Review paper

How macrophage phenotypes affect atherosclerotic plaque

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Abstract

Atherosclerosis is a chronic inflammatory pathology that raises the probability of cardiovascular events. Immune cells play a substantial role in the progression and regression of the disease, with macrophages being one of the most active types causing atherosclerotic lesions. Recent research on the role of macrophages in atherosclerosis has revealed that macrophages can act both to promote and impede plaque growth and calcification. Moreover, macrophages show flexibility in their phenotype. In response to various microenvironmental stimuli, macrophages alter their phenotype to a pro- or anti- inflammatory state. This, in turn, determines plaque stability which furthers either disease progression orregression. This article incorporates information about macrophage polarization, diverse subtypes as well as their markers, and their impact on lesions. Knowing macrophage precursors and characteristic features is essential for understanding their diverse impacts on atherosclerotic plaques and for the development of new therapeutic approaches.

Keywords
Atherosclerosis, Macrophages, Phenotypic polarization, Cytokines, Inflammatory mediators, Anti-inflammatory agents, Immunotherapy, Foam cells, Monocytes, Smooth muscle cells

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Introduction

Atherosclerosis is a significant cause of mortality and morbidity worldwide (1). Around 17.9 million deaths are due to cardiovascular diseases (CVD) in the world each year (2). An estimated 200 million people worldwide are affected with atherosclerosis, which is associated with premature CVD (3). The pathology starts when the endothelium of an artery gets damaged by physical exposure to low-density lipoproteins (LDL), toxins, or high-pressure blood. Specifically, abnormalities in the way artery walls develop are called lesions. They are often located at arterial branching points and bends because they are more likely to be exposed to local endothelial cell damage and dysfunction. Then, LDL accumulate in the intimal layer of a blood vessel.

Stored lipoproteins can be modified *via* several mechanisms, such as oxidation, enzymatic processing, desialylation, and aggregation (4). Altered lipoproteins are pro-inflammatory and activate surrounding endothelial cells, which secrete signaling molecules called chemokines and cytokines. Chemokines recruit monocytes and T-helper cells into the intimal and subintimal space of the artery, where cytokines promote differentiation of white blood cells into macrophages (5). Macrophages actively phagocytose modified LDL and become "foam cells" (6). Hence, foam cell formation positively correlates with levels of LDL. Statins are often used to lower LDL concentrations and consequently impede foam cell formation. Although the engulfment of lipoproteins by macrophages might seem beneficial, "foam cells" exacerbate inflammation through the secretion of proinflammatory mediators, such as cytokines and reactive oxygen species (ROS), and through their eventual death by necrosis or apoptosis (4). Therefore, "foam cells" are one of the main components of atherosclerotic plaques. The progression of atherosclerosis can finally result in plaque rupture and thrombosis. This is when an artery becomes blocked by the contents of a plaque. Thrombosis may lead to ischemia and myocardial infarction, a significant cause of death.

Certain microenvironmental conditions determine macrophage polarization towards distinct phenotypes. Initially, the classification of macrophage phenotypes included classically activated (M1) and alternatively activated (M2) macrophages. Inflammatory cytokines and bacterial components can induce macrophage polarization towards M1 phenotype. This phenotype is responsible for destroying microbes, eliminating tumor cells, and evoking an adaptive immune response. M2 activation occurs in response to stimulation with anti-inflammatory cytokines. Those macrophages are involved in long-term tissue repair and tumor growth and exert antiparasitic effects. Classification only between proinflammatory M1 and anti-inflammatory M2 phenotype is now considered an oversimplification, and other phenotypes have been described, including M(Hb), Mhem, Mox, and M4.

Moreover, it has been shown that plaque macrophages show remarkable plasticity and can undergo modification depending on the microenvironment along a continuous spectrum, with M1 and M2 macrophage phenotypes being the extremes. Thus, plaque stability and the probability of a cardiovascular event are affected by macrophage phenotypes, distribution, and proportion in a plaque. In consideration of all this, it is reasonable to claim that macrophages play a significant role in atherosclerosis progression and regression.

