Interactions between dyslipidemia and the immune system and their relevance as putative therapeutic targets in atherosclerosis

Michael Lacy [a], Dorothee Atzler [a,b], Rongqi Liu [a], Menno de Winther [a,c], Christian Weber [a], Esther Lutgens [a,c,*]

[a] Institute for Cardiovascular Prevention, Ludwig-Maximilians-University, Munich, Germany
[b] Walther Straub Institute for Pharmacology and Toxicology, Ludwig-Maximilians-University, Munich, Germany
[c] Department of Medical Biochemistry, Amsterdam University Medical Centre, Amsterdam Cardiovascular Sciences, University of Amsterdam, Amsterdam, the Netherlands

ARTICLE INFO

ABSTRACT

Cardiovascular disease (CVD) continues to be a leading cause of death worldwide with atherosclerosis being the major underlying pathology. The interplay between lipids and immune cells is believed to be a driving force in the chronic inflammation of the arterial wall during atherogenesis. Atherosclerosis is initiated as lipid particles accumulate and become trapped in vessel walls. The subsequent immune response, involving both adaptive and immune cells, progresses plaque development, which may be exacerbated under dyslipidemic conditions. Broad evidence, especially from animal models, clearly demonstrates the effect of lipids on immune cells from their development in the bone marrow to their phenotypic switching in circulation. Interestingly, recent research has also shown a long-lasting epigenetic signature from lipids on immune cells. Traditionally, cardiovascular therapies have approached atherosclerosis through lipid-lowering medications because, until recently, anti-inflammatory therapies have been largely unsuccessful in clinical trials. However, the recent Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) provided pivotal support of the inflammatory hypothesis of atherosclerosis in man spurring on anti-inflammatory strategies to treat atherosclerosis. In this review, we describe the interactions between lipids and immune cells along with their specific outcomes as well as discuss their future perspective as potential cardiovascular targets.

© 2018 Published by Elsevier Inc.

Contents

1. Introduction

Accounting for more than one in four deaths worldwide, cardiovascular disease (CVD) presents a major global health concern (Lozano et al., 2012). The underlying pathology involved in many forms of CVD is atherosclerosis, a slow progressing chronic inflammatory condition that causes lesions in the arterial wall as well as narrowing of the vessel lumen (Weber & Noels, 2011). Although several factors contribute to higher individual CVD risk, an overwhelming number of clinical studies have indicated dyslipidemia, or abnormal blood lipid levels, as the key modifiable risk factor in decreasing the manifestations of atherosclerosis (Castelli, Garrison, Wilson, & Levy, 1992; Walldius et al., 2001; Yusuf et al., 2004). Current therapies approach the problem of atherosclerosis through the use of lipid lowering therapeutics such as statins. Novel, clinically-approved treatments, such proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, have also emerged in recent years; however, their approach to reducing atherosclerosis focuses on lipid lowering, similar to statins (Rosenson, Hegele, Fazio, & Cannon, 2018). Although these therapies, both individually and synergistically, effectively lower CVD-risk, a large residual risk remains which needs to be addressed by additional therapies (Heidenreich et al., 2011; Hoogeveen et al., 2017).

To overcome this gap, a considerable amount of research has investigated the interplay between dyslipidemia and immune cells, particularly in genetically modified, hyperlipidemic mice models such as apolipoprotein E deficient ($Apoe^{-/-}$) or low density lipoprotein receptor deficient ($Ldlr^{-/-}$) mice (Getz & Reardon, 2014). With this in mind, it is important to note that atherogenesis is not identical in mice and humans. In both cases, plasma lipoproteins help drive plaque development; however, the lipid profiles usually differ between the species as well as between experimental models (Getz & Reardon, 2012). Therefore, dyslipidemia represents an overarching term that may be the result of diet, genetic manipulation in the case of experimental models, or genetic mutation in the case of familial hypercholesterolemias (FH), and each dyslipidemic condition can be characterized by increases in specific lipids including triglycerides, LDL, and high density lipoprotein (HDL). Nevertheless, dyslipidemia in combination with inflammatory responses drive plaque progression. While these processes represent two separate phenomena, their effects on atherosclerosis are not entirely independent. Studies have targeted both innate and adaptive immune cell types and their subsets to elucidate their function in inflammation under normal and hyperlipidemic conditions in hopes of finding novel therapeutic targets. Recent findings, such as the Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS), have provided intriguing support for the search for innovative anti-inflammation therapies (Ridker et al., 2017). In this review, we will focus on the latest findings on the interactions of lipids and lipoproteins with immune cells as well as their potential as emerging therapeutic targets.

2. Atherosclerosis

Atherosclerosis ultimately gives rise to CVD through a two-part mechanism: imbalanced lipid metabolism and maladaptive immune responses (Weber & Noels, 2011). Its pathogenesis is highly dependent on dyslipidemia as lipids accumulate in regions of disturbed blood flow in the vessel's intimal layer, which leads to innate immune cell (see Appendix A) recruitment and activation. Cellular adhesion molecules, which have be shown to be upregulated by lipoproteins including LDL, mediate the process of leukocyte attachment and diapedesis (Gebuhrer, Murphy, Bordet, Reck, & McGregor,

1995). Selectins and their ligands, such as P-selectin and P-selectin ligand, form a weak tether between immune cells and endothelial cells within the vessel. Additional molecules such as intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs) induce firm adhesion, which allows immune cells to migrate towards the deposited lipids within the intimal layer (Blankenberg, Barbaux, & Tiret, 2003). Monocytes invade early lesions to mediate lipoprotein internalization and cytokine production eventually differentiating into archetypal foam cells. In turn, adaptive immune cells such as T and B lymphocytes (see Appendix A) are attracted to the lesion site leading to a concentrated site of localized inflammation and chemokine secretion. The continuous influx of innate and adaptive immune cells, in combination with the ineffective macrophage efferocytosis of apoptotic and necrotic cells, advances necrotic core formation within plaques in the vessel wall (Doran et al., 2017; Getz & Reardon, 2014; Tse, Tse, Sidney, Sette, & Ley, 2013). Cholesterol accumulating in atherosclerotic lesions has been previously found to undergo modifications (see Glossary) such as oxidization, which exacerbates inflammatory responses. As the lesion advances, it may become destabilized with excessive amounts of lipids and immune cells (Hoogeveen et al., 2017). These vulnerable plaques have the potential to rupture leading to clinical symptoms such as thrombosis, myocardial infarction, or cerebral infarction. To better understand the mechanisms underlying the clinical manifestations of atherosclerosis, more research needs to be applied to the current knowledge we have surrounding the interactions of lipids and our immune system.

3. Dyslipidemia and immune cells

3.1. Lipids and hematopoietic stem and progenitor cells

As outlined above, the combination of abnormal lipids and activated immune cells clearly contributes to atherogenesis. However, the mechanism maintaining the chronic state of inflammation seen in the arterial wall during atherosclerosis remains unclear. Although the effects of atherosclerosis are primarily seen in the arteries and immune cells of the circulatory system, as well as in lymphoid organs, recent studies have suggested a systemic pro-inflammatory phenotype originating in hematopoietic stem and progenitor cells (HSPCs)(see Glossary) which may direct the inflammatory state in immune cells and atherosclerotic plaques (Hoogeveen et al., 2017). These HSPCs are responsible for the renewal and eventual differentiation of the myeloid, lymphoid, and erythroid cell lineages. Although HSPCs maintain our immune cell populations, major knowledge gaps exist, particularly for lymphoid and erythroid progenitors, during their interactions with lipids in the context of atherosclerosis. Therefore, we will only discuss interactions between dyslipidemia and myeloid progenitors in detail.

3.1.1. Increases in bone marrow mobilization

Typically their differentiation is a tightly controlled process responding to the bone marrow niche to replenish or expand immune effector cells; however, HSPCs have also been shown to respond to environmental stimuli in cases like dyslipidemia. For example, in both $Apoe^{-/-}$ and $Ldlr^{-/-}$ mice, studies have shown that hyperlipidemia activates HSPCs causing an increase in their proliferation and total number. In humans, bone marrow hyper-responsiveness, including increased cluster of differentiation-34+ (CD34+) progenitor populations, has been associated with both preclinical

risk factors like increased serum LDL concentrations as well as after clinical events like acute myocardial infarctions (Assmus et al., 2012; Cimato, Palka, Lang, & Young, 2013; Van Der Valk et al., 2017). As shown in Fig. 1, these increases in HSPCs have been associated with a circulating leukocytosis and, in particular, monocytosis and neutrophilia, which themselves are associated risk factors for cardiovascular disease (Coller, 2005; Dutta et al., 2013; Giugliano et al., 2010;Murphy et al., 2011; Seijkens et al., 2014). In ischemic tissue, estimates have shown the residence time for innate immune cells to be roughly 19 h necessitating the need for the observed increased bone marrow mobilization and proliferation to account for their high turnover rate (Leuschner et al., 2012).

Interestingly, this primed monocytic/granulocytotic phenotype has been reproduced in bone marrow chimeras of hyperlipidemic mice models transplanted with normal HSPCs and vice versa suggesting the effect is either on the bone marrow niche or the HSPC itself (Murphy et al., 2011; Seijkens et al., 2014). On one hand, disruptions in the CXCL12/CXCR4 axis (see Appendix A), a key regulator of HSPC mobilization, in the bone marrow niche have been suggested as the mechanism behind this phenomenon. In addition to the CXCL12/CXCR4 pathway, studies also indicated an appreciable regulatory role for cholesterol efflux pathways via ATP binding cassette A1 (ABCA1), ABCG1, and HDL in HSPC mobilization from the bone marrow. $ABCA1^{-/-}$ and $ABCG1^{-/-}$ mice, with defects in cholesterol efflux pathways, showed imbalanced cholesterol homeostasis, increased extramedullary hematopoiesis, and dysregulated expression of cytokines including interleukin (IL)-23/IL-17/ granulocyte-macrophage colony-stimulating factor (GM-CSF), which modified the bone marrow niche by decreasing CXCL12-producing cells allowing for HSPC mobilization (Gomes et al., 2011; Westerterp et al., 2012; Yvan-Charvet et al., 2010). Remarkably, hyperlipidemic $Apoe^{-/-}$ mice injected with neutralizing antibodies targeting IL-3 and granulocyte-CSF (G-CSF) showed a reduction in HSPC mobilization and a concomitant decrease in circulating myeloid populations (Robbins et al., 2012).

3.1.2. Lipid-driven reprogramming of HSPCs

Interest has been growing in the reprogramming of the myeloid progenitors within the bone marrow through epigenetic modifications allowing for long-term memory of innate cells termed 'trained immunity.' Early trained immunity studies induced memory in innate immune cells through the use of β-glucan, which after secondary challenges with LPS resulted in a primed expansion of progenitors of the myeloid lineage as well as increased signaling of immune mediators IL-1ß and GM-CSF. More recently, in the cardiovascular domain, trained immunity was induced in $Ldlr^{-/-}$ mice via a high-fat diet (HFD) resulting in a similar pro-inflammatory phenotype with increased myeloid progenitors mediated through the Nod-like receptor protein 3 (NLRP3) inflammasome (Christ et al., 2018; Mitroulis et al., 2018). Both mechanisms, either via modifications of the bone marrow niche or effects on the HSPCs themselves represent potential therapeutic targets, which we will discuss in more detail below.

