, A., Drake, C., Stordy, J., Chamberlain, J., McColl, B. W., Gram, H., Crossman, D., Francis, S., Allan, S. M., & Rothwell, N. J. (2012). Interleukin-1 Mediates Neuroinflammatory Changes Associated With Diet-Induced Atherosclerosis. *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease*, *1*(3), e002006. https://doi.org/10.1161/jaha.112.002006

Deng, X., Chu, X., Wang, P., Ma, X., Wei, C., Sun, C., Yang, J., & Li, Y. (2019). MicroRNA-29a-3p Reduces TNFα-Induced Endothelial Dysfunction by Targeting Tumor Necrosis Factor Receptor 1. *Molecular Therapy - Nucleic Acids*, *18*, 903–915. https://doi.org/10.1016/j.omtn.2019.10.014

Dhabhar, F. S., Malarkey, W. B., Neri, E., & McEwen, B. S. (2012). Stress-induced redistribution of immune cells—From barracks to boulevards to battlefields: A tale of three hormones – Curt Richter Award Winner. *Psychoneuroendocrinology*, *37*(9), 1345–1368. https://doi.org/10.1016/j.psyneuen.2012.05.008

Dimsdale, J. E. (2008). Psychological stress and cardiovascular disease. *Journal of the American College of Cardiology*, *51*(13), 1237–1246. https://doi.org/10.1016/j.jacc.2007.12.024

Dinarello, C. A. (2005). Blocking IL-1 in systemic inflammation. *The Journal of Experimental Medicine*, *201*(9), 1355–1359. https://doi.org/10.1084/jem.20050640

Dinarello, C. A. (2017). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews*, *281*(1), 8–27. https://doi.org/10.1111/imr.12621

Dinarello, C. A. (2019). The IL-1 family of cytokines and receptors in rheumatic diseases. *Nature Reviews Rheumatology*, *15*(10), 612–632. https://doi.org/10.1038/s41584-019-0277-8

Dinh, Q. N., Drummond, G. R., Sobey, C. G., & Chrissobolis, S. (2014). Roles of Inflammation, Oxidative Stress, and Vascular Dysfunction in Hypertension. *BioMed Research International*, *2014*, 1–11. https://doi.org/10.1155/2014/406960

Drechsler, M., Duchene, J., & Soehnlein, O. (2015). Chemokines Control Mobilization, Recruitment, and Fate of Monocytes in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(5), 1050–1055. https://doi.org/10.1161/atvbaha.114.304649

Drechsler, M., Megens, R. T. A., Zandvoort, M. van, Weber, C., & Soehnlein, O. (2010). Hyperlipidemia-Triggered Neutrophilia Promotes Early Atherosclerosis. *Circulation*, *122*(18), 1837–1845. https://doi.org/10.1161/circulationaha.110.961714

Duewell, P., Kono, H., Rayner, K. J., Sirois, C. M., Vladimer, G., Bauernfeind, F. G., Abela, G. S., Franchi, L., Nuñez, G., Schnurr, M., Espevik, T., Lien, E., Fitzgerald, K. A., Rock, K. L., Moore, K. J., Wright, S. D., Hornung, V., & Latz, E. (2010). NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*, *464*(7293), 1357–1361. https://doi.org/10.1038/nature08938

Dunstan, D. W., Dogra, S., Carter, S. E., & Owen, N. (2021). Sit less and move more for cardiovascular health: emerging insights and opportunities. *Nature Reviews Cardiology*, *18*(9), 637–648. https://doi.org/10.1038/s41569-021-00547-y

Dutta, P., Courties, G., Wei, Y., Leuschner, F., Gorbatov, R., Robbins, C. S., Iwamoto, Y., Thompson, B., Carlson, A. L., Heidt, T., Majmudar, M. D., Lasitschka, F., Etzrodt, M., Waterman, P., Waring, M. T., Chicoine, A. T., Laan, A. M. van der, Niessen, H. W. M., Piek, J. J., … Nahrendorf, M. (2012). Myocardial infarction accelerates atherosclerosis. *Nature*, *487*(7407), 325–329. https://doi.org/10.1038/nature11260

Dutta, P., Sager, H. B., Stengel, K. R., Naxerova, K., Courties, G., Saez, B., Silberstein, L., Heidt, T., Sebas, M., Sun, Y., Wojtkiewicz, G., Feruglio, P. F., King, K., Baker, J. N., van der Laan, A. M., Borodovsky, A., Fitzgerald, K., Hulsmans, M., Hoyer, F., … Nahrendorf, M. (2015). Myocardial Infarction Activates CCR2+ Hematopoietic Stem and Progenitor Cells. *Cell Stem Cell*, *16*(5), 477–487. https://doi.org/10.1016/j.stem.2015.04.008

Ebner, K., & Singewald, N. (2017). Individual differences in stress susceptibility and stress inhibitory mechanisms. *Current Opinion in Behavioral Sciences*, *14*, 54–64. https://doi.org/10.1016/j.cobeha.2016.11.016

Edmondson, D., Newman, J. D., Whang, W., & Davidson, K. W. (2012). Emotional triggers in myocardial infarction: do they matter? *European Heart Journal*, *34*(4), 300–306. https://doi.org/10.1093/eurheartj/ehs398

Ezzati, M., Henley, S. J., Thun, M. J., & Lopez, A. D. (2005). Role of Smoking in Global and Regional Cardiovascular Mortality. *Circulation*, *112*(4), 489–497. https://doi.org/10.1161/circulationaha.104.521708

Fernández-Ruiz, I. (2019). Neutrophil-driven SMC death destabilizes atherosclerotic plaques. *Nature Reviews Cardiology*, *16*(8), 455–455. https://doi.org/10.1038/s41569-019-0214-1

Franck, G., Mawson, T. L., Folco, E. J., Molinaro, R., Ruvkun, V., Engelbertsen, D., Liu, X., Tesmenitsky, Y., Shvartz, E., Sukhova, G. K., Michel, J.-B., Nicoletti, A., Lichtman, A., Wagner, D., Croce, K. J., & Libby, P. (2018). Roles of PAD4 and NETosis in Experimental Atherosclerosis and Arterial Injury. *Circulation Research*, *123*(1), 33–42. https://doi.org/10.1161/circresaha.117.312494

Frodermann, V., Rohde, D., Courties, G., Severe, N., Schloss, M. J., Amatullah, H., McAlpine, C. S., Cremer, S., Hoyer, F. F., Ji, F., Koeverden, I. D. van, Herisson, F., Honold, L., Masson, G. S., Zhang, S., Grune, J., Iwamoto, Y., Schmidt, S. P., Wojtkiewicz, G. R., … Nahrendorf, M. (2019). Exercise reduces inflammatory cell production and cardiovascular inflammation via instruction of hematopoietic progenitor cells. *Nature Medicine*, *25*(11), 1761–1771. https://doi.org/10.1038/s41591-019-0633-x

Fuster, J. J., MacLauchlan, S., Zuriaga, M. A., Polackal, M. N., Ostriker, A. C., Chakraborty, R., Wu, C.-L., Sano, S., Muralidharan, S., Rius, C., Vuong, J., Jacob, S., Muralidhar, V., Robertson, A. A. B., Cooper, M. A., Andrés, V., Hirschi, K. K., Martin, K. A., & Walsh, K. (2017). Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*, *355*(6327), 842–847. https://doi.org/10.1126/science.aag1381

Galkina, E., Kadl, A., Sanders, J., Varughese, D., Sarembock, I. J., & Ley, K. (2006). Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. *The Journal of Experimental Medicine*, *203*(5), 1273–1282. https://doi.org/10.1084/jem.20052205

Galkina, E., & Ley, K. (2007). Vascular Adhesion Molecules in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *27*(11), 2292–2301. https://doi.org/10.1161/atvbaha.107.149179

Garlanda, C., Dinarello, C. A., & Mantovani, A. (2013). The Interleukin-1 Family: Back to the Future. *Immunity*, *39*(6), 1003–1018. https://doi.org/10.1016/j.immuni.2013.11.010

Gaul, D. S., Stein, S., & Matter, C. M. (2017). Neutrophils in cardiovascular disease. *European Heart Journal*, *38*(22), 1702–1704. https://doi.org/10.1093/eurheartj/ehx244

Gerhardt, T., & Ley, K. (2015). Monocyte trafficking across the vessel wall. *Cardiovascular Research*, *107*(3), 321–330. https://doi.org/10.1093/cvr/cvv147

Getz, G. S., & Reardon, C. A. (2018). Animal Models of Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *32*(5), 1104–1115. https://doi.org/10.1161/atvbaha.111.237693