Where do macrophages come from?

Monocyte-derived macrophages

Monocytes make up from 2 to 10 percent of immune cells in the human body and have diverse roles in immune system functioning. They are mainly derived from the bone marrow and spleen and are released into the blood circulation for surveillance (7). In the event of inflammation, monocytes are recruited into the damaged tissue, where they phagocytose other cells or toxic molecules, including oxidized LDL, secrete proinflammatory cytokines, differentiate into macrophages or inflammatory dendritic cells, and form foam cells (8). At least three distinct monocyte subsets in human blood have been identified based on makers on their cell surface. Classical CD14++CD16− monocytes express high cluster of differentiation 14 (CD14) but no CD16 and account for 90% of all circulating monocytes. This subtype is involved in immune cell recruitment into tissue, maintenance of the vasculature, macrophage cell survival, and localization within plaques. Non-classical CD14+CD16++ monocytes with low CD14 and high CD16 expressions are less recruited in a tissue. Intermediate CD14++CD16+ monocytes with high CD14 and low CD16 expressions are primarily derived from the bone marrow. Recent research has concluded that monocytes with CD16 expression, including both non-classical and intermediate types, increase CVD risk (9). Moreover, the number of CD14++CD16+ correlates positively with the atherogenic lipids concentration and negatively with levels of anti- atherogenic high density lipoproteins (HDL) (8). In mice, two monocyte subsets are present: inflammatory Ly6Chigh monocytes and resident Ly6Clow monocytes, which are comparable to human classical and non-classical monocytes, respectively (8).

In atherosclerosis, the level of monocytes in the blood strongly correlates with disease progression. As the endothelial layer gets damaged and inflamed, different microenvironmental stimuli, for instance, lipoproteins, accelerate the production of monocytes in the bone marrow and their influx to subendothelial space. Once monocytes arrive at the tissues, they require colony-stimulating factors (CSF-1 and M-CSF) and other factors, such as interleukin (IL)-34, for differentiation into monocyte-derived macrophages and increase the survival and maintenance of this macrophage population (10). Recently, it has been discovered that CSF-1 from local smooth muscle and endothelial cells supports plaque macrophage survival (11). Additionally, rapid adaptation and differentiation of monocytes in plaques require CSF-1 receptor-mediated signaling in aortic stromal cells and macrophages.

Based on monocyte subsets from which macrophages originate, M1 and M2 macrophage phenotype functions vary. For instance, macrophages derived from classical monocytes show higher phagocytic ability than those that originate from monocytes with CD16 expression (9).

SMC-derived macrophages

Recent studies have demonstrated that not all foam cells are produced from monocyte-derived macrophages. Foam cells can also be derived from intima smooth muscle cells (SMCs). Furthermore, co-staining with SMC and foam cell-specific markers has demonstrated that about half of foam cells in human atherosclerotic lesions are SMC-derived (12). It has been concluded that SMCs can convert and transdifferentiate into macrophage-like cells and consequently into foam cells under

certain microenvironmental stimuli in plaques such as oxidized lipids, transforming growth factor-β (TGF-β), and other cytokines (13). SMC-derived foam cells share relatively non-inflammatory gene expression profiles with more classical macrophage foam cells. Under the effect of platelet-derived growth factor beta (PDGFβ) signaling, SMCs can lose their contractile phenotype and transdifferentiate into cells that produce extracellular matrices and carry a reparative function: healing and stabilizing the artery wall (14). These SMCs participate in the thickening and stabilization of a fibrous cap in atherosclerotic lesions. Moreover, SMCs are one ofthe first cell types to maintain lipoproteins in atherosclerosis progression (15).

