



Review

The influence of dysfunctional signaling and lipid homeostasis in mediating the inflammatory responses during atherosclerosis

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ARTICLE INFO

Article history:

Received 22 January 2015

Received in revised form 25 March 2015

Accepted 8 April 2015

Available online 15 April 2015

Keywords:

Atherosclerosis
Inflammation
Lipoproteins
Cytokines
Signaling
Immune cells

ABSTRACT

Atherosclerosis, the underlying cause of myocardial infarction and thrombotic cerebrovascular events, is responsible for the majority of deaths in westernized societies. Mortality from this disease is also increasing at a marked rate in developing countries due to the acquisition of a westernized lifestyle accompanied with elevated rates of obesity and diabetes. Atherosclerosis is recognized as a chronic inflammatory disorder associated with lipid accumulation and the development of fibrotic plaques within the walls of medium and large arteries. A range of immune cells, such as macrophages and T-lymphocytes, through the action of various cytokines, such as interleukins-1 and -33, transforming growth factor- β and interferon- γ , orchestrates the inflammatory response in this disease. The disease is also characterized by marked dysfunction in lipid homeostasis and signaling pathways that control the inflammatory response. This review will discuss the molecular basis of atherosclerosis with particular emphasis on the roles of the immune cells and cytokines along with the dysfunctional lipid homeostasis and cell signaling associated with this disease.

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Abbreviations: ABC, ATP-binding cassette transporter; ACAT-1, acyl-coenzyme A acyltransferase 1; AcLDL, acetylated LDL; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; ApoE, apolipoprotein E; CCL, CC-chemokine ligand; CD36, cluster of differentiation 36; CE, cholesteryl esters; CHD, coronary heart disease; CXCL, CXC-chemokine ligand; EC, endothelial cell; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GF, growth factor; GM-CSF, granulocyte-macrophage colony stimulating factor; HDL, high-density lipoprotein; HF, heart failure; HL, hepatic lipase; HMG-CoA reductase, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; I κ B, inhibitor of κ B; ICAM-1, intercellular adhesion molecule-1; IDL, intermediate-density lipoprotein; IDOL, inducible degrader of LDLR; IFN, interferon; IKK, I κ B kinase; IL, interleukin; JNK, c-Jun N-terminal kinase; LDL, low-density lipoprotein; LDLR, LDL receptor; LOX, lipoxygenase; LOX1, lectin-like oxidized LDL receptor 1; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; MARCO, macrophage receptor with collagenous structure; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; MI, myocardial infarction; mLDL, minimally modified LDL; MMP, metalloproteinase; NEMO, NF- κ B-essential modifier; NF- κ B, nuclear factor kappa B; NK, natural killer; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NOD, nucleotide-binding oligomerization domain; NPC, Niemann-Pick type C disease proteins; OxLDL, oxidized LDL; p90RSK, p90 ribosomal S6 kinase; PCSK9, proprotein convertase subtilisin/kexin type-9; PI3K, phosphoinositide 3-kinase; PRR, pattern-recognition receptor; PPAR, peroxisome proliferator-activated receptor; RCT, reverse cholesterol transport; ROS, reactive oxygen species; SMC, smooth muscle cell; SR, scavenger receptor; SREC1, scavenger receptor expressed by endothelial cells; SR-PSOX, scavenger receptor for phosphatidylserine and oxidized LDL; STAT, signal transducers and activators of transcription; TAG, triacylglycerol; TBK1, TANK-binding kinase 1; TGF, transforming growth factor; Th, T helper; TIMP, tissue inhibitors of metalloproteinase; TIR, Toll/IL-1 receptor; TLR, toll-like receptor; TNF, tumor necrosis factor; Tregs, regulatory T cells; TRIF, TIR-domain-containing adaptor protein inducing IFN- β ; VLDL, very low-density lipoprotein; VSMC, vascular SMC

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1. Introduction

Coronary heart disease (CHD) is responsible for one in three deaths in westernized countries. An estimated 23.6 million people are expected to die globally from cardiovascular related pathologies by 2030 and the disease and its complications, which include stroke and myocardial infarction (MI), have been estimated to have total costs (both direct and indirect) of approximately \$315.4 billion in 2010 [1]. Atherosclerosis, a chronic inflammatory disorder of the large and medium sized arteries, constitutes the major underlying cause of CHD [2]. Many risk factors for atherosclerosis have been identified and these are generally classified as modifiable and non-modifiable. The latter include age, gender, and genetic predisposition to hypercholesterolemia, hypertension, diabetes and systemic inflammation [2]. Modifiable risk factors include cigarette-smoking, diet rich in saturated fats, and a sedentary lifestyle [2]. It is now well accepted that atherosclerosis is initiated by a local immune response to lipid deposition within the arterial sub-endothelial compartment [2].

2. Lipid metabolism in atherosclerosis

Lipoprotein particles function as vehicles for the transport of insoluble lipids in the blood and are composed of a core region storing TAGs and cholesteryl esters (CEs), with a surrounding polar region consisting of phospholipids and apolipoproteins. Different forms of lipoproteins are involved in lipid trafficking and considerable exchange of various apolipoproteins occurs between them. For example, chylomicrons

primarily facilitate the transport of dietary triacylglycerols (TAGs) from the intestine to peripheral tissues. The non-esterified fatty acids and 2-monoacyl glycerol produced by the digestion of TAGs within chylomicrons by lipoprotein lipase (LPL) are then taken up by the adipose tissue or skeletal muscle for utilization/storage [2]. The liver can acquire the resulting chylomicron remnants via specific receptors and metabolize them [3]. In contrast to chylomicrons, very low-density lipoproteins (VLDL) are involved in the transport of TAGs synthesized by the liver [2]. Intermediate-density lipoproteins (IDL) are formed following the digestion of TAGs in VLDL by LPL and hepatic lipase (HL) [2]. Further processing and hydrolysis of TAGs in IDL by HL results in the production of low-density lipoprotein (LDL) particles [2]. LDL functions to carry cholesterol from the liver to peripheral tissues. High plasma LDL levels is a major risk factor for atherosclerosis as identified from numerous epidemiological studies and clinical trials with statins: inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase), a rate limiting step in the biosynthesis of cholesterol [2].

The LDL particles enter cells of peripheral tissues predominantly via receptor-mediated endocytosis involving its cognate receptor, LDLR (Fig. 1). The crucial involvement of LDL within atherosclerosis was discovered through studies on subjects with familial hypercholesterolemia; a condition that arises from mutations in the LDLR gene [4]. Heterozygous sufferers are relatively common (1 in 500) whereas homozygotes are less frequent (1 in a million) and exhibit six to ten times the levels of LDL within their plasma compared to non-sufferers, and are prone to MIs at an early age [4]. The clearance of plasma LDL by LDLR is critical for limiting atherosclerosis and it is therefore not surprising that considerable research and therapeutic approaches have been devoted on this receptor. For example, proprotein convertase subtilisin/kexin type-9 (PCSK9) is an emerging target for cholesterol-lowering therapies because this enzyme binds to LDLR and targets it for lysosomal degradation in cells [5]. Inducible degrader of LDLR (IDOL), an E3 ubiquitin ligase that mediates ubiquitination and subsequent degradation of LDLR, represents another promising target [6]. The pioneering work by Brown and Goldstein that demonstrated negative feedback regulation of transcription of LDLR and HMG-CoA reductase by the sterol regulatory element binding protein pathway [7] suggested that additional mechanisms mediate uncontrolled cellular

uptake of LDL in atherosclerosis. Indeed, as discussed below in detail, LDL is subject to modification, particularly oxidation, and such modified LDL is taken up in an uncontrolled manner by scavenger receptors (SRs), such as A (SR-A) and cluster of differentiation 36 (CD36), by certain plaque-resident macrophages and smooth muscle cells (SMCs) [2] (Fig. 1).

Excess intracellular cholesterol is toxic and there are essentially two main routes for its removal; either through enzymatic-driven conversion to a more soluble transportable form or through reverse cholesterol transport (RCT) [2,8]. Cholesterol is enzymatically modified through a number of processes such as hydroxylation and esterification within the endoplasmic reticulum (ER) to produce oxysterols and sterol esters respectively [2,8–10]. Esterification of cholesterol reduces the solubility of the molecule and promotes storage within cytoplasmic lipid droplets [2,8–10]. RCT is the primary pathway for the removal of excess cholesterol and involves lipid transporters such as ATP-binding cassette transporter (ABC)-A1 and -G1 that mediate the transfer of cholesterol from peripheral cells to selected extracellular acceptors such as high-density lipoproteins (HDL) and associated apolipoproteins [2,8–10] (Fig. 1). The cholesterol is then delivered to the liver for conversion to bile salts in preparation for excretion [2,8–10]. Homeostatic mechanisms exist in cells to prevent lipid overload and many act by stimulating cholesterol efflux and modulating the inflammatory response. For example, the production of oxysterols and desmosterol activates liver X receptors (LXRs) leading to induced expression of ABC-A1 and -G1 [11,12], and thereby RCT. In addition, macrophage cholesterol loading induces autophagy, a process by which double-membrane vacuoles sequester intracellular contents and targets them for degradation via fusion with secondary lysosomes, leading to RCT [13]. Furthermore, peroxisome proliferator-activated receptors (PPARs) play an important role in the control of cholesterol homeostasis [14,15].

The involvement of HDL particles within atherosclerosis has received a great level of attention [16,17]. Sufferers of Tangier disease contain mutations within the gene for ABC-A1 and are associated with drastically low levels of HDL, localized accumulation of CEs within different tissues of the body and development of premature atherosclerosis [18]. The relationship between reduced HDL levels and incidences of CHD have long been established as one of the major risk factors for the

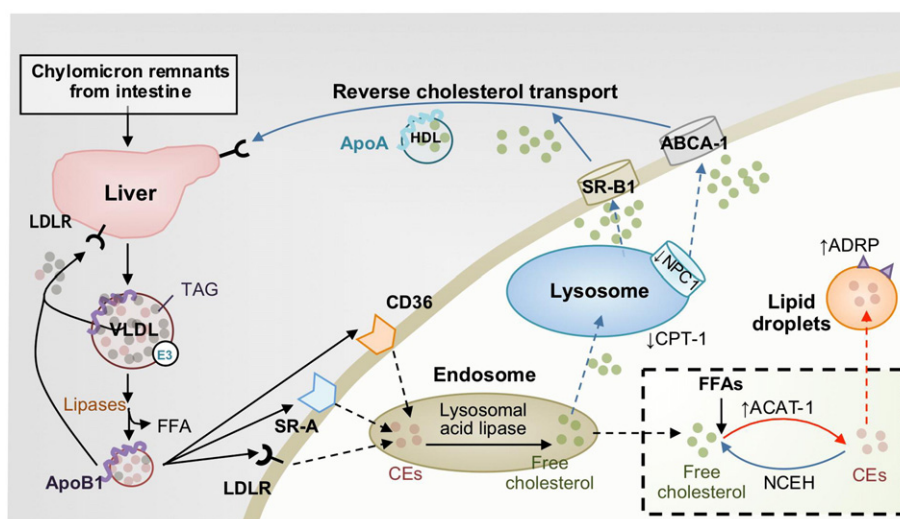


Fig. 1. Overview of cholesterol metabolism. Dietary lipids are absorbed in the intestine and transported by chylomicrons to peripheral tissues. Following lipolysis by lipases, chylomicron remnants deliver dietary lipids to the liver. Liver-derived VLDLs containing ApoB and ApoE (E3) mediate the transport of endogenously synthesized lipids. VLDLs are then hydrolyzed to IDLs and on to LDLs. ApoB facilitates LDL binding to its cognate receptor (LDLR), which are then internalized and degraded in the lysosomes. The uptake of LDL by LDLR is under negative feedback regulation. Scavenger receptors such as SR-A and CD36 predominantly facilitate the excessive, uncontrolled uptake of modified LDL particles into macrophages during the disease. Lysosomal acid lipases hydrolyze CEs to free cholesterol and free fatty acids (FFAs). The free cholesterol is either trafficked out of the cells for reverse cholesterol transport through ABC transporters, such as ABCA-1 and SR-B1, or re-esterified to CEs for storage by the action of acyl-coenzyme A acyltransferase 1 (ACAT-1) within the endoplasmic reticulum, and then stored as lipid droplets [regulated by adipocyte differentiation-related protein (ADRP)]. The accumulation of CEs depends on the FFA availability [regulated by carnitine palmitoyltransferase 1 (CPT-1)] and hydrolysis of CEs [modulated by neutral cholesterol ester hydrolase (NCEH)]. Niemann-Pick type C disease proteins (NPC)-1 and -2 regulate the intracellular trafficking of cholesterol.

disease [16–18], and the plasma concentration of HDL is often negatively affected by many other atherosclerotic risk factors such as gender, obesity and exercise [2,10,16–18]. In addition, studies in both animal model systems and humans have shown that raised plasma HDL levels are generally associated with protection against atherosclerosis [16–19]. The anti-atherosclerotic effects are thought to mostly arise from the involvement of HDLs within RCT [19]. However, the particle has additional protective properties such as inhibition of lipoprotein oxidation, attenuation of the inflammatory response, endothelial cell (EC) protection and suppression of monocyte adhesion [19]. It is therefore not surprising that augmenting HDL levels/function represents a promising therapeutic avenue against atherosclerosis. Many approaches on HDL biology are being pursued such as increasing plasma HDL levels (e.g. HDL mimetics, nicotinic acid, activators of nuclear receptors such as fibrates), preventing exchange of CEs from HDL (for RCT) with other pro-atherogenic lipoproteins (e.g. inhibitors of cholesterol ester transport protein) and mimetics or transcriptional activators of its major apolipoprotein ApoA-I [16,17]. However, HDL particles are heterogeneous in size and composition, and the complex relationship between their concentration, particle number and diverse subpopulation with anti-atherogenic effects such as RCT has been highlighted by the recent failure of some clinical trials with orally-active, HDL raising agents [16,17]. Future therapeutic approaches should therefore focus more on specific aspects of HDL biology and structure–function relationships rather than simple plasma concentration of the lipoprotein. Indeed recent studies have shown that HDL cholesterol efflux capacity rather than absolute concentration is inversely associated with cardiovascular events [16,17].