3.2. Lipids and innate immune cells

Similar to the immune response during other inflammatory disorders, innate immune cells are the first responders in the case of dyslipidemia. As described, hyperlipidemia induces increased numbers of both circulating neutrophils and monocytes in experimental mice models and patients, which can be attributed to increased HSPC proliferation and mobilization (Giugliano et al., 2010; Seijkens et al., 2014). In the cardiovascular domain, increases in both cell populations have been shown to correlate to plaque burden (Drechsler, Megens, Zandvoort, Weber, & Soehnlein, 2010; Imanishi et al., 2010; Ionita et al., 2010; Swirski et al., 2007). While there is
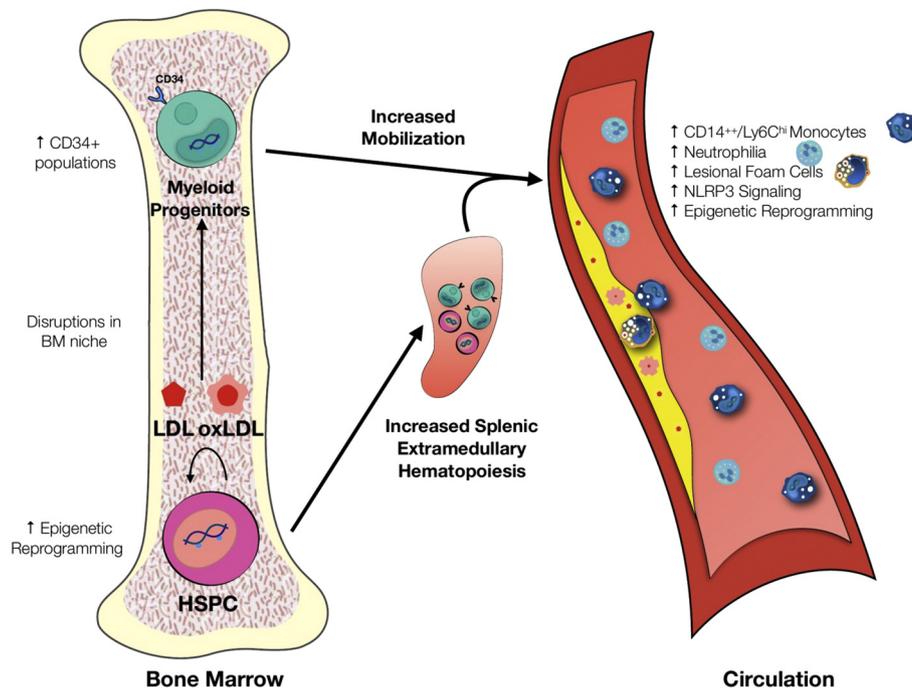
Fig. 1. Interactions between lipids, such as LDL or oxLDL, and HSPCs as well as innate immune cells. Lipids may induce changes directly on HSPCs through epigenetic changes, and they also have been associated with disruptions in the bone marrow niche allowing for increased proliferation and mobilization. The epigenetic signature of HSPCs can be altered by lipid-driven upregulation or downregulation of various epigenetic enzymes including histone methylases and demethylases. These changes can be carried from stem cells to differentiated cells in circulation and tissue. Alternatively, lipids have also been associated with disputing key recruitment and homing pathways like the CXCL12/CXCR4 pathway in the bone marrow niche. Changes induced by these disruptions can be seen in increases in extramedullary hematopoiesis in the spleen as well as increased proliferation of CD34+ progenitor cells. Both changes eventually lead to a circulating neutrophilia and monocytosis with increase signaling of the NLRP3 inflammasome.

accumulating evidence for the role of neutrophils in atherosclerosis, studies involving the specific effects of hyperlipidemia on mature neutrophils are lacking. However, the interactions between monocytes and lipids in atherosclerosis have been well-studied over the past decades.

3.2.1. Increases in circulating monocytes

In circulation, monocytes are typically divided into classical inflammatory monocytes or non-classical patrolling monocytes. Murine monocyte subsets are generally recognized depending on their expression of Ly6C, with higher expression in inflammatory monocytes. Human subsets, on the other hand, can be divided as $CD14^{++}CD16^{-}$ classical monocytes and $CD14^{dim}CD16^{+}$ non-classical monocytes (Boyette et al., 2017; Ingersoll, Platt, Potteaux, & Randolph, 2011). Although some differences exist between the human and mice subsets, they are generally considered to be orthologous based on experimental data such as gene expression analysis (Ingersoll et al., 2010). Interestingly, recent studies have indicated lipoproteins may activate circulating monocytes in plasma (Bernelot Moens et al., 2017). In both humans and mice models, hypercholesterolemia leads to a specific increase in inflammatory monocyte populations, which are thought to be highly infiltrative especially in the case of atherosclerosis (Seijkens et al., 2014; Tolani et al., 2014). Repeatedly, research has shown these elevated concentrations of murine $Ly6C^{high}$ inflammatory population more readily invade atherosclerotic lesions (Ortega-Gómez, Perretti, & Soehnlein, 2013; Swirski et al., 2007; Tacke et al., 2007). Clinical studies have even suggested using circulating levels of inflammatory $CD14^{++}CD16^{-}$ monocytes as an independent predictor of cardiovascular events in humans making them a putative target for therapeutic agents (Berg et al., 2012; Weber et al., 2011). While lipid concentrations highly affect monocytes, the effect of dyslipidemia on differentiated macrophages provides the basis for the traditional sequelae observed in atherosclerosis.

3.2.2. Activation of macrophages and foam cell formation

Lipid retention in the vessel wall acts as a key inflammatory trigger in atherosclerosis by activating pattern recognition receptors such as Toll-like receptors (TLRs) on macrophages that have infiltrated the vessel's intimal layer (Cole, Kassiteridi, & Monaco, 2013; Westerterp et al., 2014). These retained lipoproteins may undergo modifications such as oxidization and can thereby act as damage-associated molecular patterns (DAMPs) to stimulate TLRs or they can be engulfed by macrophages (Binder, Papac-Milicevic, & Witztum, 2016). TLR stimulation initiates a large immune response through the expression of several pro-inflammatory and anti-inflammatory cytokines ultimately attracting additional innate and adaptive immune cells to the plaque (Schaftenaar, Frodermann, Kuiper, & Lutgens, 2016). Lipid uptake and accumulation in macrophages can also coincide with the occurrence of cholesterol crystals, which can further activate these cells through the NLRP3 inflammasome, leading to the secretion and cleavage of IL-1 cytokines (Duewell et al., 2010).

When the balance between influx and efflux of cholesterol is disturbed, cholesterol will accumulate in the macrophages and they will take on the appearance of foam cells. Notably, cholesterol homeostasis within immune cells like macrophages may be controlled through internal cholesterol biosynthesis pathways through the regulation of the transcription factor sterol regulatory element-binding protein 2 (SREBP2) or efflux mechanisms involving liver X receptors (LXR). While investigating the effect of hyperlipidemia on peritoneal foam cell formation in $Ldlr^{-/-}$ mice, Spann et al. demonstrated that a HFD was associated with a deactivated rather than an inflammatory phenotype. The researchers attributed this phenotype to the accumulation of desmosterol, the final intermediate within the cholesterol synthesis pathway, within foam cells, which has been associated with regulation of both cholesterol homeostasis pathways through

suppression of SREBP2-coordinated biosynthesis as well as activation of LXRs (Spann et al., 2012; Yang et al., 2006). Desmosterol, unlike other LXR agonists, is decoupled from pathways that upregulate fatty acid biosynthesis that ultimately lead to hypertriglyceridemia. Therefore, desmosterol and other sterol-based regulators have been gaining attention as potential atherosclerosis therapeutics based on their reciprocal activities on cholesterol homeostasis pathways (Muse et al., 2018; Spann et al., 2012).

3.2.3. Lipid-driven reprogramming of innate immune cells

Although hyperlipidemia can account for the initial increase and infiltration of inflammatory monocytes and macrophages in atherosclerosis, the mechanisms behind the sustained pro-inflammatory state of these short-lived innate immune cells remains unresolved (Hoogeveen et al., 2017). To this end, a shift in the classical immunological paradigm has challenged the dichotomy of innate versus adaptive immunity where innate immune cells, like monocytes and macrophages, may develop memory-like features through epigenetic and metabolic reprogramming (Ifrim et al., 2014; Netea, Quintin, & Van Der Meer, 2011). In the context of atherosclerosis, several pro-atherogenic stimuli, such as oxidized LDL (oxLDL) and lipoprotein(a), have been shown to induce a primed pro-inflammatory state in innate immune cells. After in vitro incubation with oxLDL, human monocytes were found to exhibit a pro-atherogenic phenotype characterized by increased pro-inflammatory cytokine expression as well as increased trimethylation of lysine 4 at histone 3 (H3K4me3). In line with this, recent research demonstrated that a myeloid deficiency of the histone demethylase Jumonji domain-containing protein 3 (Jmjd3, or also known as Kmd6b), which counteracts increases in histone methylation similar to H3K4me3, resulted in more advanced atherosclerosis in hyperlipidemic mice (Neele et al., 2018). Furthermore, ex vivo studies of monocytes isolated from patients with elevated levels of lipoprotein(a) exhibited a trained immune phenotype consisting of increased cytokine production, cell adhesion, and migration. In this case, both metabolic reprogramming through upregulation of glycolytic enzymes and epigenetic reprogramming through increased histone methylation were identified (Van Der Valk et al., 2016). In studies outside the realm of atherosclerosis, trained or primed monocytes were able to respond to secondary stimuli in a hyper-responsive manner for up to three months (Kleinnijenhuis et al., 2012). Epigenetic modifications have gained increased attention and, in some fields like cancer, even FDA approval for treatment (Hoogeveen et al., 2017). Combined with our expanding knowledge of lipid-immune cell interactions, mechanisms behind epigenetic and metabolic reprogramming represent a wide field of research for potential therapeutics.

3.2.4. Inhibitory effects on dendritic cells

Of note, macrophages link innate and adaptive immune responses in atherosclerosis by attracting lymphocytes to the site of inflammation after expression of a myriad of cytokines (Schaftenaar et al., 2016). However, other innate immune cells such as dendritic cells (DC) play key roles in activating adaptive immune cells as well. The traditional immunological paradigm describes DCs patrolling tissue and upon activation migrating to lymph nodes to present antigens in order to activate T cells (Gil-Pulido & Zernecke, 2017). Surprisingly, in the context of atherosclerosis, hyperlipidemia has been shown to impair the migration of these antigen-presenting cells (APCs) after oxLDL induced upregulation of inhibitory signals (Angeli et al., 2004). In line with these results, a reduction in APC-mediated pathogen clearance from plaques has also been described in hyperlipidemic mice, further corroborating inhibitory effects of lipids on DCs (Roufaiel et al., 2016). While in the plaque, resident DCs exhibit enhanced lipoprotein uptake, which may adopt a foam-cell appearance similar to macrophages (Gil-Pulido & Zernecke, 2017). Given that a reduced emigration capacity of DCs has been

associated with dyslipidemia, antigen presentation by these cells may still occur in lymphoid organs, but it may also occur locally within arteries. Several studies have shown that murine aortic DCs are able to activate T cells increasing both their proliferation and expression of pro-inflammatory cytokines, which may be a driving force in the chronic inflammation seen during atherosclerosis (Choi et al., 2011; Koltsova et al., 2012; Weber et al., 2011). Further research into the communication and interactions between lipid-immobilized, lesional DCs and T cells may shed light on promising new therapeutic targets.

3.3. Lipids and adaptive immune cells

Parallel to the innate immune system, the interplay between hyperlipidemia and adaptive immune responses play a key role in atherogenesis. As touched upon, presentation of antigens such as modified LDL or its components triggers adaptive immune responses, which can be seen with the presence of both activated T cells and antibody-producing B cells associated with atherosclerotic plaques (Grivel et al., 2011; Hu et al., 2015). The interaction between lipids and adaptive immune cells as well as the co-stimulatory and co-inhibitory pathways associated with these cell types has been a major focus of cardiovascular research in recent years.

3.3.1. Lipid metabolism and cell proliferation

After interaction with APCs, T cells become activated and subsequently proliferate to expand in response to antigen exposure as shown in Fig. 2. Surprisingly, cholesterol metabolism also plays a major role in the proliferative abilities of these cells. Proliferation requires large amounts of lipids for membrane synthesis, which they can satisfy either through endogenous synthesis or exogenous uptake via the LDL receptor (Bietz, Zhu, Xue, & Xu, 2017). In case of imbalanced cholesterol homeostasis, cells upregulate LXRs to promote

cholesterol efflux or they internalize the LDL receptor for degradation (Bagley, Yuan, & Iacomini, 2017). However, when cells interact with modified lipoproteins like oxLDL, these protective mechanisms may be impaired. For example, oxLDL has been shown to direct differentiation to T effector (Teff) cells, which promote inflammation, while cholesterol efflux mechanisms involving LXRs and HDL favor differentiation towards the immunosuppressive T regulatory (Treg) population (Herold et al., 2017; Maganto-García, Tarrio, Grabie, Bu, & Lichtman, 2011; Newton & Benedict, 2014).