Recently, it has been suggested that although some SMC-derived macrophage cells continue to be macrophage-like cells in the advanced plaques, most do not continue to express macrophage markers at the late stages. Instead, some SMC-derived macrophage-like cells contribute to cells expressing fibroblast or pericyte markers in atherosclerotic plaques (16). That same study demonstrated that macrophage-like cells derived from SMCs have the potential to express markers of various cell lineages, as opposed to monocyte-derived macrophages that are terminally differentiated. Thus, SMC-derived macrophages can show plasticity during the progression of atherosclerosis. Li et al. have found that SMC-derived macrophage-like cells can revert to smooth muscle cells in fibrous caps of advanced plaques (16). In other words, the study demonstrated that macrophages and smooth muscle cells exist in an “equilibrium” in atherosclerotic lesions. Where a cell is on a differentiation spectrum from SMC to macrophage determines its role in atherosclerosis progression or regression. Furthermore, it has been found that SMCs phenotype switching to macrophage-like cells is dependent on KLF4 and is inhibited by miR-145/143 (17). Targeting the smooth muscle-macrophage communication was suggested to be a novel therapeutic approach (18). Interestingly, SMCs differentiation to macrophages may play a dual role in the disease course since not only macrophages but also SMCs can exert both anti- or pro- inflammatory effects depending on their microenvironment.

On the one hand, SMCs can contribute to atherosclerosis progression. For example, in a recent study, Dubland et. al. reported that SMCs show impeded expression of ABCA1 as well as reduced accumulation of free cholesterol following aggregated LDL. Considering that upregulation of ATP-binding cassette transporter A1 (ABCA1) impedes foam cell formation through HDL synthesis, SMCs lacking this important feature may foster atherosclerosis progression (19). The same study suggests that although SMCs endocytose excess of atherogenic lipoproteins, they lack mechanisms to process the lipids in a manner similar to macrophages consequently comprising a large proportion of potentially regression-resistant cholesterol that accumulates in atherosclerotic plaque (20).

On the other hand, smooth muscle cells are essential in exerting antiatherogenic effects as they produce extracellular matrix that stabilizes the plaque. Recent experiments have demonstrated that differentiation from SMCs to macrophages may alter the extracellular matrix by decreasing the expression of proteins, including collagen and elastin, and increasing the expression of metalloprotease MMP-9 and collagenase MMP-1 in cells. Therefore, differentiation of SMCs to macrophages may contribute to an increase in

plaque vulnerability. Statins are often used to impede the level of matrix metalloproteinases (MMPs), which are released by macrophages to degrade extracellular matrix produced by SMCs. For instance, statins are known to lower the expression of MMP-9 which contributes to inflammation and fibrosis in CVD.

However, due to current challenges in distinguishing between macrophage and SMC cell markers, the hypothesis that SMCs and SMC- derived foam cells contribute to plaque stabilization, while macrophages contribute toplaque vulnerability, may be incorrect. For instance, a recent study conducted by Allahverdian et al. demonstrated that most of the foam cells that express SMC-specific marker, SM α-actin, in advanced human coronary artery lesions also express macrophage marker CD68. Thus, in some cases, it may be challenging to identify whether such foam cells are SMC-derived or macrophage-derived (21).

It remains unclear to what extent foam cells are derived from SMCs in their expression phenotype and trajectory, and it is also still unclear whether SMC-derived macrophage-like cells adopt a stable or temporary fate during the progression of atherosclerosis. Single-cell experimental techniques and lineage tracking methods are expected to reveal more insights about SMC- derived macrophages.

Tissue-resident macrophages

Tissue-resident macrophages are present in all major organs, including arteries. They proliferate at low levels when the conditions are stable. However, tissue-resident macrophages show high proliferation when inflammation occurs (22). Tissue-resident macrophages can either develop from hematopoietic stem cells through blood monocyte intermediates and bone marrow-progenitor cells or self-renew by local proliferation of mature differentiated cells (23). The relative balance in atherosclerosis between macrophage self-proliferation and recruitment from blood is still partially unclear. While more tissue-resident macrophages are replenished by circulating LyC6high monocytes and less by Ly6Clow monocytes during homeostasis, some can exist independently of circulating monocytes (24). In atherosclerosis, newly formed lesions primarily recruit monocytes from the circulation, while advanced lesions rely more on local macrophage proliferation than on the new monocyte replenishment. Therefore, the balance between tissue-resident macrophage proliferation and recruitment from blood is crucial for disease progression.