Gilbert, J., Lekstrom-Himes, J., Donaldson, D., Lee, Y., Hu, M., Xu, J., Wyant, T., Davidson, M., & Group, M. S. (2011). Effect of CC Chemokine Receptor 2 CCR2 Blockade on Serum C-Reactive Protein in Individuals at Atherosclerotic Risk and With a Single Nucleotide Polymorphism of the Monocyte Chemoattractant Protein-1 Promoter Region. *The American Journal of Cardiology*, *107*(6), 906–911. https://doi.org/10.1016/j.amjcard.2010.11.005

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: implications for health. *Nature Reviews Immunology*, *5*(3), 243–251. https://doi.org/10.1038/nri1571

Glavin, G. B., Paré, W. P., Sandbak, T., Bakke, H.-K., & Murison, R. (1994). Restraint stress in biomedical research: An update. *Neuroscience & Biobehavioral Reviews*, *18*(2), 223–249. https://doi.org/10.1016/0149-7634(94)90027-2

Goebel, M. U., Mills, P. J., Irwin, M. R., & Ziegler, M. G. (2000). Interleukin-6 and Tumor Necrosis Factor-α Production After Acute Psychological Stress, Exercise, and Infused Isoproterenol: Differential Effects and Pathways. *Psychosomatic Medicine*, *62*(4), 591–598. https://doi.org/10.1097/00006842-200007000-00019

Grau, A. J., Boddy, A. W., Dukovic, D. A., Buggle, F., Lichy, C., Brandt, T., Hacke, W., & CAPRIE Investigators. (2004). Leukocyte Count as an Independent Predictor of Recurrent Ischemic Events. *Stroke: Journal of the American Heart Association*, *35*(5), 1147–1152. https://doi.org/10.1161/01.str.0000124122.71702.64

Grebe, A., Hoss, F., & Latz, E. (2018). NLRP3 Inflammasome and the IL-1 Pathway in Atherosclerosis. *Circulation Research*, *122*(12), 1722–1740. https://doi.org/10.1161/circresaha.118.311362

Groeschel, M., & Braam, B. (2011). Connecting chronic and recurrent stress to vascular dysfunction: no relaxed role for the renin-angiotensin system. *American Journal of Physiology-Renal Physiology*, *300*(1), F1–F10. https://doi.org/10.1152/ajprenal.00208.2010

Guo, H., Callaway, J. B., & Ting, J. P.-Y. (2015). Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nature Medicine*, *21*(7), 677–687. https://doi.org/10.1038/nm.3893

Härdtner, C., Kornemann, J., Krebs, K., Ehlert, C. A., Jander, A., Zou, J., Starz, C., Rauterberg, S., Sharipova, D., Dufner, B., Hoppe, N., Dederichs, T.-S., Willecke, F., Stachon, P., Heidt, T., Wolf, D., Mühlen, C. von zur, Madl, J., Kohl, P., … Hilgendorf, I. (2020). Inhibition of macrophage proliferation dominates plaque regression in response to cholesterol lowering. *Basic Research in Cardiology*, *115*(6), 78. https://doi.org/10.1007/s00395-020-00838-4

Harst, P. van der, & Verweij, N. (2018). Identification of 64 Novel Genetic Loci Provides an Expanded View on the Genetic Architecture of Coronary Artery Disease. *Circulation Research*, *122*(3), 433–443. https://doi.org/10.1161/circresaha.117.312086

Hedrick, C. C. (2018). Lymphocytes in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(2), 253–257. https://doi.org/10.1161/atvbaha.114.305144

Heidt, T., Sager, H. B., Courties, G., Dutta, P., Iwamoto, Y., Zaltsman, A., Muhlen, C. von zur, Bode, C., Fricchione, G. L., Denninger, J., Lin, C. P., Vinegoni, C., Libby, P., Swirski, F. K., Weissleder, R., & Nahrendorf, M. (2014). Chronic variable stress activates hematopoietic stem cells. *Nature Medicine*, *20*(7), 754–758. https://doi.org/10.1038/nm.3589

Heijden, T. van der, Kritikou, E., Venema, W., Duijn, J. van, Santbrink, P. J. van, Slütter, B., Foks, A. C., Bot, I., & Kuiper, J. (2017). NLRP3 Inflammasome Inhibition by MCC950 Reduces Atherosclerotic Lesion Development in Apolipoprotein E–Deficient Mice—Brief Report. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *37*(8), 1457–1461. https://doi.org/10.1161/atvbaha.117.309575

Heinz, A., Hermann, D., Smolka, M. N., Rieks, M., Gräf, K.-J., Pöhlau, D., Kuhn, W., & Bauer, M. (2003). Effects of acute psychological stress on adhesion molecules, interleukins and sex hormones: implications for coronary heart disease. *Psychopharmacology*, *165*(2), 111–117. https://doi.org/10.1007/s00213-002-1244-6

Higashi, Y., Kihara, Y., & Noma, K. (2012). Endothelial dysfunction and hypertension in aging. *Hypertension Research*, *35*(11), 1039–1047. https://doi.org/10.1038/hr.2012.138

Hinterdobler, J., Schunkert, H., Kessler, T., & Sager, H. B. (2021). Impact of Acute and Chronic Psychosocial Stress on Vascular Inflammation. *Antioxidants and Redox Signaling*, *35*(18), 1531–1550. https://doi.org/10.1089/ars.2021.0153

Ince, L. M., Weber, J., & Scheiermann, C. (2019). Control of Leukocyte Trafficking by Stress-Associated Hormones. *Frontiers in Immunology*, *9*, 3143. https://doi.org/10.3389/fimmu.2018.03143

Jaiswal, S., & Ebert, B. L. (2019). Clonal hematopoiesis in human aging and disease. *Science*, *366*(6465), eaan4673. https://doi.org/10.1126/science.aan4673

Jaiswal, S., Natarajan, P., Silver, A. J., Gibson, C. J., Bick, A. G., Shvartz, E., McConkey, M., Gupta, N., Gabriel, S., Ardissino, D., Baber, U., Mehran, R., Fuster, V., Danesh, J., Frossard, P., Saleheen, D., Melander, O., Sukhova, G. K., Neuberg, D., … Ebert, B. L. (2017). Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *The New England Journal of Medicine*, *377*(2), 111–121. https://doi.org/10.1056/nejmoa1701719

Juan, A. de, Ince, L. M., Pick, R., Chen, C.-S., Molica, F., Zuchtriegel, G., Wang, C., Zhang, D., Druzd, D., Hessenauer, M. E. T., Pelli, G., Kolbe, I., Oster, H., Prophete, C., Hergenhan, S. M., Albrecht, U., Ripperger, J., Montanez, E., Reichel, C. A., … Scheiermann, C. (2019). Artery-Associated Sympathetic Innervation Drives Rhythmic Vascular Inflammation of Arteries and Veins. *Circulation*, *140*(13), 1100–1114. https://doi.org/10.1161/circulationaha.119.040232

Känel, R. von. (2015). Acute mental stress and hemostasis: When physiology becomes vascular harm. *Thrombosis Research*, *135*, S52–S55. https://doi.org/10.1016/s0049-3848(15)50444-1

Känel, R. von, Bellingrath, S., & Kudielka, B. M. (2008). Association between burnout and circulating levels of pro- and anti-inflammatory cytokines in schoolteachers. *Journal of Psychosomatic Research*, *65*(1), 51–59. https://doi.org/10.1016/j.jpsychores.2008.02.007

Kang, D. O., Eo, J. S., Park, E. J., Nam, H. S., Song, J. W., Park, Y. H., Park, S. Y., Na, J. O., Choi, C. U., Kim, E. J., Rha, S.-W., Park, C. G., Seo, H. S., Kim, C. K., Yoo, H., & Kim, J. W. (2021). Stress-

associated neurobiological activity is linked with acute plaque instability via enhanced macrophage activity: a prospective serial 18F-FDG-PET/CT imaging assessment. *European Heart Journal*. https://doi.org/10.1093/eurheartj/ehaa1095

Katakami, N. (2017). Mechanism of Development of Atherosclerosis and Cardiovascular Disease in Diabetes Mellitus. *Journal of Atherosclerosis and Thrombosis*, *25*(1), RV17014. https://doi.org/10.5551/jat.rv17014

Katayama, Y., Battista, M., Kao, W.-M., Hidalgo, A., Peired, A. J., Thomas, S. A., & Frenette, P. S. (2006). Signals from the Sympathetic Nervous System Regulate Hematopoietic Stem Cell Egress from Bone Marrow. *Cell*, *124*(2), 407–421. https://doi.org/10.1016/j.cell.2005.10.041