3.3.2. T regulatory and effector cell effects

Typically, functional phenotype switching in T cells allows the immune system to react to environmental stimuli, including atherogenic stimuli like oxLDL, by modifying the ratio of effector to regulatory cells (Su, del Alcazar, Stelekati, Wherry, & Davis, 2016; Tabas & Lichtman, 2017). In hyperlipidemic $Ldlr^{-/-}$ mice, a decrease in the lesional Treg but not Teff cell populations was observed during plaque development (Maganto-García et al., 2011). Mechanistically, in vitro studies of the effect of oxLDL on Tregs showed a reduction in the key lineage-determining transcription factor forkhead box P3 (foxp3), and in their immunosuppressive abilities. Adoptive transfer studies applying Tregs established their atheroprotective role through reductions in aortic sinus plaques (Mor et al., 2007). One study suggested that the role of Tregs in modulating lipoprotein metabolism was the key to their atheroprotective nature, as Treg-deficient mice exhibited a 1.7-fold increase in plasma cholesterol that highly favored VLDL accumulation as a result of reduced expression of sortilin-1 (Klingenberg et al., 2013). Accordingly, IL-10, an anti-inflammatory cytokine associated with Tregs, has been shown to reduce both serum VLDL and LDL levels in $Ldlr^{-/-}$ mice models (Von Der Thüsen et al., 2001). Recently, Winkels et al. further elucidated the role of Tregs and their development during atherosclerosis in finding CD27 co-stimulation increased the abundance of Treg populations
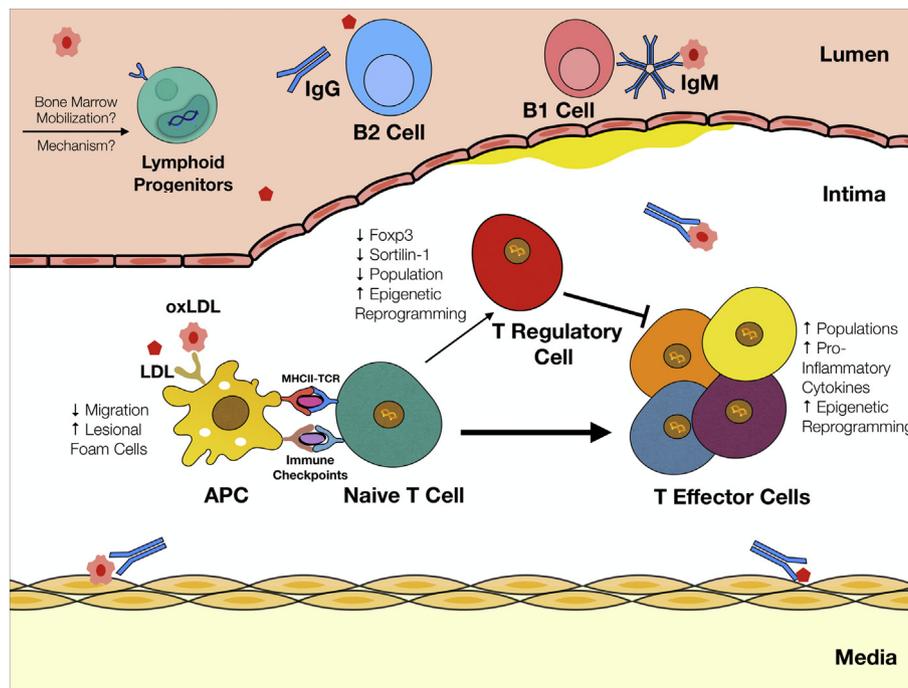
Fig. 2. Interactions between lipids, such as LDL or oxLDL, and adaptive immune cells. Major knowledge gaps exist in describing the effect of lipids on adaptive immune cell progenitors. Similar to their myeloid counterparts, mature adaptive immune cells may have increased epigenetic modifications due to interactions between lipids and HSPCs. Accumulating evidence for both differentiated B and T cells have ascribed their subsets into either pro-atherogenic or anti-atherogenic populations. B cells can be segregated into atheroprotective B1 or atherogenic B2 cells, which secrete either IgM or IgG antibodies against lipid-associated epitopes, respectively. T cell subsets, on the other hand, are still somewhat controversial. After interaction with APCs, immune checkpoint regulators will allow for the co-stimulation or co-inhibition of T cells resulting in different phenotypes such as T regulatory or effector cells. Under hyperlipidemia conditions, the immunosuppressive and atheroprotective T regulatory cell population is reduced as seen in foxp3 downregulation. Without Treg cells keeping the immune system in check, lipids drive a large increase in T effector cell populations like Th1 or Th2 cells. These cells are associated with expression of pro-inflammatory cytokines including IFN-γ and IL-17.

in circulation as well as in lymphoid organs and the aorta. Furthermore, bone marrow of $Apoe^{-/-}CD27^{-/-}$ mice reconstituted with $Apoe^{-/-}CD27^{+/+}$ Tregs exhibited reductions in lesion sizes (Winkels et al., 2017). Considering that low levels of circulating Tregs are associated with increased CVD risk, increasing either the number or function of Treg populations in patients remains a very attractive therapeutic approach (Mor, Luboshits, Planer, Keren, & George, 2006).

Modulating the pro-inflammatory effects of Teff cells during atherosclerosis, on the other hand, is an equally relevant therapeutic target. Lipid-driven cell recruitment to plaques initiates a myriad of cytokines ultimately leading to the expression of co-stimulatory molecules such as CD40 on APCs (Tuñ et al., 2018). Although still somewhat controversial, antigenic stimulation of T cells through APCs in the context of atherosclerosis has been shown to be primarily associated with lipid antigens like oxLDL as well as heat shock proteins in both humans and mice (Afek et al., 2000; Kervinen et al., 2003; Zhu et al., 2001). Afterwards, various co-stimulatory dyads can activate T cells to differentiate into Th1 (T helper), Th2, or Th17 effector cell populations. Th1 cells dominate the T cell response in plaques while promoting vascular inflammation through secretion of Interferon-γ (IFN-γ)(Laurat et al., 2001; Mallat, Taleb, Ait-Oufella, & Tedgui, 2009). To this end, injection of IFN-γ in $Apoe^{-/-}$ mice was shown to reduce serum cholesterol concentrations, but actually increase atherosclerotic lesion sizes marking it a pro-atherogenic cytokine (Whitman, Ravisankar, Elam, & Daugherty, 2000). Conversely, in $Apoe^{-/-}Tbet^{-/-}$ mice, a Th1 lineage-determining transcription factor knockout mice model, the Th1 response was downregulated and resulted in reduced plaque formation (Buono et al., 2005).

To further complicate the role of T helper cell subsets in atherosclerosis, the function of Th2 and Th17 cells in atherosclerosis are still under investigation. IL-4 is a prototypical Th2 cytokine, which has been shown to counteract the production of IFN-γ (Tse et al., 2013). It would appear that Th2 cells are athero-protective, but murine studies have indicated that IL-4 production may upregulate several atherogenic molecules including VCAM-1 and monocyte chemoattractant protein 1 (MCP-1) that are associated with plaque progression (Lee, Kühn, Hennig, Neish, & Toborek, 2001; Walch, Massade, Dufilho, Brunet, & Rendu, 2006). Studies involving Th17 and its prototypical cytokine IL-17 have reported inconsistent results as well. Taking into account that IL-17 activates pro-inflammatory pathways leading to upregulation of IL-1ß and tumor necrosis factor-α (TNF-α), Th17 cells may be viewed as an atherogenic population. $Ldlr^{-/-}IL-17A^{-/-}$ knockout mice models have repeatedly shown reductions in atherosclerosis; however, treatment with mouse anti-IL-17a antibodies found no significant reductions, ultimately blurring the role of these cells in disease development (Butcher, Gjurich, Phillips, & Galkina, 2012; Cheng et al., 2011; Taleb, Tedgui, & Mallat, 2015).

3.3.3. Lipid-driven reprogramming of adaptive immune cells

It becomes abundantly clear that T cells play a major role in atherosclerosis, but the mechanisms driving the plasticity among T helper and regulatory populations have remained largely unchallenged in recent years. The traditional immunological paradigm ascribes certain lineage-defining transcription factors and cytokines to each subset, such as foxp3 and TGF-β for Tregs (Tse et al., 2013). While this paradigm remains true, recent studies have begun investigating the effect of environmental stimuli on epigenetic landscape of T cells during their activation and eventual differentiation. DNA methylation plays an important role during differentiation as naïve T cells undergo an epigenetic shift that allows for transcription factors to bind to the necessary loci. The relevance of DNA methylation in T-cell differentiation has been shown in other chronic inflammatory conditions such as systemic lupus erythematosus where disease flares were associated with changes in helper and regulatory cell populations induced both via hypo-and hypermethylation. In

particular, Enhancer of zeste homolog 2 (EZH2), a histone methyltransferase, has been implicated in studies involving transcriptional silencing of T helper cell populations (Coit et al., 2016). Similar to the primed monocyte phenotype described above, EZH2 is associated with methylation at H3K4me3 making it an interesting therapeutic target in both innate and adaptive immune cells (Hoeksema & de Winther, 2016; Koyanagi et al., 2005; Tong et al., 2014). However, more studies need to investigate the effect of lipids and their modified counterparts, like oxLDL, on epigenetic reprogramming in T cells.

3.3.4. B cells and their subtypes

Finally, a limited effect of hyperlipidemia can also be observed when interrogating B cells. Initial studies indicated that B cells are atheroprotective, as increases in atherosclerotic lesions were found in B cell-deficient mice, achieved either through splenectomy or bone marrow transplant from B cell-deficient mice into lethally irradiated $Ldlr^{-/-}$ mice (Caligiuri, Nicoletti, Poirierand, & Hansson, 2002; Major, Fazio, & Linton, 2002). Antibodies directed against modified lipoproteins, like oxLDL, were proposed as their protective mechanism (Lewis et al., 2009). While this mechanism was confirmed for some subsets of B cells, the differing roles of B1 and B2 cells in atherosclerosis has remained controversial. B1 cells are a subset of B cells belonging to the innate immune system that generate T cell-independent natural IgM antibody responses while B2 cells mount antibody responses in a T cell dependent manner, particularly using with the co-stimulatory OX40-OX40L pathway (Schaftenaar et al., 2016). IgM oxLDL antibodies generated by B1 cells, including B1a and B1b subsets, are associated with protection against atherosclerosis by reducing the oxLDL uptake by macrophages (Ishigami et al., 2013; Rosenfeld et al., 2015). On the other hand, IgG oxLDL antibodies generated by B2 cells have been reported as pro-atherogenic, which might be explained by IgG immune complexes depositing in the plaque (Tsiantoulas, Diehl, Witztum, & Binder, 2014). In view of these results, the application of targeted oxLDL antibodies may warrant more in-depth research, before becoming a feasible therapy for atherosclerosis. However, the latest data from Que. et al. show that targeting oxidized phospholipids (OxPL) by a single-chain fragment of the IgM natural antibody E06 successfully inhibits the macrophage uptake of OxLDL and prevents OxPL-induced inflammatory signaling. This data suggest that therapies inactivating OxPL may be beneficial to inhibit inflammation and thereby the progression of atherosclerosis (Que et al., 2018).

4. Therapeutics

4.1. Lipid-lowering therapies

4.1.1. Statins and PCSK9 inhibitors

Currently, cardiovascular therapies mainly focus on lowering serum lipid levels through the use of statins; however, other lipid-lowering therapies have been developed recently which have shown moderate successes in reducing CVD risk as well. Statins target hepatocytes to effectively obstruct cholesterol synthesis by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Hepatocytes upregulate expression of cell surface LDL receptors to compensate for the statin-induced reduction in endogenous cholesterol, which successfully lowers serum lipid levels (Stancu & Sima, 2001). In practice, statin therapy aims to reduce non-HDL cholesterol significantly to reduce CVD risk. However, clinical practice has shown that statin therapy alone may not be sufficient to reduce individual risk as factors such as genetic predisposition in familial hypercholesterolemia (FH), drug intolerance, or non-adherence of statin therapy play important roles (Chaplin, 2017). Therefore, additional lipid-lowering therapies have recently been developed to further reduce cholesterol levels.

(PCSK9 inhibitors, in particular, have yielded up to 50–60% reductions in LDL when taken in combination with statins in patients suffering from FH (Blom et al., 2014). This therapy focuses on inhibiting PCSK9, which under normal conditions would signal for the degradation of the LDL receptor on hepatocytes. Similar to statins, increased availability of the LDL receptor during treatment leads to lower serum lipid levels (Page & Watts, 2018).

4.1.2. Reconstituted HDL

In addition to these FDA-approved therapies, experimental therapies using reconstituted HDL (rHDL) to lower lipids have been proposed from murine studies. We described earlier how the bone marrow niche may respond to hypercholesterolemia with increased mobilization and proliferation leading to a clinical monocytosis in patients. Studies have described the mechanism as defective cholesterol efflux, which in part could be attributed to reduced HDL levels. To test this hypothesis, researchers substantially lowered murine HDL concentration using a $LDLr^{-/-}Apoa1^{-/-}$ model, because ApoA1 is the major protein component of HDL particles. After six weeks of HFD, increases were observed in circulating neutrophils and monocytes, inflammatory monocytes populations, and HSPC proliferation in $LDLr^{-/-}Apoa1^{-/-}$ mice when compared to $LDLr^{-/-}$ littermates suggesting HDL is implicated in hyperlipidemic myeloid proliferation (Tolani et al., 2014). In rheumatoid arthritis, a chronic inflammatory disease with similarities to atherosclerosis, mouse models have shown increased HSPC proliferation with monocytosis and neutrophilia as well. Although enhanced inflammatory monocyte populations that preferentially infiltrated atherosclerotic plaques were observed, this phenotype could be reversed with the administration of rHDL (Dragoljevic et al., 2018). Unfortunately, most clinical studies targeting HDL failed to improve CV outcome. However, differences in HDL subclasses may explain failures of previous clinical studies (Woudberg et al., 2018). Further exacerbating this problem, recent research has demonstrated a pro-inflammatory effect of HDL on macrophages contradicting its long held anti-inflammatory role (Van Der Vorst et al., 2017). Targeting cellular cholesterol efflux mechanisms via subclasses of HDL remains a therapeutic target with great potential; however, additional research is needed to elucidate and expand our knowledge of the role of HDL in humans.