Resident macrophages can alter their phenotype and carry various functions in response to different tissue microenvironments, including IL-4, IL-13, and M-CSF cytokine secretion, damage-associated molecular patterns, and pathogen-associated molecular patterns. Consequently, tissue-resident macrophages serve a dual role. They can inhibit tissue inflammation without adverse effects but rapidly alert the immune system if they encounter pathogens, signs of ischemia, or any other stress (25). Moreover, tissue-resident macrophages are involved in the organization of matrix metabolism, as they interact with fibroblasts that produce collagen and provide proteases that degrade the extracellular matrix (26).

Collectively, recent research confirms that knowing the difference between monocyte-derived, SMC-derived, and tissue-resident macrophages is essential to understand pathways of foam cells

formation, plaque accumulation, and the progression of atherosclerosis.

The most common macrophage phenotypes

Macrophage polarization is a process during which macrophages attain a phenotype with specific functions in response to microenvironmental stimuli. Theoretically, macrophage subtypes can be identified by their surface markers and chemokine receptor expression. However, some markers are identical among different macrophage subtypes, and only a few markers are specific for a given phenotype, which increases the complexity of the macrophage classification proposal (27). For that reason, macrophage phenotypes are generally defined based on both their surface markers and possible functions.

Initially, macrophage classification included classically activated (M1) and alternatively activated (M2) macrophages. M1 macrophages can be induced by the tumor necrosis factor-α (TNF-α), pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS), interferon-γ (IFN-γ), and toll-like receptor ligands (28). Classically activated macrophages eliminate tumor cells and microbes and present antigens to T cells to provoke an adaptive immune response. Moreover, M1 macrophages damage the tissue and produce proatherogenic substances. For instance, M1 macrophages release pro-inflammatory reactive nitrogen intermediates (RNI) and ROS which are involved in bacterial killing (29). In addition, classically activated macrophages express nitric oxide synthase (iNOS) to produce nitric oxide (NO) with the substrate L-arginine in response to inflammatory mediators. Generation of both ROS and NO results in tissue destruction and impaired wound healing (30). Furthermore, M1 macrophages secrete pro-inflammatory cytokines, including TNF-α, interleukin-6 (IL-6), and IL-1β, to resist infections and promote Th1 responses (4). Inflammatory cytokines recruit more immune cells, including macrophages, to the lesion site. Thus, more foam cells form by phagocytosis and eventually die to make up the plaque (31).

M2 macrophages are polarized in response to stimulation with IL-4, IL-10, IL-13, and IL-33 anti-inflammatory cytokines, immunocomplexes, collagen, and certain lipid products (32). These macrophages display scavenger, mannose, and galactose-type surface receptors responsible for debris clearance. M2 phenotype is immunosuppressive due to decreased antigen presentation to T cells and release of cytokines that stimulate a Th2 response. Moreover, alternatively activated macrophages express the enzyme Arginase 1 (ARG1), which hydrolyzes L-arginine to L-ornithine. L-ornithine is the precursor for polyamines which are crucial for cell survival and essential amino acids for collagen production, such as proline and hydroxyproline.

Thus, M2 macrophages play a significant role in atherosclerosis regression as they participate in long-term tissue repair, exert antiparasitic effects and favor tumor growth (4). In addition, it has been reported that M2 macrophages are less susceptible to transform into foam cells than M1 macrophages (33). Nevertheless, it is important to emphasize that alternatively activated macrophages are not solely antiatherogenic (34). For instance, engulfment of apoptotic cells results in macrophage polarization towards an M2-like phenotype, characterized by the secretion of anti-inflammatory mediators TGFβ and IL10 as well as prostaglandin E2 which can exert both pro- and anti- inflammatory effects, depending on relative EP receptor subtype expression (35).