Kiecolt-Glaser, J. K., Preacher, K. J., MacCallum, R. C., Atkinson, C., Malarkey, W. B., & Glaser, R. (2003). Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proceedings of the National Academy of Sciences*, *100*(15), 9090–9095. https://doi.org/10.1073/pnas.1531903100

Kim, J. H., Almuwaqqat, Z., Hammadah, M., Liu, C., Ko, Y.-A., Lima, B., Sullivan, S., Alkhoder, A., Abdulbaki, R., Ward, L., Bremner, J. D., Sheps, D. S., Raggi, P., Sun, Y. V., Shah, A. J., Vaccarino, V., & Quyyumi, A. A. (2019). Peripheral Vasoconstriction During Mental Stress and Adverse Cardiovascular Outcomes in Patients With Coronary Artery Disease. *Circulation Research*, *125*(10), 874–883. https://doi.org/10.1161/circresaha.119.315005

Kivimäki, M., & Steptoe, A. (2018). Effects of stress on the development and progression of cardiovascular disease. *Nature Reviews Cardiology*, *15*(4), 215–229. https://doi.org/10.1038/nrcardio.2017.189

Koelsch, S., Boehlig, A., Hohenadel, M., Nitsche, I., Bauer, K., & Sack, U. (2016). The impact of acute stress on hormones and cytokines and how their recovery is affected by music-evoked positive mood. *Scientific Reports*, *6*(1), 23008. https://doi.org/10.1038/srep23008

Koenig, W. (2020). Persistent inflammatory residual risk despite aggressive cholesterol-lowering therapy: further evidence fuelling the dual target concept. *European Heart Journal*, *41*(31), 2962–2964. https://doi.org/10.1093/eurheartj/ehaa186

Koudouovoh-Tripp, P., Hüfner, K., Egeter, J., Kandler, C., Giesinger, J. M., Sopper, S., Humpel, C., & Sperner-Unterweger, B. (2020). Stress Enhances Proinflammatory Platelet Activity: the Impact of Acute and Chronic Mental Stress. *Journal of Neuroimmune Pharmacology*, 1–13. https://doi.org/10.1007/s11481-020-09945-4

Kröller-Schön, S., Daiber, A., Steven, S., Oelze, M., Frenis, K., Kalinovic, S., Heimann, A., Schmidt, F. P., Pinto, A., Kvandova, M., Vujacic-Mirski, K., Filippou, K., Dudek, M., Bosmann, M., Klein, M., Bopp, T., Hahad, O., Wild, P. S., Frauenknecht, K., … Münzel, T. (2018). Crucial role for Nox2 and sleep deprivation in aircraft noise-induced vascular and cerebral oxidative stress, inflammation, and gene regulation. *European Heart Journal*, *39*(38), 3528–3539. https://doi.org/10.1093/eurheartj/ehy333

Kuebler, U., Zuccarella-Hackl, C., Arpagaus, A., Wolf, J. M., Farahmand, F., Känel, R. von, Ehlert, U., & Wirtz, P. H. (2015). Stress-induced modulation of NF-κB activation, inflammation-associated gene expression, and cytokine levels in blood of healthy men. *Brain, Behavior, and Immunity*, *46*, 87–95. https://doi.org/10.1016/j.bbi.2014.12.024

Kwong, J. C., Schwartz, K. L., Campitelli, M. A., Chung, H., Crowcroft, N. S., Karnauchow, T., Katz, K., Ko, D. T., McGeer, A. J., McNally, D., Richardson, D. C., Rosella, L. C., Simor, A., Smieja, M., Zahariadis, G., & Gubbay, J. B. (2018). Acute Myocardial Infarction after Laboratory-Confirmed

Influenza Infection. *The New England Journal of Medicine*, *378*(4), 345–353. https://doi.org/10.1056/nejmoa1702090

Lagraauw, H. M., Kuiper, J., & Bot, I. (2015). Acute and chronic psychological stress as risk factors for cardiovascular disease: Insights gained from epidemiological, clinical and experimental studies. *Brain, Behavior, and Immunity*, *50*, 18–30. https://doi.org/10.1016/j.bbi.2015.08.007

Lagraauw, H. M., Wezel, A., Velden, D. van der, Kuiper, J., & Bot, I. (2019). Stress-induced mast cell activation contributes to atherosclerotic plaque destabilization. *Scientific Reports*, *9*(1), 2134. https://doi.org/10.1038/s41598-019-38679-4

Lavie, C. J., Ozemek, C., Carbone, S., Katzmarzyk, P. T., & Blair, S. N. (2019). Sedentary Behavior, Exercise, and Cardiovascular Health. *Circulation Research*, *124*(5), 799–815. https://doi.org/10.1161/circresaha.118.312669

Leeka, J., Schwartz, B. G., & Kloner, R. A. (2010). Sporting Events Affect Spectators' Cardiovascular Mortality: It Is Not Just a Game. *The American Journal of Medicine*, *123*(11), 972–977. https://doi.org/10.1016/j.amjmed.2010.03.026

Lelieveld, J., Klingmüller, K., Pozzer, A., Pöschl, U., Fnais, M., Daiber, A., & Münzel, T. (2019). Cardiovascular disease burden from ambient air pollution in Europe reassessed using novel hazard ratio functions. *European Heart Journal*, *40*(20), 1590–1596. https://doi.org/10.1093/eurheartj/ehz135

Leor, J., Poole, W. K., & Kloner, R. A. (1996). Sudden Cardiac Death Triggered by an Earthquake. *New England Journal of Medicine*, *334*(7), 413–419. https://doi.org/10.1056/nejm199602153340701

Levin, M. G., & Rader, D. J. (2020). Polygenic Risk Scores and Coronary Artery Disease. *Circulation*, *141*(8), 637–640. https://doi.org/10.1161/circulationaha.119.044770

Levitan, I., Volkov, S., & Subbaiah, P. V. (2010). Oxidized LDL: Diversity, Patterns of Recognition, and Pathophysiology. *Antioxidants & Redox Signaling*, *13*(1), 39–75. https://doi.org/10.1089/ars.2009.2733

Ley, K., Laudanna, C., Cybulsky, M. I., & Nourshargh, S. (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nature Reviews Immunology*, *7*(9), 678–689. https://doi.org/10.1038/nri2156

Ley, K., Miller, Y. I., & Hedrick, C. C. (2011). Monocyte and Macrophage Dynamics During Atherogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*(7), 1506–1516. https://doi.org/10.1161/atvbaha.110.221127

Li, A. C., & Glass, C. K. (2002). The macrophage foam cell as a target for therapeutic intervention. *Nature Medicine*, *8*(11), 1235–1242. https://doi.org/10.1038/nm1102-1235

Li, H., Cybulsky, M. I., Gimbrone, M. A., & Libby, P. (1993). An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*, *13*(2), 197–204. https://doi.org/10.1161/01.atv.13.2.197

Libby, P. (2002). Inflammation in atherosclerosis. *Nature*, *420*(6917), 868–874. https://doi.org/10.1038/nature01323

Libby, P. (2013). Mechanisms of acute coronary syndromes and their implications for therapy. *The New England Journal of Medicine*, *368*(21), 2004–2013. https://doi.org/10.1056/nejmra1216063

Libby, P. (2017). Interleukin-1 Beta as a Target for Atherosclerosis Therapy Biological Basis of CANTOS and Beyond. *Journal of the American College of Cardiology*, *70*(18), 2278–2289. https://doi.org/10.1016/j.jacc.2017.09.028

Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., Tokgözoğlu, L., & Lewis, E. F. (2019). Atherosclerosis. *Nature Reviews Disease Primers*, *5*(1), 56. https://doi.org/10.1038/s41572-019-0106-z

Libby, P., & Everett, B. M. (2019). Novel Antiatherosclerotic Therapies. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *39*(4), 538–545. https://doi.org/10.1161/atvbaha.118.310958

Libby, P., Pasterkamp, G., Crea, F., & Jang, I.-K. (2019). Reassessing the Mechanisms of Acute Coronary Syndromes. *Circulation Research*, *124*(1), 150–160. https://doi.org/10.1161/circresaha.118.311098

Libby, P., Ridker, P. M., & Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature*, *473*(7347), 317–325. https://doi.org/10.1038/nature10146