3. The development and progression of atherosclerosis

Advances in murine models capable of developing advanced atherosclerotic lesions, particularly apolipoprotein E (ApoE)^{−/−} and LDLR^{−/−} knockout mice, have markedly facilitated our understanding of the molecular basis of this disease [20,21]. ApoE is produced prominently within the liver and by macrophages and is a component of lipoprotein particles where it functions as a ligand for lipoprotein receptors [22]. ApoE^{−/−} mice are markedly hypercholesterolemic and develop spontaneous atherosclerotic lesions that can be speeded up by feeding of a high-fat diet [20–22]. LDLR^{−/−} mice are mildly hypercholesterolemic due to defective clearance of plasma LDL and, following feeding on a high-fat diet, develop atherosclerotic lesions accompanied with increased levels of cholesterol within the plasma and raised amounts of pro-atherogenic lipoproteins [20,21]. Bone marrow transplantation approaches in these mice allow determination of whether a given phenotype is governed by hematopoietic or non-hematopoietic cells [20,21].

The use of mouse model systems has some key limitations. For instance, the disruption of the genes for ApoE or LDLR may affect other crucial cellular processes; for example, ApoE also functions as an anti-oxidant and modulator of immune responses [22]. In addition, recent comparisons of the expression profiles of 15 tissues have revealed considerable diversity in RNA expression between humans and mice [23]. Furthermore, mice have a distinct lipoprotein profile from humans and the majority of plasma cholesterol is carried on HDL particles whereas, in humans, 75% of cholesterol is carried on LDL particles [20,21]. Moreover, immunological responses differ as the immune system has partly evolved due to selective pressure from microbial exposure; as such mice are more resilient to inflammatory damage than humans [20,24]. Despite these potential caveats, the use of these mice has greatly progressed our understanding of the disease, and there are many advantages associated with the use of murine models such as environmental conditions and dietary intake can be carefully controlled, the generation time is short therefore facilitating cross-breeding with mice that have deficiency in specific genes, and the evaluation of disease progression can be undertaken within a reasonable time frame [20,21].

3.1. Initiation of atherosclerosis

Fig. 2 summarizes the key steps in the different stages involved in the pathogenesis of atherosclerosis that develops during the life span of an individual. A number of potential initiators of atherosclerosis have been identified including intimal lipid accumulation, changes in hemodynamic forces and a response to injury of the endothelium [2]. Atherosclerotic lesions tends to develop within areas of curvature, such as branching points, which are prone to disturbed laminar flow within the large and medium sized arteries [2,25]. Whilst the arterial endothelium is typically impermeable to large biomolecules such as LDL, physiological and pathophysiological changes can activate them leading to an increase in the permeability of the EC layer [25,26]. As a result the expression of adhesion molecules on the cell surface increases in addition to the production of extracellular matrix (ECM) proteins and the secretion of chemokines and growth factors (GFs) including macrophage colony-stimulating factor (M-CSF) [2,25,26].

LDL particles containing ApoB diffuse between EC junctions and accumulate within the subendothelial space [2]. The LDL particles associate with ECM components through interactions with ApoB and LPL and proteoglycans, becoming trapped and susceptible to a range of enzymatic and non-enzymatic chemical modifications, particularly oxidation, mediated through the activities of myeloperoxidases, lipoxygenases (LOX), reactive oxygen species (ROS), peroxynitrite and nitric oxide [2]. Additionally, non-oxidized LDL is likely taken up by macrophages where it may be subjected to oxidation and subsequent aggregation within lysosomes [27].

Modified LDL particles are immunogenic as the peroxidation of phospholipids, CEs and TAGs creates reactive species capable of stimulating inflammatory processes that promote the activation of ECs, platelets and macrophages [28–30]. The presence of oxidized LDL (OxLDLs) in the intima also aggravates surrounding cells and induces SMC mitogenesis [2]. The oxidation of LDL occurs in stages with minimally modified LDL (mmLDL), which is not recognized by SRs, being a major initiator of the immune response [2,28,30]. For example, mmLDL stimulates the production of ROS and pro-inflammatory mediators via a mechanism that requires toll-like receptor (TLR) 4 [31]. In addition, mmLDL and its active components such as CE hydroperoxides, induce TLR4-dependent fluid phase uptake, and hence lipoprotein accumulation, in macrophages [32].

The recruitment of immune cells, including monocytes, neutrophils, T cells, B cells, dendritic cells and mast cells, to lesion sites is controlled by chemokines released in particular by activated ECs and SMCs, and their corresponding G protein-coupled receptors present on leukocytes [33–36]. The roles of numerous chemokines and their receptors in atherosclerosis along with the recruitment of diverse sets of immune cells to the lesion have been investigated. A detailed coverage of these findings as well as those aimed at targeting chemokine interactions in atherosclerosis is beyond the scope of this article, and the reader is directed to several excellent recent reviews on this topic [33–36]. The leukocyte adhesion cascade is comprised of three main stages; rolling, activation and arrest [33–36]. In the case of LY6C^{high} mouse monocytes (the precursors to most of the macrophages present in atherosclerotic lesions), their tethering and rolling on the EC surface is dependent on the immobilization of chemokines, such as CXC-chemokine ligand (CXCL)-1 and CC-chemokine ligand (CCL)-5, on EC glycosaminoglycans and their receptors (R) expressed on the surface of monocytes [35]. In addition, the rolling of the monocytes along the endothelium is coordinated by weak interactions between P-selectin glycoprotein ligand-1 found on the monocyte surface to P-selectin and E-selectin expressed by the endothelium [35]. The monocytes are immobilized through associations between integrins on the monocyte surface and adhesion proteins expressed on ECs [very late antigen 4 with vascular cell adhesion molecule 1 and lymphocyte function-associated antigen 1 with intercellular adhesion molecule 1 (ICAM-1)] [35]. The transmigration of monocytes across the endothelium is governed by chemokine:chemokine

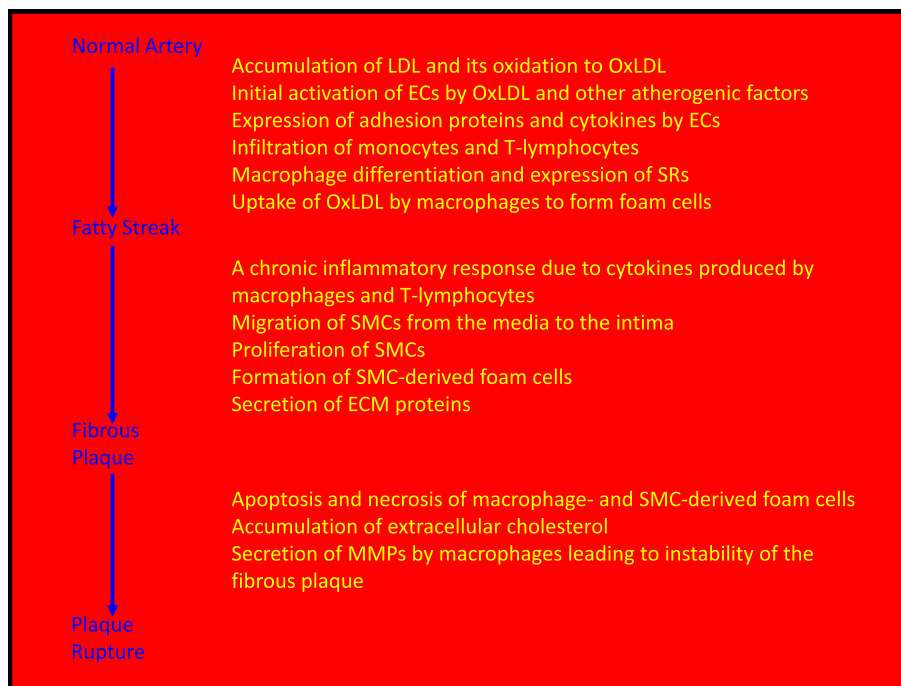


Fig. 2. Overview of different stages of atherosclerosis. See text for more details. Abbreviations: ECs, endothelial cells; ECM, extracellular matrix; LDL, low-density lipoprotein; OxLDL, oxidized low-density lipoprotein; SMC, smooth muscle cells; SR, scavenger receptor.

receptor interactions with CCL2-CCR2, CX3CL1: CX3CR1 and CCL5-CCR5 being particularly important [35]. Following transdiapedesis into the intima, monocytes differentiate into macrophages under the influence of M-CSF or granulocyte-macrophage colony stimulating factor (GM-CSF) [33–36].

Macrophages utilize a variety of pattern-recognition receptors (PRRs), including SRs, TLRs and nucleotide-binding oligomerization domain (NOD)-like receptors, to elicit rapid responses against foreign particles (or endogenous danger signals) to mount an inflammatory response [35,37]. The role of TLR4 in the promotion of foam cell formation and the inflammatory response [31,32] was described above and studies in mouse model systems have shown that the absence of TLR2 or TLR4 along with the adapter proteins used by TLRs [IL-1 receptor associated kinase 4, tumor necrosis factor (TNF) receptor-associated factor 6, Toll/interleukin (IL)-1 receptor (TIR)-domain-containing adaptor protein inducing interferon (IFN)- β (TRIF) and myeloid differentiation primary response protein 88] results in an athero-protective phenotype [37–43]. The atherosclerotic plaques also contain cholesterol crystals and their uptake by macrophages via macropinocytosis leads to activation of the NOD-, leucine-rich repeat- and pyrin domain containing 3 (NLRP3) inflammasome and production of IL-1 β [44]. The pro-atherogenic role of IL-1 β is well established (see Section 6) though the link between NLRP3 inflammasome and atherosclerosis is not clear-cut [44,45]. SRs, which includes SR-A1, SR-B1, CD36, macrophage receptor with collagenous structure (MARCO), lectin-like oxidized LDL receptor 1 (LOX1), scavenger receptor for phosphatidylserine and oxidized LDL (SR-PSOX) and scavenger receptor expressed by endothelial cells-1 (SREC1), form part of the innate immune system tasked with the recognition of a wide range of ligands associated with pathogenic classes of molecular patterns, and support the elimination of foreign agents [46, 47]. Several SRs contain multiple LDL-specific binding sites that facilitate the uptake of modified forms of this lipoprotein and subsequent foam cell formation [46,47]. SR-A1 and CD36 have the highest affinity for acetylated LDL (AcLDL) and OxLDL respectively, and are responsible for up to 90% uptake of modified LDL *in vitro* [46,47]. However, the role of SR-A1 and CD36 in atherosclerosis *in vivo* is less clear with often-conflicting outcomes from studies involving their genetic disruption in

mouse model systems [46–51]. Functional redundancy or compensatory mechanisms are potential contributors and indeed Makinen et al., [49] have shown that targeted down-regulation of either CD36 or SR-A1 hinders atherosclerotic development and silencing of one receptor results in the up-regulation of the other. Furthermore, macrophages can accumulate LDL, VLDL and modified LDL through several receptor-independent processes such as phagocytosis and macropinocytosis [2, 10,52]. The CEs in the lipoprotein particles are hydrolyzed to fatty acids and free cholesterol in the endolysosomal compartment [2]. The latter then moves to the ER where they can be re-esterified by acyl-coenzyme A acyltransferase 1 (ACAT-1) to form CEs “droplets” characteristic of foam cells [2].

SMCs within the plaque are also capable of foam cell transformation mediated mainly through the uptake of modified LDLs by SRs expressed at their cell surface [2,46,47]. Small, asymptomatic lesions are comprised of macrophage- and SMC-foam cells along with T cells, and are referred to as fatty streaks due to the high concentration of foam cells [2,30,35]. The fatty streak may regress or progress into a clinically relevant plaque [2,30,35].

3.2. Disease progression

The formation of an intermediate lesion arises due to enhanced migration and proliferation of vascular SMCs (VSMCs) from the tunica media into the inflamed area in response to GFs released from plaque-resident cells [2,10]. The SMCs proliferate and release ECM proteins contributing towards the production of a fibrotic cap [2,10]. Stable plaques are associated with the presence of a fibrous cap, containing a matrix enriched with type I and III collagen, and the absence of a necrotic core [2,10,53].

The cholesterol homeostatic mechanisms become dysfunction during excessive cellular uptake of this sterol leading to the accumulation of free cholesterol, which unlike CEs, is toxic to the cells. Excessive levels of free cholesterol in the ER leads to defective esterification by ACAT-1, and in the plasma membrane results in an inflammatory response via the activation of nuclear factor-kappa B (NF- κ B) [2,35,54,55]. Such dysfunctional lipid metabolism triggers an unfolded protein response

within the ER and, together with other insults, initiates apoptotic pathways [56,57]. During the earlier stages of the disease, effective engulfment of apoptosing cells by neighbouring phagocytes (efferocytosis) helps to resolve pro-inflammatory processes and maintain stability within the plaque [56–59]. However, dysfunctional efferocytosis is a key feature of advanced lesions and as the disease progresses, the rate of apoptosis within the necrotic core likely overwhelms the phagocytic capabilities of residing phagocytes [56–59]. Efficient clearance of apoptotic cells by surrounding macrophages requires intact lipid metabolism to deal with the ingested lipids, and hence defective lipid homeostasis is likely to contribute to dysfunctional efferocytosis [56–59]. In addition, *in vitro* experiments have demonstrated that modified LDL serves as a substrate for phagocytes and so may competitively hinder the efferocytosis of dying cells [56–59]. Ineffective efferocytosis also stimulates secondary necrosis of lesion-resident cells and, in the case of macrophages, leads to the release of oxidized lipids and pro-inflammatory propagators [56–59]. As the disease continues, the plaque becomes increasingly unstable and vulnerable as a result of reduced efferocytosis, chronic inflammation and the ineffective egress of immune cells [56–59]. When residing within the plaque, the migration of macrophages is limited and therefore compromises potential resolution of inflammation thereby favouring pathogenic processes [35,56,60]. Lesion-resident macrophages contribute towards the inflammatory state through the secretion of protease enzymes and pro-inflammatory cytokines [35,56,60].

3.3. Advanced plaque formation and rupture

Towards the later stages of the disease, the atherosclerotic lesion is characterized by an abundance of disorganized cells, lipids, matrix components and minerals [2]. Clinical symptoms may occur during this phase of the disease as the intimal region is thickened and the area of the arterial lumen may be reduced in size [2,56,60]. Unstable plaques are associated with a high proportion of macrophages to SMCs and a lipid-rich necrotic core [2,56,60]. The dying foam cells release their cytoplasmic contents causing a build-up of extracellular lipids and GFs that exacerbates inflammation and triggers secondary necrosis [2,56,60]. Excessive levels of cholesterol also promote the formation of solid crystals that are toxic to cells and initiate a pro-inflammatory response [44].

Macrophages induce the expression of matrix metalloproteinases (MMPs) that promote the degradation of collagen. The MMP family encompasses a range of proteolytic enzymes, including collagenases, gelatinases, matrilysins and membrane-type MMPs [61–63]. In the healthy state, MMPs are carefully regulated through the production of precursor zymogens, the activities of endogenous tissue inhibitors of metalloproteinases (TIMPs) and associations with the ECM [61–63]. However, an imbalance in the ratio of MMPs to TIMPs is thought to support excessive ECM breakdown [61–63]. MMPs are overexpressed within unstable lesions and localize to vulnerable regions within the plaque that are prone to rupture and inhabited by macrophage foam cells [61–63]. The degradation of the connective tissue is detrimental to

plaque stability and the resulting products are deposited within the arterial intima where they promote vasculitis [61–63].