Since ARG1 is a biomarker of alternatively activated macrophages and iNOS is a biomarker of classically activated macrophages, the iNOS to ARG1 ratio is often used to determine the M1 to M2 ratio. However, classification only between M1 and M2 macrophage phenotypes is now considered oversimplified, and other phenotypes have been described (Figure 1). For instance, Mox macrophages comprise about 30 % of plaque macrophages in hypercholesterolemic mice. Oxidized phospholipids, such as oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), activate macrophage gene expression pattern and induce polarization towards Mox subset (36). Mox gene expression resembles that of M1 more than M2, since there are more M1/Mox overlapping genes, which are mainly proinflammatory, such as IL-1β and cyclooxygenase-2 (COX-2) (37). Moreover, Mox macrophages show reduced phagocytic and chemotactic abilities compared with M1 and M2 macrophages, which contributes to the tissue damage and inflammation exacerbation (38).

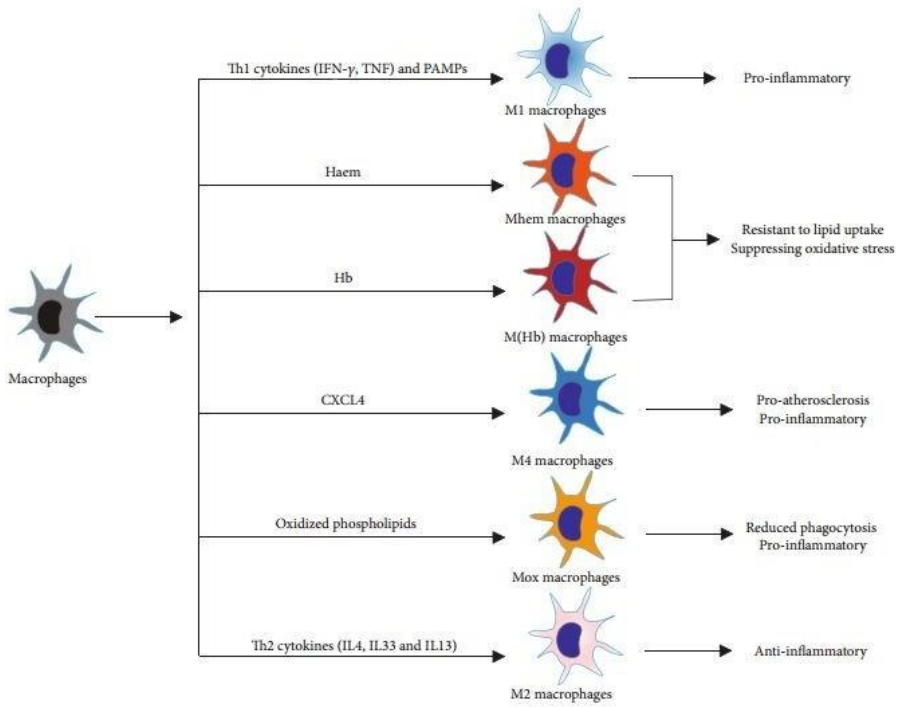

Figure 1: Macrophage subsets (30).

Therefore, Mox phenotype can be proatherogenic. On the other hand, Mox macrophages exclusively express genes for antioxidant enzymes, including heme oxygenase 1 (HO-1), sulfiredoxin-1 (Srxn 1), and thioredoxin reductase 1 (TrxR1), which are upregulated by nuclear factor erythroid 2-related factor 2 (Nrf2) (31). These enzymes prevent tissue damage from oxidative stress. Thus, Mox

macrophages are vital in redox regulation and antioxidant activity and may exert anti-inflammatory effects. Collectively, this data shows that the proinflammatory Mox phenotype can also have antiatherogenic effects.