4.1.3. Anti-inflammation benefits

There is also increasing evidence that lipid-lowering therapies have anti-inflammatory and immunomodulatory benefits as well. Statins have been shown to decrease the activity of NF-kB, which is a key transcription factor associated with inflammation induced through the NLRP3 inflammasome during atherosclerosis (Duewell et al., 2010; Hölschermann et al., 2006). Importantly, this reduction in activity has also been accompanied by decreased production of inflammatory cytokines such as IL-6 and IL-8 (Iwata et al., 2012). In line with this, statins have also been associated with a decrease in inflammatory biomarkers, namely C-reactive protein (CRP), but there may be great inter-individual variation between patients (Ridker et al., 2005). While lacking reductions in serum CRP, PCSK9 inhibitor therapy has nevertheless revealed other anti-inflammatory side effects (Baruch et al., 2017). In FH patients, PCSK9 monoclonal antibodies successfully reduced lipoprotein(a) as wells as pro-inflammatory cytokine, TNF-α, production while increasing anti-inflammatory cytokine, IL-10, production. Changes in both macrophage phenotype and migration were also associated with PCSK9 therapy (Bernelot Moens et al., 2017). Anti-inflammatory benefits from experimental therapies, like rHDL, remain to be demonstrated in human trials.

Unfortunately, these lipid-lowering therapies, particularly statins, leave a large residual risk of cardiovascular complications (Heidenreich et al., 2011; Hoogeveen et al., 2017). Throughout this paper, we have discussed the interplay between lipids and immune cells, which synergistically drive plaque progression in atherosclerosis. Mechanistically, this idea is not new and many clinical trials, as shown in Table 1, have

also tested the association of anti-inflammatory treatments with CVD risk. Until recently, though, many large clinical studies could provide no beneficial evidence for the widespread implantation of non-steroidal anti-inflammatory therapies to reduce CVD risk (Antman et al., 2007; McGettigan & Henry, 2011). However, the recent CANTOS trial provided intriguing support for the inflammatory hypothesis of atherosclerosis. As the first proof-of-principle cardiovascular intervention trial specifically targeting inflammation in humans, patients with previous myocardial infarction and a high-sensitivity CRP level > 2 mg/L treated with monoclonal antibodies against IL-1ß, a master cytokine of innate immunity, witnessed a reduction in both circulating pro-inflammatory biomarkers and incidence rates for cardiovascular events (Ridker et al., 2017). Through this, the search for therapeutics within inflammatory processes has been validated in the context of atherosclerosis.

4.2. Anti-inflammation and immune modulating therapies

As we discussed in earlier sections, the combination of innate and adaptive immune cell responses plays a critical role in the progression of atherosclerosis in response to dyslipidemia. In light of the CANTOS trial, these two separate branches of immunity present great potential for a variety of options of different targeted therapeutics to prevent plaque development. In order to direct therapeutics against specific immune responses, researchers have targeted particular cytokines, cell markers, and cell populations in both experimental mice models and ongoing clinical trials.

4.2.1. Cytokine directed therapies

Currently, the most prominent and anticipated clinical studies involving potential anti-inflammatory cardiovascular therapeutics revolve around innate immune cell secreted cytokines. As we described previously, lipid-driven activation of the NLRP3 inflammasome can influence the maturation and expression of IL-1ß, which itself has been shown to induce IL-6 expression (Duewell et al., 2010; Tosato & Jones, 1990). While the nature of IL-6 remains somewhat controversial, both cytokines have been linked with inflammation during atherosclerosis in animal models. Knockout models involving IL-1RN, an inhibitor of IL-1 and its downstream cytokines such as IL-1ß, resulted in severe inflammation with increased macrophage populations in inflammatory lesions in the adventitia under hyperlipidemic conditions (Merhi-Soussi et al., 2005). Furthermore, targeted treatment with monoclonal antibodies against IL-1ß in $Apoe^{-/-}$ mice showed a significant reduction in plaque formation (Bhaskar et al., 2011). Similarly, IL-6 has been shown to be highly expressed in atherosclerotic plaques, and elevated plasma levels of the cytokine are associated with increased cardiovascular risk (Nus & Mallat, 2016; Reiss, Siegart, & De Leon, 2017). Therefore, both IL-1ß and IL-6 are targets of two separate, randomized placebo-controlled clinical trials. We have already discussed the success of CANTOS targeting IL-1ß in the previous section, and we expect to see the results of Cardiovascular Inflammation Reduction Trial (CIRT) by the end of 2018. Unlike CANTOS, the CIRT study administers a low-dose of methotrexate (15–20 mg/week) to inhibit the pro-inflammatory effects of IL-6. Methotrexate is routinely used to treat other chronic inflammatory disorders such as rheumatoid arthritis; therefore, its transition to a cardiovascular application appears warranted (Everett et al., 2013; Yamashita, Sasaki, Kasahara, & Hirata, 2015). Furthermore, the Colchicine Cardiovascular Outcomes Trial (COLCOT) also plans to evaluate whether long-term treatment with colchicine, an anti-inflammatory drug already approved for acute use in patients with gout, may reduce CV events. If successful, both clinical trials would further support evidence for the inflammatory hypothesis of atherosclerosis alongside the results of the CANTOS trial.

4.2.2. Small molecular inhibitors

In addition to cytokines, researchers have begun to direct targeted therapeutics to specific immune cell populations using cell markers.

Table 1
Ongoing and completed clinical trials involving therapeutic agents targeting various aspects of the interactions between lipids and immune cells.

Therapeutic/study name	Agent	Method of action	Sponsor	Clinical trial number	Status
PCSK9 inhibitors					
ODYSSEY	Alirocumab	PCSK9 Antibody	Sanofi/Regeneron	NCT01663402	Approved
FOURIER	Evolocumab	PCSK9 Antibody	Amgen	NCT01764633	Approved
ORION-10	Inclisiran	siRNA	The Medicines Company	NCT03399370	Phase III
rHDL					
AEGIS-II	CSL-112	Recombinant lipoprotein	CSL Behring	NCT03473223	Phase III
ERASE	CSL-111	Recombinant lipoprotein	CSL Limited	NCT00225719	Discontinued
Anti-inflammatory therapies					
CANTOS	Canakinumab	IL-1ß antibody	Novartis	NCT01327846	Phase III
CIRT	Methotrexate	Purine metabolism inhibitor	Icahn School of Medicine at Mount Sinai	NCT02576067	Phase III
COLCOT	Colchicine	Tubulin disruption	Montreal Heart Institute	NCT02551094	Phase III
Inflammation and Vascular Function in Atherosclerosis	Salsalate	IkB kinase inhibitor	Brigham and Women's Hospital	NCT00760019	Completed
Immune modulating therapies					
ABC	Autologous transplantation	Bone marrow derived mononuclear cells	Leiden University Medical Center	NCT00539266	Completed
LDL immunization					
V6	V6	Vaccination	Immunitor LLC	NCT03042741	Phase III
Epigenetic therapies					
BETonMACE	RVX000222 (apabetalone)	Epigenetic BET protein inhibitor	Resverlogix Corp	NCT02586155	Phase III

Co-stimulatory dyads such as CD40-CD40L signaling between DCs or monocytes and T cells have long been established as pro-inflammatory pathways (Elgueta et al., 2009; Schönbeck, Sukhova, Shimizu, Mach, & Libby, 2000). Unsurprisingly though, long-term blockade of these pathways leads to severe immune deficiencies as their roles are quite complex between cell types, which makes therapeutic development challenging. However, recent approaches using small molecule inhibitors to specifically block downstream targets of these co-stimulatory dyads have been very promising. For example, Seijkens et al. developed a small molecule inhibitor termed TRAF-STOP intended to disrupt the pro-inflammatory CD40-tumor necrosis factor receptor-associated factor (TRAF) 6 signaling pathway during atherosclerosis. Although the TRAF-STOPs were most efficient in blocking inflammation in the myeloid lineage, when given as bare compound, researchers also used rHDL nanoparticles to carry these TRAF-STOPs directly to macrophages for uptake. After engulfment, the TRAF-STOPs effectively abolished CD40-TRAF6 signaling. Significant reductions in the progression of both early and established atherosclerosis, leukocyte recruitment, and macrophage activation were accomplished, and, favorably, classical CD40 signaling such as DC-mediated T-cell activation was unimpaired thereby circumventing immune suppressive side effects (Lameijer et al., 2018; Seijkens et al., 2018). A cell specific approach using targeted therapeutics directed against cell markers without complete signaling ablation is an innovative strategy that has great potential considering the wide variety of immune cell phenotypes seen throughout the progression of atherosclerosis. Blockade of other cell markers such CD47, which inhibits cell efferocytosis within plaques, or Triggering Receptor Expressed on Myeloid cells-1 (TREM-1), which amplifies the innate immune response, through the use of similar therapeutic agents could be promising targets as well (Barclay & van den Berg, 2014; Y. S. Wang, Li, & Zhao, 2012). Potentially, specific immune cell responses could be better controlled through similar therapeutics allowing physicians to regulate cytokine production and immune cell interactions that drive atherogenesis, like some macrophage and Teff cell populations.

4.2.3. Immunomodulation therapies

Interestingly, research into augmenting atheroprotective cell populations like Tregs has shown moderate successes as a potential therapeutic as well. As we described previously, Tregs play a key role in suppressing excessive immune responses especially in inhibiting the progression of atherosclerosis. In fact, a recent clinical study showed a decrease in Treg population in comparison to Teff cell population in coronary artery disease (Emoto et al., 2014). In order to expand Treg populations in vivo, induction with IL-2, a key cytokine for the differentiation and proliferation of Tregs, has been suggested as a potential therapy. Using hyperlipidemic $Apoe^{-/-}$ mice, researchers were able to show both a reduction in atherosclerosis and an expansion of the CD4 + CD25 + Foxp3+ Treg population using a IL-2/anti-IL-2 monoclonal antibody complex (Dinh, Kyaw, Kanellakis, Tipping, et al., 2012). Interestingly, another study using a similar IL-2/anti-IL-2 complex in addition to anti-CD3 vaccine also showed remarkable reductions in atherosclerosis in $Apoe^{-/-}$ mice, which they attributed to an improved Treg to Teff cell ratio (Kasahara et al., 2014). Augmentation of the Treg population has been shown to be possible though adoptive transfer studies in mice, which also lead to reduced atherosclerosis (Ait-Oufella et al., 2006). Clinical trials have begun testing the effect of ex vivo expanded autologous Treg transplantations on several disease states, but human data is still lacking for atherosclerosis (Bullenkamp, Dinkla, Kaski, & Dumitriu, 2016). Animal studies have shown that Treg therapies are an intriguing strategy for CVD treatment; however, without translational studies this therapy is severely lacking.

4.2.4. Vaccination

Vaccination therapies have also gained attention for expanding atheroprotective immune functions. Lipids, especially LDL and its modified counterparts, contribute significantly to the progression of atherosclerosis; therefore, several studies have investigated and reported the inhibitory effects of LDL immunization on plaque development (Ameli et al., 1996; Chyu et al., 2004). However, recent research has begun to investigate specific atheroprotective epitopes of lipoproteins because whole molecules of LDL are too impractical for clinical vaccines considering their size and complexity (Chyu, Dimayuga, & Shah, 2017). In particular, epitopes from apolipoprotein B100 (ApoB100), the protein component of LDL, have emerged as prime therapeutic candidates. Several studies have reported reductions in atherosclerosis, including reduced plaque size as well as macrophage content, after vaccination

with T-cell ApoB100 epitopes (Gisterå et al., 2017; Kimura et al., 2017). Further immunization experiments with ApoB100 peptide 18 resulted in an enriched Treg phenotype as well as reductions in lesion size (Kimura et al., 2018). While these therapies are advantageous in the fact that they leave host defense mechanisms untouched, induction of immune responses through self-antigens could have severe consequences. Currently, the V6 vaccine, containing various pooled adipose tissue antigens, is under evaluation in a clinical trial aiming to reduce atherosclerosis in overweight and obese patients (Bourinbaiar & Jirathitikal, 2010).

Overall, anti-inflammatory and immune modulating therapies are a very promising strategy for cardiovascular treatment. Targeting specific immune cells, either atherogenic or atheroprotective, to balance our immune response during chronic inflammation seems reasonable. While the effects of atherosclerosis are initiated by lipids, these therapies may be targeting our immune system rather than the interaction between immune cells and lipids to resolve this problem. New strategies are being developed which target the cellular, or primarily epigenetic, effects of lipids and dyslipidemia on immune cells.

