Atherosclerosis: the interplay between lipids and immune cells

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Purpose of review

Cardiovascular disease is the leading cause of mortality worldwide. The underlying cause of the majority of cardiovascular disease is atherosclerosis. In the past, atherosclerosis was considered to be the result of passive lipid accumulation in the vessel wall. However, today's picture of the pathogenesis of atherosclerosis is much more complex, with a key role for immune cells and inflammation in conjunction with hyperlipidemia, especially elevated (modified) LDL levels. Knowledge on immune cells and immune responses in atherosclerosis has progressed tremendously over the past decades, and the same is true for the role of lipid metabolism and the different lipid components. However, it is largely unknown how lipids and the immune system interact. In this review, we will describe the effect of lipids on immune cell development and function, and the effects of immune cells on lipid metabolism.

Recent findings

Recently, novel data have emerged that show that immune cells are affected, and behave differently in a hyperlipidemic environment. Moreover, immune cells have reported to be able to affect lipid metabolism.

Summary

In this review, we will summarize the latest findings on the interactions between lipids and the immune system, and we will discuss the potential consequences of these novel insights for future therapies for atherosclerosis.

Keywords

atherosclerosis, immune system, lipids

Key points

- Dyslipidemia affects the adaptive immune response.
- T cells specific for modified lipoproteins aggravate atherosclerosis.
- The adaptive immune response modulates lipoprotein metabolism.
- Immune responses and lipid metabolism interact in a unique metabolic pathway underlying atherosclerosis.

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, accounting for 16.7 million deaths each year. The underlying cause of the majority of CVD is atherosclerosis, a disease that is characterized by the formation of lipid and (immune)-cell containing plaques in the intima of large and mid-sized arteries *(1)*. In the past decades, it was found that the immune system plays a crucial role in the development and progression of atherosclerotic plaques. By transforming immune cells into proinflammatory and anti-inflammatory chemokine and cytokine producing units, and by guiding the interactions between the different immune cells, the immune system decisively influences the propensity of a given plaque to rupture and cause clinical symptoms like myocardial infarction and stroke *(1–3)*. Although knowledge on immune cells and immune responses has progressed tremendously over the past decades, and has provided novel insights for many diseases, including atherosclerosis, it has become clear that atherosclerosis is not a 'standard' immunological disease. Recently, novel data have emerged that show that immune cells are affected, and behave differently in a hyperlipidemic environment. In this review, we will summarize the current knowledge on the effects of hyperlipidemia, and especially hypercholesterolemia and the effects of modified LDL, on immune cell development and function. The way the mature immune system reacts to challenges such as inflammation is largely defined by the self-renewal and multilineage capacity of a rare population of hematopoietic stem and progenitor cells (HSPCs), defined as $Lin^{-}Sca^{+}cKit^{+}$ cells, in the bone marrow *(4, 5)*. These HSPCs differentiate into leukocytes (including lymphocytes), dendritic cells, erythrocytes, and platelets, but also to endothelial progenitor cells. The self-renewal capacity and differentiation of HSPCs into mature blood cell lineages and the subset distribution of these separate blood lineages are tightly regulated by a combination of intrinsic and extrinsic signals such as growth factors, chemokines, and cell cycle proteins *(6)*. HSPCs are located in specialized microenvironments: the bone marrow niche. The bone marrow niche is composed of many cell types, including mesenchymal stem cells, CXCL12-abundant reticular (CAR) cells, osteoclasts, osteoblasts, adipocytes, and endothelial cells. The niche plays a critical role in the regulation of HSPC self-renewal, quiescence, and differentiation during hematopoiesis *(7)*. HSPCs are also activated during immunological challenges to replenish exhausted immune effector cells. These HSPC responses include expansion, mobilization, and differentiation and are regulated by systemic (cytokines, chemokines) and local, nichederived signals (chemokines, growth factors) *(6, 8, 9)*. In hypercholesterolemic ApoE $^{-/-}$ and $Ldlr^{-/-}$ mice, hyperlipidemia induces a substantial increase in the number of HSPCs, and a preference for myeloid skewing, which results in aggravated atherosclerosis *(10–12)*. Hypercholesterolemia causes HSPCs to lose their quiescence, characterized by increased proliferation and expression of cell cycle proteins such as cyclin B1, C1, and D1 *(10)*. Moreover, when hypercholesterolemia-primed HSPCs are transplanted into normocholesterolemic mice, they maintain their proliferative capacity and their preference to differentiate towards the myeloid lineage, resulting in monocytosis and granulocytosis *(10,*