Locally produced cytokines, such as IFN- γ secreted by T cells, reduce the proliferation of SMCs and also inhibit the synthesis of integral ECM components like collagen types I and III [64]. The fibrous cap undergoes thinning prior to rupture, which undermines the stability of the structure [2,10,63]. At this stage of the disease, the plaque contains depleted levels of fibrous material and may show signs of calcification, ulceration and hemorrhaging from small vessels, which grow in from the adventia and are leaky [2,10,63].

A number of factors contribute towards the disruption of the cap including the presence of inflammatory cells, building toxicity, the activities of proteolytic enzymes released from macrophages, coronary spasms and physical vulnerabilities and stresses arising from the altered composition of the lesion [2,10,63]. The exposure of tissue factor from the plaque with the arterial lumen promotes coagulation and the formation of a thrombus [2,10,63]. The thrombus may instantly obstruct the lumen or may detach in the form of an embolus and block blood flow at a downstream site [63]. The usual cause of a MI is the rupture of an advanced atherosclerotic lesion, which exposes collagen and tissue factor leading to platelet aggregation and coagulation [63].

4. Key cellular signaling events in atherosclerosis

The dysregulation of key signaling pathways during atherosclerosis leads to altered gene expression that facilitates the disease processes. Several signaling pathways have been implicated within the atherosclerotic state and some of the key ones are associated with the inflammatory response such as mitogen-activated protein kinases (MAPKs), nuclear factor kappa B (NF- κ B) and phosphoinositide 3-kinase (PI3K) [65–68]. Table 1 summarizes the roles of some key components of these pathways in atherosclerosis.

4.1. MAPKs: extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK)

The ERK family includes ERK-1, -2 and -5. The exact roles of ERK-1 and -2 in atherosclerosis are still not properly understood but studies have shown that pathways involving these enzymes regulate the proliferation and differentiation of SMCs in the lesion [65]. OxLDL promotes the proliferation of cultured aortic SMCs in a signaling mechanism involving ERK1/2 [65]. A study by Zhou et al., [69] demonstrated that inhibition of ERK1/2 activity leads to increased efflux of cholesterol to ApoA-I and HDL acceptors in macrophage-derived foam cells because of induced expression of ABC-A1. Furthermore, we have shown that ERK1/2 are integral to the IFN- γ -mediated activation of signal transducer and activator of transcription (STAT)-1; a key regulator of many genes implicated in atherosclerosis such as ICAM-1 and monocyte chemoattractant protein-1 (MCP-1), and the uptake of modified LDL by macrophages [70]. In addition to the pro-atherogenic actions described, ERK1/2 are also likely involved in protective effects. A disintegrin and

Table 1
Role of components of key signaling pathways in atherosclerosis.

Signaling pathway/protein	Role in atherosclerosis	Ref.
ERK-1/2	Regulates the proliferation and differentiation of VSMC, foam cell formation and IFN- γ signaling.	[65,69,70]
ERK5	Activated by statins. Promotes efferocytosis and regulates both EC function and inflammation.	[73–75]
p38	Regulates foam cell formation and cellular apoptosis in macrophages. Modulates chemokine and adhesion molecule expression in EC.	[65,76–79]
JNK	Regulates macrophage foam cell formation, EC apoptosis and expression of MMPs and ECM proteins in VSMC.	[65,80–83]
NF- κ B	Regulates expression of pro-inflammatory cytokines, chemokines and adhesion proteins. Modulates vascular inflammation, the recruitment of immune cells and foam cell formation.	[67,84–88,97,98]
PI3K γ	Modulates foam cell formation, macrophage proliferation and recruitment of immune cells.	[101–103]
Akt1	Modulates pro-inflammatory gene expression, apoptosis, and VSMC proliferation and migration.	[105,106]
Akt2	Modulates migration and proliferation of VSMC, expression of protease and ECM proteins, and macrophage polarization.	[107,108]
Akt3	Regulates foam cell formation.	[109]

metalloproteinase with thrombospondin motifs (ADAMTS) are structurally related to MMPs and potentially involved in remodelling of the ECM within the plaque [71]. The anti-atherogenic cytokine IL-33 (see Section 6) down-regulates the expression of ADAMTS-1 and -4 in human macrophages through a signaling mechanism dependent on ERK1/2 [72].

In contrast to ERK1/2, ERK5 is a less characterized isoform but has received interest because of its robust activation by statins [73]. Recent studies in mouse model systems have demonstrated that ERK5 activation in macrophages promotes efferocytosis and inhibits the development of atherosclerosis [73]. In addition, ERK5 regulates EC function by increasing the expression of endothelial nitric oxide synthase (eNOS) and by inhibiting inflammation in these cells [74]. Reduction of ERK5 action by p90 ribosomal S6 kinase (RSK) is associated with endothelial dysfunction and atherosclerosis [74]. Furthermore, ERK5 inhibits endothelial migration via Krüppel-like factor-2-dependent down regulation of p21-activated kinase 1 [75].

Another MAPK, p38, has also been implicated in the control of macrophage foam cell formation through the inhibition of macroautophagy [76]. In response to the accumulation of free cholesterol within the ER, p38 co-ordinates signaling events that induce cellular apoptosis [77]. However, p38 can have both pro- and anti-apoptotic actions depending on the stimulus/cellular conditions [78]. For example, macrophage deficiency of p38 α in ApoE^{-/-} mice results in increased levels of apoptosing macrophages and enhanced necrosis within the plaque [78]. Lesions from these mice are also characterized by a reduced content of collagen and a thin fibrous cap [78]. The MAPK is thought to inhibit ER-stress-induced apoptosis because apoptosis arising in response to ER stressors is enhanced following knockdown of p38 α in primary mouse macrophages [78]. It therefore seems likely that during atherosclerosis this MAPK is involved in protective signaling pathways against ER-stress responses and apoptosis in advanced plaques [78]. Interestingly a study by Kardakaris et al., [79] indicated that ablation of p38 α in macrophages and ECs did not affect atherosclerotic plaque formation in ApoE^{-/-} mice following feeding on a high cholesterol diet. Nevertheless p38 α deficiency inhibited OxLDL-induced chemokine and adhesion molecule expression in *in vitro* cultures of ECs [79].

c-Jun N terminal kinases (JNKs) also belong to the MAPK superfamily. These proteins regulate the maturation and activity of T cells and the synthesis of pro-inflammatory cytokines such IL-2, IL-6 and TNF- α [65, 66]. Within ApoE^{-/-} mice, JNK is activated in response to a high cholesterol diet [65,66,80]. Interestingly, JNK2^{-/-} mice crossed with ApoE^{-/-} mice contained smaller plaques, whereas ablation of JNK1 had little effect on the size of the lesions in these experiments [80]. The differences in results implicate the differential roles of JNK isoforms within the atherosclerotic disease state [80]. The lack of JNK2 within these mice is also associated with a halved number of macrophage foam cells despite enhanced uptake of fluorescently labelled AcLDL in peritoneal macrophages from ApoE^{-/-}/JNK2^{-/-} mice [80]. Additionally, although the levels of SR-A were raised, the efflux of cholesterol to ApoA-I acceptors was enhanced and the amount of phosphorylated, activated SR-A was reduced indicating that the knockdown of JNK2 in macrophages compromises cholesterol homeostasis [80]. In contrast to such specific action of JNK2, recent studies have shown that genetic deletion of JNK1 reduces EC apoptosis in the hypercholesterolemic LDLR^{-/-} model system [81]. The JNK signaling pathway has also been implicated in later stages of the disease. In cultured primary rat aortic SMCs, JNK is activated in response to oxidative stresses and induces the expression of matrix degrading MMPs whilst down-regulating the expression of genes involved in ECM biosynthesis such as lysyl hydroxylase [82]. The results indicate that the signaling component exerts destabilizing effects on the architecture of the aortic tissue that could compromise the integrity of the atherosclerotic plaque [82]. More recently, the use of the JNK inhibitor SP600125 in the ApoE^{-/-} model showed that this kinase pathway might play a critical role in low shear stress-induced atherosclerosis by a mechanism involving platelet endothelial cell adhesion molecule-

1-mediated modulation of NF- κ B activity and expression of adhesion proteins [83].

4.2. NF- κ B

NF- κ B consists of homo- or hetero-dimers of Rel-domain containing proteins NF- κ B1 (p50 and its precursor p105), NF- κ B2 (p52 and its precursor p100), RelA (p65), RelB and c-Rel [67]. The NF- κ B dimer resides in the cytoplasm in the absence of any activating signals because its nuclear localization signal is masked by association with inhibitors belonging to the inhibitor of kappa B (I κ B) family consisting of I κ B α , I κ B β , I κ B γ , I κ B ϵ or Bcl-3 [67]. Inflammatory stimulus activates signaling cascades leading to phosphorylation of I κ B by a complex consisting of I κ B kinase (IKK) 1 (IKK1 or IKK α), IKK2 (or IKK β) and NF- κ B-essential modulator (NEMO or IKK γ) [67]. Recent studies show that IKK α and IKK β possess catalytic activity whereas IKK γ plays a more regulatory role [67]. In addition, IKK-related kinases such as IKK ϵ and TANK-binding kinase 1 (TBK1) are involved in NF- κ B signaling [67]. The phosphorylation of I κ B leads to its ubiquitination and subsequent proteasomal degradation, thereby leaving NF- κ B free to translocate to the nucleus [67].

The NF- κ B signaling pathway regulates the expression of a number of genes implicated in atherosclerosis, including TNF- α , IL-6, MCP-1 and ICAM-1 [67]. Furthermore, the pathway is activated by several factors associated with the disease such as mmOxLDL and hemodynamic forces [67]. High levels of activated NF- κ B subunits are found within the nuclei of intima-resident SMCs, macrophages and ECs within human atheromas [67]. Indeed, inhibition of the NF- κ B pathway in mouse model systems using cell-permeable peptide inhibitors of nuclear import or other effectors (e.g. acetyl-11-keto-beta-boswellic acid) reduces atherosclerotic development and the expression of pro-inflammatory markers, including chemokines and adhesion molecules [84,85]. In addition, A20 (also called TNF- α -induced protein 3) reduces atherosclerosis in the ApoE^{-/-} model by decreasing NF- κ B activity and thereby the pro-inflammatory state in the lesion [86]. Furthermore, systemic delivery of miR-181b inhibits NF- κ B activation, vascular inflammation and atherosclerosis in ApoE^{-/-} mice [87]. More recently, macrophage mitochondrial oxidative stress has been found to promote atherosclerosis and NF- κ B-mediated inflammation in macrophages [88].

A pro-atherogenic role for NF- κ B has been identified for some but not all studies aimed at manipulating the action of individual subunits or their upstream activators in mouse model systems, and this probably reflects the complex function of the different proteins. For example, endothelial-cell specific inhibition of NF- κ B, arising from the deletion of NEMO/IKK γ or expression of dominant-negative I κ B α in these cells, reduces the size of atherosclerotic plaques in ApoE^{-/-} mice and is accompanied with impaired macrophage recruitment and reduced expression of cytokines and chemokines [89]. In contrast, myeloid-specific deficiency of I κ B α was found to promote atherosclerosis in the LDLR^{-/-} model system via induced recruitment of leukocytes to the plaque [90]. Studies involving bone marrow-specific knock-in of a non-activatable IKK α kinase mutant in the ApoE^{-/-} model found that this affected hematopoiesis but not atherosclerosis [91], whereas deficiency of IKK ϵ reduced atherosclerosis in the ApoE^{-/-} model [92]. The role of myeloid-specific deficiency of IKK β , however, is unclear with an earlier study in the LDLR^{-/-} model system demonstrating increased atherosclerosis in part via reduction in the levels of the anti-inflammatory cytokine IL-10 [93] whereas a more recent study in the same mouse model showed reduced lesion development due to decreased adhesion, migration and lipid uptake by macrophages [94]. Deficiency of IKK β in SMCs reduced atherosclerosis and vascular inflammation in the LDLR^{-/-} model [95]. Consistent with such findings, hepatocyte-specific IKK β expression in the ApoE*3-Leiden model promoted atherosclerosis in part by increasing the sensitivity to pro-inflammatory effectors and via transient increase in plasma cholesterol levels [96].

Experiments utilizing the transfer of bone marrow from p50-deficient mice to LDLR^{-/-} mice have shown that a decrease in plaque size obtained by the ablation of NF-κB is associated with a reduction in both the number of lesional macrophage foam cells and expression of SR-A [97]. The resulting lesion had an inflammatory phenotype characterized by increased numbers of macrophages and T cells [97]. In contrast to these findings, overexpression of the NF-κB subunit p65 in macrophages inhibited atherosclerotic development in ApoE^{-/-} mice associated with a reduction in foam cell numbers and SR-A expression [98].

In conclusion, the precise roles of individual components of the NF-κB signaling pathways in atherosclerosis are unclear with both pro- and anti-atherogenic actions being identified. This probably reflects the complex roles that this family plays in the control of the inflammatory response. For example, in addition to the well-established action of activating pro-inflammatory gene expression at the onset of inflammation, NF-κB also plays a key role in the resolution phase of inflammation [99,100].

4.3. PI3K

The PI3K signaling cascade is responsible for the regulation of many cellular functions, including glycogen and lipid metabolism, cell growth, calcium signaling, inflammation and apoptosis [68]. Due to the diverse role of these proteins, the pathway has been implicated in many atherosclerotic processes [68]. Ablation of the PI3K catalytic subunit, p110γ, decreases atherosclerotic development in genetically prone mice [101, 102]. The resulting lesions are characterized by a reduced number of proliferating plaque-resident macrophages [101,102]. Furthermore, atherosclerotic lesions within humans and mice display raised levels of the PI3K isoform; PI3K-γ, and transplantation of bone marrow from PI3K-γ^{-/-} mice into irradiated LDLR^{-/-} mice demonstrates that the expression of PI3K-γ by hematopoietic cells is integral for atherosclerotic development [103]. PI3K-γ is also a key regulator of reparative neovascularization and infarction size following a MI event within mice [104]. As such inhibition of PI3K-γ impairs the angiogenic properties of ECs and compromises cardiac function [104].