Liu, Y., Zhu, Y., Jia, W., Sun, D., Zhao, L., Zhang, C., Wang, C., Lyu, Q., Chen, Y., Chen, G., Bo, Y., & Xing, Y. (2020). Association of the Total White Blood Cell, Neutrophils, and Monocytes Count With the Presence, Severity, and Types of Carotid Atherosclerotic Plaque. *Frontiers in Medicine*, *7*, 313. https://doi.org/10.3389/fmed.2020.00313

Lloyd-Jones, D. M. (2010). Cardiovascular Risk Prediction. *Circulation*, *121*(15), 1768–1777. https://doi.org/10.1161/circulationaha.109.849166

Lusis, A. J. (2000). Atherosclerosis. *Nature*, *407*(6801), 233–241. https://doi.org/10.1038/35025203

Ma, L., Yang, J., Runesha, H. B., Tanaka, T., Ferrucci, L., Bandinelli, S., & Da, Y. (2010). Genome-wide association analysis of total cholesterol and high-density lipoprotein cholesterol levels using the Framingham Heart Study data. *BMC Medical Genetics*, *11*(1), 55. https://doi.org/10.1186/1471-2350-11-55

Mackay, F., Loetscher, H., Stueber, D., Gehr, G., & Lesslauer, W. (1993). Tumor necrosis factor alpha (TNF-alpha)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *The Journal of Experimental Medicine*, *177*(5), 1277–1286. https://doi.org/10.1084/jem.177.5.1277

Man, J. J., Beckman, J. A., & Jaffe, I. Z. (2020). Sex as a Biological Variable in Atherosclerosis. *Circulation Research*, *126*(9), 1297–1319. https://doi.org/10.1161/circresaha.120.315930

Mannu, G. S., Zaman, M. J. S., Gupta, A., Rehman, H. U., & Myint, P. K. (2013). Evidence of Lifestyle Modification in the Management of Hypercholesterolemia. *Current Cardiology Reviews*, *9*(1), 2–14. https://doi.org/10.2174/157340313805076313

Marchini, T., Mitre, L. S., & Wolf, D. (2021). Inflammatory Cell Recruitment in Cardiovascular Disease. *Frontiers in Cell and Developmental Biology*, *9*, 635527. https://doi.org/10.3389/fcell.2021.635527

Mateo, T., Nabah, Y. N. A., Taha, M. A., Mata, M., Cerdá-Nicolás, M., Proudfoot, A. E. I., Stahl, R. A. K., Issekutz, A. C., Cortijo, J., Morcillo, E. J., Jose, P. J., & Sanz, M.-J. (2006). Angiotensin II-

Induced Mononuclear Leukocyte Interactions with Arteriolar and Venular Endothelium Are Mediated by the Release of Different CC Chemokines. *The Journal of Immunology*, *176*(9), 5577–5586. https://doi.org/10.4049/jimmunol.176.9.5577

Mauersberger, C., Schunkert, H., & Sager, H. B. (2021). Inflammation-Related Risk Loci in Genome-Wide Association Studies of Coronary Artery Disease. *Cells*, *10*(2), 440. https://doi.org/10.3390/cells10020440

Mawhin, M.-A., Tilly, P., Zirka, G., Charles, A.-L., Slimani, F., Vonesch, J.-L., Michel, J.-B., Bäck, M., Norel, X., & Fabre, J.-E. (2018). Neutrophils recruited by leukotriene B4 induce features of plaque destabilization during endotoxaemia. *Cardiovascular Research*, *114*(12), 1656–1666. https://doi.org/10.1093/cvr/cvy130

McAlpine, C. S., Kiss, M. G., Rattik, S., He, S., Vassalli, A., Valet, C., Anzai, A., Chan, C. T., Mindur, J. E., Kahles, F., Poller, W. C., Frodermann, V., Fenn, A. M., Gregory, A. F., Halle, L., Iwamoto, Y., Hoyer, F. F., Binder, C. J., Libby, P., … Swirski, F. K. (2019). Sleep modulates haematopoiesis and protects against atherosclerosis. *Nature*, *566*(7744), 383–387. https://doi.org/10.1038/s41586-019-0948-2

McGorrian, C., Yusuf, S., Islam, S., Jung, H., Rangarajan, S., Avezum, A., Prabhakaran, D., Almahmeed, W., Rumboldt, Z., Budaj, A., Dans, A. L., Gerstein, H. C., Teo, K., Anand, S. S., & INTERHEART Investigators. (2011). Estimating modifiable coronary heart disease risk in multiple regions of the world: the INTERHEART Modifiable Risk Score. *European Heart Journal*, *32*(5), 581–589. https://doi.org/10.1093/eurheartj/ehq448

Mefford, M. T., Mittleman, M. A., Li, B. H., Qian, L. X., Reynolds, K., Zhou, H., Harrison, T. N., Geller, A. C., Sidney, S., Sloan, R. P., Mostofsky, E., & Williams, D. R. (2020). Sociopolitical stress and acute cardiovascular disease hospitalizations around the 2016 presidential election. *Proceedings of the National Academy of Sciences*, *117*(43), 27054–27058. https://doi.org/10.1073/pnas.2012096117

Meisel, S. R., Dayan, K. I., Pauzner, H., Chetboun, I., Arbel, Y., David, D., & Kutz, I. (1991). Effect of Iraqi missile war on incidence of acute myocardial infarction and sudden death in Israeli civilians. *The Lancet*, *338*(8768), 660–661. https://doi.org/10.1016/0140-6736(91)91234-l

Messner, B., & Bernhard, D. (2014). Smoking and Cardiovascular Disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(3), 509–515. https://doi.org/10.1161/atvbaha.113.300156

Miller, E. S., Apple, C. G., Kannan, K. B., Funk, Z. M., Plazas, J. M., Efron, P. A., & Mohr, A. M. (2019). Chronic stress induces persistent low-grade inflammation. *The American Journal of Surgery*, *218*(4), 677–683. https://doi.org/10.1016/j.amjsurg.2019.07.006

Miller, M. R., & Newby, D. E. (2019). Air pollution and cardiovascular disease: car sick. *Cardiovascular Research*, *116*(2), 279–294. https://doi.org/10.1093/cvr/cvz228

Mittleman, M. A., & Mostofsky, E. (2011). Physical, Psychological and Chemical Triggers of Acute Cardiovascular Events. *Circulation*, *124*(3), 346–354. https://doi.org/10.1161/circulationaha.110.968776

Moore, K. J., & Tabas, I. (2011). Macrophages in the Pathogenesis of Atherosclerosis. *Cell*, *145*(3), 341–355. https://doi.org/10.1016/j.cell.2011.04.005

Mosca, L., Barrett-Connor, E., & Wenger, N. K. (2011). Sex/Gender Differences in Cardiovascular Disease Prevention. *Circulation*, *124*(19), 2145–2154. https://doi.org/10.1161/circulationaha.110.968792

Mostofsky, E., Penner, E. A., & Mittleman, M. A. (2014). Outbursts of anger as a trigger of acute cardiovascular events: a systematic review and meta-analysis. *European Heart Journal*, *35*(21), 1404–1410. https://doi.org/10.1093/eurheartj/ehu033

Münzel, T., Daiber, A., Steven, S., Tran, L. P., Ullmann, E., Kossmann, S., Schmidt, F. P., Oelze, M., Xia, N., Li, H., Pinto, A., Wild, P., Pies, K., Schmidt, E. R., Rapp, S., & Kröller-Schön, S. (2017). Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice. *European Heart Journal*, *38*(37), ehx081. https://doi.org/10.1093/eurheartj/ehx081

Münzel, T., Gori, T., Al-Kindi, S., Deanfield, J., Lelieveld, J., Daiber, A., & Rajagopalan, S. (2018). Effects of gaseous and solid constituents of air pollution on endothelial function. *European Heart Journal*, *39*(38), 3543–3550. https://doi.org/10.1093/eurheartj/ehy481

Münzel, T., Hahad, O., Kuntic, M., Keaney, J. F., Deanfield, J. E., & Daiber, A. (2020). Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on endothelial function and clinical outcomes. *European Heart Journal*, *41*(41), ehaa460-. https://doi.org/10.1093/eurheartj/ehaa460

Münzel, T., Miller, M. R., Sørensen, M., Lelieveld, J., Daiber, A., & Rajagopalan, S. (2020). Reduction of environmental pollutants for prevention of cardiovascular disease: it's time to act. *European Heart Journal*, *41*(41), ehaa745-. https://doi.org/10.1093/eurheartj/ehaa745