11). Likewise, when normal HSPCs are transplanted into hypercholesterolemic mice, which contain a hypercholesterolemia-primed bone marrow niche, similar results were observed, proving that priming of either the HSPC itself or the bone marrow niche induces these effects *(10)*. Surprisingly, hypercholesterolemia also induces extramedullary hematopoiesis, and increased myelopoiesis was also found in the spleen *(13)*. Surprisingly, important mediators of HSPC biology are lipid mediators. For example, a predominant role for the cholesterol efflux pathways (ATP binding cassette transporters A1 and G1) and HDL was found for the maintenance of HSPC quiescence. Absence of ABCA1 and G1 induces HSPC mobilization, proliferation, and differentiation towards the myeloid lineage, and results in extramedullary hematopoiesis, revealing that disruption of cholesterol-efflux mechanisms play a major role in HSPC biology *(14)*. Moreover, this process is also mediated via proteoglycan bound apolipoprotein E that promotes cholesterol efflux via ABCA1 and ABCG1, thereby inhibiting HSPC proliferation *(11)*.

Lipids and innate immune responses

Monocytes

As described above, increased numbers of circulating monocytes are found in patients suffering from hyperlipidemia and atherosclerosis, as well as in experimental animal models of atherosclerosis, and are correlated with plaque size and plaque stage *(15–18)*. Monocytes can be divided into inflammatory monocytes (characterized by the expression of $CD14^{++}CD16^{-}$ or $CD14^{++}CD16^{+}$ in humans and $Ly6C^{high}$ in mice) and patrolling monocytes (which are $CD14^{+}CD16^{++}$ in humans and $Ly6C^{low}$ in mice) *(19)*. Hypercholesterolemia causes a profound increase in $Ly6C^{high}$ and $CD14^{++}$ monocytes, which is partly generated via extramedullary hematopoiesis *(10, 16–18, 20)*. Their fate to differentiate towards $Ly6C^{high}$ monocytes is driven by high cholesterol levels, as competitive bone marrow transplantation studies show that hypercholesterolemia-primed HSPC or a hypercholesterolemia primed bone marrow niche results in an increased fraction of $Ly6C^{high}$ monocytes *(10)*. Increasing evidence points out that the $Ly6C^{high}/CD14^{++}CD16^{-}$ monocytes do not only increase in number, they are also the monocyte subset that preferentially adheres to the endothelium, infiltrates the arterial wall, and is responsible for the generation of plaque macrophages. The role for the patrolling, $Ly6C^{low}/ CD14^{+}CD16^{+/+}$ monocytes is less clear. They are longer-lived, scan the endothelium for activation markers, and pathogens are able to phagocytose oxidative lipids, but do not seem to infiltrate atherosclerotic plaques *(17, 18)*.

Macrophages

Once monocytes have infiltrated the arterial wall, they differentiate into macrophages and become a key component of the atherosclerotic plaque. When exposed to a hyperlipidemic milieu, macrophages ingest and process (modified) lipids, predominantly modified LDL, which is a complex mixture of oxidation products and proteins, and store it in lipid droplets in their cytoplasm *(1)*. The uptake of LDL is mainly mediated by scavenger receptors such as

scavenger receptor A and CD36, and the efflux is mediated by ABC transporters, in particular, ABCA1 and G1 *(21)*. When lipid uptake exceeds efflux, or efflux is disturbed, lipids accumulate and macrophages become 'foam cells'. Initially, lipid uptake including oxLDL and phospholipids results in the activation of macrophages via pathogen associated molecular patterns (PAMPs) or danger associated molecular patterns (DAMPS), predominantly using TLR2 and TLR4, resulting in the release of a myriad of proinflammatory (i.e. interleukin-1 (IL-1), IL-6, IL-12, IL-15, IL-18, TNF-a, MCP-1) and anti-inflammatory (i.e. IL-10, TGF-b) cytokines and growth factors, thereby initiating/enhancing an inflammatory response which further regulates immune cell infiltration into the atherosclerotic lesion *(1, 22)*. Although many studies have found that proinflammatory cytokines prevail upon lipid loading *(1, 22)*, others claim that macrophage foam cell formation is associated with an anti-inflammatory response. Spann et al. *(23)* found that desmosterol plays a key role in the homeostatic response of peritoneal macrophages upon lipid loading, including activation of LXR target genes, inhibition of SREBP target genes, and suppression of inflammatory response genes. These results imply that macrophage activation in atherosclerosis results from extrinsic stimuli such as (lipid) debris, and inflammatory mediators derived from other cell types in the arterial wall. However, the stage of foam cell formation and the environment may also exert differential roles in macrophage activation. After massive uptake of lipids, cholesterol crystals can form in the macrophage foam cells. This crystalline material, but also the increased oxidative stress can lead to the formation of an inflammasome complex in macrophages. Inflammasome formation leads to activation of caspase-1 that rapidly cleaves pro-IL1b and pro-IL18 into their mature forms, which are both pathogenic inflammatory cytokines that drive atherosclerosis. Cholesterol crystals induce the nlrp3 inflammasome, which has been found to play a major role in atherosclerosis *(24)*. Within the atherosclerotic lesion, macrophages are exposed to sustained inflammation and oxidative stress, resulting in activation of endoplasmic reticulum stress pathways resulting in macrophage apoptosis and necrosis. The unfolded protein response (UPR), with factors like CCAAT-enhancerbinding protein homologous protein, $Ca2^{+}$/calmodulin-dependent protein kinase II, signal transducer and activator of transcription 1, and nitric oxides, play a major role in this process. Necrosis and apoptosis, and the subsequent defective efferocytosis of macrophage cell-rich and lipid-rich, sometimes crystalline, debris results in the formation of a necrotic lipid core and sustained atherosclerotic plaque inflammation *(25)*.