In the event of intraplaque hemorrhage, which is a common complication linked to atherosclerosis progression, red blood cells lyse rapidly and release hemoglobin and free heme. When hemoglobin binds to plasma protein haptoglobin, a hemoglobin-haptoglobin complex forms. Free heme and hemoglobin directly polarize macrophages toward the Mhem phenotype. Mhem phenotype can phagocytose damaged erythrocytes and iron. Moreover, Mhem subtype has a low iron accumulation and is resistant to lipid uptake, thus, precluding foam cell formation (39). In addition, Mhem phenotype expresses high levels of CD163 scavenger receptor, which uptakes hemoglobin-haptoglobin complexes and consequently induces the release of anti-inflammatory IL10 and HO-1 (40). Due to the expression of HO-1 regulated by Nrf2 Mhem macrophages suppress oxidative stress (41).

Hemoglobin-haptoglobin complex induces macrophage polarization to the M(Hb). Similar to Mhem phenotype, M(Hb) subset shows increased levels of the scavenger receptors CD163 (the hemoglobin-haptoglobin complex receptor) and suppresses oxidative stress. M(Hb) macrophages show reduced levels of ROS and express low levels of intracellular iron due to upregulation of ferroportin (FPN). The M(Hb) subtype is also characterized by elevated levels of liver X receptor which induces cholesterol efflux. Importantly, M(Hb) phenotype can release both proinflammatory molecules, including IL-1β cytokine and vascular endothelial growth factor (VEGF), and anti-inflammatory factors, such as IL-10 and IL-1 receptor antagonist (42). Collectively, M(Hb) and Mhem macrophages can coexist in areas of neovascularization or hemorrhage and generally display atheroprotective properties, such as oxidative stress suppression and resistance to foam cell formation (43).

Gleissner et al. have subsequently identified a new macrophage phenotype. The M4 subset is induced by CXCL4 (platelet factor 4) and combines both the M1 and M2 characteristics. M4 macrophages are often found in advanced human atherosclerotic lesions and express exclusive markers metalloproteinase 7 (MMP 7) and calcium binding protein S100A8. CXCL4-induced phenotype is characterized by the absence of the hemoglobin-haptoglobin scavenger receptor CD163, required for hemoglobin clearance after plaque hemorrhage (44). Consequently, M4 subset expresses low levels of anti-inflammatory HO-1 when exposed to a hemoglobin-haptoglobincomplex.

Moreover, CXCL4-induced macrophages show low levels of CD36 and scavenger receptor-A, which are both responsible for the majority of modified LDL uptake (45). The M4 phenotype produces the pro-inflammatory cytokines TNF-α and IL-6 and expresses reduced phagocytic properties (44). Furthermore, M4 macrophages can be involved in complications of late atherosclerosis, including acute coronary syndrome and arterial thrombosis (38). In addition, CXCL4- induced phenotype produces the matrix metalloproteinase-12 (MMP12) enzyme, which is involved in the degradation of the fibrous cap, thus leading to plaque destabilization. Therefore, M4 subtype can be considered to be pro-atherogenic.

Macrophage plasticity and distribution in an atherosclerotic plaque

Recent investigations have demonstrated that macrophages are plastic cells and can modify their phenotype in response to microenvironmental stimuli along a continuous spectrum with M1 and M2 subsets as the extremes. Factors affecting macrophage polarization include cholesterol and lipid loading, inflammatory stimuli, and systemic factors, such as infection, dyslipidemia, and low-grade inflammation associated with diabetes or autoimmune diseases (46). However, whether macrophages can alter their phenotype dramatically after they have already attained one, for instance, from a pro-inflammatory to an atheroprotective subtype, remains controversial and unclear. Interestingly, it was reported that the polarization of CXCL-4-induced macrophages is irreversible (44).