Münzel, T., Sørensen, M., & Daiber, A. (2021). Transportation noise pollution and cardiovascular disease. *Nature Reviews Cardiology*, *18*(9), 619–636. https://doi.org/10.1038/s41569-021-00532-5

Münzel, T., Sørensen, M., Gori, T., Schmidt, F. P., Rao, X., Brook, F. R., Chen, L. C., Brook, R. D., & Rajagopalan, S. (2017). Environmental stressors and cardio-metabolic disease: part II–mechanistic insights. *European Heart Journal*, *38*(8), 557–564. https://doi.org/10.1093/eurheartj/ehw294

Musher, D. M., Abers, M. S., & Corrales-Medina, V. F. (2019). Acute Infection and Myocardial Infarction. *New England Journal of Medicine*, *380*(2), 171–176. https://doi.org/10.1056/nejmra1808137

Nahrendorf, M. (2020). Multiorgan Imaging of Comorbidity and Cardiovascular Risk. *JACC. Cardiovascular Imaging*, *13*, 478–480. https://doi.org/10.1016/j.jcmg.2018.09.017

Nahrendorf, M., & Swirski, F. K. (2015). Lifestyle effects on hematopoiesis and atherosclerosis. *Circulation Research*, *116*(5), 884–894. https://doi.org/10.1161/circresaha.116.303550

Najafi, A. H., Aghili, N., Tilan, J. U., Andrews, J. A., Peng, X., Lassance-Soares, R. M., Sood, S., Alderman, L. O., Abe, K., Li, L., Kolodgie, F. D., Virmani, R., Zukowska, Z., Epstein, S. E., & Burnett, M. S. (2013). A new murine model of stress-induced complex atherosclerotic lesions. *Disease Models & Mechanisms*, *6*(2), 323–331. https://doi.org/10.1242/dmm.009977

Nidorf, S. M., Eikelboom, J. W., Budgeon, C. A., & Thompson, P. L. (2013). Low-Dose Colchicine for Secondary Prevention of Cardiovascular Disease. *Journal of the American College of Cardiology*, *61*(4), 404–410. https://doi.org/10.1016/j.jacc.2012.10.027

Nidorf, S. M., Fiolet, A. T. L., Mosterd, A., Eikelboom, J. W., Schut, A., Opstal, T. S. J., The, S. H. K., Xu, X.-F., Ireland, M. A., Lenderink, T., Latchem, D., Hoogslag, P., Jerzewski, A., Nierop, P., Whelan, A., Hendriks, R., Swart, H., Schaap, J., Kuijper, A. F. M., … LoDoCo2 Trial Investigators.

(2020). Colchicine in Patients with Chronic Coronary Disease. *New England Journal of Medicine*. https://doi.org/10.1056/nejmoa2021372

Nigro, P., Abe, J., & Berk, B. C. (2011). Flow Shear Stress and Atherosclerosis: A Matter of Site Specificity. *Antioxidants & Redox Signaling*, *15*(5), 1405–1414. https://doi.org/10.1089/ars.2010.3679

North, B. J., & Sinclair, D. A. (2012). The Intersection Between Aging and Cardiovascular Disease. *Circulation Research*, *110*(8), 1097–1108. https://doi.org/10.1161/circresaha.111.246876

Ortega, F. B., Lavie, C. J., & Blair, S. N. (2016). Obesity and Cardiovascular Disease. *Circulation Research*, *118*(11), 1752–1770. https://doi.org/10.1161/circresaha.115.306883

Osborne, M. T., Shin, L. M., Mehta, N. N., Pitman, R. K., Fayad, Z. A., & Tawakol, A. (2020). Disentangling the Links Between Psychosocial Stress and Cardiovascular Disease. *Circulation: Cardiovascular Imaging*, *13*(8), e010931. https://doi.org/10.1161/circimaging.120.010931

Paneni, F., Cañestro, C. D., Libby, P., Lüscher, T. F., & Camici, G. G. (2017). The Aging Cardiovascular System Understanding It at the Cellular and Clinical Levels. *Journal of the American College of Cardiology*, *69*(15), 1952–1967. https://doi.org/10.1016/j.jacc.2017.01.064

Pennathur, S., & Heinecke, J. W. (2007). Mechanisms for Oxidative Stress in Diabetic Cardiovascular Disease. *Antioxidants & Redox Signaling*, *9*(7), 955–969. https://doi.org/10.1089/ars.2007.1595

Piepoli, M. F., Hoes, A. W., Agewall, S., Albus, C., Brotons, C., Catapano, A. L., Cooney, M.-T., Corrà, U., Cosyns, B., Deaton, C., Graham, I., Hall, M. S., Hobbs, F. D. R., Løchen, M.-L., Löllgen, H., Marques-Vidal, P., Perk, J., Prescott, E., Redon, J., … Gale, C. (2016). 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *European Heart Journal*, *37*(29), 2315–2381. https://doi.org/10.1093/eurheartj/ehw106

Pierce, H., Zhang, D., Magnon, C., Lucas, D., Christin, J. R., Huggins, M., Schwartz, G. J., & Frenette, P. S. (2017). Cholinergic Signals from the CNS Regulate G-CSF-Mediated HSC Mobilization from Bone Marrow via a Glucocorticoid Signaling Relay. *Cell Stem Cell*, *20*(5), 648-658.e4. https://doi.org/10.1016/j.stem.2017.01.002

Pietras, E. M., Mirantes-Barbeito, C., Fong, S., Loeffler, D., Kovtonyuk, L. V., Zhang, S., Lakshminarasimhan, R., Chin, C. P., Techner, J.-M., Will, B., Nerlov, C., Steidl, U., Manz, M. G., Schroeder, T., & Passegué, E. (2016). Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nature Cell Biology*, *18*(6), 607–618. https://doi.org/10.1038/ncb3346

Poller, W. C., Nahrendorf, M., & Swirski, F. K. (2020). Hematopoiesis and Cardiovascular Disease. *Circulation Research*, *126*(8), 1061–1085. https://doi.org/10.1161/circresaha.120.315895

Powell, N. D., Sloan, E. K., Bailey, M. T., Arevalo, J. M. G., Miller, G. E., Chen, E., Kobor, M. S., Reader, B. F., Sheridan, J. F., & Cole, S. W. (2013). Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoiesis. *Proceedings of the National Academy of Sciences*, *110*(41), 16574–16579. https://doi.org/10.1073/pnas.1310655110

Puhlmann, M., Weinreich, D. M., Farma, J. M., Carroll, N. M., Turner, E. M., & Alexander, H. R. (2005). Interleukin-1β induced vascular permeability is dependent on induction of endothelial Tissue Factor (TF) activity. *Journal of Translational Medicine*, *3*(1), 37. https://doi.org/10.1186/1479-5876-3-37

Qing, H., Desrouleaux, R., Israni-Winger, K., Mineur, Y. S., Fogelman, N., Zhang, C., Rashed, S., Palm, N. W., Sinha, R., Picciotto, M. R., Perry, R. J., & Wang, A. (2020). Origin and Function of Stress-Induced IL-6 in Murine Models. *Cell*, *182*(2), 372-387.e14. https://doi.org/10.1016/j.cell.2020.05.054

Randolph, G. J. (2014). Mechanisms That Regulate Macrophage Burden in Atherosclerosis. *Circulation Research*, *114*(11), 1757–1771. https://doi.org/10.1161/circresaha.114.301174

Ridker, P. M. (2016). Residual inflammatory risk: addressing the obverse side of the atherosclerosis prevention coin. *European Heart Journal*, *37*(22), 1720–1722. https://doi.org/10.1093/eurheartj/ehw024

Ridker, P. M. (2021). Inhibiting Interleukin-6 to Reduce Cardiovascular Event Rates A Next Step for Atherothrombosis Treatment and Prevention ∗. *Journal of the American College of Cardiology*, *77*(15), 1856–1858. https://doi.org/10.1016/j.jacc.2021.02.060

Ridker, P. M., Everett, B. M., Pradhan, A., MacFadyen, J. G., Solomon, D. H., Zaharris, E., Mam, V., Hasan, A., Rosenberg, Y., Iturriaga, E., Gupta, M., Tsigoulis, M., Verma, S., Clearfield, M., Libby, P., Goldhaber, S. Z., Seagle, R., Ofori, C., Saklayen, M., … CIRT Investigators. (2019). Low-Dose Methotrexate for the Prevention of Atherosclerotic Events. *New England Journal of Medicine*, *380*(8), 752–762. https://doi.org/10.1056/nejmoa1809798