4.3. Epigenetic therapies

The emerging field of epigenetics has become increasingly prevalent in the search for potential therapeutics for atherosclerosis as our understanding of regulators of immune cell function and differentiation improves. Epigenetics can be defined as any heritable change not associated with alterations in DNA sequence. Although the most well studied epigenetic modulation remains DNA methylation, additional modulations such as histone post-translational modifications and non-coding RNA have also been shown to be associated with disease conditions like atherosclerosis (Feinberg & Moore, 2016; Greißel et al., 2015). These modifications serve to disseminate cellular memory; however, one key feature of epigenetic modifications is their dynamic nature. Depending on environmental stimuli, these modifications have the ability to alter gene expression as well as cell phenotype by allowing chromosomal regions to change their accessibility to transcription factors (Khyzha, Alizada, Wilson, & Fish, 2017). Known risk factors for CVD, such as diet and smoking, may alter environmental stimuli that immune cells interact with thus influencing both epigenetic modifications and further cell differentiation. Within those cells, particular genes may be effectively turned on or off depending on its chromosomal accessibility leading to alterations in downstream gene products and cell phenotypes. As described above, this phenotype switching and priming of both innate and adaptive immune cells in the presence of atherogenic stimuli, such as oxLDL, could help explain the strong inflammatory response seen in the arterial wall during atherosclerosis (Hoogeveen et al., 2017).

4.3.1. Chromatin-modifying enzymes

Many epigenetic processes are tightly regulated by writer and eraser complexes, which have the ability to propagate or silence certain pathways. As discussed previously, histone methyltransferases as well as demethylases, e.g. EZH2 and JMJD3, play important and opposing roles in epigenetic modulations. Considering this fact, chromatin-modifying enzymes have become an interesting therapeutic target in many disease states (Coit et al., 2016; Tong et al., 2014). Although drug development for inhibitors of modulators such as histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) has rapidly expanded, research in atherosclerosis is still poor (Hoogeveen et al., 2017). One prime target for epigenetic modulation is HDAC3, which is upregulated in human plaques. A pro-fibrotic plaque phenotype was observed in mice deficient in myeloid HDAC3, which researchers linked to regulation of the *Tgfb1* loci. Murine macrophages were skewed to an anti-inflammatory phenotype with a reduction in foam cells found in plaques (Hoeksema et al., 2014). Similarly, HDAC9 has also been associated with increases in atherosclerotic lesion sizes in *Apoe*$^{-/-}$ mice suggesting it may be an attractive therapeutic target as well (Azghandi et al., 2015). Additional

research studying the relationship between lipids and immune cells may uncover more epigenetic enzymes that directly affect plaque progression and stability.

4.3.2. Methylation patterns

Interestingly, several genome wide methylation studies have discovered atherosclerosis-specific methylation patterns (Aavik et al., 2015; Z. Wang & Patel, 2014; Zaina et al., 2014). While these studies have provided the basis for exploring epigenetic targets in atherosclerosis, their results have been inconsistent when reporting the level of methylation in human plaques. Further studies have suggested differences in methylation patterns between early and late stage human plaques, which may help explain the discrepancies seen in earlier studies (Greißel et al., 2015). Under hyperlipidemic conditions, several studies have found changes in the methylation pattern of trimethylation of lysine 4 at histone 3 in both monocytes and T cells, which may represent a key to their epigenetic remodeling (Bekkering et al., 2014; Tie, Yan, Khair, Messina, & Deng, 2017). Although a definitive methylation pattern in atherosclerotic plaques remains elusive, future studies focusing on both stage and cell-specific epigenetic signatures might lead to targeted anti-inflammatory therapies.

5. Conclusion

In this review, we outlined the current knowledge surrounding the cross talk between lipids and immune cells, particularly in cases of dyslipidemia. Throughout the decades of cardiovascular research, it has become abundantly clear that lipids and immune cells cooperatively drive atherosclerosis. Surprisingly, though, current treatment recommendations take a one-sided approach to CVD through lipid-lowering therapeutics leaving a large residual risk. However in light of the success of the CANTOS trial, anti-inflammation therapies targeting specific aspects of the immune system may be poised to address this additional need. Although we have described what is currently known about these issues, the mechanisms driving both anti- and pro-atherogenic changes during lipid-immune cell interactions still need to be clarified. As our understanding of immune cell phenotypic switching and epigenetic signatures improves, this knowledge can be applied to multiple chronic inflammatory conditions including atherosclerosis. A combination therapy involving both lipid-lowering and anti-inflammatory treatments may be the first step towards personalized medicine for patients suffering from cardiovascular disease.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Appendix A. Glossary of specific terms and their functions in the context of atherosclerosis

Term	Function during atherosclerosis
Adaptive immune cells	
B Lymphocytes	• Secretion of antibodies targeting pro-atherogenic material such as oxLDL or oxPL • Facilitation of cellular memory and rapid response to previously encountered antigens • Antigen presentation to T cells Subsets: • B1 cells: atheroprotective IgM antibodies • B2 cells: pro-atherogenic IgG antibodies
T Lymphocytes	• Form specific responses after presentation with pro-atherogenic epitopes from oxLDL and oxPL • Assist with the maturation of other immune cells including B cells and activation of macrophages through differential cytokine expression • Maintain immunologic tolerance of autoreactive cells • Facilitation of cellular memory and rapid response to previously encountered antigens Subsets: • Teff: regulation and assistance in immune response • Treg: suppression of immune response
Innate Immune Cells	
Dendritic Cells	• Act as intermediate between innate and adaptive immune systems • Antigen processing and presentation • After activation, they migrate to lymph nodes to interact with adaptive immune cells
Monocytes/Macrophages	• First responder to antigenic material through phagocytosis • Antigen processing and presentation • Cytokine production to assist with immune response Subsets: • Classical: inflammatory immune responses • Non-classical: tissue patrol
Neutrophils	• First responder to antigenic material through phagocytosis • Extracellular traps using DNA to activate innate immune signaling
LDL Modifications	
Aggregation	• Interaction with proteoglycans, especially in the sub-endothelial layer of the vessel intima, may destabilize apoB conformation causing aggregation and increase foam cell formation.
Lipolysis	• Various enzymes, including phospholipase A and sphingomyelinase, may be found in the sub-endothelial layer of the vessel intima, which can modify deposited LDL. These enzymes may hydrolyze LDL lipoproteins allowing for additional aggregation, as well as fusion of LDL particles in the case of sphingomyelinase, in the presence of proteoglycans ultimately increasing foam cell formation.
Oxidation	• Endothelial cells may induce oxidative changes to LDL through free radical modifications producing a wide range of antigenic epitopes in both its lipid and protein portions.
Progenitor and Stem Cells	
CXCR4/CXCL12 axis	• Chemokines, like CXCL12, are small chemoattractant molecules. Although expressed ubiquitously, it forms a homing system through its cognate receptor, CXCR4, in the bone marrow. Typically, decreases in the expression of CXCL12 allow for increased bone mobilization.
Hematopoietic Progenitor and Stem Cells	• Progenitor cells for all cells involved in the immune system through hematopoiesis. HSPCs are found in the bone marrow of most large bones of adults and ultimately migrate to outside locations for further differentiation.

Reference

Aavik, E., Lumivuori, H., Leppänen, O., Wirth, T., Häkkinen, S.K., Bräsen, J.H., ... Ylä-Herttuala, S. (2015). Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. *European Heart Journal 36*(16), 993–1000. https://doi.org/10.1093/eurheartj/ehu437.

Afek, A., George, J., Gilburd, B., Rauova, L., Goldberg, I., Kopolovic, J., ... Shoenfeld, Y. (2000). Immunization of low-density lipoprotein receptor deficient (LDL-RD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis. *Journal of Autoimmunity 14*(2), 115–121. https://doi.org/10.1006/jaut.1999.0351.

Ait-Oufella, H., Salomon, B.L., Potteaux, S., Robertson, A. -K.L., Gourdy, P., Zoll, J., ... Mallat, Z. (2006). Natural regulatory T cells control the development of atherosclerosis in mice. *Nature Medicine 12*(2), 178–180. https://doi.org/10.1038/nm1343.

Ameli, S., Hultgårdh-Nilsson, A., Regnström, J., Calara, F., Yano, J., Cercek, B., ... Nilsson, J. (1996). Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arteriosclerosis, Thrombosis, and Vascular Biology 16*(8), 1074–1079. https://doi.org/10.1161/01.ATV.16.8.1074.

Angeli, V., Llodrá, J., Rong, J.X., Satoh, K., Ishii, S., Shimizu, T., ... Randolph, G.J. (2004). Dyslipidemia associated with atherosclerotic disease systemically alters dendritic cell mobilization. *Immunity 21*(4), 561–574. https://doi.org/10.1016/j.immuni.2004.09.003.

Antman, E.M., Bennett, J.S., Daugherty, A., Furberg, C., Roberts, H., & Taubert, K.A. (2007). Use of nonsteroidal antiinflammatory drugs: An update for clinicians: A scientific statement from the American Heart Association. *Circulation 115*(12), 1634–1642. https://doi.org/10.1161/CIRCULATIONAHA.106.181424.

Assmus, B., Iwasaki, M., Schächinger, V., Roexe, T., Koyanagi, M., Iekushi, K., ... Zeiher, A.M. (2012). Acute myocardial infarction activates progenitor cells and increases Wnt signaling in the bone marrow. *European Heart Journal 33*(15), 1911–1919. https://doi.org/10.1093/eurheartj/ehr388.

Azghandi, S., Prell, C., Van Der Laan, S.W., Schneider, M., Malik, R., Berer, K., ... Dichgans, M. (2015). Deficiency of the stroke relevant HDAC9 gene attenuates atherosclerosis in accord with allele-specific effects at 7p21. 1. *Stroke 46*(1), 197–202. https://doi.org/10.1161/STROKEAHA.114.007213.

Bagley, J., Yuan, J., & Iacomini, J. (2017). Impact of hyperlipidemia on alloimmunity. *Current Opinion in Organ Transplantation 22*(1), 14–21. https://doi.org/10.1097/MOT.0000000000000381.

Barclay, A.N., & van den Berg, T.K. (2014). The interaction between signal regulatory protein alpha (SIRP-α) and CD47: Structure, function, and therapeutic target. *Annual Review of Immunology 32*(1), 25–50. https://doi.org/10.1146/annurev-immunol-032713-120142.

Baruch, A., Mosesova, S., Davis, J.D., Budha, N., Vilimovskij, A., Kahn, R., ... Tingley, W.G. (2017). Effects of RG7652, a monoclonal antibody against PCSK9, on LDL-C, LDL-C subfractions, and inflammatory biomarkers in patients at high risk of or with established coronary heart disease (from the Phase 2 EQUATOR Study). *American Journal of Cardiology 119*(10), 1576–1583. https://doi.org/10.1016/j.amjcard.2017.02.020.

Bekkering, S., Quintin, J., Joosten, L.A.B., Van Der Meer, J.W.M., Netea, M.G., & Riksen, N.P. (2014). Oxidized low-density lipoprotein induces long-term proinflammatory

cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology 34*(8), 1731–1738. https://doi.org/10.1161/ATVBAHA.114.303887.

Berg, K.E., Ljungcrantz, I., Andersson, L., Bryngelsson, C., Hedblad, B., Fredrikson, G.N., & Björkbacka, H. (2012). Elevated CD14++CD16-monocytes predict cardiovascular events. *Circulation: Cardiovascular Genetics 5*(1), 122–131. https://doi.org/10.1161/CIRCGENETICS.111.960385.

Bernelot Moens, S.J., Neele, A.E., Kroon, J., Van Der Valk, F.M., Van Den Bossche, J., Hoeksema, M.A., ... Stroes, E.S.G. (2017). PCSK9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia. *European Heart Journal 38*(20), 1584–1593. https://doi.org/10.1093/eurheartj/ehx002.

Bhaskar, V., Yin, J., Mirza, A.M., Phan, D., Vanegas, S., Issafras, H., & Kantak, S.S. (2011). Monoclonal antibodies targeting IL-1 beta reduce biomarkers of atherosclerosis in vitro and inhibit atherosclerotic plaque formation in Apolipoprotein *E*-deficient mice. *Atherosclerosis 216*(2), 313–320. https://doi.org/10.1016/j.atherosclerosis.2011.02.026.

Bietz, A., Zhu, H., Xue, M., & Xu, C. (2017). Cholesterol metabolism in T cells. *Frontiers in Immunology 8*, 1–8. https://doi.org/10.3389/fimmu.2017.01664.

Binder, C.J., Papac-Milicevic, N., & Witztum, J.L. (2016). Innate sensing of oxidation-specific epitopes in health and disease. *Nature Reviews Immunology 16*(8), 485–497. https://doi.org/10.1038/nri.2016.63.

Blankenberg, S., Barbaux, S., & Tiret, L. (2003). Adhesion molecules and atherosclerosis. *Atherosclerosis 170*(2), 191–203.

Blom, D.J., Hala, T., Bolognese, M., Lillestol, M.J., Toth, P.D., Burgess, L., ... Stein, E.A. (2014). A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. *New England Journal of Medicine 370*(19), 1809–1819. https://doi.org/10.1056/NEJMoa1316222.

Bourinbaiar, A.S., & Jirathitikal, V. (2010). Effect of oral immunization with pooled antigens derived from adipose tissue on atherosclerosis and obesity indices. *Vaccine 28* (15), 2763–2768. https://doi.org/10.1016/j.vaccine.2010.01.032.