Akt/protein kinase B (called Akt hereafter) is a major downstream target of PI3K action. There are three Akt isoforms (1-3) and recent studies have started to unravel their roles in atherosclerosis [105–109]. Deficiency of Akt1 in the ApoE^{-/-} model system increased atherosclerosis due to enhanced expression of pro-inflammatory genes and increased apoptosis of both ECs and macrophages [105]. In addition, deficiency of Akt1 reduced VSMC proliferation and migration, and produced features of plaque vulnerability and cardiac dysfunction [106]. A recent study, however, showed that myeloid-specific deficiency of Akt1 in the LDLR^{-/-} model had little effect on atherosclerotic development [107]. Deficiency of Akt2 in this model system impaired glucose tolerance and resulted in complex atherosclerotic lesions associated with reduced collagen content, larger necrotic cores, reduced migration and proliferation of VSMCs, and disturbed balance of MMP and TIMP expression [108]. In contrast, myeloid-specific deficiency of Akt2 in the LDLR^{-/-} model reduced atherosclerosis associated with decreased numbers of pro-inflammatory monocytes expressing Ly-6C^{hi} and CCR2, and more polarization of macrophages to an M2 phenotype (see Section 5.1) [107]. Finally, deficiency of Akt3 in macrophages promotes foam cell formation and atherosclerosis in the LDLR^{-/-} model [109].

5. The role of immune cells in atherogenesis

Atherosclerosis is now recognized as a chronic inflammatory disorder that involves both the innate and adaptive immune responses [2, 28,30,35,60]. Immunohistochemical staining had earlier shown that regions of the intima prone to atherosclerotic development are marked by an accumulation of macrophages, dendritic cells and activated T cells [2,

28,30,35,60]. As the disease progresses a range of additional immune cells are recruited and reside within the plaque including B-lymphocytes, mast cells and natural killer (NK) cells [2,28,30,35,60]. The role of immune cells during the disease is vast and varied. The following sections will provide a brief overview of some of the key immune cells involved in atherogenesis.

5.1. Macrophage heterogeneity within the plaque

During the early stages of atherosclerosis, monocytes are the primary group of leukocytes recruited to the lesion [35,56,110]. There are two categories of monocytes referred to as CD14^{hi}CD16⁻ and CD14⁺CD16⁺ in humans and respectively termed Ly6C^{hi} (inflammatory) and Ly6C^{lo} (patrolling) within mice [35,56,110]. Hypercholesterolemic ApoE^{-/-} mice fed on a high-fat diet exhibit raised levels of Ly6C^{hi} monocytes that adhere to the activated endothelium and enter the atherosclerotic lesion [111]. Interestingly, within these mice the conversion of Ly6C^{hi} to the Ly6C^{lo} phenotype is impaired and the migration of Ly6C^{lo} monocytes into the plaque is also reduced [35,56,110,111]. Following recruitment to the lesion, the monocytes differentiate into either macrophages or myeloid dendritic cells under the influence of M-CSF, GM-CSF and other differentiating agents [112]. It should be noted that emerging evidence also suggests that the accumulation of macrophages in atherosclerotic lesions primarily depends on local macrophage proliferation instead of recruitment of circulating monocytes [113].

Macrophages were the first immune cells identified within the plaque [35,56,110,112]. Different subsets of macrophages arise due to the exposure of circulating monocytes to specific priming agents with the most common phenotype classified as either M1 or M2 [114, 115]. Ly6C^{hi} monocytes in mice are thought to function as the precursors for M1 macrophages whereas Ly6C^{lo} monocytes give rise to M2 macrophages [35,114,115]. Monocyte differentiation into macrophages is accompanied by an increase in the expression of PRRs, such as SRs, that facilitate the uptake of endotoxins, microbial products, apoptotic bodies and LDL particles [114,115]. Microbial products including lipopolysaccharide (LPS), and cytokines like IFN-γ stimulate classically activated M1 macrophages [114,115]. Within the disease state, M1 macrophages are pro-atherosclerotic and release ROS and pro-inflammatory cytokines such as TNF-α (see Section 6 for roles of cytokines in atherosclerosis) [35,56,114,115]. Conversely, alternatively activated M2 macrophages aid the resolution of inflammatory responses through the synthesis of ECM components and anti-inflammatory cytokines like IL-10 [35,56,114,115]. M2 macrophages are induced by T helper (Th) 2 cytokines like IL-4 and are abundant in regressing plaques [35,56,114,115]. In contrast, advanced lesions display an imbalanced ratio of M1 to M2 macrophages supporting defective resolution and augmentation of the chronic inflammatory state [35,56,114,115].

The M1 and M2 macrophage categories are not absolute as the population of infiltrating monocytes seen during the disease state contain a combination of M1 and M2 markers and, although macrophages are mainly categorized under these two broad labels, additional sub classifications and macrophage phenotypes exist [114,115]. For example M2 macrophages are further subdivided based on the polarizing agent along with gene expression and chemokine profile [114,115]. Other macrophage subtypes have also been identified, including Mox, Mhem and M4 [116]. For example, stimulation of monocytes with platelet factor CXCL4 gives rise to M4 macrophages which express a mixture of M1- and M2-associated genes, display limited phagocytic capabilities and express a transcriptome distinct from M1 and M2 macrophages [117]. Although M4 markers are found within human atherosclerotic coronary arteries, the exact contribution of this subtype is not clear as M4 macrophages express both pro- and anti-atherogenic genes [117]. However, the deletion of the gene encoding CXCL4 decreases the size of atherosclerotic lesions in mouse model systems [118].

5.2. Dendritic cells

The exposure of circulating monocytes to inflammatory stimuli like GM-CSF promotes dendritic cell formation [119]. Ablation of GM-CSF in LDLR^{-/-} mice causes a substantial reduction in the number of plaque-residing dendritic cells in addition to a significant decrease in lesion size [120]. Although macrophages and dendritic cells share common origins, the cells can be distinguished by differences in functionality [119]. Macrophages are mainly involved in responding to injury whereas dendritic cells favour the presentation of antigens on major histocompatibility complex (MHC) molecules to direct a T cell response [35,110,119]. Similar to monocytes, subsets of dendritic cells exist with conventional and plasmacytoid being the most common [121,122]. The accumulation of dendritic cells, although not as abundant as macrophages and T cells, into areas prone to atherosclerosis correlates with disease progression and inflammation [121,122]. In addition, some studies in mouse model systems have demonstrated a key role for plasmacytoid dendritic cells in the promotion of atherosclerosis [123,124] though anti-atherogenic actions have also been identified [125].

A range of receptors, including SRs, are expressed at the surface of dendritic cells, and these facilitate the uptake of antigens and also participate in foam cell formation [126]. The antigen peptides are then translocated onto MHC molecules in preparation for T cell presentation [28,60,123,124]. Danger-associated molecular patterns released during atherosclerosis are recognized through such receptors and promote a shift to adaptive immune responses [28,60,121,122]. The importance of dendritic cells in the regulation of an adaptive immune response is highlighted by the success of vaccination strategies involving these cells against atherosclerosis in mouse model systems [127,128]. Dendritic cells are key primers of T cell responses and modulators of immune tolerance [28,60,121,122]. The maturation state of dendritic cells dictates their function; immature dendritic cells within peripheral tissues drive tolerance through the deletion of autoreactive T cells, suppression of naïve T cell activation and induction of athero-protective regulatory T cells (Tregs) [28,60,121,122]. Yet, in response to inflammatory and other atherogenic stimuli, dendritic cells undergo phenotypic and functional changes, and mature and migrate to T cell-rich areas to activate naïve T- and B- cells [28,60,121,122]. The change is also accompanied by enhanced expression of costimulatory molecules, chemokine receptors, adhesion molecules and the release of cytokines that influence the formation of different T cell subtypes [28,60,121,122]. For example, the production of IL-12 promotes Th1 differentiation whereas IL-6 stimulates a Th17 response [28,60,121,122]. Indeed, a recent study has shown that MHC class II-restricted antigen presentation by plasmacytoid dendritic cells drives pro-atherogenic T cell immunity [124].

5.3. T cells

The number of T cells present within the plaque is far fewer than that of macrophages but have an important modulating role during pathology [60,122]. Antigen presenting cells such as macrophages and dendritic cells interact with T cells to activate the adaptive immune system [60,122]. These cells also influence the activities of T cells through the secretion of cytokines like IL-12 that modulates the infiltration of T cells into the plaque [60,122]. As such the function of T cells is closely tied to that of dendritic cells and macrophages [60,122].

A range of T-cell subsets exist that are characterized by cell surface markers, cytokines produced, and key transcription factors and co-factors that drive their differentiation and function [60,122]. Th1 cells are the most abundant subtype in human atherosclerotic plaques [60,122]. These cells release pro-inflammatory cytokines such as the classical Th1 cytokine IFN- γ [60,64,122]. A pro-atherogenic role for this cytokine has been demonstrated by numerous studies [64]. IFN- γ mediates its cellular effects via STAT1, and a pro-atherogenic role for this transcription factor has also been identified from studies using mouse

model systems [129,130]. Similar studies with other markers or approaches aimed at manipulating their function have revealed that Th1 cells promote atherosclerosis [60,122]. The Th2 response is conversely believed to be associated with attenuated atherosclerotic formation, however its exact contribution in the disease state is not as clear-cut [60,122]. Th2 cell cytokines, including IL-4, and the transcription factor PPAR- γ bias macrophage differentiation towards the M2 phenotype [35,56,114–116]. However, the abundances of Th2 cytokines such as IL-4, IL-5 and IL-13 are markedly under-represented in comparison to Th1 cytokines within the plaque [60,114–116,122]. In addition, studies that have analyzed the effect of IL-4 deficiency in mouse model systems have not always revealed a protective role for this cytokine [122]. Th17 cells represent a more recently identified T-cell subset associated with the production of the cytokine IL-17A [122]. Although the Th17-IL17A axis has many pro-inflammatory actions [122], studies investigating the role of IL-17A in mouse model systems have revealed conflicting results with both pro- and anti-atherogenic effects identified by different researchers [122,131]. The precise reasons for such contradictory results is currently unclear but it is possible that the action of IL-17A might be context-dependent and be influenced by the nature of the cell type producing it and the other cytokines (e.g. IFN- γ) present in the local environment [131]. As mentioned above, Tregs are a specialized subset that suppress pathogenic responses by the immune system towards foreign and self-antigens [122]. Many studies suggest a protective role for Tregs in atherosclerosis, including the principal cytokines produced by these cells [transforming growth factor (TGF)- β and IL-10], and that such protection is hampered during the disease [122,132]. Immunomodulatory strategies that increase the levels/action of Tregs or stimulate immune tolerance to atherosclerosis antigens are currently being evaluated [122,133].

5.4. Other immune cells

In contrast to monocytes/macrophages, T-cells and dendritic cells, limited research has been carried out on the roles of other immune cells in atherogenesis. NKT cells are pro-atherogenic [122,134]. On the other hand, the role of NK cells is not clear-cut [122] though a recent study demonstrated augmentation of atherosclerosis by a cytotoxic-dependent manner [135]. The presence of neutrophils in atherosclerotic plaques has been identified and a causal link to atherogenesis has been suggested, at least in mice, however the role in humans remains poorly understood [136]. Recent studies are beginning to reveal the roles of different B-cell subsets in atherosclerosis with IgM-producing B1a cells preventing the disease and B2 cells and innate response activator B cells promoting it [60,122,137–139]. Mast cells amass at sites prone to rupture and are persistently activated during the disease aiding plaque development within mice [140,141]. The actions of these cells destabilize the plaque by inducing intraplaque hemorrhaging, macrophage apoptosis and vascular damage [140,141].

6. Cytokine involvement in the atherosclerotic state

Cytokines are important mediators of the innate and adaptive immune system and play a key role at every stage of atherosclerosis, from early events involving dysfunction of the endothelium and lipid metabolism, and later phase actions such as enhancing MMP secretion [142–144]. Within the disease state, the production of many cytokines is auto-inducible through autocrine and paracrine signaling, which helps to augment and sustain inflammation [142–144]. Extracellular GFs and cytokines are highly expressed during atherosclerosis and mediate the proliferation and survival of cells involved in plaque formation [60,142–144]. Furthermore, the synergistic actions of cytokines and GFs can function to amplify their responses. For example, the effects of IL-1 α and TNF- α on MMP activation is enhanced through costimulation with platelet-derived growth factor and fibroblast growth factor-2 in rabbit VSMCs and human SMCs [145].

Cytokines exert a dual role during atherosclerosis and a complex interplay between pro- and anti-inflammatory cytokines arises which influences the development and stability of the plaque. Table 2 gives a summary of the outcome of studies aimed at delineating the roles of key cytokines in atherosclerosis using mouse model systems. The prevalence of pro-inflammatory cytokines within the plaque drives Th1-related processes and augments disease progression [2]. A variety of pro-inflammatory cytokines such as IFN- γ , TNF- α and IL-1 β support Th1 responses and promote foam cell formation [2]. Conversely, anti-inflammatory cytokines predominantly promote Th2-type responses that function to resolve inflammation and limit foam cell formation [2]. For instance, our previous studies have shown that TGF- β inhibits SR expression and reduces macrophage foam cell formation [132,172].

The role of various cytokines in atherosclerosis has been the subject of several excellent reviews [142–144]. Amongst these cytokines, IL-33 has been more recently identified and will be discussed here in detail. IL-33 belongs to the IL-1 family of cytokines and is not expressed by the majority of human hematopoietic cells; with the exception of activated dendritic cells and macrophages where it is present at low levels [173–175]. IL-33 interacts with the ST2 receptor that is expressed on the surface of immune cells, including mast cells, dendritic cells, Th2 cells and macrophages [173–175]. Alternative splicing of the ST2 gene gives rise to at least eight isoforms of the receptor, including ST2L (functional full-length transmembrane form), ST2V (variant), ST2LV and sST2 (secreted, soluble decoy receptor) [173,174].

Family members such as IL-1 and IL-18 contain prodomains that are proteolytically cleaved to produce the mature form of the cytokine [173, 174]. Interestingly, IL-33 is also secreted and contains a prodomain, and *in vitro* studies showed that the cytokine is susceptible to cleavage by caspase-1 yielding a mature product [176]. Initial theories proposed that IL-33 may be processed in a fashion similar to other IL-1 β members and that caspase activity may be a prerequisite for activation [173–176]. However, recent studies have demonstrated that full length IL-33 is biologically active and processing by caspases results in inactivation rather than activation of the cytokine [177,178]. IL-33 can also act in an intracrine manner by interacting with the heterochromatin and regulating gene expression [179,180].

Several studies have described a protective role for IL-33 within atherosclerosis and cardiovascular diseases. In experiments undertaken by Miller et al. [170], injections of recombinant IL-33 into ApoE^{-/-} mice decreased the generation of atherosclerotic lesions. Additionally, the cytokine reduced the number of lesional macrophages and promoted a Th1 to Th2 phenotypic switch within the plaque, accompanied with

enhanced production of the Th2 cytokines IL-4 and IL-13 and secretion of antibodies against OxLDL [170]. Treatment with the decoy receptor reversed the protective effects of IL-33 and also increased the size of the lesion [170].