Ridker, P. M., Everett, B. M., Thuren, T., MacFadyen, J. G., Chang, W. H., Ballantyne, C., Fonseca, F., Nicolau, J., Koenig, W., Anker, S. D., Kastelein, J. J. P., Cornel, J. H., Pais, P., Pella, D., Genest, J., Cifkova, R., Lorenzatti, A., Forster, T., Kobalava, Z., … CANTOS Trial Group. (2017). Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *The New England Journal of Medicine*, *377*(12), 1119–1131. https://doi.org/10.1056/nejmoa1707914

Ridker, P. M., MacFadyen, J. G., Thuren, T., & Libby, P. (2019). Residual inflammatory risk associated with interleukin-18 and interleukin-6 after successful interleukin-1β inhibition with canakinumab: further rationale for the development of targeted anti-cytokine therapies for the treatment of atherothrombosis. *European Heart Journal*, *41*(23), 2153–2163. https://doi.org/10.1093/eurheartj/ehz542

Ridker, P. M., Thuren, T., Zalewski, A., Libby, P., & Cantos Study Group. (2011). Interleukin-1 beta inhibition and the prevention of recurrent cardiovascular events: Rationale and Design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *American Heart Journal*, *162*(4), 597–605. https://doi.org/10.1016/j.ahj.2011.06.012

Robbins, C. S., Hilgendorf, I., Weber, G. F., Theurl, I., Iwamoto, Y., Figueiredo, J.-L., Gorbatov, R., Sukhova, G. K., Gerhardt, L. M. S., Smyth, D., Zavitz, C. C. J., Shikatani, E. A., Parsons, M., Rooijen, N. van, Lin, H. Y., Husain, M., Libby, P., Nahrendorf, M., Weissleder, R., & Swirski, F. K. (2013). Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nature Medicine*, *19*(9), 1166–1172. https://doi.org/10.1038/nm.3258

Robertson, A.-K. L., Rudling, M., Zhou, X., Gorelik, L., Flavell, R. A., & Hansson, G. K. (2003). Disruption of TGF-β signaling in T cells accelerates atherosclerosis. *Journal of Clinical Investigation*, *112*(9), 1342–1350. https://doi.org/10.1172/jci18607

Rocha, V. Z., & Libby, P. (2009). Obesity, inflammation, and atherosclerosis. *Nature Reviews Cardiology*, *6*(6), 399–409. https://doi.org/10.1038/nrcardio.2009.55

Roth, G. A., Mensah, G. A., Johnson, C. O., Addolorato, G., Ammirati, E., Baddour, L. M., Barengo, N. C., Beaton, A. Z., Benjamin, E. J., Benziger, C. P., Bonny, A., Brauer, M., Brodmann, M., Cahill, T. J., Carapetis, J., Catapano, A. L., Chugh, S. S., Cooper, L. T., Coresh, J., … Fuster, V. (2020). Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019 Update From the GBD 2019 Study. *Journal of the American College of Cardiology*, *76*(25), 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010

Roth, L., Rombouts, M., Schrijvers, D. M., Lemmens, K., Keulenaer, G. W. D., Martinet, W., & Meyer, G. R. Y. D. (2015). Chronic intermittent mental stress promotes atherosclerotic plaque vulnerability, myocardial infarction and sudden death in mice. *Atherosclerosis*, *242*(1), 288–294. https://doi.org/10.1016/j.atherosclerosis.2015.07.025

Rozanski, A., Blumenthal, J. A., & Kaplan, J. (1999). Impact of Psychological Factors on the Pathogenesis of Cardiovascular Disease and Implications for Therapy. *Circulation*, *99*(16), 2192–2217. https://doi.org/10.1161/01.cir.99.16.2192

Ruparelia, N., Chai, J. T., Fisher, E. A., & Choudhury, R. P. (2017). Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nature Reviews Cardiology*, *14*(3), 133–144. https://doi.org/10.1038/nrcardio.2016.185

Sager, H. B., Dutta, P., Dahlman, J. E., Hulsmans, M., Courties, G., Sun, Y., Heidt, T., Vinegoni, C., Borodovsky, A., Fitzgerald, K., Wojtkiewicz, G. R., Iwamoto, Y., Tricot, B., Khan, O. F., Kauffman, K. J., Xing, Y., Shaw, T. E., Libby, P., Langer, R., … Nahrendorf, M. (2016). RNAi targeting multiple cell adhesion molecules reduces immune cell recruitment and vascular inflammation after myocardial infarction. *Science Translational Medicine*, *8*(342), 342ra80. https://doi.org/10.1126/scitranslmed.aaf1435

Sager, H. B., Heidt, T., Hulsmans, M., Dutta, P., Courties, G., Sebas, M., Wojtkiewicz, G. R., Tricot, B., Iwamoto, Y., Sun, Y., Weissleder, R., Libby, P., Swirski, F. K., & Nahrendorf, M. (2015). Targeting Interleukin-1β Reduces Leukocyte Production After Acute Myocardial Infarction. *Circulation*, *132*(20), 1880–1890. https://doi.org/10.1161/circulationaha.115.016160

Sager, H. B., & Koenig, W. (2017). Acute inflammation and long-term cardiovascular risk: Identifying an unrecognised vulnerable gap. *European Journal of Preventive Cardiology*, *24*(18), 1956–1957. https://doi.org/10.1177/2047487317736869

Sager, H. B., & Nahrendorf, M. (2016). Inflammation: a trigger for acute coronary syndrome. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging : Official Publication of the Italian Association of Nuclear Medicine (AIMN) [and] the International Association of Radiopharmacology (IAR), [and] Section of the Society of Radiopharmaceutical Chemistry and Biology*, *60*(3), 185–193.

Salvador, A. F., Lima, K. A. de, & Kipnis, J. (2021). Neuromodulation by the immune system: a focus on cytokines. *Nature Reviews Immunology*, 1–16. https://doi.org/10.1038/s41577-021-00508-z

Scally, C., Abbas, H., Ahearn, T., Srinivasan, J., Mezincescu, A., Rudd, A., Spath, N., Yucel-Finn, A., Yuecel, R., Oldroyd, K., Dospinescu, C., Horgan, G., Broadhurst, P., Henning, A., Newby, D. E., Semple, S., Wilson, H., & Dawson, D. K. (2019). Myocardial and Systemic Inflammation in Acute Stress-Induced (Takotsubo) Cardiomyopathy. *Circulation*, *139*(13), 1581–1592. https://doi.org/10.1161/circulationaha.118.037975

Scheiermann, C., Kunisaki, Y., Lucas, D., Chow, A., Zhang, J. E., Zhang, D., Hashimoto, D., M., M., & Frenette, P. S. (2012). Adrenergic Nerves Govern Circadian Leukocyte Recruitment to Tissues. *Immunity*, *37*(2), 290–301. https://doi.org/10.1016/j.immuni.2012.05.021

Schloss, M. J., Swirski, F. K., & Nahrendorf, M. (2020). Modifiable Cardiovascular Risk, Hematopoiesis, and Innate Immunity. *Circulation Research*, *126*(9), 1242–1259. https://doi.org/10.1161/circresaha.120.315936

Sheedy, F. J., Grebe, A., Rayner, K. J., Kalantari, P., Ramkhelawon, B., Carpenter, S. B., Becker, C. E., Ediriweera, H. N., Mullick, A. E., Golenbock, D. T., Stuart, L. M., Latz, E., Fitzgerald, K. A., & Moore, K. J. (2013). CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. *Nature Immunology*, *14*(8), 812–820. https://doi.org/10.1038/ni.2639

Shemesh, S., Kamari, Y., Shaish, A., Olteanu, S., Kandel-Kfir, M., Almog, T., Grosskopf, I., Apte, R. N., & Harats, D. (2012). Interleukin-1 receptor type-1 in non-hematopoietic cells is the target for the pro-atherogenic effects of interleukin-1 in apoE-deficient mice. *Atherosclerosis*, *222*(2), 329–336. https://doi.org/10.1016/j.atherosclerosis.2011.12.010

Sher, L. D., Geddie, H., Olivier, L., Cairns, M., Truter, N., Beselaar, L., & Essop, M. F. (2020). Chronic stress and endothelial dysfunction: mechanisms, experimental challenges, and the way ahead. *American Journal of Physiology-Heart and Circulatory Physiology*, *319*(2), H488–H506. https://doi.org/10.1152/ajpheart.00244.2020

Siggins, R. W., Hossain, F., Rehman, T., Melvan, J. N., Zhang, P., & Welsh, D. A. (2014). Cigarette Smoke Alters the Hematopoietic Stem Cell Niche. *Medical Sciences*, *2*(1), 37–50. https://doi.org/10.3390/medsci2010037