Boyette, L.B., MacEdo, C., Hadi, K., Elinoff, B.D., Walters, J.T., Ramaswami, B., ... Metes, D.M. (2017). Phenotype, function, and differentiation potential of human monocyte subsets. *PLoS ONE 12*(4), 1–20 https://doi.org/10.1371/journal.pone.0176460.

Bullenkamp, J., Dinkla, S., Kaski, J.C., & Dumitriu, I.E. (2016). Targeting T cells to treat atherosclerosis: odyssey from bench to bedside. *European Heart Journal - Cardiovascular Pharmacotherapy 2*(3), 194–199. https://doi.org/10.1093/ehjcvp/pvw001.

Buono, C., Binder, C.J., Stavrakis, G., Witztum, J.L., Glimcher, L.H., & Lichtman, A.H. (2005). T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. *Proceedings of the National Academy of Sciences 102*(5), 1596–1601. https://doi.org/10.1073/pnas.0409015102.

Butcher, M.J., Gjurich, B.N., Phillips, T., & Galkina, E.V. (2012). The IL-17A/IL-17RA axis plays a proatherogenic role via the regulation of aortic myeloid cell recruitment. *Circulation Research 110*(5), 675–687. https://doi.org/10.1161/CIRCRESAHA.111.261784.

Caligiuri, G., Nicoletti, A., Poirierand, B., & Hansson, G.K. (2002). Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. *Journal of Clinical Investigation 109*(6), 745–753. https://doi.org/10.1172/JCI200207272.

Castelli, W.P., Garrison, R.J., Wilson, P.W., & Levy, D. (1992). Lipids and risk of coronary heart disease: The Framingham Study. *Annals of Epidemiology 2*, 23–28.

Chaplin, S. (2017). PCSK9 inhibitors: when statins aren't enough. *Prescriber* (pp. 21–26). https://doi.org/10.1002/psb.1616.

Cheng, X., Taleb, S., Wang, J., Tang, T.T., Chen, J., Gao, X.L., ... Liao, Y.H. (2011). Inhibition of IL-17A in atherosclerosis. *Atherosclerosis 215*(2), 471–474. https://doi.org/10.1016/j.atherosclerosis.2010.12.034.

Choi, J.H., Cheong, C., Dandamudi, D.B., Park, C.G., Rodriguez, A., Mehandru, S., ... Steinman, R.M. (2011). Flt3 signaling-dependent dendritic cells protect against atherosclerosis. *Immunity 35*(5), 819–831. https://doi.org/10.1016/j.immuni.2011.09.014.

Christ, A., Günther, P., Lauterbach, M.A.R., Duewell, P., Biswas, D., Pelka, K., ... Latz, E. (2018). Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell 172*(1-2), 162–175. https://doi.org/10.1016/j.cell.2017.12.013.

Chyu, K.Y., Dimayuga, P.C., & Shah, P.K. (2017). Vaccine against arteriosclerosis: an update. *Therapeutic Advances in Vaccines 5*(2), 39–47. https://doi.org/10.1177/2051013617693753.

Chyu, K.Y., Reyes, O.S., Zhao, X., Yano, J., Dimayuga, P., Nilsson, J., ... Shah, P.K. (2004). Timing affects the efficacy of LDL immunization on atherosclerotic lesions in apo e (-/-) mice. *Atherosclerosis 176*(1), 27–35.

Cimato, T.R., Palka, B.A., Lang, J.K., & Young, R.F. (2013). LDL cholesterol modulates human CD34+ HSPCs through effects on proliferation and the IL-17 G-CSF axis. *PLoS ONE 8* (8), 1–11. https://doi.org/10.1371/journal.pone.0073861.

Coit, P., Dozmorov, M.G., Merrill, J.T., McCune, W.J., Maksimowicz-McKinnon, K., Wren, J. D., & Sawalha, A.H. (2016). Epigenetic reprogramming in naive CD4+ T cells favoring T cell activation and non-Th1 effector T cell immune response as an early event in lupus flares. *Arthritis & Rheumatology 68*(9), 2200–2209. https://doi.org/10.1002/art.39720.

Cole, J.E., Kassiteridi, C., & Monaco, C. (2013). Toll-like receptors in atherosclerosis: A "Pandora's box" of advances and controversies. *Trends in Pharmacological Sciences 34*(11), 629–636. https://doi.org/10.1016/j.tips.2013.09.008.

Coller, B.S. (2005). Leukocytosis and ischemic vascular disease morbidity and mortality: Is it time to intervene? *Arteriosclerosis, Thrombosis, and Vascular Biology 25*(4), 658–670. https://doi.org/10.1161/01.ATV.0000156877.94472.a5.

Dinh, T.N., Kyaw, T.S., Kanellakis, P., Tipping, P., ... Agrotis, A. (2012). Cytokine therapy with interleukin-2/anti-interleukin-2 monoclonal antibody complexes expands CD4 + CD25 + Foxp3+ regulatory T cells and attenuates development and progression of atherosclerosis. *Circulation 126*(10), 1256–1266. https://doi.org/10.1161/CIRCULATIONAHA.112.099044.

Doran, A.C., Ozcan, L., Cai, B., Zheng, Z., Fredman, G., Rymond, C.C., & Tabas, I. (2017). CAMKIIγ suppresses an efferocytosis pathway in macrophages and promotes atherosclerotic plaque necrosis. *Journal of Clinical Investigation 127*(11), 4075–4089. https://doi.org/10.1172/JCI94735.

Dragoljevic, D., Kraakman, M.J., Nagareddy, P.R., Ngo, D., Shihata, W., Kammoun, H.L., ... Murphy, A.J. (2018). Defective cholesterol metabolism in hematopoietic stem cells promotes monocyte-driven atherosclerosis in rheumatoid arthritis. *European Heart Journal*, 1–11 https://doi.org/10.1093/eurheartj/ehy119.

Drechsler, M., Megens, R.T.A., Van Zandvoort, M., Weber, C., & Soehnlein, O. (2010). Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation 122* (18), 1837–1845 https://doi.org/10.1161/CIRCULATIONAHA.110.961714.

Duewell, P., Kono, H., Rayner, K.J., Sirois, C.M., Bauernfeind, F.G., Abela, G.S., ... Latz, E. (2010). NLRP3 inflammsomes are required for atherogenesis and activated by cholesterol crystals that form early in deisease. *Nature 464*(7293), 1357–1361. https://doi.org/10.1038/nature08938.

Dutta, P., Courties, G., Wei, Y., Leuschner, F., Gorbatov, R., Robbins, C., ... Nahrendorf, M. (2013). Myocardial infarction accelerates atherosclerosis. *Nature 487*(7407), 325–329. https://doi.org/10.1038/nature11260.

Elgueta, R., Benson, M.J., de Vries, V.C., Wasiuk, A., Guo, Y., & Noelle, R.J. (2009). Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunology Review*(1), 229. https://doi.org/10.1111/j.1600-065X.2009.00782.x.

Emoto, T., Sasaki, N., Yamashita, T., Kasahara, K., Yodoi, K., Sasaki, Y., ... Hirata, K. (2014). Regulatory/Effector T-Cell Ratio Is Reduced in Coronary Artery Disease. *Circulation Journal 78*(12), 2935–2941. https://doi.org/10.1253/circj.CJ-14-0644.

Everett, B.M., Pradhan, A.D., Solomon, D.H., Paynter, N., MacFadyen, J., Zaharris, E., ... Ridker, P.M. (2013). Rationale and design of the cardiovascular inflammation reduction trial (CIRT): A test of the inflammatory hypothesis of atherothrombosis. *American Heart Journal 166*(2), 199–207. https://doi.org/10.1016/j.ahj.2013.03.018.

Feinberg, M.W., & Moore, K.J. (2016). MicroRNA regulation of atherosclerosis. *Circulation Research 118*(4), 703–720. https://doi.org/10.1161/CIRCRESAHA.115.306300.

Gebuhrer, V., Murphy, J.F., Bordet, J.C., Reck, M.P., & McGregor, J.L. (1995). Oxidized low-density lipoprotein induces the expression of P-selectin (GMP140/PADGEM/CD62) on human endothelial cells. *Biochemical Journal 306*, 293–298.

Getz, G.S., & Reardon, C. a. (2012). Brief review animal models of atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology 32*(5), 1104–1115 https://doi.org/10.1161/ATVBAHA.111.237693.

Getz, G.S., & Reardon, C.A. (2014). The mutual interplay of lipid metabolism and the cells of the immune system in relation to atherosclerosis. *Clinical Lipidology 9*(6), 657–671. https://doi.org/10.2217/clp.14.50.

Gil-Pulido, J., & Zernecke, A. (2017). Antigen-presenting dendritic cells in atherosclerosis. *European Journal of Pharmacology 816*, 25–31. https://doi.org/10.1016/j.ejphar.2017.08.016.

Gisterå, A., Hermansson, A., Strodthoff, D., Klement, M.L., Hedin, U., Fredrikson, G.N., ... Ketelhuth, D.F.J. (2017). Vaccination against T-cell epitopes of native ApoB100 reduces vascular inflammation and disease in a humanized mouse model of atherosclerosis. *Journal of Internal Medicine 281*(4), 383–397. https://doi.org/10.1111/joim.12589.

Giugliano, G., Brevetti, G., Lanero, S., Schiano, V., Laurenzano, E., & Chiariello, M. (2010). Leukocyte count in peripheral arterial disease: A simple, reliable, inexpensive approach to cardiovascular risk prediction. *Atherosclerosis 210*(1), 288–293. https://doi.org/10.1016/j.atherosclerosis.2009.11.009.

Gomes, A.L., Carvalho, T., Serpa, J., Torre, C., Dias, S., & Dc, W. (2011). Hypercholesterolemia promotes bone marrow cell mobilization by perturbing the SDF-1: CXCR4 axis. *Blood 115*(19), 3886–3894. https://doi.org/10.1182/blood-2009-08-240580.

Greißel, A., Culmes, M., Napieralski, R., Wagner, E., Gebhard, H., Schmitt, M., & Pelisek, J. (2015). Alternation of histone and DNA methylation in human atherosclerotic carotid plaques. *Thrombosis and Haemostasis 114*(2), 390–402. https://doi.org/10.1160/TH14-10-0852.

Grivel, J.-C., Ivanova, O., Pinegina, N., Blank, P.S., Shpektor, A., Margolis, L.B., & Vasilieva, E. (2011). Activation of T lymphocytes in atherosclerotic plaques. *Arteriosclerosis, Thrombosis, and Vascular Biology 31*(12), 2929–2937. https://doi.org/10.1161/ATVBAHA.111.237081.

Heidenreich, P.A., Trogdon, J.G., Khavjou, O.A., Butler, J., Dracup, K., Ezekowitz, M.D., ... Woo, Y.J. (2011). Forecasting the future of cardiovascular disease in the United States: A policy statement from the American Heart Association. *Circulation 123*(8), 933–944. https://doi.org/10.1161/CIR.0b013e31820a55f5.

Herold, M., Breuer, J., Hucke, S., Knolle, P., Schwab, N., Wiendl, H., & Klotz, L. (2017). Liver X receptor activation promotes differentiation of regulatory T cells. *PLoS ONE 12*(9), 1–13. https://doi.org/10.1371/journal.pone.0184985.

Hoeksema, M.A., & de Winther, M.P.J. (2016). Epigenetic regulation of monocyte and macrophage function. *Antioxidants & Redox Signaling 25*(14), 758–774. https://doi.org/10.1089/ars.2016.6695.

Hoeksema, M.A., Gijbels, M.J., Van den Bossche, J., van der Velden, S., Sijm, A., Neele, A.E., ... de Winther, M.P. (2014). Targeting macrophage Histone deacetylase 3 stabilizes atherosclerotic lesions. *EMBO Molecular Medicine 6*(9), 1124–1132. https://doi.org/10.15252/emmm.201404170.

Hölschermann, H., Schuster, D., Parviz, B., Haberbosch, W., Tillmanns, H., & Muth, H. (2006). Statins prevent NF-κB transactivation independently of the IKK-pathway in human endothelial cells. *Atherosclerosis 185*(2), 240–245. https://doi.org/10.1016/j.atherosclerosis.2005.06.019.

Hoogeveen, R.M., Nahrendorf, M., Riksen, N.P., Netea, M.G., de Winther, M.P.J., Lutgens, E., ... Bekkering, S. (2017). Monocyte and hematopoietic progenitor reprogramming as common mechanism underlying chronic inflammatory and cardiovascular diseases. *European Heart Journal*, 1–10. https://doi.org/10.1093/eurheartj/ehx581.

Hu, D., Mohanta, S.K., Yin, C., Peng, L., Ma, Z., Srikakulapu, P., ... Habenicht, A.J.R. (2015). Artery tertiary lymphoid organs control aorta immunity and protect against atherosclerosis via vascular smooth muscle cell lymphotoxin β receptors. *Immunity 42*(6), 1100–1115. https://doi.org/10.1016/j.immuni.2015.05.015.