We have recently demonstrated that IL-33 acts through the ST2 receptor to decrease foam cell formation *in vivo* [171]. Also, treatment of human macrophages with IL-33 reduced the uptake of AcLDL and OxLDL [171]. The cytokine decreased the expression of genes implicated in lipid uptake and storage, such as SR-A1 and CD36, and cholesterol esterification like ACAT-1 [171]. In contrast, the expression of the cholesterol efflux transporters; ABC-A1 and ABC-G1 was up regulated by the cytokine [171]. We have also shown that IL-33 inhibits the uptake of Lucifer yellow, a fluorescent dye used as an indicator of macropinocytosis [52]. As macropinocytosis is attributed as a contributor to plaque formation through constitutive and passive uptake of LDL particles, the study demonstrates a novel mechanism by which IL-33 may reduce macrophage foam cell formation *in vitro* [2].

The cytokine also has a wide range of effects on different cell types that reside within the atherosclerotic plaque. A study by Wasserman et al., [181] showed that IL-33 increases the number of Tregs in wild type control mice. During normal physiological conditions Tregs promote a switch from Th1 to Th2 response but during atherosclerosis the number of Tregs within the plaque is reduced [122]. Interestingly, within ApoE^{-/-} mice, IL-33 treatment had no effect on the number of Tregs [181]. However within these animals, the levels of the decoy receptor sST2 were elevated whilst the amount of ST2 was reduced [181]. The authors suggest that attenuation of signaling through the IL-33/ST2 axis contributes to the repressed number of regulatory T cells observed during the atherosclerotic state and therefore promotes the Th1 state [181].

IL-33 protects against cardiomyocyte apoptosis in *in vitro* and *in vivo* systems [182]. These effects were shown to be ST2-dependent as ischemic mice displayed reduced infarction volume and improved ventricular function when treated with IL-33 but the same effects were not observed in ST2^{-/-} mice [182]. The cytokine also defends cells against mechanical stresses [183]. In response to mechanical stretch cardiomyocytes undergo hypertrophy characterized by the enlargement of cells in the absence of cell division. Sustained hypertrophy can lead to compromised contractile functionality and arrhythmia and therefore often serves as a precursor to heart disease. However, IL-33 is released by cardiomyocytes in response to biomechanical stress and inhibits the actions of hypertrophic effectors such as angiotensin II and phenylephrine [183]. The cytokine functions as a protector against

Table 2
The role of key cytokines in atherosclerosis.

Cytokine	Outcome of studies using mouse model systems	Ref.
IFN- γ	Pro-atherogenic. Deficiency of the cytokine or its receptor decreased atherosclerosis associated with reduction of lesion cellularity and lipid accumulation. Injection of the cytokine augmented disease development. Post-natal blocking of cytokine function by expression of a soluble mutant decoy receptor attenuated lesion formation and produced a stable plaque phenotype.	[64,146–149]
TNF- α	Pro-atherogenic. Deficiency of the cytokine or its receptor (p55) reduced atherosclerosis with attenuated expression of several pro-inflammatory cytokines and adhesion molecules along with decreased uptake of modified LDL.	[150–152]
IL-1 β	Pro-atherogenic. Deficiency of the cytokine or its receptor reduced atherosclerosis associated with decreased arterial inflammation and oxidative stress. Administration of recombinant IL-1 receptor antagonist or its overexpression reduced atherosclerosis and modulated lipoprotein metabolism and foam cell formation.	[153–156]
IL-18	Pro-atherogenic. Deficiency of the cytokine decreased atherosclerosis, reduced IFN- γ action and produced a more stable plaque phenotype. Administration of the cytokine increased atherosclerosis via an IFN- γ dependent manner. <i>In vivo</i> electrotransfer of an expression plasmid for IL-18 binding protein reduced lesion development and produced a stable plaque phenotype.	[157–160]
IL-10	Anti-atherogenic. Deficiency of the cytokine increased atherosclerosis associated with augmented inflammatory response, LDL levels, MMP and tissue factor activity, and markers of systemic coagulation. Local or systemic overexpression of the cytokine attenuated atherosclerosis and reduced inflammation, oxidative stress, cholesterol levels and Th1 response.	[161–164]
TGF- β	Anti-atherogenic. Disruption of signaling accelerates atherosclerotic development associated with increased inflammation and decreased collagen content. Disruption of TGF- β signaling in T cells also accelerates atherosclerosis and is associated with increased T cells, activated macrophages and reduced collagen content. TGF- β -mediated plaque stabilization was mediated through an IL-17 dependent pathway. Overexpression of TGF- β attenuates atherosclerosis, oxidative stress and inflammation, and stabilizes plaques.	[132,165–169]
IL-33	Anti-atherogenic. Administration of the cytokine reduced atherosclerosis associated with increased levels of IL-4, IL-5 and IL-13, higher levels of anti-OxLDL antibodies and a Th1 to Th2 shift. Inhibition of cytokine action using a soluble decoy receptor increased plaque development. The cytokine inhibited macrophage foam cell formation <i>in vitro</i> and <i>in vivo</i> .	[170,171]

mechanical overload by reducing cardiac fibrosis and cardiomyocyte hypertrophy *in vivo* [183]. These responses were shown to be dependent on ST2 as administration of sST2 reversed the effects of IL-33 [183].

Epidemiological studies have also revealed a relationship between the cytokine and cardiac disorders [184,185]. For example, levels of IL-33 are decreased in the serum of patients who suffer from acute coronary syndrome and stable angina pectoris [184]. Another study found that serum levels of IL-33 were elevated in patients with chronic heart failure (HF) and were positively associated with several markers of oxidative stress such as erythrocyte superoxide dismutase activity [185]. Interestingly, levels of sST2 were also high and contributed to a depressed ratio of IL-33/sST2, and hence reduced IL-33 bioactivity. The results suggest that IL-33 may be released in response to HF and exert anti-oxidative effects but such cardio-protective effects of the cytokine are negated by an increased presence of the decoy receptor [185]. Indeed, the levels of sST2 have been proposed to function as an accurate prognostic tool for predicting cardiovascular risk [186–188]. For instance, circulating sST2 is transiently boosted following a MI event within human patients and in mice with experimental MI [186]. Furthermore, the levels of sST2 correlate with other inflammatory markers, total mortality and cardiovascular mortality in low risk populations and in patients with acute MI [187,188]. There is a relationship between levels of serum sST2 and the extent and volume of infarctions within MI patients [188]. Additionally, sST2 has been proposed to function as a reliable biomarker of HF in patients with acute dyspnea indicating that circulating sST2 could function as a robust marker for different manifestations of cardiovascular disease [189].

7. Concluding remarks

Atherosclerosis is now considered as an inflammatory disorder of the vasculature initiated by various risk factors, particularly high plasma LDL levels. Our understanding of the molecular basis of the disease has advanced considerably from studies using mouse model systems. However, doubts remain whether the various findings will translate into humans given the key differences in lipoprotein metabolism and inflammation. Educational drives at reducing risk factors via dietary and other changes, such as cessation of smoking and moderate exercise, has clearly had a positive impact in reducing mortality from atherosclerosis and its complications in many countries. However, this is expected to reverse in the future because of a global increase in diabetes and obesity. It is therefore essential that different aspects of the disease are fully understood, particularly in humans, reliable biomarkers are identified, and new therapeutic avenues are investigated and evaluated. The impact of statins in reducing mortality from atherosclerosis and its complications is well known [190,191]. However, the significance of residual risk in patients on statin therapy is a major limitation. Many lipid-modifying therapies are being analyzed ranging from inhibition of cholesterol absorption, modulating lipoprotein metabolism and clearance, to stimulating RCT [191]. Manipulating inflammation either at the cellular level or through the use of molecules that are involved in regulating the processes, such as cytokines and various activators/co-stimulators/modulators, represents another avenue particularly for high risk factors [60,190]. Indeed, some of these, such as anti-inflammatory drugs (e.g. methotrexate) and anti-cytokine therapies (anti-IL-1 β antibodies), have now progressed to the clinical trial stage [190]. It should also be noted that many lipid lowering therapies such as statins also have pleiotropic effects, including acting in an anti-inflammatory manner [2]. In addition, agonists of PPARs and LXRs not only modulate lipid and glucose homeostasis but also attenuate the inflammatory response [11, 14,15]. The regulators of atherosclerosis are not just restricted to proteins as various non-coding RNA, particularly microRNAs, are emerging as key modulators of inflammation and lipid homeostasis in this disease and represent promising future targets [192]. The next few years will be exciting in advancing our understanding of the molecular mechanisms and translation to the clinic.

Transparency Document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

We apologize to all the authors whose work could not be cited because of space restrictions. Melanie Buckley was supported by a PhD studentship from the Medical Research Council (Grant G1000385).

References

- [1] A.S. Go, D. Mozaffarian, V.L. Roger, E.J. Benjamin, J.D. Berry, M.J. Blaha, et al., Heart disease and stroke statistics-2014 update: A report from the American Heart Association, *Circulation* 129 (2014) 399–410.
- [2] J.E. McLaren, D.R. Michael, T.G. Ashlin, D.P. Ramji, Cytokines, macrophage lipid metabolism and foam cells: Implications for cardiovascular disease therapy, *Prog. Lipid Res.* 50 (2011) 331–347.
- [3] G.H. Tomkin, D. Owens, The chylomicron: relationship to atherosclerosis, *Int. J. Vasc. Med.* 2012 (2012) 784536.
- [4] S. Kovacic, M. Bakran, Genetic susceptibility to atherosclerosis, *Stroke Res. Treat.* 2012 (2012) 362941.
- [5] T.A. Lagace, PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells, *Curr. Opin. Lipidol.* 25 (2014) 387–393.
- [6] L. Zhang, K. Reue, L.G. Fong, S.G. Young, P. Tontonoz, Feedback regulation of cholesterol uptake by the LXR-IDOL-LDLR axis, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 2541–2546.
- [7] J.L. Golstein, M.S. Brown, The LDL receptor, *Arterioscler. Thromb. Vasc. Biol.* 29 (2009) 431–438.
- [8] K. Ono, Current concept of reverse cholesterol transport and novel strategy for atheroprotection, *J. Cardiol.* 60 (2012) 339–343.
- [9] M.C. Phillips, Molecular mechanism of cellular cholesterol efflux, *J. Biol. Chem.* 29 (2014) 24020–24029.
- [10] D.R. Michael, T.G. Ashlin, M.L. Buckley, D.P. Ramji, Macrophages, lipid metabolism and gene expression in atherogenesis: A therapeutic target of the future? *J. Clin. Lipidol.* 7 (2012) 37–48.
- [11] D.R. Michael, T.G. Ashlin, M.L. Buckley, D.P. Ramji, Liver X receptors, atherosclerosis and inflammation, *Curr. Atheroscler. Rep.* 14 (2012) 284–293.
- [12] N.J. Spann, L.X. Garmire, J.G. McDonald, D.S. Myers, S.B. Milne, N. Shibata, et al., Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses, *Cell* 15 (2012) 138–152.
- [13] M. Ouimet, V. Franklin, E. Mak, X. Liao, I. Tabas, Y.L. Marcel, Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase, *Cell Metab.* 13 (2011) 655–667.
- [14] J. Plutzky, The PPAR-RXR transcriptional complex in the vasculature: energy in the balance, *Circ. Res.* 108 (2011) 1002–1016.
- [15] W.S. Cheang, X. Fang, X.Y. Tian, Pleiotropic effects of peroxisome proliferator-activated receptor γ and δ in vascular diseases, *Circ. J.* 77 (2013) 2664–2671.
- [16] D.J. Rader, G.K. Hovingh, HDL and cardiovascular disease, *Lancet* 384 (2014) 618–625.
- [17] B.A. Kingwell, M.J. Chapman, A. Konhish, N.E. Miller, HDL-targeted therapies: progress, failures and future, *Nat. Rev. Drug Discov.* 13 (2014) 445–464.
- [18] M. Van Eck, ATP-binding cassette transporter A1: key player in cardiovascular and metabolic disease at local and systemic level, *Curr. Opin. Lipidol.* 25 (2014) 297–303.
- [19] L. Calabresi, M. Gomasrasi, S. Simonelli, F. Bernini, G. Franceschini, HDL and atherosclerosis: Insights from inherited HDL disorders, *Biochim. Biophys. Acta* 185 (2015) 13–18.
- [20] G.S. Getz, C.A. Reardon, Animal models of atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 1104–1115.
- [21] S. Zedelaa, R. Kleemann, L. Verschuren, J. de Vries-Van der Weij, J. van der Hoorn, H.M. Princen, T. Kooistra, Mouse models for atherosclerosis and pharmaceutical modifiers, *Arterioscler. Thromb. Vasc. Biol.* 27 (2007) 1706–1721.
- [22] K. Greenow, N.J. Pearce, D.P. Ramji, The key role of apolipoprotein E in atherosclerosis, *J. Mol. Med.* 83 (2005) 329–342.
- [23] S. Lin, Y. Lin, J.R. Nery, M.A. Ulrich, A. Breschi, C.A. Davis, et al., Comparison of the transcriptional landscapes between human and mouse tissues, *Proc. Natl. Acad. Sci. U. S. A.* 111 (2014) 17224–17229.
- [24] J. Seok, H.S. Warren, A.G. Cuenca, M.N. Mindrinos, H.V. Baker, W. Xu, et al., Genomic responses in mouse models poorly mimic human inflammatory diseases, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 3507–3512.
- [25] M.T. Bryan, H. Duckles, S. Feng, S.T. Hsiao, H.R. Kim, J. Serbanovic-Canic, P.C. Evans, Mechanoresponsive networks controlling vascular inflammation, *Arterioscler. Thromb. Vasc. Biol.* 34 (2014) 2199–2205.
- [26] D. Tousoulis, C. Simpoulou, N. Papageorgiou, E. Oikonomou, G. Hatzis, G. Siasos, E. Tsiamis, C. Stefanadis, Endothelial dysfunction in conduit arteries and in microcirculation, Novel therapeutic approaches, *Pharmacol. Ther.* 144 (2014) 253–267.
- [27] Y. Wen, D.S. Leake, Low density lipoprotein undergoes oxidation within lysosomes in cells, *Circ. Res.* 100 (2007) 1337–1343.
- [28] J. Frostegård, Immunity, atherosclerosis and cardiovascular disease, *BMC Med.* 11 (2013) 117.