A large number of studies has proved that different macrophage phenotypes are associated with a specific location within a plaque. For instance, classically activated macrophages are predominantly located in rupture-prone shoulder regions of a plaque. In contrast, the alternatively activated phenotype is more common in adventitia and stable regions of a plaque which contain many cells (47). Equal numbers of M1 and M2 macrophages are distributed in a fibrous cap of relatively stable plaques close to necrotic core (47). Furthermore, both M1 and M2 numbers rise through atherosclerosis progression. In advanced plaques, M1 macrophages are mainly located near the lipid core, while M2 macrophages are localizedin neo-angiogenic regions (regions where new blood vessels form from pre-existing vessels) (25). In mice, M2 macrophages are commonly found in early plaques, while M1 macrophages are abundant in advanced lesions (48). In atherosclerosis progression, M1 macrophages dominate in vulnerable plaques, whereas the polarization of macrophages to the M2 phenotype results in plaque stabilization and disease regression (25). Cho et al. studied the impact of M1 and M2 macrophages on plaque vulnerability in patients with carotid artery disease. They found that M1 macrophages are present in symptomatic plaques exclusively, while M2 macrophages are present in plaques of both symptomatic and asymptomatic patients. Collectively, the M1/M2 macrophage ratio and specific location in the lesion determine atherosclerotic plaque stability (49). When and where during the course of the disease, the M2 to M1 ratio should be increased remains to be investigated to improve current atherosclerosis treatment protocols.

In human lesions, Mhem and M(Hb) macrophages are present in iron-rich regions and regions of previous hemorrhage or angiogenesis (43). M4 phenotype is common in the intima and adventitia of a blood vessel and contributes to plaque instability (50). Mox macrophages were found to colocalize with oxidized phospholipids in plaques (51). In addition, Mox macrophages contribute to lesion development since they display low phagocytic and chemotactic abilities and upregulate VEGF.

Macrophage-targeting therapy and treatment

Atherosclerosis therapy is currently under a strong research focus. Nanoparticle-based therapy aimed to increase HDL levels may impede atherosclerotic plaque development by inhibiting lipid deposition (52). Nevertheless, the nanoparticle-based approach remains to be further investigated to access its effectiveness. In addition, cell therapy has shown promising results. However, due to limitations of the method, such as complexity requiring control of many variables and high cost,

there has been no breakthrough in cell therapy research in the past few years (53). Other successful and emerging therapeutic strategies include nanomedicine aimed at macrophages and stem cell transplantation (53).

There is a significant focus on atherosclerosis treatment research as well. Lipid-lowering drugs, such as statins, have been the primary option to reduce LDL levels in blood and treat atherosclerosis thus far (54). Statins work to inhibitthe activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase – an enzyme responsible for cholesterol synthesis in the body. Furthermore, it has been evidenced that statins can exert anti-atherosclerotic effects associated with macrophages. For instance, atorvastatin together with IL-4 treatment during macrophage differentiation may lead to elevated levels of M2 macrophages polarized via the peroxisome proliferator-activated receptor γ (PPARγ) pathway (55). Similarly, a recent study by Zhang et al. demonstrated that pravastatin enhances polarization toward an M2 phenotype (56). In addition, statins can suppress the CIITA gene transcription and consequently inhibit major histocompatibility complex class II receptor (MHC-II) expression by IFN-γ characteristic to M1-like macrophages (57). Kauerova et. al. investigated the influence of statin treatment on macrophage polarization in human adipose tissue and reported that statin therapy increases the proportion of M2 to M1 phenotypes in macrophages (58).

Nevertheless, statins may also contribute to atherosclerosis progression. As such, statin treatment during macrophage differentiation stage may enhance LPS-induced IL-1β and IL-6 proinflammatory cytokine secretion (57). Furthermore, statins have also been reported to exert proinflammatory effects by altering macrophage polarization. For instance, lovastatin treatment was found to inhibit M2-like polarization and favor that towards an M1 phenotype (59).

Collectively, recent studies demonstrated that all statins examined to date exert both pro- and anti-inflammatory immunomodulatory effects *in vitro*, with the exception of pravastatin. These contradictory findings are partially due to differences between animal and human models, differences in experimental design, and in various treatment regimens (57). Importantly, mechanisms following which statins affect macrophages have not been studied. Thus, the effect of statins on human macrophages remains to be elucidated.