Lipids and adaptive immune responses

In addition to innate immune responses, hyperlipidemia can also trigger adaptive immune responses as illustrated by the presence of activated T cells in the atherosclerotic lesion as well as by the T-cell dependent induction of antibody production by B cells towards modified LDL *(1)*. Adaptive immune responses are initiated through antigen presentation, and in atherosclerosis, the antigen is often considered to be a (neo-)epitope of modified LDL.

Another proof that modified LDL drives adaptive immune responses is the existence of oxLDL specific T cells in experimental animal models and in patients *(1)*.

Dendritic cells

Although part of the innate immune system, dendritic cells have an important role in initiating the adaptive immune response towards atherosclerosis related antigens. In addition, dendritic cells also take up lipids (via scavenger receptors and efferocytosis) and form foam cells, contributing to atherosclerotic lesion development *(26–28)*. As they share phenotypic and functional properties with macrophages, which attain a dendritic cell-like phenotype upon foam cell formation *(29)*, it is complicated to dissect the role of dendritic cells and macrophages in the lesion. The uptake of oxLDL results in dendritic cells maturation, migration, and antigen presentation to T cells in the draining lymph nodes *(30, 31)*. On the other hand, oxidized phospholipids can impair maturation of dendritic cells *(32)*, possibly limiting excessive dendritic cell Interplay between lipids and immune activation *(32)*, and desmosterol may induce an anti-inflammatory response via LXR activation *(23, 33)*. Interestingly, circulating cholesterol levels and dendritic cells numbers do correlate as shown in DC-hBcl2 mice, which express the antiapoptotic Bcl-2 under the CD11c promoter *(34)*. Conversely, reduced levels of dendritic cells in ApoE$^{-/-}$ mice result in enhanced systemic cholesterol levels Conventional dendritic cells at the crossroads between immunity and cholesterol homeostasis in atherosclerosis *(34)*, whereas lesional lipid accumulation decreases *(27)*. The mechanisms behind this effect of dendritic cells on cholesterol metabolism have yet to be identified. Dendritic cells have been frequently used to modify the outcome of atherosclerosis. Adoptive transfer of dendritic cells pulsed with modified LDL into atherogenic mice may aggravate atherosclerosis via activation of T cells *(35)*, but may also inhibit atherosclerosis via the induction of antibodies specific for modified LDL. In addition adoptive transfer of tolerogenic dendritic cells (ApoB100-pulsed) can protect against atherosclerosis *(36)*. Similarly, transfer of oxLDL-induced apoptotic dendritic cells may form a novel therapy for both initial and advanced atherosclerosis since it induces tolerogenic dendritic cells, enhances regulatory T cells (Treg) numbers, and reduces inflammatory monocyte responses. T cells Initial reports on the presence of T cells in human atherosclerotic lesions initiated research into the role of T cells in atherosclerosis. T cells can differentiate into various subsets of T cells and a main driving force into their activation is the presentation of antigen by dendritic cells within the lymph nodes and their reactivation within the atherosclerotic lesion by the interaction of effector T cells with macrophages presenting antigen, which often is a (neo)-epitope of (modified) LDL.