Silvestre-Roig, C., Braster, Q., Ortega-Gomez, A., & Soehnlein, O. (2020). Neutrophils as regulators of cardiovascular inflammation. *Nature Reviews Cardiology*, *17*(6), 327–340. https://doi.org/10.1038/s41569-019-0326-7

Silvestre-Roig, C., Braster, Q., Wichapong, K., Lee, E. Y., Teulon, J. M., Berrebeh, N., Winter, J., Adrover, J. M., Santos, G. S., Froese, A., Lemnitzer, P., Ortega-Gómez, A., Chevre, R., Marschner, J., Schumski, A., Winter, C., Perez-Olivares, L., Pan, C., Paulin, N., … Soehnlein, O. (2019). Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death. *Nature*, *569*(7755), 236–240. https://doi.org/10.1038/s41586-019-1167-6

Silvestre-Roig, C., Winther, M. P. de, Weber, C., Daemen, M. J., Lutgens, E., & Soehnlein, O. (2014). Atherosclerotic Plaque Destabilization. *Circulation Research*, *114*(1), 214–226. https://doi.org/10.1161/circresaha.114.302355

Skeoch, S., & Bruce, I. N. (2015). Atherosclerosis in rheumatoid arthritis: is it all about inflammation? *Nature Reviews Rheumatology*, *11*(7), 390–400. https://doi.org/10.1038/nrrheum.2015.40

Smyth, A., O'Donnell, M., Lamelas, P., Teo, K., Rangarajan, S., Yusuf, S., & INTERHEART Investigators. (2016). Physical Activity and Anger or Emotional Upset as Triggers of Acute Myocardial Infarction. *Circulation*, *134*(15), 1059–1067. https://doi.org/10.1161/circulationaha.116.023142

Soehnlein, O. (2012). Multiple Roles for Neutrophils in Atherosclerosis. *Circulation Research*, *110*(6), 875–888. https://doi.org/10.1161/circresaha.111.257535

Soehnlein, O., Drechsler, M., Döring, Y., Lievens, D., Hartwig, H., Kemmerich, K., Ortega-Gómez, A., Mandl, M., Vijayan, S., Projahn, D., Garlichs, C. D., Koenen, R. R., Hristov, M., Lutgens, E., Zernecke, A., & Weber, C. (2013). Distinct functions of chemokine receptor axes in the atherogenic mobilization and recruitment of classical monocytes. *EMBO Molecular Medicine*, *5*(3), 471–481. https://doi.org/10.1002/emmm.201201717

Soehnlein, O., & Libby, P. (2021). Targeting inflammation in atherosclerosis — from experimental insights to the clinic. *Nature Reviews Drug Discovery*, *20*(8), 589–610. https://doi.org/10.1038/s41573-021-00198-1

Soehnlein, O., & Swirski, F. K. (2013). Hypercholesterolemia links hematopoiesis with atherosclerosis. *Trends in Endocrinology & Metabolism*, *24*(3), 129–136. https://doi.org/10.1016/j.tem.2012.10.008

Steptoe, A., & Kivimäki, M. (2012). Stress and cardiovascular disease. *Nature Reviews Cardiology*, *9*(6), 360–370. https://doi.org/10.1038/nrcardio.2012.45

Tabas, I., García-Cardeña, G., & Owens, G. K. (2015). Recent insights into the cellular biology of atherosclerosisThe cellular biology of atherosclerosis. *The Journal of Cell Biology*, *209*(1), 13–22. https://doi.org/10.1083/jcb.201412052

Tall, A. R., & Jelic, S. (2019). How broken sleep promotes cardiovascular disease. *Nature*, *566*(7744), 329–330. https://doi.org/10.1038/d41586-019-00393-6

Tardif, J.-C., Kouz, S., Waters, D. D., Bertrand, O. F., Diaz, R., Maggioni, A. P., Pinto, F. J., Ibrahim, R., Gamra, H., Kiwan, G. S., Berry, C., López-Sendón, J., Ostadal, P., Koenig, W., Angoulvant, D., Grégoire, J. C., Lavoie, M.-A., Dubé, M.-P., Rhainds, D., … Roubille, F. (2019). Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *New England Journal of Medicine*, *381*(26), 2497–2505. https://doi.org/10.1056/nejmoa1912388

Tawakol, A., Ishai, A., Takx, R. A., Figueroa, A. L., Ali, A., Kaiser, Y., Truong, Q. A., Solomon, C. J., Calcagno, C., Mani, V., Tang, C. Y., Mulder, W. J., Murrough, J. W., Hoffmann, U., Nahrendorf, M., Shin, L. M., Fayad, Z. A., & Pitman, R. K. (2017). Relation between resting amygdalar activity and cardiovascular events: a longitudinal and cohort study. *Lancet (London, England)*, *389*(10071), 834–845. https://doi.org/10.1016/s0140-6736(16)31714-7

The CARDIoGRAMplusC4D Consortium, DIAGRAM Consortium, CARDIOGENICS Consortium, MuTHER Consortium, Wellcome Trust Case Control Consortium, Deloukas, P., Kanoni, S., Willenborg, C., Farrall, M., Assimes, T. L., Thompson, J. R., Ingelsson, E., Saleheen, D., Erdmann, J., Goldstein, B. A., Stirrups, K., König, I. R., Cazier, J.-B., Johansson, Å., … Samani, N. J. (2013). Large-scale association analysis identifies new risk loci for coronary artery disease. *Nature Genetics*, *45*(1), 25–33. https://doi.org/10.1038/ng.2480

Tobaldini, E., Fiorelli, E. M., Solbiati, M., Costantino, G., Nobili, L., & Montano, N. (2019). Short sleep duration and cardiometabolic risk: from pathophysiology to clinical evidence. *Nature Reviews Cardiology*, *16*(4), 213–224. https://doi.org/10.1038/s41569-018-0109-6

Townsend, N., Wilson, L., Bhatnagar, P., Wickramasinghe, K., Rayner, M., & Nichols, M. (2016). Cardiovascular disease in Europe: epidemiological update 2016. *European Heart Journal*, *37*(42), 3232–3245. https://doi.org/10.1093/eurheartj/ehw334

Tsou, C.-L., Peters, W., Si, Y., Slaymaker, S., Aslanian, A. M., Weisberg, S. P., Mack, M., & Charo, I. F. (2007). Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *Journal of Clinical Investigation*, *117*(4), 902–909. https://doi.org/10.1172/jci29919

Tyrrell, D. J., & Goldstein, D. R. (2021). Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6. *Nature Reviews Cardiology*, *18*(1), 58–68. https://doi.org/10.1038/s41569-020-0431-7

Understanding Society Scientific Group, International Consortium for Blood Pressure, Blood Pressure-International Consortium of Exome Chip Studies, Million Veteran Program, Giri, A., Hellwege, J. N., Keaton, J. M., Park, J., Qiu, C., Warren, H. R., Torstenson, E. S., Kovesdy, C. P., Sun, Y. V., Wilson, O. D., Robinson-Cohen, C., Roumie, C. L., Chung, C. P., Birdwell, K. A., Damrauer, S. M., … Edwards, T. L. (2019). Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. *Nature Genetics*, *51*(1), 51–62. https://doi.org/10.1038/s41588-018-0303-9

Vaccarino, V., Sullivan, S., Hammadah, M., Wilmot, K., Mheid, I. A., Ramadan, R., Elon, L., Pimple, P. M., Garcia, E. V., Nye, J., Shah, A. J., Alkhoder, A., Levantsevych, O., Gay, H., Obideen, M., Huang, M., Lewis, T. T., Bremner, J. D., Quyyumi, A. A., & Raggi, P. (2018). Mental Stress–Induced-Myocardial Ischemia in Young Patients With Recent Myocardial Infarction. *Circulation*, *137*(8), 794–805. https://doi.org/10.1161/circulationaha.117.030849

VanderLaan, P. A., Reardon, C. A., & Getz, G. S. (2004). Site Specificity of Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*(1), 12–22. https://doi.org/10.1161/01.atv.0000105054.43931.f0

Vasamsetti, S. B., Florentin, J., Coppin, E., Stiekema, L. C. A., Zheng, K. H., Nisar, M. U., Sembrat, J., Levinthal, D. J., Rojas, M., Stroes, E. S. G., Kim, K., & Dutta, P. (2018). Sympathetic Neuronal Activation Triggers Myeloid Progenitor Proliferation and Differentiation. *Immunity*, *49*(1), 93-106.e7. https://doi.org/10.1016/j.immuni.2018.05.004