- [29] K.S. Wraith, S. Magwenzi, A. Aburima, Y. Wen, D.S. Leake, K.M. Naseem, Oxidized low-density lipoproteins induce rapid platelet activation and shape change through tyrosine kinase and Rho kinase-signaling pathways, *Blood* 122 (2013) 580–589.
- [30] P. Libby, A.H. Lichtman, G.K. Hansson, Immune effector mechanisms implicated in atherosclerosis: From mice to humans, *Immunity* 38 (2013) 1092–1104.
- [31] Y.S. Bae, J.H. Lee, S.H. Choi, S. Kim, F. Almazan, J.L. Witztum, Y.I. Miller, Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: toll-like receptor 4- and spleen tyrosine kinase-dependent activation of NADPH oxidase 2, *Circ. Res.* 104 (2009) 201–218.
- [32] S.H. Choi, R. Harkewicz, J.H. Lee, A. Boullier, F. Almazan, A.C. Li, J.L. Witztum, Y.S. Bae, Y.I. Miller, Lipoprotein accumulation in macrophages via toll-like receptor-4 dependent fluid phase uptake, *Circ. Res.* 104 (2009) 1355–1363.
- [33] R.R. Koenen, C. Weber, Therapeutic targeting of chemokine interactions in atherosclerosis, *Nat. Rev. Drug Discov.* 141 (2010) 141–153.
- [34] C. Weber, H. Noels, Atherosclerosis: current pathogenesis and therapeutic options, *Nat. Med.* 17 (2011) 1410–1422.
- [35] K.J. Moore, F.J. Sheedy, E.A. Fisher, Macrophages in atherosclerosis: a dynamic balance, *Nat. Rev. Immunol.* 13 (2013) 709–721.
- [36] A. Zerneck, C. Weber, Chemokines in atherosclerosis: proceedings resumed, *Arterioscler. Thromb. Vasc. Biol.* 34 (2014) 742–750.
- [37] J.E. Cole, C. Kassiteridi, C. Monaco, Toll-like receptors in atherosclerosis: a “Pandora’s box” of advances and controversies, *Trends Pharmacol. Sci.* 34 (2013) 629–636.
- [38] K.S. Michelsen, M.H. Wong, P.K. Shah, W. Zhang, J. Yano, T.M. Doherty, S. Akira, T.B. Rajavashisth, M. Ardity, Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 10679–10684.
- [39] A.E. Mullick, P.S. Tobias, L.K. Curtiss, Modulation of atherosclerosis in mice by Toll-like receptor 2, *J. Clin. Invest.* 115 (2005) 3149–3156.
- [40] T.W. Kim, M. Febbraio, P. Robinet, B. Dugar, D. Greene, A. Cerny, et al., The critical role of IL-1 receptor-associated kinase 4-mediated NF- κ B activation in modified low-density lipoprotein-induced inflammatory gene expression and atherosclerosis, *J. Immunol.* 186 (2011) 2871–2880.
- [41] E. Lutgens, D. Lievens, L. Beckers, E. Wijnands, O. Soehnlein, A. Zerneck, et al., Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile, *J. Exp. Med.* 207 (2010) 391–404.
- [42] M.R. Richards, A.S. Black, D.J. Bonnet, G.D. Barish, C.W. Woo, I. Tabas, L.K. Curtiss, P.S. Tobias, The LPS2 mutation in TRIF is atheroprotective in hyperlipidemic low density lipoprotein receptor knockout mice, *Innate Immun.* 19 (2013) 20–29.
- [43] H. Björkbacka, V.V. Kunjathoor, K.J. Moore, S. Koehn, C.M. Ordija, M.A. Lee, T. Means, K. Halmen, A.D. Luster, D.T. Golenbock, M.W. Freeman, Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways, *Nat. Med.* 10 (2004) 416–421.
- [44] P. Dweuwel, H. Kono, K.J. Rayner, C.M. Sirois, G. Vladimer, F.G. Bauernfeind, et al., NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals, *Nature* 464 (2010) 1357–1361.
- [45] P. Menu, M. Pellegrin, J.F. Aubert, K. Bouzourene, A. Tardivel, L. Mazzolai, J. Tschopp, Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome, *Cell Death Dis.* 2 (2011) e137.
- [46] J. Canton, D. Neculai, S. Grinstead, Scavenger receptors in homeostasis and immunity, *Nat. Rev. Immunol.* 13 (2013) 621–636.
- [47] J. Kzyshkowska, C. Neyen, S. Gordon, Role of macrophage scavenger receptors in atherosclerosis, *Immunobiology* 217 (2012) 492–502.
- [48] M. Febbraio, E.A. Podrez, J.D. Smith, D.P. Hajjar, S.L. Hazen, H.F. Hoff, K. Sharma, R.L. Silverstein, Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice, *J. Clin. Invest.* 105 (2000) 1049–1056.
- [49] P.I. Makinen, J.P. Lappalainen, S.E. Heinonen, P. Leppanen, M.T. Lahtenvuo, J.V. Aarnio, J. Heikkila, M.P. Turunen, S. Ylä-Herttua, Silencing of either SR-A or CD36 reduces atherosclerosis in hyperlipidaemic mice and reveals reciprocal up-regulation of these receptors, *Cardiovasc. Res.* 88 (2010) 530–538.
- [50] J.J. Manning-Tobin, K.J. Moore, T.A. Seimon, S.A. Bell, M. Sharuk, J.I. Alvarez-Leite, M.P. de Winther, I. Tabas, M.W. Freeman, Loss of SR-A and CD36 activity reduces atherosclerotic lesion complexity without abrogating foam cell formation in hyperlipidemic mice, *Arterioscler. Thromb. Vasc. Biol.* 29 (2009) 19–26.
- [51] K.J. Moore, V. Kunjathoor, S.L. Koehn, J.J. Manning, A.A. Tseng, J.M. Silver, M. McKee, M.W. Freeman, Loss of receptor-mediated uptake via scavenger receptor A or CD36 pathways does not ameliorate atherosclerosis in hyperlipidemic mice, *J. Clin. Invest.* 115 (2005) 2192–2201.
- [52] D.R. Michael, T.G. Ashlin, C.S. Davies, H. Gallagher, T.W. Stoneman, M.L. Buckley, D.P. Ramji, Differential regulation of macrophage apoptosis in macrophages by cytokines: Implications for foam cell formation and atherosclerosis, *Cytokine* 64 (2013) 357–361.
- [53] J.F. Bentzon, F. Otsuka, R. Virmani, E. Falk, Mechanisms of plaque formation and rupture, *Circ. Res.* 114 (2014) 1852–1866.
- [54] X. Zhu, J.S. Owen, M.D. Wilson, H. Li, G.L. Griffiths, M.J. Thomas, E.M. Hiltbold, M.B. Fessler, J.S. Parks, Macrophage ABCA1 reduces Myd88-dependent Toll-like receptor trafficking to lipid rafts by reduction of lipid raft cholesterol, *J. Lipid Res.* 51 (2010) 3196–3206.
- [55] L. Yuan-Charvet, C. Welch, T.A. Pagler, M. Ranalletta, M. Lamkanfi, S. Han, M. Ishibashi, R. Li, N. Wang, A.R. Tall, Increased inflammatory gene expression in ABC transporter-deficient macrophages: free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions, *Circulation* 118 (2008) 1837–1847 (118).
- [56] I. Tabas, Macrophage death and defective inflammation resolution in atherosclerosis, *Nat. Rev. Immunol.* 10 (2010) 36–46.
- [57] B. Feng, P.M. Yao, Y.K. Li, C.M. Devlin, D.J. Zhang, H.P. Harding, M. Sweeney, et al., The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages, *Nat. Cell Biol.* 5 (2003) 781–792.
- [58] E.A. Van Vre, H. Ait-Oufella, A. Tedgui, Z. Mallat, Apoptotic cell death and efferocytosis in atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 887–893.
- [59] Z. Szondy, E. Garabuczi, G. Joós, G.J. Tsay, Z. Sarang, Impaired clearance of apoptotic cells in chronic inflammatory diseases: therapeutic implications, *Front. Immunol.* 5 (2014) 354.
- [60] J.L. Witztum, A.H. Lichtman, The influence of innate and adaptive immune responses on atherosclerosis, *Annu. Rev. Pathol.* 9 (2014) 73–102.
- [61] A. Newby, Proteinases and plaque rupture: unblocking the road to translation, *Curr. Opin. Lipidol.* 25 (2014) 358–366.
- [62] A. Newby, Matrix metalloproteinase inhibition therapy for vascular diseases, *Vasc. Pharmacol.* 56 (2012) 232–244.
- [63] C. Silvestre-Roig, M.P. de Winther, C. Weber, M.J. Daemen, E. Lutgens, O. Soehnlein, Atherosclerotic plaque destabilization: mechanisms, models, and therapeutic strategies, *Circ. Res.* 114 (2014) 214–226.
- [64] J.E. McLaren, D.P. Ramji, Interferon gamma: A master regulator of atherosclerosis, *Cytokine Growth Factor Rev.* 20 (2009) 125–135.
- [65] A.J. Muslin, MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets, *Clin. Sci.* 115 (2008) 203–218.
- [66] J.M. Kyriakis, J. Avruch, Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update, *Physiol. Rev.* 92 (2012) 689–737.
- [67] B. Pamukcu, G.Y. Lip, E. Shantsila, The nuclear factor-kappaB pathway in atherosclerosis: a potential therapeutic target for atherothrombotic vascular disease, *Thromb. Res.* 128 (2011) 117–123.
- [68] A. Ghigo, F. Morello, A. Perino, E. Hirsch, Therapeutic applications of PI3K inhibitors in cardiovascular diseases, *Future Med. Chem.* 5 (2013) 479–492.
- [69] X. Zhou, Z. Yin, X. Guo, D.P. Hajjar, J. Han, Inhibition of ERK1/2 and activation of liver X receptor synergistically induce macrophage ABCA1 expression and cholesterol efflux, *J. Biol. Chem.* 285 (2010) 6316–6326.
- [70] N. Li, J.E. McLaren, D.R. Michael, M. Clement, C.A. Fielding, D.P. Ramji, ERK is integral to the IFN-gamma-mediated activation of STAT1, the expression of key genes implicated in atherosclerosis, and the uptake of modified lipoproteins by human macrophages, *J. Immunol.* 185 (2010) 3041–3048.
- [71] R.C. Salter, T.G. Ashlin, A.P.L. Kwan, D.P. Ramji, ADAMTS proteases: key roles in atherosclerosis? *J. Mol. Med.* 88 (2010) 1203–1211.
- [72] T.G. Ashlin, M.L. Buckley, R.C. Salter, J.L. Johnston, A.P.L. Kwan, D.P. Ramji, The anti-atherogenic cytokine interleukin-33 inhibits the expression of a disintegrin and metalloproteinase with thrombospondin motifs-1, -4 and -5 in human macrophages: Requirement of extracellular signal-regulated kinase, c-Jun N-terminal kinase and phosphoinositide 3-kinase signaling pathways, *Int. J. Biochem. Cell Biol.* 46 (2014) 113–123.
- [73] K.S. Heo, H.J. Cushman, M. Akaike, C.H. Woo, X. Wang, X. Qiu, K. Fujiwara, J. Abe, ERK5 activation in macrophages promotes efferocytosis and inhibits atherosclerosis, *Circulation* 130 (2014) 180–191.
- [74] N.T. Le, K.S. Heo, Y. Takei, H. Lee, C.H. Woo, E. Chang, et al., A crucial role for p90RSK-mediated reduction of ERK5 transcriptional activity in endothelial dysfunction and atherosclerosis, *Circulation* 127 (2013) 486–499.
- [75] R.K. Komaravolu, C. Adam, J.R. Moonen, M.C. Harsmen, M. Goebeler, M. Schmidt, Erk5 inhibits endothelial migration via KLF2-dependent down-regulation of PAK1, *Cardiovasc. Res.* 105 (2015) 86–95.
- [76] S. Mei, H. Gu, A. Ward, X. Yang, H. Guo, K. He, Z. Liu, W. Cao, p38 mitogen-activated protein kinase (MAPK) promotes cholesterol ester accumulation in macrophages through inhibition of macroautophagy, *J. Biol. Chem.* 287 (2012) 11761–11768.
- [77] T. DeVries-Seimon, Y.K. Li, P.M. Yao, E. Stone, Y.B. Wang, R.J. Davis, R. Flavell, I. Tabas, Cholesterol-induced macrophage apoptosis requires ER stress pathways and engagement of the type A scavenger receptor, *J. Cell Biol.* 171 (2005) 61–73.
- [78] T.A. Seimon, Y. Wang, S. Han, T. Senokuchi, D.M. Schrijvers, G. Kuriakose, A.R. Tall, I.A. Tabas, Macrophage deficiency of p38 alpha MAPK promotes apoptosis and plaque necrosis in advanced atherosclerotic lesions in mice, *J. Clin. Invest.* 119 (2009) 886–898.
- [79] R. Kardakaris, R. Gareus, S. Xanthoulea, M. Pasparakis, Endothelial and macrophage-specific deficiency of p38 α MAPK does not affect the pathogenesis of atherosclerosis in ApoE^{-/-} Mice, *PLoS ONE* 6 (2011) e21055.
- [80] R. Ricci, G. Sumara, I. Sumara, I. Rozenberg, M. Kurrer, A. Akhmedov, et al., Requirement of JNK2 for scavenger receptor A-mediated foam cell formation in atherosclerosis, *Science* 306 (2004) 1558–1561.
- [81] N. Amini, J.J. Boyle, B. Moers, C.M. Warboys, T.H. Malik, M. Zakkar, S.E. Francis, J.C. Mason, D.O. Haskard, P.C. Evans, Requirement of JNK1 for endothelial cell injury in atherosclerosis, *Atherosclerosis* 235 (2014) 613–618.
- [82] K. Yoshimura, H. Aoki, Y. Ikeda, K. Fujii, N. Akiyama, A. Furutani, et al., Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase, *Nat. Med.* 11 (2005) 1330–1338.
- [83] J. Wang, F.S. An, W. Zhang, L. Gong, S.J. Wie, W.-D. Qin, X.P. Wang, Y.X. Zhao, Y. Zhang, C. Zhang, M.X. Zhang, Inhibition of c-Jun N-terminal kinase attenuates low shear stress-induced atherogenesis in apolipoprotein E-deficient mice, *Mol. Med.* 17 (2011) 990–991.
- [84] B. Mallavia, C. Recio, A. Oguiza, G. Ortiz-Muñoz, I. Lazaro, V. Lopez-Parra, O. Lopez-Franco, S. Schindler, R. Depping, J. Egido, C. Gomez-Guerrero, Peptide inhibitor of NF- κ B translocation ameliorates experimental atherosclerosis, *Am. J. Pathol.* 182 (2013) 1910–1921.
- [85] C. Cuaiz-Pérolin, L. Billiet, E. Baugé, C. Copin, D. Scott-Algara, F. Genze, B. Büchele, T. Syrovets, T. Simmet, M. Rouis, Antiinflammatory and antiatherogenic effects of the