Although statins have a significant effect on atherosclerotic lesions, a recent investigation has proved that the effect of statins is not sufficient for decreasing mortality associated with the disease. In a randomized, double-blind, placebo-controlled trial, patients between 40 and 85 years old with fasting LDL-C or non-HDL-C levels were treated with lipid-lowering drugs, preferably high-intensity statins (60). 10% of all patients in the investigation experienced a cardiac event during a median follow-up of 26 months. The results of the study demonstrate that many patients receiving lipid-lowering treatment still experience cardiovascular events. Hence, this data as well as other trials suggest that the effect of lipid-lowering therapy on atherosclerosis regression is limited. In fact, specific targets such as macrophage-mediated inflammation that can be reduced through targeting macrophage cytokines or promoting macrophage efferocytosis, emigration, and polarization to an anti-inflammatory phenotype may benefit atherosclerosis regression and have a more significant effect when combined with statins (61).

For instance, a recent randomized, double-blind trial was conducted on the effectiveness of the anti-inflammatory treatment with Canakinumab for atherosclerosis (62). Canakinumab is a therapeutic monoclonal antibody that targets an inflammatory cytokine IL-1β released by macrophage subsets promoting atherogenesis. The investigation included 10,061 patients with a C-reactive protein (CRP) level of 2 mg per liter or more who previously had myocardial infarction. The trial demonstrated that Canakinumab therapy targeting the interleukin-1β innate immunity pathway at a dose of 150 mg every 3 months significantly reduced the C-reactive protein level as well as the rate of recurrent cardiovascular events, independent of lipid-level lowering.

Metformin is a hypoglycemic drug which plays a protective role in diabetes-related CVD and potential CVD in patients without diabetes. Metformin was suggested to improve macrophage atheroprotective functions in combination with other drugs, such as hypoglycemic agent-sodium glucose cotransporter 2 inhibitors (SGLT2i), statins, and anti-inflammatory drug IL-β inhibitors (63). Empagliflozin is one of the SGLT2i which induces polarization towards M2 phenotype in the liver and reduces levels of M1 macrophages and proinflammatory TNF-α in the blood. Moreover, empagliflozin inhibits resistance to insulin, promotes utilization of lipids and reduces obesity-related inflammation. Importantly, treatment of people with diabetes and an elevated risk of CVD with a combination of Metformin with empagliflozin and glucagon-like peptide-1 (GLP- 1) receptor agonists resulted in lower all-cause mortality and cardiovascular death. Collectively, combining Canakinumab and Metformin therapies may be more effective in treating CVD, in particular atherosclerosis. Further research is expected to demonstrate whether combining lipid-lowering drugs with therapy against macrophage proatherogenic effects is necessary.

Conclusion

Macrophage polarization to various phenotypes as well as subsets' plasticity provide prospects for new methods of treating atherosclerosis, the primary precursor of cardiovascular events. Macrophage subsets have diverse impacts on plaque stability and, thus, may participate in pathology progression or regression. Stable plaques are associated with disease regression, as they are not exposed to rupture. Rupture of unstable plaques results in cells that make up the lesion, blocking an artery and, subsequently, restricting blood flow. M1 and M4 macrophages promote atherogenesis mainly due to inflammatory cytokine expression. M2, M(Hb), and Mhem subtypes contribute to pathology regression by exerting anti-inflammatory effects. The Mox phenotype can exert both proatherogenic and antiatherogenic impacts on lesions depending on microenvironmental conditions. Therefore, understanding the inducers and markers of macrophage phenotypes as well as their distribution in atherosclerotic lesions will be beneficial in the development of new therapies and treatments. Targeting proinflammatory mediators or altering macrophage polarization towards antiatherogenic subtypes by modulating microenvironmental stimuli may be a productive future approach in atherosclerosis therapy.

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