Vergallo, R., & Crea, F. (2020). Atherosclerotic Plaque Healing. *New England Journal of Medicine*, *383*(9), 846–857. https://doi.org/10.1056/nejmra2000317

Virtanen, M., & Kivimäki, M. (2018). Long Working Hours and Risk of Cardiovascular Disease. *Current Cardiology Reports*, *20*(11), 123. https://doi.org/10.1007/s11886-018-1049-9

Viswanathan, K., & Dhabhar, F. S. (2005). Stress-induced enhancement of leukocyte trafficking into sites of surgery or immune activation. *Proceedings of the National Academy of Sciences*, *102*(16), 5808–5813. https://doi.org/10.1073/pnas.0501650102

Vromman, A., Ruvkun, V., Shvartz, E., Wojtkiewicz, G., Masson, G. S., Tesmenitsky, Y., Folco, E., Gram, H., Nahrendorf, M., Swirski, F. K., Sukhova, G. K., & Libby, P. (2019). Stage-dependent differential effects of interleukin-1 isoforms on experimental atherosclerosis. *European Heart Journal*, *40*(30), 2482–2491. https://doi.org/10.1093/eurheartj/ehz008

Walli-Attaei, M., Joseph, P., Rosengren, A., Chow, C. K., Rangarajan, S., Lear, S. A., AlHabib, K. F., Davletov, K., Dans, A., Lanas, F., Yeates, K., Poirier, P., Teo, K. K., Bahonar, A., Camilo, F., Chifamba, J., Diaz, R., Didkowska, J. A., Irazola, V., … Yusuf, S. (2020). Variations between women and men in risk factors, treatments, cardiovascular disease incidence, and death in 27 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *The Lancet*, *396*(10244), 97–109. https://doi.org/10.1016/s0140-6736(20)30543-2

Wang, J. C., & Bennett, M. (2012). Aging and Atherosclerosis. *Circulation Research*, *111*(2), 245–259. https://doi.org/10.1161/circresaha.111.261388

Wang, Z., Bergeron, N., Levison, B. S., Li, X. S., Chiu, S., Jia, X., Koeth, R. A., Li, L., Wu, Y., Tang, W. H. W., Krauss, R. M., & Hazen, S. L. (2018). Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. *European Heart Journal*, *40*(7), 583–594. https://doi.org/10.1093/eurheartj/ehy799

Weber, C., & Hundelshausen, P. von. (2017). CANTOS Trial Validates the Inflammatory Pathogenesis of Atherosclerosis. *Circulation Research*, *121*(10), 1119–1121. https://doi.org/10.1161/circresaha.117.311984

Wieduwild, E., Girard-Madoux, M. J., Quatrini, L., Laprie, C., Chasson, L., Rossignol, R., Bernat, C., Guia, S., & Ugolini, S. (2020). β2-adrenergic signals downregulate the innate immune response and reduce host resistance to viral infection. *The Journal of Experimental Medicine*, *217*(4). https://doi.org/10.1084/jem.20190554

Wilbert-Lampen, U., Leistner, D., Greven, S., Pohl, T., Sper, S., Völker, C., Güthlin, D., Plasse, A., Knez, A., Küchenhoff, H., & Steinbeck, G. (2008). Cardiovascular Events during World Cup Soccer. *New England Journal of Medicine*, *358*(5), 475–483. https://doi.org/10.1056/nejmoa0707427

Wilbert-Lampen, U., Nickel, T., Leistner, D., Güthlin, D., Matis, T., Völker, C., Sper, S., Küchenhoff, H., Kääb, S., & Steinbeck, G. (2010). Modified Serum Profiles of Inflammatory and Vasoconstrictive Factors in Patients With Emotional Stress-Induced Acute Coronary Syndrome During World Cup Soccer 2006. *Journal of the American College of Cardiology*, *55*(7), 637–642. https://doi.org/10.1016/j.jacc.2009.07.073

Wolf, D., & Ley, K. (2019). Immunity and Inflammation in Atherosclerosis. *Circulation Research*, *124*(2), 315–327. https://doi.org/10.1161/circresaha.118.313591

Wouw, M. van de, Lyte, J. M., Boehme, M., Sichetti, M., Moloney, G., Goodson, M. S., Kelley-Loughnane, N., Dinan, T. G., Clarke, G., & Cryan, J. F. (2020). The role of the microbiota in acute stress-induced myeloid immune cell trafficking. *Brain, Behavior, and Immunity*, *84*, 209–217. https://doi.org/10.1016/j.bbi.2019.12.003

Xu, C., Lee, S. K., Zhang, D., & Frenette, P. S. (2020). The Gut Microbiome Regulates Psychological-Stress-Induced Inflammation. *Immunity*, *53*(2), 417-428.e4. https://doi.org/10.1016/j.immuni.2020.06.025

Yuan, T., Yang, T., Chen, H., Fu, D., Hu, Y., Wang, J., Yuan, Q., Yu, H., Xu, W., & Xie, X. (2018). New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox Biology*, *20*, 247–260. https://doi.org/10.1016/j.redox.2018.09.025

Yusuf, S., Hawken, S., Ôunpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., Lisheng, L., & on behalf of the INTERHEART Study Investigators. (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *The Lancet*, *364*(9438), 937–952. https://doi.org/10.1016/s0140-6736(04)17018-9

Yvan-Charvet, L., Pagler, T., Gautier, E. L., Avagyan, S., Siry, R. L., Han, S., Welch, C. L., Wang, N., Randolph, G. J., Snoeck, H. W., & Tall, A. R. (2010). ATP-Binding Cassette Transporters and HDL Suppress Hematopoietic Stem Cell Proliferation. *Science*, *328*(5986), 1689–1693. https://doi.org/10.1126/science.1189731

Zernecke, A., & Weber, C. (2014). Chemokines in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(4), 742–750. https://doi.org/10.1161/atvbaha.113.301655

Zhang, B., Ma, S., Rachmin, I., He, M., Baral, P., Choi, S., Gonçalves, W. A., Shwartz, Y., Fast, E. M., Su, Y., Zon, L. I., Regev, A., Buenrostro, J. D., Cunha, T. M., Chiu, I. M., Fisher, D. E., & Hsu, Y.-C. (2020). Hyperactivation of sympathetic nerves drives depletion of melanocyte stem cells. *Nature*, *577*(7792), 676–681. https://doi.org/10.1038/s41586-020-1935-3

Zhang, T., Zhai, Y., Chen, Y., Zhou, Z., Yang, J., & Liu, H. (2011). Effects of emotional and physiological stress on plaque instability in apolipoprotein E knockout mice. *Journal of Physiology and Biochemistry*, *67*(3), 401. https://doi.org/10.1007/s13105-011-0090-6

Zheng, X., Wang, Q., Zhang, Y., Yang, D., Li, D., Tang, B., L, X., Yang, Y., & Ma, S. (2014). Intermittent cold stress enhances features of atherosclerotic plaque instability in apolipoprotein E-deficient mice. *Molecular Medicine Reports*, *10*(4), 1679–1684. https://doi.org/10.3892/mmr.2014.2464

Zhou, X., Robertson, A.-K. L., Rudling, M., Parini, P., & Hansson, G. K. (2005). Lesion Development and Response to Immunization Reveal a Complex Role for CD4 in Atherosclerosis. *Circulation Research*, *96*(4), 427–434. https://doi.org/10.1161/01.res.0000156889.22364.f1

Appendix

Publication 1

Hettwer J*, **Hinterdobler J***, Miritsch B, Deutsch MA, Li X, Mauersberger C, Moggio A, Braster Q, Gram H, Robertson AAB, Cooper MA, Groß O, Krane M, Weber C, Koenig W, Soehnlein O, Ridker P, Schunkert H, Libby P, Kessler T**, Sager HB**. Interleukin-1β suppression dampens inflammatory leukocyte production and uptake in atherosclerosis. *Cardiovasc Res* 2021.

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Publication 2

Hinterdobler J*, Schott S*, Jin H, Meesmann A, Steinsiek AL, Zimmermann AS, Wobst J, Müller P, Mauersberger C, Vilne B, Baecklund A, Chen CS, Moggio A, Braster Q, Molitor M, Krane M, Kempf WE, Ladwig KH, Hristov M, Hulsmans M, Hilgendorf I, Weber C, Wenzel P, Scheiermann S, Maegdefessel L, Soehnlein O, Libby P, Nahrendorf M, Schunkert H, Kessler T, Sager HB. Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis. *Eur Heart J* 2021. 42: 4077-4088.

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