Que, X., Hung, M., Yeang, C., Gonen, A., Prohaska, T.A., Sun, X., ... Witztum, J.L. (2018). Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice. *Nature 558*. https://doi.org/10.1038/s41586-018-0198-8.

Ifrim, D.C., Quintin, J., Joosten, L.A.B. Jacobs, C., Jansen, T., Jacobs, L., ... Netea, M.G. (2014). Trained immunity or tolerance: Opposing functional programs induced in human monocytes after engagement of various pattern recognition receptors. *Clinical and Vaccine Immunology 21*(4), 534–545. https://doi.org/10.1128/CVI.00688-13.

Imanishi, T., Ikejima, H., Tsujioka, H., Kuroi, A., Ishibashi, K., Komukai, K., ... Akasaka, T. (2010). Association of monocyte subset counts with coronary fibrous cap thickness in patients with unstable angina pectoris. *Atherosclerosis 212*(2), 628–635. https://doi.org/10.1016/j.atherosclerosis.2010.06.025.

Ingersoll, M.A., Platt, A.M., Potteaux, S., & Randolph, G.J. (2011). Monocyte trafficking in acute and chronic inflammation. *Trends in Immunology 32*(10), 470–477. https://doi.org/10.1016/j.it.2011.05.001.

Ingersoll, M.A., Spanbroek, R., Lottaz, C., Gautier, E.L., Frankenberger, M., Hoffmann, R., & Randolph, G.J. (2010). Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood 115*(3), 10–20. https://doi.org/10.1182/blood-2009-07-235028.

Ionita, M.G., Van Den Borne, P., Catanzariti, L.M., Moll, F.L., De Vries, J.P.P.M., Pasterkamp, G., ... De Kleijn, D.P.V. (2010). High neutrophil numbers in human carotid atherosclerotic plaques are associated with characteristics of rupture-prone lesions. *Arteriosclerosis, Thrombosis, and Vascular Biology 30*(9), 1842–1848. https://doi.org/10.1161/ATVBAHA.110.209296.

Ishigami, T., Abe, K., Aoki, I., Minegishi, S., Ryo, A., Matsunaga, S., ... Endo, Y. (2013). Anti-interleukin-5 and multiple autoantibodies are associated with human atherosclerotic diseases and serum interleukin-5 levels. *FASEB Journal 27*(9), 3437–3445. https://doi.org/10.1096/fj.12-222653.

Iwata, A., Shirai, R., Ishii, H., Kushima, H., Otani, S., Hashinaga, K., ... Kadota, J. (2012). Inhibitory effect of statins on inflammatory cytokine production from human bronchial epithelial cells. *Clinical and Experimental Immunology 168*(2), 234–240. https://doi.org/10.1111/j.1365-2249.2012.04564.x.

Kasahara, K., Sasaki, N., Yamashita, T., Kita, T., Yodoi, K., Sasaki, Y., ... Hirata, K.I. (2014). CD3 antibody and IL-2 complex combination therapy inhibits atherosclerosis by augmenting a regulatory immune response. *Journal of the American Heart Association 3*(2), 1–12. https://doi.org/10.1161/JAHA.113.000719.

Kervinen, H., Huittinen, T., Vaarala, O., Leinonen, M., Saikku, P., Manninen, V., & Mänttäri, M. (2003). Antibodies to human heat shock protein 60, hypertension and dyslipidemia. A study of joint effects on coronary risk. *Atherosclerosis 169*(2), 339–344 https://doi.org/10.1016/S0021-9150(03)00229-6.

Khyzha, N., Alizada, A., Wilson, M.D., & Fish, J.E. (2017). Epigenetics of Atherosclerosis: Emerging Mechanisms and Methods. *Trends in Molecular Medicine 23*(4), 332–347. https://doi.org/10.1016/j.molmed.2017.02.004.

Kimura, T., Kobiyama, K., Winkels, H., Tse, K., Miller, J., Vassallo, M., ... Ley, K. (2018). Regulatory CD4 + T cells recognize MHC-II-restricted peptide epitopes of apolipoprotein B. *Circulation 138*(1). https://doi.org/10.1161/CIRCULATIONAHA.117.031420.

Kimura, T., Tse, K., McArdle, S., Gerhardt, T., Miller, J., Mikulski, Z., ... Ley, K. (2017). Atheroprotective vaccination with MHC-II-restricted ApoB peptides induces peritoneal IL-10-producing CD4 T cells. *American Journal of Physiology - Heart and Circulatory Physiology 312*(4), H781–H790. https://doi.org/10.1152/ajpheart.00798.2016.

Kleinnijenhuis, J., Quintin, J., Preijers, F., Joosten, L.A.B., Ifrim, D.C., Saeed, S., ... Netea, M.G. (2012). Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proceedings of the National Academy of Sciences 109*(43), 17537–17542. https://doi.org/10.1073/pnas.1202870109.

Klingenberg, R., Gerdes, N., Badeau, R.M., Gisterå, A., Strodthoff, D., Ketelhuth, D.F.J., ... Hansson, G.K. (2013). Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. *The Journal of Clinical ... 123*(3), 1323–1334. https://doi.org/10.1172/JCI63891DS1.

Koltsova, E.K., Garcia, Z., Chodaczek, G., Landau, M., Mcardle, S., Scott, S.R., ... Ley, K. (2012). Dynamic T cell – APC interactions sustain chronic inflammation in atherosclerosis. *The Journal of Clinical Investigation 122*(9), 3114–3126. https://doi.org/10.1172/JCI61758.

Koyanagi, M., Baguet, A., Martens, J., Margueron, R., Jenuwein, T., & Bix, M. (2005). EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in TH1 cells. *Journal of Biological Chemistry 280*(36), 31470–31477. https://doi.org/10.1074/jbc.M504766200.

Lameijer, M., Binderup, T., Van Leent, M.M.T., Senders, M.L., Fay, F., Malkus, J., ... Duivenvoorden, R. (2018). Efficacy and safety assessment of a TRAF6-targeted nanoimmunotherapy in atherosclerotic mice and non-human primates. *Nature Biomedical Engineering 2*(5), 279–292. https://doi.org/10.1038/s41551-018-0221-2.

Laurat, E., Poirier, B., Tupin, E., Caligiuri, G., Hansson, G.K., Bariéty, J., & Nicoletti, A. (2001). In vivo downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation 104*(2), 197–202. https://doi.org/10.1161/01.CIR.104.2.197.

Lee, Y.W., Kühn, H., Hennig, B., Neish, A.S., & Toborek, M. (2001). IL-4-induced oxidative stress upregulates VCAM-1 gene expression in human endothelial cells. *Journal of Molecular and Cellular Cardiology 33*(1), 83–94. https://doi.org/10.1006/jmcc.2000.1278.

Leuschner, F., Rauch, P.J., Ueno, T., Gorbatov, R., Marinelli, B., Lee, W.W., ... Nahrendorf, M. (2012). Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. *The Journal of Experimental Medicine 209*(1), 123–137. https://doi.org/10.1084/jem.20111009.

Lewis, M.J., Malik, T.H., Ehrenstein, M.R., Boyle, J.J., Botto, M., & Haskard, D.O. (2009). Immunoglobulin M is required for protection against atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation 120*(5), 417–426. https://doi.org/10.1161/CIRCULATIONAHA.109.868158.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., ... Murray, C.J.L. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet 380*(9859), 2095–2128.

Maganto-García, E., Tarrio, M.L., Grabie, N., Bu, D.X., & Lichtman, A.H. (2011). Dynamic changes in regulatory T cells are linked to levels of diet-induced hypercholesterolemia. *Circulation 124*(2), 185–195. https://doi.org/10.1161/CIRCULATIONAHA.110.006411.

Major, A.S., Fazio, S., & Linton, M.F. (2002). B-lymphocyte deficiency increases atherosclerosis in LDL receptor-null mice. *Arteriosclerosis, Thrombosis, and Vascular Biology 22*(11), 1892–1898. https://doi.org/10.1161/01.ATV.0000039169.47943.EE.

Mallat, Z., Taleb, S., Ait-Oufella, H., & Tedgui, A. (2009). The role of adaptive T cell immunity in atherosclerosis. *Journal of Lipid Research 50*(Supplement), S364–S369 https://doi.org/10.1194/jlr.R800092-JLR200.

McGettigan, P., & Henry, D. (2011). Cardiovascular risk with non-steroidal anti-inflammatory drugs: Systematic review of population-based controlled observational studies. *PLoS Medicine 8*(9). https://doi.org/10.1371/journal.pmed.1001098.

Merhi-Soussi, F., Kwak, B.R., Magne, D., Chadjichristos, C., Berti, M., Pelli, G., ... Gabay, C. (2005). Interleukin-1 plays a major role in vascular inflammation and atherosclerosis in male apolipoprotein E-knockout mice. *Cardiovascular Research 66*(3), 583–593. https://doi.org/10.1016/j.cardiores.2005.01.008.

Mitroulis, I., Ruppova, K., Wang, B., Chen, L.S., Grzybek, M., Grinenko, T., ... Chavakis, T. (2018). Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell 172*(1–2), 147–161. https://doi.org/10.1016/j.cell.2017.11.034.

Mor, A., Luboshits, G., Planer, D., Keren, G., & George, J. (2006). Altered status of CD4 + CD25+ regulatory T cells in patients with acute coronary syndromes. *European Heart Journal 27*(21), 2530–2537. https://doi.org/10.1093/eurheartj/ehl222.

Mor, A., Planer, D., Luboshits, G., Afek, A., Metzger, S., Chajek-Shaul, T., ... George, J. (2007). Role of naturally occurring CD4 + CD25+ regulatory T cells in experimental atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology 27*(4), 893–900. https://doi.org/10.1161/01.ATV.0000259365.31469.89.

Murphy, A.J., Akhtari, M., Tolani, S., Pagler, T., Bijl, N., Kuo, C., ... Tall, A.R. (2011). ApoE regulates hematopoeitic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. *Journal of Clinical Investigation 121*(10), 4138–4149. https://doi.org/10.1172/JCI57559DS1.

Muse, E.D., Yu, S., Edillor, C.R., Tao, J., Spann, N.J., Troutman, T.D., ... Glass, C.K. (2018). Cell-specific discrimination of desmosterol and desmosterol mimetics confers selective regulation of LXR and SREBP in macrophages. *Proceedings of the National Academy of Sciences 115*(20), 201714518. https://doi.org/10.1073/pnas.1714518115.

Neele, A.E., Gijbels, M.J.J., van der Velden, S., Hoeksema, M.A., Boshuizen, M.C.S., Prange, K. H.M., ... de Winther, M.P.J. (2018). Myeloid Kdm6b deficiency results in advanced atherosclerosis. *Atherosclerosis 275*, 156–165. https://doi.org/10.1016/j.atherosclerosis.2018.05.052.

Netea, M.G., Quintin, J., & Van Der Meer, J.W.M. (2011). Trained immunity: A memory for innate host defense. *Cell Host and Microbe 9*(5), 355–361. https://doi.org/10.1016/j.chom.2011.04.006.

Newton, A.H., & Benedict, S.H. (2014). Low density lipoprotein promotes human naive T cell differentiation to Th1 cells. *Human Immunology 75*(7), 621–628. https://doi.org/10.1016/j.humimm.2014.04.017.

Nus, M., & Mallat, Z. (2016). Immune-mediated mechanisms of atherosclerosis and implications for the clinic. *Expert Review of Clinical Immunology 12*(11), 1217–1237. https://doi.org/10.1080/1744666X.2016.1195686.

Ortega-Gómez, A., Perretti, M., & Soehnlein, O. (2013). Resolution of inflammation: An integrated view. *EMBO Molecular Medicine 5*(5), 661–674. https://doi.org/10.1002/emmm.201202382.

Page, M.M., & Watts, G.F. (2018). PCSK9 in context: A contemporary review of an important biological target for the prevention and treatment of atherosclerotic cardiovascular disease. *Diabetes, Obesity and Metabolism 20*(2), 270–282. https://doi.org/10.1111/dom.13070.

Reiss, A.B., Siegart, N.M., & De Leon, J. (2017). Interleukin-6 in atherosclerosis: atherogenic or atheroprotective? *Clinical Lipidologypidology 12*(1), 14–23. https://doi.org/10.1080/17584299.2017.1319787.

Ridker, P.M., Cannon, C.P., Morrow, D., Rifai, N., Rose, L.M., McCabe, C.H., ... Braunwald, E. (2005). C-reactive protein levels and outcomes after statin therapy. *N Engl J Med 352*(1), 20–28. https://doi.org/10.1056/NEJMoa042378.

Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., ... Glynn, R.J. (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. *New England Journal of Medicine* https://doi.org/10.1056/NEJMoa1707914.