- NF-kappaB inhibitor acetyl-11-keto-beta-boswellic acid in LPS-challenged ApoE^{-/-} mice, *Arterioscler. Thromb. Vasc. Biol.* 28 (2008) 272–277.
- [86] S. Wolfrum, D. Teupser, M. Tan, K.Y. Chen, J.L. Breslow, The protective effect of A20 on atherosclerosis in apolipoprotein E-deficient mice is associated with reduced expression of NF-kappaB target genes, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 18601–18606.
- [87] X. Sun, S. He, A.K. Wara, B. Icli, E. Shvartz, Y. Tesmenitsky, et al., Systemic delivery of microRNA-181b inhibits nuclear factor- κ B activation, vascular inflammation, and atherosclerosis in apolipoprotein E-deficient mice, *Circ. Res.* 114 (2014) 32–40.
- [88] Y. Wang, G.Z. Wang, P.S. Rabinovitch, I. Tabas, Macrophage mitochondrial oxidative stress promotes atherosclerosis and nuclear factor- κ B-mediated inflammation in macrophages, *Circ. Res.* 114 (2014) 421–433.
- [89] R. Gareus, E. Kotsaki, S. Xanthoulea, I. van der Made, M.J.J. Gijbels, R. Kardakaris, A. Polykratis, G. Kollias, M.P. de Winther, M. Pasparakis, Endothelial cell-specific NF-kappa B inhibition protects mice from atherosclerosis, *Cell Metab.* 8 (2008) 372–383.
- [90] P. Goossens, M.N. Vergouwe, M.J. Gijbels, D.M. Curfs, J.H. van Woelk, M.A. Hoeksema, S. Xanthoulea, P.J. Leenen, R.A. Rupec, M.H. Hofker, M.P. de Winther, Myeloid I κ B α deficiency promotes atherogenesis by enhancing leukocyte recruitment to the plaques, *PLoS ONE* 6 (2011) e22327.
- [91] P.V. Tilstam, M.J. Gijbels, M. Habbaddine, C. Cudejko, Y. Asare, W. Theelen, B. Zhou, et al., Bone marrow-specific knock-in of a non-activatable I κ k α kinase mutant influences haematopoiesis but not atherosclerosis in ApoE-deficient mice, *PLoS ONE* 9 (2014) e87452.
- [92] C. Cao, Y. Zhu, W. Chen, L. Li, Y. Qi, X. Wang, Y. Zhao, X. Wan, X. Chen, IKK ϵ knock-out prevents high fat diet induced arterial atherosclerosis and NF- κ B signaling in mice, *PLoS ONE* 8 (2013) e64930.
- [93] E. Kanters, M. Pasparakis, M.J. Gijbels, M.N. Vergouwe, I. Partouns-Hedriks, R.J. Fijneman, et al., Inhibition of NF-kappaB activation in macrophages increases atherosclerosis in LDL receptor-deficient mice, *J. Clin. Invest.* 112 (2003) 1176–1185.
- [94] S.H. Park, Y. Sui, F. Gizard, J. Xu, J. Rios-Pilier, R.N. Helsley, S.S. Han, C. Zhou, Myeloid-specific I κ B kinase β deficiency decreases atherosclerosis in low-density lipoprotein receptor-deficient mice, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 2869–2876.
- [95] Y. Sui, S.H. Park, J. Xu, S. Monette, R.N. Helsley, S.S. Han, C. Zhou, IKK β links vascular inflammation to obesity and atherosclerosis, *J. Exp. Med.* 211 (2014) 869–886.
- [96] M.C. Wong, J.A. van Diepen, L. Hu, B. Guigas, H.C. de Boer, G.H. van Puijvelde, et al., Hepatocyte-specific IKK β expression aggravates atherosclerosis development in APOE*3-Leiden mice, *Atherosclerosis* 220 (2012) 362–368.
- [97] E. Kanters, M.J.J. Gijbels, I. van der Made, M.N. Vergouwe, P. Heeringa, G. Kraal, M.H. Hofker, M.P. de Winther, Hematopoietic NF-kappa B1 deficiency results in small atherosclerotic lesions with an inflammatory phenotype, *Blood* 103 (2004) 934–940.
- [98] X. Ye, X. Jiang, W. Guo, K. Clark, Z. Gao, Overexpression of NF-kappa B p65 in macrophages ameliorates atherosclerosis in apoE-knockout mice, *Am. J. Physiol. Endocrinol. Metab.* 305 (2013) E1375–E1383.
- [99] T. Lawrence, D.W. Gilroy, P.R. Colville-Nash, D.A. Willoughby, Possible new role for NF-kappaB in the resolution of inflammation, *Nat. Med.* 7 (2001) 1291–1297.
- [100] T. Lawrence, C. Fong, The resolution of inflammation: Anti-inflammatory roles for NF- κ B, *Int. J. Biochem. Cell Biol.* 42 (2010) 519–523.
- [101] J.D. Chang, G.K. Sukhova, P. Libby, E. Schvartz, A.H. Lichtenstein, S.J. Field, C. Kennedy, S. Madhavarapu, J. Luo, D. Wu, L.C. Cantley, Deletion of the phosphoinositide 3-kinase p110 gamma gene attenuates murine atherosclerosis, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 8077–8082.
- [102] T.M. Zotes, C.F. Arias, J.J. Fuster, R. Spada, S. Perez-Yaguee, E. Hirsch, M. Wymann, A.C. Carrera, V. Andrés, D.F. Barber, PI3K p110 gamma deletion attenuates murine atherosclerosis by reducing macrophage proliferation but not polarization or apoptosis in lesions, *PLoS ONE* 8 (2013) e72674.
- [103] A. Fougerat, S. Gayral, P. Gourdy, A. Schambourg, T. Rueckle, M.K. Schwarz, et al., Genetic and pharmacological targeting of phosphoinositide 3-kinase-gamma reduces atherosclerosis and favors plaque stability by modulating inflammatory processes, *Circulation* 117 (2008) 1310–1317.
- [104] M. Siragusa, R. Katare, M. Meloni, F. Damilano, E. Hirsch, C. Emanuelli, P. Madeddu, Involvement of phosphoinositide 3-kinase gamma in angiogenesis and healing of experimental myocardial infarction in mice, *Circ. Res.* 106 (2010) 757–768.
- [105] C. Fernández-Hernando, E. Ackah, J. Yu, Y. Suárez, T. Murata, Y. Iwakiri, J. Prendergast, R.Q. Miao, M.J. Birnbaum, W.C. Sessa, Loss of Akt1 leads to severe atherosclerosis and occlusive coronary artery disease, *Cell Metab.* 6 (2007) 446–457.
- [106] C. Fernández-Hernando, L. József, D. Jenkins, A. Di Lorenzo, W.C. Sessa, Absence of Akt1 reduces vascular smooth muscle cell migration and survival and induces features of plaque vulnerability and cardiac dysfunction during atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* 29 (2009) 2033–2040.
- [107] V.R. Babaev, K.E. Hebron, C.B. Wiese, C.L. Toth, L. Ding, Y. Zhang, J.M. May, S. Fazio, K.C. Vickers, M.F. Linton, Macrophage deficiency of Akt2 reduces atherosclerosis in Ldlr null mice, *J. Lipid Res.* 55 (2014) 2296–2308.
- [108] K.L. Rensing, S.C. de Jager, E.S. Stroes, M. Vos, M.T. Twickler, G.M. Dallinga-Thie, C.J. de Vries, J. Kuiper, I. Bot, J.H. von der Thüsen, Akt2/LDLr double knockout mice display impaired glucose tolerance and develop more complex atherosclerotic plaques than LDLr knockout mice, *Cardiovasc. Res.* 101 (2014) 277–287.
- [109] L. Ding, S. Biswas, R.E. Morton, J.D. Smith, N. Hay, T.V. Byzova, M. Febbraio, E.A. Podrez, Akt3 deficiency in macrophages promotes foam cell formation and atherosclerosis in mice, *Cell Metab.* 15 (2012) 861–872.
- [110] G.J. Randolph, Mechanisms that regulate macrophage burden in atherosclerosis, *Circ. Res.* 114 (2014) 1757–1771.
- [111] F.K. Swirski, P. Libby, E. Aikawa, P. Alcaide, F.W. Lusinskas, R. Weissleder, M.J. Pittet, Ly-6C(hi) monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata, *J. Clin. Invest.* 117 (2007) 195–205.
- [112] T. Gui, A. Shimokado, Y. Sun, T. Akasaka, Y. Muragaki, Diverse roles of macrophages in atherosclerosis: From inflammatory biology to biomarker discovery, *Mediat. Inflamm.* 2012 (2012) 693083.
- [113] C.S. Robbins, I. Hilgendorf, G.F. Weber, I. Theurl, Y. Iwamoto, J.L. Figueiredo, et al., Local proliferation dominates lesional macrophage accumulation in atherosclerosis, *Nat. Med.* 19 (2013) 1166–1172.
- [114] N. Letinger, I.G. Schulman, Phenotypic polarization of macrophages in atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* 33 (2013) 1120–1126.
- [115] I.M.J. Wolfs, M.M.P.C. Donners, M.P.J. de Winther, Differentiation factors and cytokines in the atherosclerotic plaque micro-environment as a trigger for macrophage polarisation, *Thromb. Haemost.* 106 (2011) 763–771.
- [116] S. Colin, G. Chinett-Gbaguidi, B. Staels, Macrophage phenotypes in atherosclerosis, *Immunol. Rev.* 262 (2014) 153–166.
- [117] C.A. Gleissner, I. Shaked, K. Little, K. Ley, CXC chemokine ligand 4 induces a unique transcriptome in monocyte-derived macrophages, *J. Immunol.* 184 (2010) 4810–4818.
- [118] B.S. Sachais, T. Turrentine, J.M.D. McKenna, A.H. Rux, D. Rader, M.A. Kowalska, Elimination of platelet factor 4 (PF4) from platelets reduces atherosclerosis in C57Bl/6 and apoE(–/–) mice, *Thromb. Haemost.* 98 (2007) 1108–1113.
- [119] A. Niessner, C.M. Weyand, Dendritic cells in atherosclerotic disease, *Clin. Immunol.* 134 (2010) 25–32.
- [120] Z. Shaposhnik, X. Wang, M. Weinstein, B.J. Bennett, A.J. Lusis, Granulocyte macrophage colony-stimulating factor regulates dendritic cell content of atherosclerotic lesions, *Arterioscler. Thromb. Vasc. Biol.* 27 (2007) 621–627.
- [121] D.A. Chistiakov, I.A. Sobenin, A.N. Orekhov, Y.V. Bobryshev, Dendritic cells in atherosclerotic inflammation: the complexity of functions and the peculiarities of pathophysiological effects, *Front. Physiol.* 5 (2014) 196.
- [122] H. Ait-Oufella, A.P. Sage, Z. Mallat, A. Tedgui, Adaptive (T and B cells) immunity and control by dendritic cells in atherosclerosis, *Circ. Res.* 114 (2014) 1640–1660.
- [123] N. Macritchie, G. Grassia, S.R. Sabir, M. Maddaluno, P. Welsh, N. Sattar, et al., Plasmacytoid dendritic cells play a key role in promoting atherosclerosis in apolipoprotein E-deficient mice, *Arterioscler. Thromb. Vasc. Biol.* 32 (2012) 2569–2579.
- [124] A. Sage, D.M. Murphy, P. Maffia, L.M. Masters, S.R. Sabir, L.L. Baker, et al., MHC class II-restricted antigen presentation by plasmacytoid dendritic cells drives pro-atherogenic T cell immunity, *Circulation* 130 (2014) 1363–1373.
- [125] I.T. Daissormont, A. Christ, L. Temmerman, S. Sampedro Millares, T. Seijkens, M. Manca, et al., Plasmacytoid dendritic cells protect against atherosclerosis by tuning T-cell proliferation and activity, *Circ. Res.* 109 (2011) 1387–1395.
- [126] K.E. Paulson, S.N. Zhu, M. Chen, S. Nurmohamed, J. Jongstra-Bilen, M.L. Cybulsky, Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis, *Circ. Res.* 106 (2010) 383–390.
- [127] K.L. Habets, G.H. van Puijvelde, L.M. van Duivenvoorde, E.J. van Wanrooij, P. de Vos, J.W. Tervaert, T.J. van Berkel, R.E. Toes, J. Kuiper, Vaccination using oxidized low-density lipoprotein-pulsed dendritic cells reduces atherosclerosis in LDL receptor-deficient mice, *Cardiovasc. Res.* 85 (2010) 622–630.
- [128] A. Hermansson, D.K. Johansson, D.F. Ketelhuth, J. Andersson, X. Zhou, G.K. Hansson, Immunotherapy with tolerogenic apolipoprotein B-100-loaded dendritic cells attenuates atherosclerosis in hypercholesteremic mice, *Circulation* 123 (2011) 1083–1091.
- [129] S. Agrawal, M. Febbraio, E. Podrez, M.K. Cathcart, G.R. Stark, G.M. Chisolm, Signal transducer and activator of transcription 1 is required for optimal foam cell formation and atherosclerotic lesion development, *Circulation* 115 (2007) 2939–2947.
- [130] W.S. Lim, J.M. Timmins, T.A. Seimon, A. Sadler, F.D. Koldziej, R. Virmani, I. Tabas, Signal transducer and activator of transcription-1 is critical for apoptosis in macrophages subjected to endoplasmic reticulum stress *in vitro* and in advanced atherosclerotic lesions *in vivo*, *Circulation* 117 (2008) 940–951.
- [131] S. Taleb, A. Tedgui, Z. Mallat, IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 258–264.
- [132] N.N. Singh, D.P. Ramji, The role of transforming growth factor-beta in atherosclerosis, *Cytokine Growth Factor Rev.* 17 (2006) 487–499.
- [133] P.K. Shah, K. Chyu, P.C. Dimayuga, J. Nilsson, Vaccine for atherosclerosis, *J. Am. Coll. Cardiol.* 64 (2014) 2779–2791.
- [134] K. To, A. Agotis, G. Besra, A. Bobik, B.H. Toh, NKT cell subsets mediate differential proatherogenic effects in ApoE^{-/-} mice, *Arterioscler. Thromb. Vasc. Biol.* 29 (2009) 671–677.
- [135] A. Selathurai, V. Deswaerte, P. Kanellakis, P. Tipping, B.H. Toh, A. Bobik, T. Kyaw, Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms, *Cardiovasc. Res.* 102 (2014) 128–137.
- [136] Y. Döring, M. Drechsler, O. Soehnlein, C. Weber, Neutrophils in atherosclerosis: From mice to man, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 288–295.
- [137] D. Tsiantoulas, C.J. Diehl, J.L. Witztum, C.J. Binder, B cells and humoral immunity in atherosclerosis, *Circ. Res.* 114 (2014) 1743–1756.
- [138] T. Kyaw, P. Tipping, A. Bobik, B.H. Toh, Protective role of natural IgM-producing B1a cells in atherosclerosis, *Trends Cardiovasc. Med.* 22 (2012) 48–53.
- [139] I. Hilgendorf, I. Theurl, L.M. Gerhardt, C.S. Robbins, G.F. Weber, A. Gonen, et al., Innate response activator B cells aggravate atherosclerosis by stimulating T helper-1 adaptive immunity, *Circulation* 129 (2014) 1677–1687.
- [140] I. Bot, S.C.A. de Jager, A. Zernecke, K.A. Lindstedt, T.J.C. van Berkel, C. Weber, E.A.L. Biessen, Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice, *Circulation* 115 (2007) 2516–2525.
- [141] J. Sun, G.K. Sukhova, P.J. Wolters, M. Yang, S. Kitamoto, P. Libby, L.A. MacFarlane, et al., Mast cells promote atherosclerosis by releasing proinflammatory cytokines, *Nat. Med.* 13 (2007) 719–724.