Th1 cells

The predominant type of CD4^{+} T cells *(37–39)* within the atherosclerotic lesion is the Th1 cell. Th1 cells produce a plethora of proinflammatory cytokines (e.g. TNF-a, IFN-g, IL-2, and IL-12) and express the transcription factor T-bet. IFN-g promotes vascular inflammation by activating antigen-presenting cells (APCs), enhancing their lipid uptake, reducing collagen

production by smooth muscle cells, and enhancing leukocyte recruitment *(40–43)*. Th1 cells have been suggested to drive antigen specific immune responses in the atherosclerotic lesion and both oxidized LDL and heat shock proteins have been suggested as antigens *(44, 45)*. Initial reports show that oxLDL is recognized by T cells in human lesions, while in addition the adoptive transfer of T cells from atherogenic mice aggravates atherosclerosis. In line with the observation that these $CD4^+$ T cells recognize oxLDL in a human leukocyte antigen-antigen D related restricted manner, Paulsson et al. *(46, 47)* described the oligoclonal expansion of T cells in atherosclerotic lesions indicating the response towards modified LDL. More recent data identify T cells expressing TRBV31 that react to native LDL and ApoB100, possibly more than to modified LDL *(48)*. Interestingly next to antigen specific activation, hyperlipidemia may lead to lipid accumulation in T cells and activation of LXR leading to a decrease in the production of proinflammatory cytokines *(49)*.

Th2 cells

Th2 cells are known for their B cell help and the presence of immunoglobulin (Ig)G that recognizes native and modified LDL implies that T cells actively support isotype switching from IgM to IgG in B cells *(50)*. Isotype switching is also dependent on the co-stimulatory IL-4 and OX40-OX40L pathway and blockade of this pathway reduces atherosclerosis *(51)*. The importance of this pathway is demonstrated by the identification of immune response network associated with blood lipid levels *(52)*. This network shows a gene module, the lipid leukocyte module, which is replicated in T cells. Genetic variation driving lipid leukocyte module expression associate with serum IgE levels, which relate to mast cell activity. Lesional mast cell numbers and their activity are strongly related to the complexity of lesions and the outcome of CVD *(53)*. In addition to their effect on isotype switching, Th2 cells produce IL-5 and IL-13. IL-5 has been shown to be anti-atherogenic by promoting the development of B-1 cells that produce protective IgM antibodies, resulting in reduced atherosclerosis *(54)*. Recently anti-IL-5 autoantibodies have been shown to be associated with human atherosclerosis *(55)*. IL-13 has also been shown to reduce atherosclerotic lesion development by skewing macrophages towards an M2 phenotype *(56)*. Tregs Tregs are regulators of immune responses and their main function is inhibition of self-reactive T cells in the periphery, but Tregs have also a distinct effect on hyperlipidemia. Low levels of Tregs are associated with increased risk for myocardial infarction *(57)* and coronary syndromes *(58)* and during murine atherosclerosis Treg numbers significantly decrease with lesion progression *(59, 60)*. Interestingly, oxLDL negatively affects the suppressive capacity of Tregs *(59, 60)*. On the other hand, Tregs specific for oxLDL or peptides derived from apoB100 can be induced via oral or nasal tolerance induction and these induced Tregs inhibit lesion formation and progression. Overexpression of IL-10, a hallmark cytokine of Tregs reduces VLDL and LDL levels in serum of $LDLr^{-/-}$ mice *(61)*. Recent studies have revealed a direct role for Tregs in cholesterol metabolism because depletion of Tregs using DEREG mice significantly increases atherosclerosis associated with a 1.7-fold increase in plasma cholesterol levels. More specifically, VLDL levels were increased because the clearance of

VLDL and chylomicron remnants was inhibited in the absence of Tregs. They found reduced expression of sortilin-1 in the liver and increased plasma enzyme activity of lipoprotein lipase, hepatic lipase, and phospholipid transfer protein in Treg-depleted mice. In addition, Treg expansion in a regression model of atherosclerosis significantly reduced cholesterol levels when compared with control mice *(62)*.

B cells

Mature B cells can be categorized into B1 cells and B2 cells. The former are innate T-cell independent B cells capable of producing natural IgM antibodies. Conventional B2 cells are T-cell dependent and are important in adaptive immunity by production of specific IgG antibodies to their cognate antigen. OxLDL is highly immunogenic and anti-oxLDL antibodies can be detected in atherosclerotic plaques as well as in the circulation of mice and men. OxLDL-specific IgM titers, produced by natural antibodies, are associated with protection against atherosclerosis *(54)*. In experimental animal models, this protective role of natural anti-oxLDL antibodies produced by B1 cells was found to be mediated by IL-5 *(63)*. In contrast to B1 cells, B2 cells, T-cell-dependent antibody-producing cells, promote atherosclerosis, which is in line with the aforementioned role of the co-stimulatory OX40-OX40L pathway and the role of Th2 cells *(51)*. When B2 cells are depleted using anti-CD20, atherosclerosis decreases, and when B2 cells are transferred to atherosclerotic mice, atherosclerosis increases *(64)*. This also is consistent with the observation that anti-oxLDL IgG antibodies, derived from B2 cells correlate with the presence of CVD (54, 65).