Robbins, C., Chudnovskiy, A., Rauch, P.J., Figueiredo, J. -L., Iwamoto, Y., Gorbatov, R., ... Swirski, F.K. (2012). Extramedullary hematopoiesis generates Ly-6Chigh monocytes that infiltrate atherosclerotic lesions. *Circulation 125*(2), 364–374. https://doi.org/10.1161/CIRCULATIONAHA.111.061986.

Rosenfeld, S.M., Perry, H.M., Gonen, A., Prohaska, T.A., Srikakulapu, P., Grewel, S., ... McNamara, C.A. (2015). B1-b cells secrete atheroprotective IgM and attenuate atherosclerosis. *Circulation Research 117*(3), e28–e39. https://doi.org/10.1161/CIRCRESAHA.117.306044.

Rosenson, R.S., Hegele, R.A., Fazio, S., & Cannon, C.P. (2018). The evolving future of PCSK9 inhibitors. *Journal of the American College of Cardiology 72*(3), 314–329. https://doi.org/10.1016/j.jacc.2018.04.054.

Roufaiel, M., Gracey, E., Siu, A., Zhu, S.N., Lau, A., Ibrahim, H., ... Cybulsky, M.I. (2016). CCL19-CCR7-dependent reverse transendothelial migration of myeloid cells clears Chlamydia muridarum from the arterial intima. *Nature Immunology 17*(11), 1263–1272. https://doi.org/10.1038/ni.3564.

Schaftenaar, F., Frodermann, V., Kuiper, J., & Lutgens, E. (2016). Atherosclerosis: The interplay between lipids and immune cells. *Current Opinion in Lipidology 27*(3), 209–215. https://doi.org/10.1097/MOL.0000000000000302.

Schönbeck, U., Sukhova, G.K., Shimizu, K., Mach, F., & Libby, P. (2000). Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. _Proceedings of the National Academy of Sciences of the United States of America. 97._ (pp. 7458–7463). https://doi.org/10.1073/pnas.97.13.7458.

Seijkens, T., Hoeksema, M.A., Beckers, L., Smeets, E., Meiler, S., Levels, J., ... Lutgens, E. (2014). Hypercholesterolemia-induced priming of hematopoietic stem and progenitor cells aggravates atherosclerosis. _FASEB Journal 28_(5), 2202–2213. https://doi.org/10.1096/fj.13-243105.

Seijkens, T.T.P., van Tiel, C.M., Kusters, P.J.H., Atzler, D., Soehnlein, O., Zarzycka, B., ... Lutgens, E. (2018). Targeting CD40-induced TRAF6 signaling in macrophages reduces atherosclerosis. _Journal of the American College of Cardiology 71_(5), 527–542. https://doi.org/10.1016/j.jacc.2017.11.055.

Spann, N.J., Garmire, L.X., Mcdonald, J.G., Myers, D.S., Milne, B., Shibata, N., ... Glass, C.K. (2012). Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. _Cell 151_(1), 138–152. https://doi.org/10.1016/j.cell.2012.06.054.

Stancu, C., & Sima, A. (2001). Statins: mechanism of action and effects. _Journal of Cellular and Molecular Medicine 5_(4), 378–387. https://doi.org/10.1111/j.1582-4934.2001.tb00172.x.

Su, L.F., del Alcazar, D., Stelekati, E., Wherry, E.J., & Davis, M.M. (2016). Antigen exposure shapes the ratio between antigen-specific Tregs and conventional T cells in human peripheral blood. _Proceedings of the National Academy of Sciences 113_(41), E6192–E6198. https://doi.org/10.1073/pnas.1611723113.

Swirski, F.K., Libby, P., Aikawa, E., Alcaide, P., Luscinskas, F.W., Weissleder, R., & Pittet, M.J. (2007). Ly-6Chi monocytes dominate hypercholesterolemi. _Journal of Clinical Investigation 117_(1), 195–205. https://doi.org/10.1172/JCI29950.

Tabas, I., & Lichtman, A.H. (2017). Monocyte-macrophages and T cells in atherosclerosis. _Immunity 47_(4), 621–634. https://doi.org/10.1016/j.immuni.2017.09.008.

Tacke, F., Alvarez, D., Kaplan, T.J., Jakubzick, C., Spanbroek, R., Llodra, J., ... Randolph, G.J. (2007). Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. _The Journal of Clinical Investigations 117_(1), 185–194. https://doi.org/10.1172/JCI28549.

Taleb, S., Tedgui, A., & Mallat, Z. (2015). IL-17 and Th17 cells in atherosclerosis: Subtle and contextual roles. _Arteriosclerosis, Thrombosis, and Vascular Biology 35_(2), 258–264. https://doi.org/10.1161/ATVBAHA.114.303567.

Tie, A.G., Yan, J., Khair, L., Messina, J.A., & Deng, A. (2017). Hypercholesterolemia increases colorectal cancer incidence by reducing production of NKT and γδ T cells from hematopoietic stem cells. _Cancer Research_(9), 77. https://doi.org/10.1158/0008-5472.CAN-16-1916.

Tolani, S., Pagler, T.A., Murphy, A.J., Bochem, A.E., Welch, C., Nagareddy, P.R., ... Tall, A.R. (2014). Hypercholesterolemia and reducedHDL-C promote hematopoietic stem cell proliferation and monocytosis: Studies in mice and FH. _Children 229_(1), 79–85. https://doi.org/10.1016/j.atherosclerosis.2013.03.031.

Tong, Q., He, S., Xie, F., Mochizuki, K., Liu, Y., Mochizuki, I., ... Zhang, Y. (2014). Ezh2 regulates transcriptional and posttranslational expression of T-bet and promotes Th1 cell responses mediating aplastic anemia in mice. _The Journal of Immunology 192_(11), 5012–5022. https://doi.org/10.4049/jimmunol.1302943.

Tosato, G., & Jones, K.D. (1990). Interleukin-1 induces interleukin-6 production in peripheral blood monocytes. _Blood 75_(6), 1305–1310.

Tse, K., Tse, H., Sidney, J., Sette, A., & Ley, K. (2013). T cells in atherosclerosis. _International Immunology 25_(11), 615–622. https://doi.org/10.1093/intimm/dxt043.

Tsiantoulas, D., Diehl, C.J., Witztum, J.L., & Binder, C.J. (2014). B cells and humoral immunity in atherosclerosis. _Circulation Research 114_(11), 1743–1756. https://doi.org/10.1161/CIRCRESAHA.113.301145.

Tuñ, Ó. N.J., Bäck, M., Badimó, N.L., Bochaton-Piallat, M. -L., Cariou, B., Daemen, M.J., ... Tuñó, J. (2018). Interplay between hypercholesterolaemia and inflammation in atherosclerosis: Translating experimental targets into clinical practice. _European Journal of Preventive Cardiology 0_(00), 1–8. https://doi.org/10.1177/2047487318773384.

Van Der Valk, F.M., Bekkering, S., Kroon, J., Yeang, C., Van Den Bossche, J., Van Buul, J.D., ... Stroes, E.S.G. (2016). Oxidized phospholipids on Lipoprotein(a) elicit arterial wall inflammation and an inflammatory monocyte response in humans. _Circulation 134_(8), 611–624. https://doi.org/10.1161/CIRCULATIONAHA.116.020838.

Van Der Valk, F.M., Kuijk, C., Verweij, S.L., Stiekema, L.C.A., Kaiser, Y., Zeerleder, S., ... Stroes, E.S.G. (2017). Increased hematopoietic activity in patients with atherosclerosis. _European Heart Journal 38_(6), 425–432a. https://doi.org/10.1093/eurheartj/ehw246.

Van Der Vorst, E.P.C., Theodorou, K., Wu, Y., Hoeksema, M.A., Goossens, P., Bursill, C.A., ... Donners, M.M.P.C. (2017). High-density lipoproteins exert pro-inflammatory effects on macrophages via passive cholesterol depletion and PKC-NF-κB/STAT1-IRF1 signaling. _Cell Metabolism 25_(1), 197–207 https://doi.org/10.1016/j.cmet.2016.10.013.

Von Der Thüsen, J.H., Kuiper, J., Fekkes, M.L., De Vos, P., Van Berkel, T.J., & Biessen, E.A. (2001). Attenuation of atherogenesis by systemic and local adenovirus-mediated gene transfer of interleukin-10 in LDLr-/- mice. _The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 15_(14), 2730–2732. https://doi.org/10.1096/fj.01-0483fje.

Walch, L., Massade, L., Dufilho, M., Brunet, A., & Rendu, F. (2006). Pro-atherogenic effect of interleukin-4 in endothelial cells: Modulation of oxidative stress, nitric oxide and monocyte chemoattractant protein-1 expression. _Atherosclerosis 187_(2), 285–291. https://doi.org/10.1016/j.atherosclerosis.2005.09.016.

Walldius, G., Jungner, I., Holme, I., Aastveit, A.H., Kolar, W., & Steiner, E. (2001). High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): A prospective study. _Lancet 358_(9298), 2026–2033.

Wang, Y.S., Li, X.J., & Zhao, W.O. (2012). TREM-1 is a positive regulator of TNF-alpha and IL-8 production in U937 foam cells. _Bostann Journal of Basic Medical Science 12_(2), 94–101.

Wang, Z., & Patel, D.J. (2014). Small molecule epigenetic inhibitors targeted to histone lysine methyltransferases and demethylases. _Quarterly Reviews of Biophysics 46_(4), 349–373. https://doi.org/10.1017/S0033583513000085.

Weber, C., Meiler, S., Döring, Y., Koch, M., Drechsler, M., Megens, R.T.A., ... Zernecke, A. (2011). CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. _The Journal of Clinical Investigations_(7), 121. https://doi.org/10.1172/JCI44925DS1.

Weber, C., & Noels, H. (2011). Atherosclerosis: current pathogenesis and therapeutic options. _Nature Medicine 17_(11), 1410–1422. https://doi.org/10.1038/nm.2538.

Westerterp, M., Bochem, A.E., Yvan-Charvet, L., Murphy, A.J., Wang, N., & Tall, A.R. (2014). ATP-binding cassette transporters, atherosclerosis, and inflammation. _Circulation Research 114_(1), 157–170. https://doi.org/10.1161/CIRCRESAHA.114.300738.

Westerterp, M., Gourion-Arsiquaud, S., Murphy, A.J., Shih, A., Cremers, S., Levine, R.L., ... Yvan-Charvet, L. (2012). Regulation of hematopoietic stem and progenitor cell mobilization by cholesterol efflux pathways. _Cell Stem Cell 11_(2), 195–206. https://doi.org/10.1016/j.stem.2012.04.024.

Whitman, S.C., Ravisankar, P., Elam, H., & Daugherty, A. (2000). Exogenous interferon-γ enhances atherosclerosis in apolipoprotein E−/− Mice. _The American Journal of Pathology 157_(6), 1819–1824 https://doi.org/10.1016/S0002-9440(10)64820-1.

Winkels, H., Meiler, S., Lievens, D., Engel, D., Spitz, C., Bürger, C., ... Gerdes, N. (2017). CD27 co-stimulation increases the abundance of regulatory T cells and reduces atherosclerosis in hyperlipidaemic mice. _European Heart Journal 38_(48), 3590–3599. https://doi.org/10.1093/eurheartj/ehx517.

Woudberg, N.J., Pedretti, S., Lecour, S., Schulz, R., Vuilleumier, N., James, R.W., & Frias, M.A. (2018). Pharmacological intervention to modulate HDL: What do we target? _Frontiers in Pharmacology 8_, 1–16. https://doi.org/10.3389/fphar.2017.00989.

Yamashita, T., Sasaki, N., Kasahara, K., & Hirata, K. i. (2015). Anti-inflammatory and immune-modulatory therapies for preventing atherosclerotic cardiovascular disease. _Journal of Cardiology 66_(1), 1–8. https://doi.org/10.1016/j.jjcc.2015.02.002.

Yang, C., McDonald, J.G., Patel, A., Zhang, Y., Umetani, M., Xu, F., ... Hobbs, H.H. (2006). Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. _Journal of Biological Chemistry 281_(38), 27816–27826. https://doi.org/10.1074/jbc.M603781200.

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., & Lisheng, L. (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case control study. _The Lancet 364_(9438), 937–952.

Yvan-Charvet, L., Pagler, T., Gautier, E.L., Avagyan, S., Siry, R.L., Han, S., ... Tall, A.R. (2010). ATP-binding cassette transporters and HDL supress hematopoietic stem cell proliferation. _Science 328_(5986), 1689–1693. https://doi.org/10.1126/science.1189731.

Zaina, S., Heyn, H., Carmona, F.J., Varol, N., Sayols, S., Condom, E., & Esteller, M. (2014). DNA methylation map of human atherosclerosis. _Circulation: Cardiovascular Genetics 7_(5), 692–700. https://doi.org/10.1161/CIRCGENETICS.113.000441.

Zhu, J., Quyyumi, A.A., Rott, D., Csako, G., Wu, H., Halcox, J., & Epstein, S.E. (2001). Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease. _Circulation 103_(8), 1071 LP–1075 Retrieved from http://circ.ahajournals.org/content/103/8/1071.abstract.