- [142] H. Ait-Oufella, S. Taleb, Z. Mallat, A. Tedgui, Recent advances on the role of cytokines in atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* 31 (2011) 969–979.
- [143] R. Kleemann, S. Zadelara, T. Kooistra, Cytokines and atherosclerosis: a comprehensive review of studies in mice, *Cardiovasc. Res.* 79 (2008) 360–376.
- [144] A. Tedgui, Z. Mallat, Cytokines in atherosclerosis: Pathogenic and regulatory pathways, *Physiol. Rev.* 86 (2006) 515–581.
- [145] M. Bond, A.J. Chase, A.H. Baker, A.C. Newby, Inhibition of transcription factor NF-kappa B reduces matrix metalloproteinase-1,-3 and-9 production by vascular smooth muscle cells, *Cardiovasc. Res.* 50 (2001) 556–565.
- [146] S. Gupta, A.M. Pablo, X.C. Jiang, N. Wang, A.R. Tall, C. Schindler, IFN-gamma potentiates atherosclerosis in apoE knock-out mice, *J. Clin. Invest.* 99 (1997) 2752–2761.
- [147] C. Buono, C.E. Come, G. Stavrakis, G.G. Maguire, P.W. Connelly, A.H. Lichtman, Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLR-deficient mouse, *Arterioscler. Thromb. Vasc. Biol.* 23 (2003) 454–460.
- [148] S.C. Whitman, P. Ravisankar, H. Elam, A. Daugherty, Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein E^{-/-} mice, *Am. J. Pathol.* 157 (2000) 1819–1824.
- [149] M. Koga, H. Kai, H. Yasukawa, T. Yamamoto, Y. Kawai, S. Kato, K. Kusaba, M. Kai, K. Egashira, Y. Kataoka, T. Imaizumi, Inhibition of progression and stabilization of plaques by postnatal interferon-gamma function blocking in ApoE-knockout mice, *Circ. Res.* 101 (2007) 348–356.
- [150] S. Xanthoulea, M. Thelen, C. Pöttgens, M.J. Gijbels, E. Lutgens, M.P. de Winther, Absence of p55 TNF receptor reduces atherosclerosis, but has no major effect on angiotensin II induced aneurysms in LDL receptor deficient mice, *PLoS ONE* 7 (2009) e6113.
- [151] N. Xiao, M. Yin, L. Zhang, X. Qu, H. Du, X. Sun, et al., Tumor necrosis factor-alpha deficiency retards early fatty-streak lesion by influencing the expression of inflammatory factors in apoE-null mice, *Mol. Genet. Metab.* 96 (2009) 239–244.
- [152] H. Ohta, H. Wada, T. Niwa, H. Kirii, N. Iwamoto, H. Fujii, K. Saito, K. Sekikawa, M. Seishima, Disruption of tumor necrosis factor-alpha gene diminishes the development of atherosclerosis in ApoE-deficient mice, *Atherosclerosis* 180 (2005) 11–17.
- [153] H. Kirii, T. Niwa, Y. Yamada, H. Wada, K. Saito, Y. Iwakura, M. Asano, H. Moriwaki, M. Seishima, Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice, *Arterioscler. Thromb. Vasc. Biol.* 23 (2003) 656–660.
- [154] J. Chamberlain, S. Francis, Z. Brookes, G. Shaw, D. Graham, N.J. Alp, S. Dower, D.C. Crossman, Interleukin-1 regulates multiple atherogenic mechanisms in response to fat feeding, *PLoS ONE* 4 (2009) e5073.
- [155] C.M. Devlin, G. Kuriakose, E. Hirsch, I. Tabas, Genetic alterations of IL-1 receptor antagonist in mice affect plasma cholesterol level and foam cell lesion size, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 6280–6285.
- [156] F. Merhi-Soussi, B.R. Kwak, D. Magne, C. Chadjichristos, M. Berti, G. Pelli, R.W. James, F. Mach, C. Gabay, Interleukin-1 plays a major role in vascular inflammation and atherosclerosis in male apolipoprotein E-knockout mice, *Cardiovasc. Res.* 66 (2005) 583–593.
- [157] R. Elhage, J. Jawien, M. Rudling, H.G. Ljunggren, K. Takeda, S. Akira, F. Bayard, G.K. Hansson, Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice, *Cardiovasc. Res.* 59 (2003) 234–240.
- [158] C. Tenger, A. Sundborger, J. Jawien, X. Zhou, IL-18 accelerates atherosclerosis accompanied by elevation of IFN-gamma and CXCL16 expression independently of T cells, *Arterioscler. Thromb. Vasc. Biol.* 25 (2005) 791–796.
- [159] S.C. Whitman, P. Ravisankar, A. Daugherty, Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma, *Circ. Res.* 90 (2002) E34–E38.
- [160] Z. Mallat, A. Corbaz, A. Scoazec, P. Graber, S. Alouani, B. Esposito, Y. Humbert, Y. Chvatchko, A. Tedgui, Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability, *Circ. Res.* 89 (2001) E41–E45.
- [161] C. Caligiuri, M. Rudling, V. Ollivier, M.P. Jacob, J.B. Michel, G.K. Hansson, A. Nicoletti, Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice, *Mol. Med.* 9 (2003) 10–17.
- [162] Y. Liu, D. Li, J. Chen, J. Xie, S. Bandyopadhyay, D. Zhang, A.R. Nemarkommula, H. Liu, J.L. Mehta, P.L. Hermonat, Inhibition of atherogenesis in LDLR knockout mice by systemic delivery of adeno-associated virus type 2-hIL-10, *Atherosclerosis* 188 (2006) 19–27.
- [163] T. Yoshioka, T. Okada, Y. Maeda, U. Ikeda, M. Shimpo, T. Nomoto, et al., Adeno-associated virus vector-mediated interleukin-10 gene transfer inhibits atherosclerosis in apolipoprotein E-deficient mice, *Gene Ther.* 11 (2004) 1772–1779.
- [164] M. Namiki, S. Kawashima, T. Yamashita, M. Ozaki, T. Sakoda, N. Inoue, K. Hirata, R. Morishita, Y. Kaneda, M. Yokoyama, Intramuscular gene transfer of interleukin-10 cDNA reduces atherosclerosis in apolipoprotein E-knockout mice, *Atherosclerosis* 172 (2004) 21–29.
- [165] Z. Mallat, A. Gojova, C. Marchiol-Fournigault, B. Esposito, C. Kamatè, R. Merval, D. Fradelizi, A. Tedgui, Inhibition of transforming growth factor-beta signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice, *Circ. Res.* 89 (2001) 930–934.
- [166] A.K. Robertson, M. Rudling, X. Zhou, L. Gorelik, R.A. Flavell, G.K. Hansson, Disruption of TGF-beta signaling in T cells accelerates atherosclerosis, *J. Clin. Invest.* 112 (2003) 1342–1350.
- [167] A. Gisterá, A.K. Robertson, J. Andersson, D.F. Ketelhuth, O. Ovchinnikova, S.K. Nilsson, A.M. Lundberg, M.O. Li, R.A. Flavell, G.K. Hansson, Transforming growth factor-beta signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17-dependent pathway, *Sci. Transl. Med.* 5 (2013) (196ra100).
- [168] K. Reifenberg, F. Cheng, C. Orning, J. Crain, I. Küpper, E. Wiese, M. Protschka, M. Blessing, K.J. Lackner, M. Torzewski, Overexpression of TGF-beta1 in macrophages reduces and stabilizes atherosclerotic plaques in ApoE-deficient mice, *PLoS ONE* 7 (2012) e40990.
- [169] A.D. Frutkin, G. Otsuka, A. Stempien-Otero, C. Sesti, L. Du, M. Jaffe, et al., TGF-beta1 limits plaque growth, stabilizes plaque structure, and prevents aortic dilation in apolipoprotein E-null mice, *Arterioscler. Thromb. Vasc. Biol.* 29 (2009) 1251–1257.
- [170] A.M. Miller, D. Xu, D.L. Asquith, L. Denby, Y. Li, N. Sattar, et al., IL-33 reduces the development of atherosclerosis, *J. Exp. Med.* 205 (2008) 339–346.
- [171] J.E. McLaren, D.R. Michael, R.C. Salter, T.G. Ashlin, C.J. Calder, A.M. Miller, F.Y. Liew, D.P. Ramji, IL-33 reduces macrophage foam cell formation, *J. Immunol.* 185 (2010) 1222–1229.
- [172] D.R. Michael, R.C. Salter, D.P. Ramji, TGF-beta inhibits the uptake of modified low density lipoprotein by human macrophages through a Smad-dependent pathway: a dominant role for Smad-2, *Biochim. Biophys. Acta* 1822 (2012) 1608–1616.
- [173] A.M. Miller, Role of IL-33 in inflammation and disease, *J. Inflamm.* 8 (2011) 22.
- [174] A.M. Miller, F.Y. Liew, The IL-33/ST2 pathway – A new therapeutic target in cardiovascular disease, *Pharmacol. Ther.* 131 (2011) 179–186.
- [175] J. Schmitz, A. Owyang, E. Oldham, Y.L. Song, E. Murphy, T.K. McClanahan, et al., IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines, *Immunity* 23 (2005) 479–490.
- [176] C.A. Dinarello, An IL-1 family member requires caspase-1 processing and signals through the ST2 receptor, *Immunity* 23 (2005) 461–462.
- [177] E. Lefrançois, C. Cayrol, Mechanisms of IL-33 processing and secretion: differences and similarities between IL-1 family members, *Eur. Cytokine Netw.* 23 (2012) 120–127.
- [178] A.U. Lüthi, S.P. Cullen, E.A. McNeela, P.J. Duriez, I.S. Afonina, C. Sheridan, et al., Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases, *Immunity* 31 (2009) 84–98.
- [179] S. Ali, A. Mohs, M. Thomas, J. Klare, R. Ross, M.L. Schmitz, M.U. Martin, The dual function cytokine IL-33 interacts with the transcription factor NF-kappa B to dampen NF-kappa B-stimulated gene transcription, *J. Immunol.* 187 (2011) 1609–1616.
- [180] V. Carriere, L. Roussel, N. Ortega, D.A. Lacorre, L. Americh, L. Aguilar, G. Bouche, J.P. Girard, IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 282–287.
- [181] A. Wasserman, J. Ben-Shoshan, M. Entin-Meer, S. Maysel-Auslender, H. Guznern-Gur, G. Keren, Interleukin-33 augments Treg cell levels: a flaw mechanism in atherosclerosis, *Isr. Med. Assoc. J.* 14 (2012) 620–623.
- [182] K. Seki, S. Sanada, A.Y. Kudinova, M.L. Steinhauser, V. Handa, J. Gannon, R.T. Lee, Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling, *Circ. Heart Fail.* 2 (2009) 684–691.
- [183] S. Sanada, D. Hakuno, L.J. Higgins, E.R. Schreiter, A.N.J. McKenzie, R.T. Lee, IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system, *J. Clin. Invest.* 117 (2007) 1538–1549.
- [184] C.-L. Liu, D.-L. Shen, Z. Kui, J.-N. Tang, Q.-M. Hai, J.-Y. Zhang, Levels of interleukin-33 and interleukin-6 in patients with acute coronary syndrome or stable angina, *Clin. Invest. Med.* 36 (2013) E234–E241.
- [185] H.-F. Zhang, S.-L. Xie, Y.-X. Chen, J.-T. Mai, J.-F. Wang, W.-L. Zhu, L.-G. Zhu, Altered serum levels of IL-33 in patients with advanced systolic chronic heart failure: correlation with oxidative stress, *J. Transl. Med.* 10 (2012) 120.
- [186] E.O. Weinberg, M. Shimpo, G.W. De Keulenaer, C. MacGillivray, S. Tominaga, S.D. Solomon, J.L. Rouleau, R.T. Lee, Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction, *Circulation* 106 (2002) 2961–2966.
- [187] L.Q. Chen, J.A. de Lemos, S.R. Das, C.R. Ayers, A. Rohatgi, Soluble ST2 is associated with all-cause and cardiovascular mortality in a population-based cohort: The Dallas Heart Study, *Clin. Chem.* 59 (2013) 536–546.
- [188] R.A.P. Weir, A.M. Miller, G.E.J. Murphy, S. Clements, T. Steedman, J.M.C. Connell, I.B. McInnes, H.J. Dargie, J.J. McMurray, Serum soluble ST2 a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction, *J. Am. Coll. Cardiol.* 55 (2010) 243–250.
- [189] J.L. Januzzi Jr., W.F. Peacock, A.S. Maisel, C.U. Chae, R.L. Jesse, A.L. Baggish, et al., Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (pro-brain natriuretic peptide investigation of dyspnea in the emergency department) study, *J. Am. Coll. Cardiol.* 50 (2007) 607–613.
- [190] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, *Nature* 473 (2011) 317–325.
- [191] R.Q. Do, S.J. Nicholls, G.G. Schwartz, Evolving targets for lipid-modifying therapy, *EMBO Mol. Med.* 6 (2014) 1215–1230.
- [192] B. Aryal, N. Rotllan, C. Fernández-Hernando, Noncoding RNAs and atherosclerosis, *Curr. Atheroscler. Rep.* 16 (2014) 407.