Lipid-induced epigenetic changes in immune cells

In the past decade, it has become increasingly clear that many epigenetic pathways govern differentiation and activation patterns of immune cells. It was found that chromatin modifying enzymes, such as histone deacetylases (HDACs) or histone methyltransferases (HMTs) can modify lipid metabolism and inflammatory responses of macrophages upon oxLDL exposure *(66)*. For example, myeloid specific deletion of HDAC3 results in an anti-inflammatory macrophage phenotype that produces high amounts of TGF-b and thereby induces collagen production by SMCs and fibrous cap formation in an in-vivo atherosclerosis model *(67)*. Interestingly, when HSPCs, monocytes, and macrophages are exposed to oxLDL or hypercholesterolemia in vivo *(10, 68)*, differentiated macrophages exhibit a higher inflammatory status than the unexposed control groups. This 'trained immunity' response could be reversed by pretreatment with the methyltransferase inhibitor methylthioadenosine, suggesting an important role for epigenetic histone modifications in this process *(68)*. Also other epigenetic modulators such as micro-RNAs *(69)* and long non-coding RNAs *(70)* can be modulated upon lipid loading in monocytes/macrophages. Similar findings have been reported for other immune cells, such as T cells *(71)*, suggesting that lipid challenges induce epigenetic changes in immune cells that can mediate their differentiation, polarization or activation status, and thereby affect atherosclerosis (see also: Heijmans et al.,

The multifaceted interplay between circulating lipids and epigenetics. Curr Opin Lipidol, pp. 288–294).

Conclusion

In this review, we have outlined the current knowledge on how hyperlipidemia, and especially modified LDL, affects the immune system, and vice versa. As has become clear during the last decades, the immune system reacts to lipids and lipid modifiers, which drives the progression of atherosclerosis. However, although we know that both lipids and the immune system are major determinants of atherosclerosis, the majority of the mechanisms and pathways mediating the crosstalk between lipids and immune cells have still not been identified. However, understanding how the immune system is regulated in hyperlipidemic conditions and in the different stages of atherosclerosis, and how immune cells regulate lipid metabolism is of utmost importance to identify potential therapeutic targets to prevent or stabilize the disease process. During the last decades, treatment of atherosclerosis was predominantly focused on lipid lowering. Although our insights in cholesterol metabolism and the development of lipid lowering drugs, in particular 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins), has helped to lower the incidence of CVD, a substantial part of the population still suffers from CVD notwithstanding optimal lipid-modulating therapy *(72)*. Therefore, next to lipid lowering strategies, developing and testing of new anti-inflammatory protocols is needed in the future therapeutic approach of atherosclerosis. It may be anticipated that specific immunomodulatory therapies may not only correct derailed immune responses, but also correct dyslipidemia. Current therapies using statins effectively lower plasma LDL cholesterol but are also reported to have an anti-inflammatory effect, such as reducing intimal inflammation, lowering the lesional macrophage content *(73)*. From a scientific viewpoint it is interesting to dissect whether the anti-inflammatory effects of statins are the consequence of their lipid lowering effect or the consequence of, for example, inhibiting the mevalonate pathway. In this respect it will be of major interest to determine the effect of the strong lipid-lowering anti-PCSK9 treatment on the inflammatory status of patients. At present, various clinical trials are ongoing that directly focus on inhibiting the low-grade inflammation in CVD patients, which is illustrated by enhanced levels of IL-1, IL-6, and high sensitivity C reactive protein (hsCRP) *(1)*. The strategies include the use of a low dose of methotrexate or colchicine, comparable to the approach taken for the treatment of, for example, rheumatoid arthritis *(74)*. Currently the largest trial, the CANTOS trial (trial.gov: NCT01327846), is focusing on blocking IL-1b using canakinumab *(75)*. Experimental mouse data have shown that IL-1b blockade does diminish atherosclerosis and mechanistically it may be that reduction in IL-1b leads to lower IL-6 and hsCRP levels *(76)*. It is anticipated that once we understand the interactions between lipids and the immune system, the current anti-inflammatory approaches to address the low-grade inflammation will be combined with lipid lowering approaches to provide optimal treatment regimens for atherosclerosis.

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Conflicts of interest

There are no conflicts of interest.

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